Systematics, evolutionary distinctiveness, and conservation priority of the speciose lizard genus *Cyrtodactylus* in Vietnam

Doctoral thesis

for

the award of the doctoral degree

of the Faculty of Mathematics and Natural Sciences

of the University of Cologne

submitted by

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accepted October in the year 2025

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Abstract

Cyrtodactylus is the most diverse genus in the family Gekkonidae and ranks as the third-largest vertebrate genus in the world. In the past two decades, the number of new species has increased more than fourfold, particularly with a significant surge in new discoveries in Vietnam. However, as new species in this genus are described at a rapid rate, several taxa have been synonymized as a result of additional data. For example, *C. paradoxus* and *C. thochuensis* have been shown to be junior synonyms of *C. condorensis* and *C. leegrismeri*, respectively. This highlights the pressing need to review the taxonomic progress. Moreover, many areas in Vietnam where this group likely occurs are still poorly studied, emphasizing the necessity for additional field surveys to better understand the diversity of *Cyrtodactylus* in these regions.

Furthermore, although some phylogenetic relationships and biogeographic studies have been conducted, phylogenetic analyses have yet included all members of the genus in Vietnam. Previous studies have primarily focused on just one or two mitochondrial genes (COI and/or ND2). Additionally, since the beginning of the dissertation, only one study has been carried out that provides information on the population status and main anthropogenic threats to one threatened species, and none of the *Cyrtodactylus* species, especially the most threatened taxa, have been investigated for their microhabitat preferences. Therefore, we conducted this study to (1) investigate the taxonomic status of described *Cyrtodactylus* species in Vietnam and discover new taxa (2) recover phylogenetic relationships, evolutionary processes, and biogeographic history of *Cyrtodactylus* and (3) assess ecological traits and population status of two endemic and critically endangered species.

The results of the dissertation show that *C. thuongae* and *C. rufford* are junior synonyms of *C. dati* and *C. lomyenensis*, respectively. During four years of this study, seven new species have also been described and at least ten new lineages have been discovered. Our study reveals that the *C. angularis* and *C. chauquangensis* are monophyletic groups and several lineages within these groups diverged during the Miocene era when the East Asia monsoon was developed and increased precipitation in the region. Biogeographic analysis suggests that the Mekong Lowlands is likely the ancestral area of the *C. angularis* group while *C. chauquangensis* might have originated from the Northwest Uplands (including northern Laos and part of northern Vietnam).

Our ecological analyses confirm that *C. takouensis* is a granite specialist. In contrast, *C. gialaiensis* likely has a broader distribution across the Gia Lai District rather than

restricted to a single locality. It has had to adapt to various habitats, including soil cliffs, tree trunks, and shrubs found between coffee plantations and roads, as well as within coffee plantation areas. Recognizing the significance of demographic information for species conservation, population monitoring studies were conducted to estimate the population size of two Vietnamese *Cyrtodactylus* species, namely, *C. takouensis* and *C. gialaiensis*. According to the study results, their population size and density are extremely small and negatively correlated with the increasing severity of human impacts. The diet of *C. nigriocularis* was also for the first time investigated in this study to support *ex situ* conservation measures.

To provide a reference for future studies, we also assess the suitability of genetic markers for molecular systematics and species identification of *Cyrtodactylus* based on twelve complete mitochondrial genomes of ten *Cyrtodactylus* species. Overall, phylogenies inferred from ND5 most frequently recovered a topology similar to those based on the 13 protein coding genes.

In conclusion, this study helps clarify issues related to the systematics and species diversity, and provide robust phylogenetic relationships, evolutionary hypotheses, and successfully reconstruct biogeographic history for two *Cyrtodactylus* groups, namely the *C*. *angularis* and *C*. *chauquangensis* groups. Together with assessments of ecology, demography and anthropogenic threats, the dissertation highlights the urgent need of conservation actions for at least two threatened species, *C*. *gialaiensis* and *C*. *takouensis*.

1 General Introduction

1.1. The Vietnamese landscape

Vietnam, with a total mainland area of about 330,591 km^2, borders with China in the North, with Laos and Cambodia in the West and with the Eastern Sea in the East (Averyanov et al. 2003; Sterling et al. 2006; Nguyen 2011; Ngo 2022). The country is characterized by mountains and hills, which cover three quarters of its territory. The mountain system extends from the northwestern border to the eastern side of the South. Two largest mountain ranges of Vietnam are the Hoang Lien Son, which runs from northwest to southeast, and the Truong Son Range (also called the Annamites), which runs along Vietnam's western border with Laos, ending in south-central Vietnam (Averyanov et al. 2003; Sterling et al. 2006). Vietnam's high peaks include Fansipan (3,143 meters above sea level), Ta Giang Pinh (3,096 m above sea level), Pu Luong (2,985 meters above sea level), and several others are in the northern and central regions of the country (Averyanov et al. 2003; Nguyen 2011, 2017). They are composed mainly of karst and granite formations (Averyanov et al. 2003; Sterling et al. 2006).

Vietnam has a diverse monsoon tropical climate. In northern Vietnam, the seasons are clearly defined: the dry and cold winter from November to April contrasts sharply with the hot, humid, and rainy summer that stretch from May to October (Averyanov et al. 2003; Sterling et al. 2006). Further south, temperatures rise, and the climate becomes less seasonal, with rainfall extending from summer into winter. Southern Vietnam experiences consistently warm temperatures year-round and heavy rainfall during the summer months (Sterling et al. 2006).

Due to its shape, topography, position, and climate, Vietnam is considered one of the world's top biodiversity hotspots, classified by the presence of high numbers of endemic and endangered species (Myers et al. 2000). Vietnam is also ranked as the 16^{th} out of 25 countries with the richest biodiversity in the world, especially in terms of reptile diversity (Mittermeier et al. 2004; van Schingen 2017). However, the biodiversity of reptiles in Vietnam remains underestimated (Nguyen et al. 2009). Many areas in the country have not been studied thoroughly, especially the northern limestone, the Truong Son Range and the Central Highlands (Sterling et al. 2006). Therefore, more surveys in the areas will certainly yield more new species for science (Sterling et al. 2006; Ngo et al. 2022).

On the other hand, its remaining biodiversity and tropical forests face enormous anthropogenic threats. These include illegal wildlife trade, habitat loss and degradation, and pollution caused by infrastructure development, tourism, and agricultural expansion (Sterling et al. 2006; van Schingen et al. 2014; van Schingen 2017; Le et al. 2020; Ngo 2022). These factors severely imperil reptile survival, particularly for threatened endemic species, even in areas designated as protected. According to the IUCN Red List, several species of reptile in Vietnam are facing exceedingly high extinction risks, as there are 21 species listed as Critically Endangered (CR), 23 as Endangered (EN), 34 as Vulnerable (VU), and 13 as Near Threatened (NT), of which 40 species are endemic to Vietnam (including six CR species, ten EN species, 19 VU species and five NT species) (IUCN 2025). However, the population status of many threatened and endemic reptile species in Vietnam has not been surveyed to develop appropriate conservation measures. As a result, there is an urgent need to fill this critical gap of knowledge to safeguard wild populations of threatened and endemic species in Vietnam.

1.2. An overview of the genus *Cyrtodactylus*

1.2.1. Taxonomy, distribution, and natural history

Cyrtodactylus Gray, 1827 is the most diverse genus of Gekkonidae and the third largest vertebrate genus on the planet, including at least 381 nominal species (Grismer et al. 2021, 2022; Uetz et al. 2025). They have a broad distribution, extending from tropical

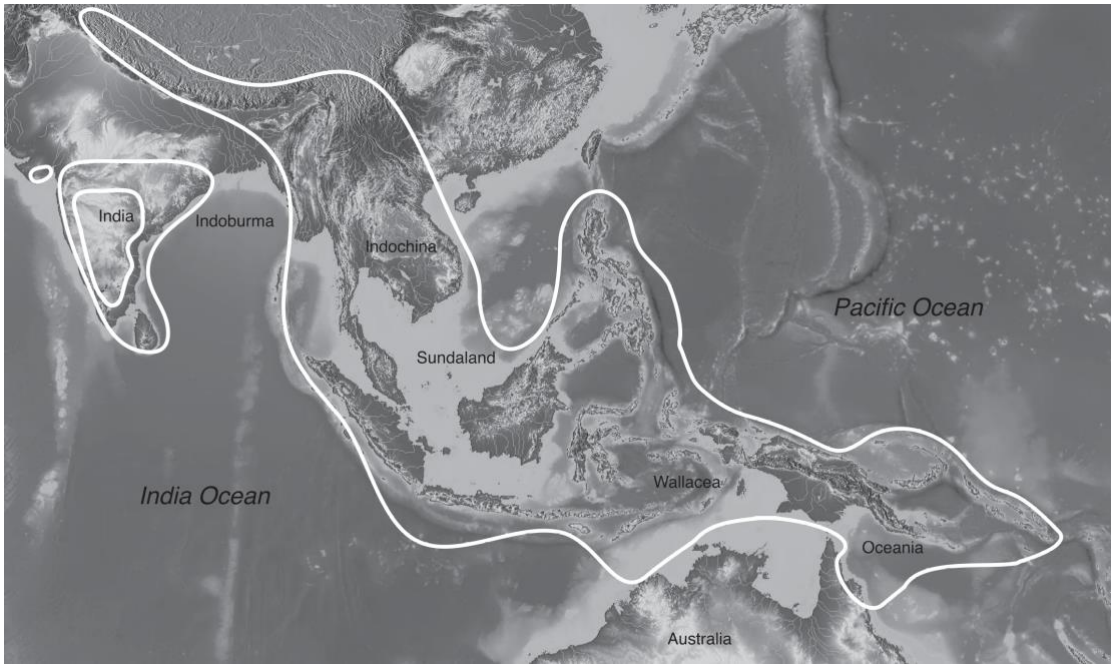

Figure 1. General distribution of the genus *Cyrtodactylus* (Grismer et al. 2021).

South Asia, southern China, Indochina, the Philippines, the Indo-Australian Archipelago, and the Solomon Islands (Wood et al. 2012; Grismer et al. 2015, 2021, 2022; Ngo et al. 2022; Riedel et al. 2024) (Fig. 1). Species in the genus can live in diverse habitat types. According to Grismer et al. (2021, 2022), species in the genus can adapt to 11 different microhabitat types, encompassing general, trunk, karst, cave, terrestrial, arboreal, granite, swamp, intertidal, volcanic and sandstone. Interestingly, several species have been observed in sympatry, e.g., *C. badenensis* and *C. nigriocularis* inhabiting granite habitat in Ba Den Mountain in Vietnam (Nguyen et al. 2006) or *C. chungi* and *C. takouensis* occur in the granite habitat in Ta Kou Mountain in Vietnam (Ostrowski et al. 2021). Even more intriguing is the situation in karst limestones in Phong Nha – Ke Bang National Park in central Vietnam, where at least three species are found broadly syntopytic (*C. cryptus, C phongnhakebangensis,* and *C. roesleri*) (Loos et al. 2012; Duong et al. 2024). Moreover, with many new species of *Cyrtodactylus* described over the last ten years, *Cyrtodactylus* is recognized as ideal group for taxonomic, biogeographic research as well as a model group for lizard evolution (Grismer et al. 2015, 2021, 2022; Ngo et al. 2022).

Vietnam has long been recognized as a hotspot of new bent-toed gecko discoveries (*e.g.,* Ziegler et al. 2010; Luu et al. 2011; Schneider et al. 2014; Nguyen et al. 2015; Luu et al. 2017; Le et al. 2021). Until 2021, 46 species have been described in the country (Uetz et al. 2021) (Fig. 2). However, several areas in the country where this group is distributed are still poorly studied. Therefore, further studies are required to provide more information about the diversity of *Cyrtodactylus* in Vietnam. In addition, many species complexes and/or cryptic species have been recognized (Nguyen et al. 2017a). For example, *C. irregularis* was split into more than 30 species (Nguyen et al. 2017a; Do et al. 2023; Ngo et al. 2024). As a result, a comprehensive taxonomic study and phylogenetic analysis of *Cyrtodactylus* in Vietnam is crucial to assess status of all recognized species.

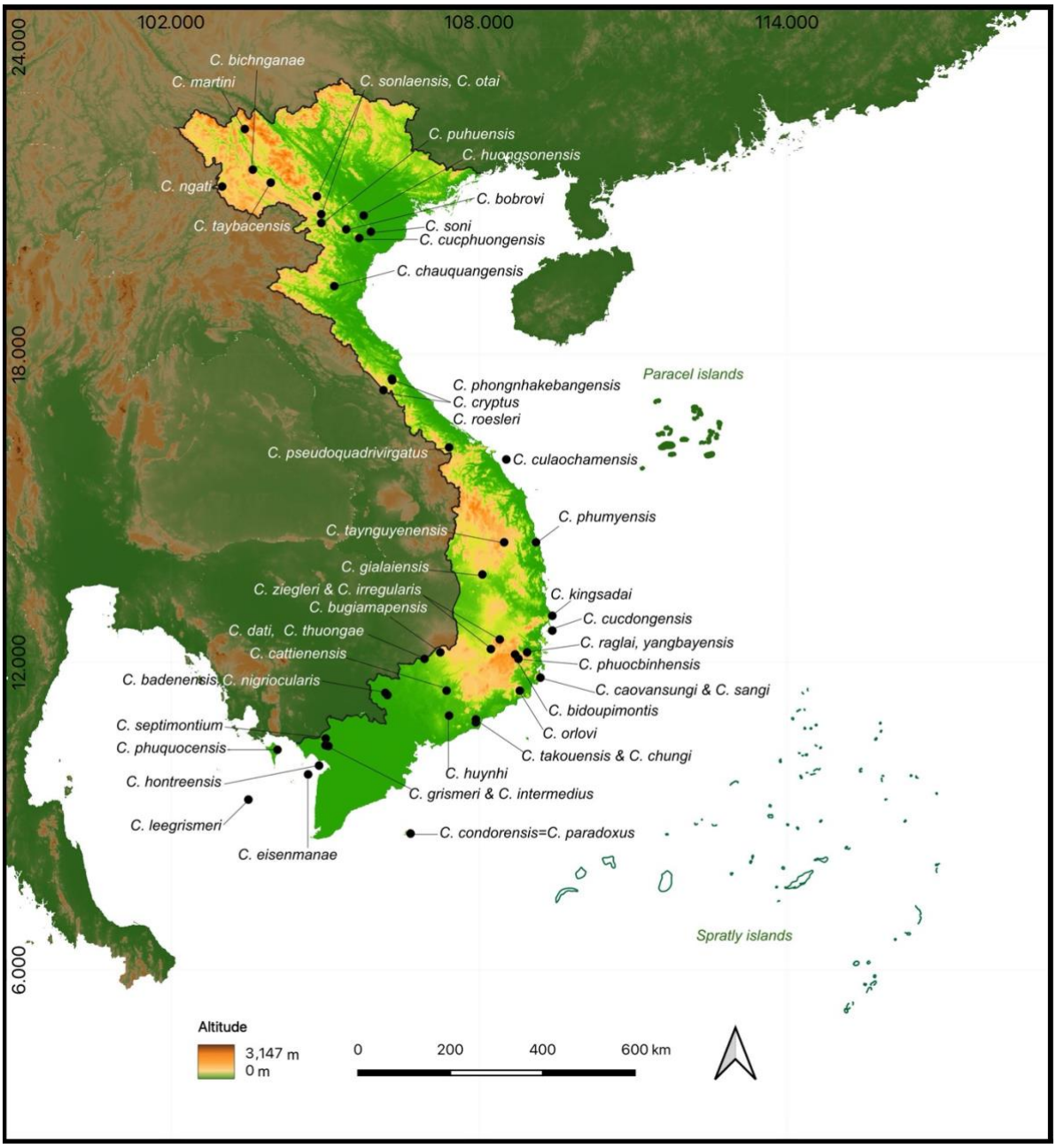

Fig. 2. Type localities of all *Cyrtodactylus* taxa occurring in Vietnam (Uetz et al. 2021).

1.2.2. Phylogeny, evolution, and biogeography

Although there have been several studies attempted to construct phylogenetic hypotheses and estimate divergence times and historical diversification of some *Cyrtodactylus* species groups *(e.g.,* Wood et al. 2012; Agarwal et al. 2014; Grismer et al. 2014; Agarwal and Karanth, 2015; Brennan et al. 2017; Nguyen et al. 2013, 2017b; Nielsen & Oliver 2018; Grismer and Davis 2018, 2020, 2021, 2022), the analyses only offered a sparse sampling of members from Vietnam. For example, although Wood et al. (2012) represented the phylogenetic relationships and biogeographic patterns that encompass all major clades of *Cyrtodactylus* across its range, their study did not

incorporate taxa from the *C. chauquangensis* group and contained only five species from Vietnam. In one of the most comprehensive works in terms of taxon sampling, Grismer et al. (2021) provided the phylogenetic partitioning of 310 species within the genus *Cyrtodactylus*. Nonetheless, this study only covered 22/45 species distributed in Vietnam (number of known species in 2021, Uet et al. 2021). Additionally, previous studies have primarily been restricted to the use of one or two mitochondrial genes (Brennan et al. 2017; Grismer et al. 2021, 2022). Therefore, to improve our understanding of the phylogenetic relationships and radiation of this genus in Vietnam, more in-depth studies are needed to clarify several issues related to biogeographic and evolutionary history of the genus in Vietnam, especially with the inclusion of additional nuclear markers.

1.2.3. Conservation status of bent-toed geckos

Cyrtodactylus is one of the most neglected vertebrate groups in terms of conservation attention since only little has been done to assess population status and main anthropogenic threats to threatened species. To date, 381 species of this genus have been discovered, but as many as 142 species, equating to approximately 37.3%, have not been assessed by the International Union for Conservation of Nature's (IUCN) Red List of Threatened species (IUCN 2025; Uetz et al. 2025). Among those assessed, 13.39% (51 species) of species have been categorized as Data Deficient, 33.07% (126 species) of species as Least Concern, 7.87% (30 species) of species as Near Threatened and Vulnerable, 4.99% (19 species) of species as Endangered, and 2.89% (11 species) of species listed as Critically Endangered (IUCN 2025). Nevertheless, current assessments have largely been based on the results of general herpetofauna surveys with a focus on their relative commonness or rarity and the coverage their habitats by protected areas (*e.g.,* Nguyen et al. 2018; Sumontha & Cota 2018a; Iskandar & Stubbs 2021; Ukuwela et al. 2021; IUCN 2025). Only few of the assessed species have been evaluated for the population status, distribution range, and habitat suitability (Luu et al. 2020; Grismer & Quah 2018; Brown & Siler 2022; IUCN 2025). Furthermore, threats have mostly been recorded by observation during the general surveys and interviews have barely been undertaken to better understand their extinction risks (*e.g.,* Sumontha & Cota 2018b; Riyanto et al. 2021; IUCN 2025). Therefore, there is a significant gap in our knowledge to design effective conservation measures. Specifically, no conservation actions have been implemented to improve the protection of threatened *Cyrtodactylus* in Vietnam.

In Vietnam, almost all *Cyrtodactylus* species have been only found at their type localities within their specialized microhabitats or isolated islands. For example, *C. takouensis* is restricted to a few small granite caves in Ta Kou Nature Reserve; *C. phuquocensis* is only recorded in Phu Quoc Island; and *C. culaochamensis* only occurs in Cu Lao Cham Island (Ngo et al. 2008, 2010, 2020; Uetz et al. 2025). Consequently, many of them are exceptionally vulnerable to anthropogenic threats, such as habitat loss and degradation. According to the IUCN Red List, in Vietnam, there are three species of *Cyrtodactylus* listed as Critically Endangered, three as Endangered and six as Vulnerable (IUCN 2025). However, only one of them (*C. gialaiensis*) has been surveyed to preliminarily estimate its population size (Luu et al. 2020). Therefore, there is a severe lack of data essential to support conservation efforts of the threatened taxa.

Furthermore, there is limited data available on the ecology and population biology of these threatened species. Until now, very few studies have been conducted to examine their microhabitat preferences or to investigate their diets (Loos et al. 2012; Luu et al. 2020). This information is crucial for understanding their ecological roles and developing effective *ex situ* conservation measures. Thus, further research is needed to better understand their microhabitats and dietary ecology. This knowledge will support future conservation breeding programs, especially if anthropogenic threats continue to significantly impact their populations.

1.3. Aim and objectives

To fill these knowledge gaps, this project attempts to elucidate systematics, evolutionary history, biogeography, and conservation priority of the genus *Cyrtodactylus* in Vietnam. It has the following objectives:

1. Investigating taxonomic status of all described *Cyrtodactylus* species in Vietnam and discover new taxa.
2. Investigating phylogenetic relationships, evolutionary and biogeographic history of *Cyrtodactylus.*
3. Study ecological traits and population status of two endemic and critically endangered species

The following hypotheses are postulated:

1. The rapid rate of *Cyrtodactylus* species discoveries in Vietnam can lead to taxonomic confusion.

2. The diversity of *Cyrtodactylus* in Vietnam is substantially underestimated.
3. Integrating mitochondrial and nuclear data leads to improved statistical nodal values, particularly for the deeper nodes in the phylogenetic analyses.
4. Asia monsoons play a major role in the evolution of the *Cyrtodactylus* genus.
5. Karst promotes speciation rate of *Cyrtodactylus* in Vietnam, as shown in other regions in Southeast Asia.
6. Natural populations of the most threatened species in Vietnam are extremely small and geographically restricted.
7. Anthropogenic threats to population of the most threatened species are caused by habitat loss and degradation.

2 Material and methods

1. Study areas and sampling

For taxonomy studies, previous field surveys were conducted during a period from 2010 – 2021 in 32 provinces of Vietnam (black dot in Fig. 3) and additional surveys between 2022 to 2024 were carried out in 10 provinces of Vietnam (red dot in Fig. 3).

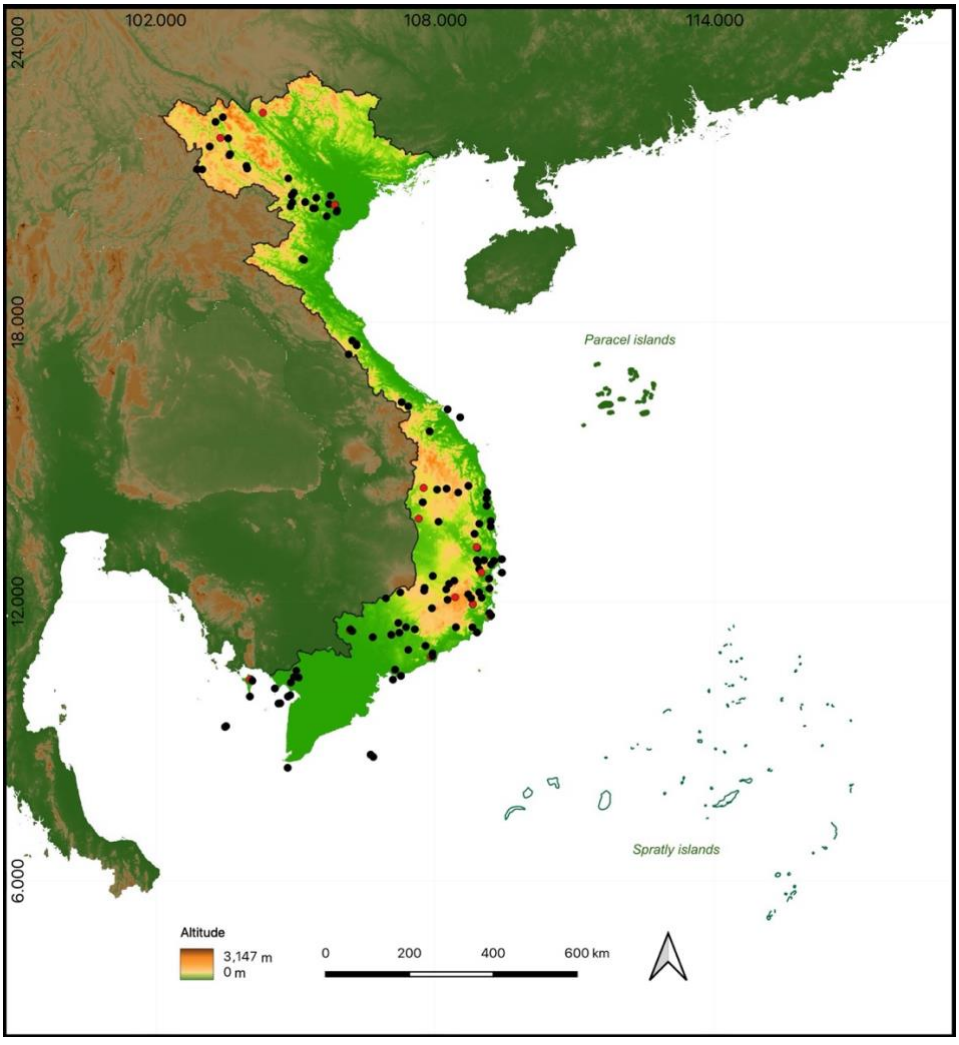

Fig. 3. Map of survey sites in Vietnam. Black and red dots denote sites visited between 2010–2021 and 2022–2024, respectively.

In total, more than 750 DNA samples of described and undescribed species were collected. Surveys were undertaken after sunset to guarantee the highest detection probability between 18:00 and 24:00. For voucher specimens, 3–5 individuals of new populations and potential candidate species were collected. Specimens were subsequently anaesthetized and fixed in approximately 85% ethanol, then later transferred to 70% ethanol for permanent storage. For molecular analysis, tissue

samples of muscle or liver or tail tissue samples were preserved in 70% ethanol (Merck, Germany).

To study population status and ecological features of two endemic and critically endangered species, field surveys were documented during both dry and rainy season in Gia Lai and Binh Thuan provinces from 2022 to 2024 (Fig. 4). At least three researchers and one local ranger or local people were conducted each survey. All geckos were captured by hand and subsequently released at the collecting site after taking photos, recording habitat characteristics, taking a measurement and marking coordinates with the Garmin (GPSmap62s in the WGS84 datum).

Fig. 4. Collecting of *Cyrtodactylus* specimens and ecological features in the field (A. L.T. Nguyen; B,C & D. H.Q. Nguyen)

2. Data analysis

2.2.1 Morphological characters

Measurements were taken with a digital caliper to the nearest 0.1 mm. Morphological characters were followed Nguyen et al. (2017b) and Do et al. (2021). Abbreviations are as follows: SVL = snout-vent length, from tip of snout to vent; TaL = tail length, from vent to tip of tail (* regenerated); HL = head length, from tip of snout to retroarticular process of jaw; HW = head width, maximum width of head; HH = head

height, from occiput to underside of jaws; OrbD = orbital diameter, greatest diameter of orbit; SnE = snout to eye distance, from tip of snout to anterior-most point of eye; EE = eye to ear distance, from anterior edge of ear opening to posterior corner of eye; NarEye = nares to eye distance, from anterior-most point of eye to posterior-most point of nostril; EarL = ear length, longest dimension of ear; ForeaL = forearm length, from base of palm to tip of elbow; CrusL = crus length, from base of heel to knee; AG = axilla- groin distance, from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; BW = body width, the widest distance of body; Internar = internarial distance, distance between nares; Interorb = Interorbital distance, shortest distance between left and right supraciliary scale rows; RW = maximum rostral width; RH = maximum rostral height; MW = maximum mental width; ML = maximum mental length.

Scale counts were taken as follows: SL = supralabials, counted from the first labial scale to corner of mouth; IL = infralabials, counted from the first labial scale to corner of mouth; N = nasal scales surrounding nare; IN = postrostrals or internasals; PM = postmentals; GST = granular scales surrounding dorsal tubercles; V = ventral scales in longitudinal rows at midbody; FP = femoral pores, number of total femoral pores on both thigh; PP = precloacal pores; PAT = postcloacal tubercles; TubR = the number of dorsal longitudinal rows of tubercles at midbody between the lateral folds; EFS = enlarged femoral scales, number of enlarged femoral scale beneath each thigh; LD1 = number of subdigital lamellae on I fingers; LD4 = number of subdigital lamellae on IV fingers; LT1 = number of subdigital lamellae on I toes; LT4 = number of subdigital lamellae on IV toes. Bilateral scale counts were given as left/right.

2.2.2 DNA extraction, amplification, and sequencing

DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Germany) or the GenJet Genomic DNA Purification kit (Thermo Fisher Scientific, Lithuania) following the manufacturer's instructions. Seven molecular markers, comprising three mitochondrial loci, cytochrome c oxidase subunit I (COI), cytochrome b (cytb), and NADH dehydrogenase subunit 2 (ND2) (including tRNA) and four nuclear loci, Cmos, phosducin (PDC), recombination activating protein 1 (Rag1), ribosomal protein L35 (Rpl35) were used in this study. PCR amplification was performed in a total volume of 21 µl that contained 2 µl template DNA, 2 µl of each primer and 10 µl DreamTaq Mastermix (Thermo Fisher Scientific, Lithuania) or HotStarTaq Mastermix (Qiagen,

Germany). The primers used to amplify the fragment's loci are listed in Table 1. The reaction was carried out with an initial denaturation at 95°C for 15 min with HotStar Taq Mastermix or 5 min with Dream Taq Mastermix, followed by 35 cycles of amplification (denaturation at 95°C for 30 s, annealing at 48°C – 58°C for 45 s, and extension at 72°C for 1 min), with final extension at 72°C for 10 min. Negative and positive controls were also used in all amplifications and extractions to check for possible contamination. The PCR products were visualized by agarose gel electrophoresis and stored at -4°C after visualization. The PCR products were purified using GeneJET PCR Purification kit (Thermo Fisher Scientific, Lithuania), in accordance with the manufacturer's instructions. The sequences of the forward and reverse strands were determined for all taxa using the sequencing service from 1st Base (Malaysia).

Table 1. PCR primers used in this study

Gene	Primer	Primer sequences (5' – 3')	Reference
COI	VF1d	TTCTCAACCAACCACAARGAYATYGG	Nazarov et al. (2012)
	VR1d	TAGACTTCTGGGTGGCCRAARAAYCA	
Cytb	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink et al. (2000)
	H16064	CTTTGGTTTACAAGAACAATGCTTTA	
ND2 + tRNA	MetF1	AAGCTTTCGGGCCCATACC	Macey et al. (1997)
	COIR1	AGRGTGCCAATGTCTTTGTGRTT	Arevalo et al. (1994)
Rpl35	N66	GCTAAACAAGCACAGAGTTGATCC	Siler et al. (2010)
	N67	TCAGGCTCAGAAAGRACTATTATGG	
Rag1	R13	TCTGAATGGAAATTCAAGCTGTT	Groth & Barrowclough (1999)
	CyrRag1	CTCCTTGTGRCTAGAAAGAT	This study
PDC	PHOF1	AGATGAGCATGCAGGAGTATGA	Bauet et al. (2007)
	PHOR1	TCCACATCCACAGCAAAAAACTCCT	
Cmos	G73	GCGGTAAAGCAGGTGAAGAAA	Sant et al. (1998)
	G74	TGAGCATCCAAAGTCTCCAATC	

2.2.3 Phylogenetic analyses

The resulting sequences were edited using Sequencher v5.4.6 (Gene Codes Corporation). Each gene was initially aligned separately using ClustalX v1.8.3 (Thompson et al. 1994, 1997; Larkin et al. 2007) with default settings for complete alignment. Phylogenetic relationships among mitochondrial and/or nuclear sequences were inferred based on partitioned and combined data matrix using maximum likelihood as implemented in IQtree v2.4.0 (Minh et al. 2020) and Bayesian analysis as implemented in MrBayes v3.2.7 (Ronquist et al. 2012). The best model of molecular evolution will be selected using the Akaike Information Criterion (AIC) as

implemented in jModeltest v2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). For combined Bayesian analysis, the optimal model determined by jModeltest with parameters estimated by MrBayes v3.2.7 was used. For partitioned analyses, best partition scheme and the best evolutionary model for each partition were selected for the phylogenetic analyses using the program PARTITIONFINDER v.2.1.1 (Lanfear et al. 2016). Two simultaneous analyses with four Markov chains (one cold and three heated) were run for 10^7 generations with a random starting tree and sample every 1,000 generations. Log-likelihood scores of samples points was plotted against generated time to determine stationarity of Markov chains. Tree generated prior to stationarity will be removed from the final analyses using the burn-in function. For ML analysis, 10,000 ultrafast bootstrap replications (UFBP) were run. We regard BP $\geq$ 70% and UFB $\geq$ 95 and PP $\geq$ 0.95 as strong support (Hillis and Bull 1993; Ronquist et al. 2012; Hoang et al. 2018). We considered nodes with UFB and PP values of 90–94 or 0.90–0.94 as well-supported.

2.2.4 Divergence time estimation

Three mitochondrial and four nuclear genes of each sample for each species were used to estimate the divergence time of the *Cyrtodactylus* group using a Bayesian MCMC approach to co-estimate topology, substitution rates and node ages as implemented in BEAST v2.7.6 (Drummond et al. 2012; Bouckaert et al. 2019). BEAUti was used to set criteria for the analysis, in which the substitution models were unlinked, but the molecular clock and trees were linked for each gene partition. A Yule tree prior model was implemented in the analysis, with rate variation across branches assumed to be uncorrelated and lognormally distributed (Drummond et al. 2006). The dating analysis was run for 200,000,000 generations, with sampling every 1,000 generations. After the dataset with the above settings was analyzed in BEAST, the resulting likelihood profile was then examined by the program Tracer v1.7.2 (Rambaut et al. 2018) to confirm the ESS > 200 for all parameters. The final tree with calibration estimates was computed using the program TreeAnnotator v2.7.6 as recommended by the program manual.

For the fossil calibrations, we followed the strategy adopted by Wood et al. (2012) and Grismer et al. (2022). Two calibration point was selected. The substitution models applied to the data matrix were determined using jModeltest v2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012).

2.2.5 Inferring historical biogeography

Considering the areas of endemism and tectonic history of region and subregions suggested by Bain and Hurley (2011), each sample was assigned to its respective area according to its contemporary distribution range. Biogeographic inferences were obtained by applying both Bayesian binary MCMC analysis (BBM) implemented in RASP v4.3 (Yu et al. 2010, 2015) and *BioGeoBears* package (Matzke 2018) implemented in R Core Team (2024). For BBM analysis, a subset of 1,000 randomly selected trees from the posterior distribution output of BEAST and a final tree from TreeAnnotator v2.7.4 were used. The probability of dispersal between areas was maintained as equal. For the BioGeoBears, all biogeographic models, dispersal-vicariance (DIVA); dispersal-extinction-clado-genesis (DEC); and Bayesian analysis of biogeography when the number of areas is large (BayArea), with and without the jump dispersal parameter (j) was tested. Afterward, the best-fit model for the data was applied to reconstruct the time-calibrated biogeographic of *Cyrtodactylus* group.

2.2.6 Population estimation

A “capture-mark-recapture” method was applied. In particular, each new individual was marked with a permanent pen to identify recaptured ones in each monthly seasonal survey. Each survey transect was visited in intervals of at least twice. In case of a one-time mark and one recapture event, the “Petersen-Lincoln Index” was used following the formula: $P = (n_1 \times n_2)/m_2$, whereof P is the estimated population size, n_1 is marked individuals re-leased, n_2 is the size of the second sample, m_2 is marked animal’s recapture. In case of a one-time mark and at least two recapture events, a method of “Schnabel Index” was applied to estimate the population size ($\widehat{N}$) with following the formula (Lincoln 1930; Schnabel 1938; Petersen 1896; Allendorf et al. 2013):

$$\widehat{\mathrm{N}} = \frac{\sum_{\mathrm{i}=1}^{\mathrm{t}}(\mathrm{C_i M_i})}{\sum_{\mathrm{i}=1}^{\mathrm{t}} \mathrm{R_i}}$$

Variance

$$s^2\left(\frac{1}{\widehat{N}}\right) = \frac{\sum_{i=1}^{t} R_i}{\left(\sum_{i=1}^{t}(C_i M_i)\right)^2}$$

Standard error of

$$s_{\bar{x}}\left(\frac{1}{\widehat{N}}\right) = \sqrt{s^2\left(\frac{1}{\widehat{N}}\right)}$$

Whereof M_i is the total number of previously marked animals at time i, C_i is the number of animals caught at time i, and R_i is the number of marked animals caught at time i.

A 95% confidence limits on ΣR_i was also obtained to reduce the bias as follows (Crow et al. 1959; Krebs 2015):

$$\text{Lower 95\% confidence limit} = \frac{\sum(C_i M_i)}{\sum R_i} \qquad \text{Upper 95\% confidence limit} = \frac{\sum(C_i M_i)}{\sum R_i}$$

2.2.7 Microhabitat characterization

Regarding microclimatic parameters, the air temperature (°C) and relative air humidity (%) were measured with a digital thermometer (TFA Dostmann/Wertheim Kat. No. 30.5015, Germany) at each location where animals were captured. A digital infrared thermometer (Mestek, China) was also used to measure temperatures (°C) at the substrate surface and at the ventral body surface of animals. Moreover, two HOBO pendant dataloggers temperature (HOBO, Germany) and one Elitech RC-51H USB temperature humidity data logger, 3200 (Elitech, UK) were also used to record twice per day of the air temperature (°C) at the transects where animals were found.

Other microhabitat characteristics were also obtained: substrate type (classified as dead leaves, branch, dead wood, rock, root, soil, trunk), whereof rocky surface (classified as bare, moss and lichen, root), position (outside or inside rocky cave/crevice), canopy (percentage of vegetation coverage above each animal – estimated by direct observation), substrate condition (dry or wet), substrate angle (between the substrate surface axis and the horizontal axis, ranging from 0° to 180°) and the elevation of captured positions using the GPS. The animal status (hanging or standing), activity (resting, feeding or moving), and the encountered time was further documented.

2.2.8 Statistical analysis

Statistical analysis was performed using Rv4.4.3 (R Core Team 2024). A Chi^2 test was applied to test the difference in population structure between dry and rainy seasons. Given microhabitat characteristics, six categorical variables (i.e. substrate type, rocky surface, position, substrate condition, animal status and activity) were also seasonally compared using Chi^2 tests, and other microhabitat traits (i.e. canopy, air temperature, substrate temperature, animal temperature and humidity) was checked the normal distribution using Shaprio's tests and tested differences by Wilcoxon tests. For all these tests, a significant difference was declared with p-value ($p < 0.05$).

A Multiple Factor Analysis (MFA) was further performed to illustrate the spaces of ecological niche between dry and rainy seasons, using a microhabitat data set of four

qualitative groups, including “substrate” (e.g. substrate type and rocky surface), “position” (e.g. animal status and position), “status” (activity) and “substrate surface” (e.g. substrate condition) together with three quantitative groups, including “humidity”, “temperature” (e.g. air and substrate temperatures) and “canopy”. This ordination test was performed using the packages ‘*Factoextra*’ and ‘*FactoMinerR*’ in the software Rstudio (Le et al. 2008; Kassambara and Mundt 2020). T-tests were further applied to test differences in these Dim values between the dry and rainy seasons.

2.2.9 Threat assessment

To evaluate impacts of human activities on the *Cyrtodactylus* species in Vietnam, the local markets were visited, and local people and rangers were also interviewed to identify the local use of the species. Surveys in the daytime and nighttime were also carried out to obtain evidence of human activities (such as traps, deforestation, direct-burning incense and rubbish within transects surrounding areas). Additionally, direct effects on the species were collected during observation in the field surveys.

3. Results

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Chapter 1. Another new species of the Cyrtodactylus (Squamata: Gekkonidae) from Binh Dinh Province, south-central Vietnam

Zootaxa 5446 (1): 105–120
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5446.1.6
http://zoobank.org/urn:lsid:zoobank.org:pub:F78635F8-6BE6-4F5B-BB7C-13BF84E8E4AA

Another new species of *Cyrtodactylus* (Squamata: Gekkonidae) from Binh Dinh Province, south-central Vietnam

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Abstract

We describe a new species of the *Cyrtodactylus irregularis* complex based on six adult specimens from Phu Cat District, Binh Dinh Province, Vietnam. *Cyrtodactylus binhdinhensis* **sp. nov.** is morphologically distinguished from the remaining congeners of the *C. irregularis* group by a combination of the following characteristics: Size medium (SVL up to 80.4 mm); nasal scales 4; internasal single; ventral scales in 39–42 longitudinal rows at midbody; ventrolateral folds present or absent without interspersed tubercles; precloacal pores 6 or 7 in males; 5 or 6 enlarged femoral scales on each thigh; femoral pores 10 in males; postcloacal tubercles 2–4; lamellae under toe IV 18–21; dorsal pattern consisting of slightly clear transverse banding formed by shaped dark brown bands, a continuous neckband with U-shape or triangle shape in the middle, dorsal head surface with small dark brown blotches; subcaudal scales transversely enlarged. In the phylogenetic analyses, the new species is recovered as a sister taxon to *C. badenensis* with approximately 15.34–16.15% genetic divergence between the two species based on a fragment of the COI gene.

Key words: *Cyrtodactylus binhdinhensis* **sp. nov.**, COI, molecular phylogeny, morphology, Binh Dinh Province

Introduction

The *Cyrtodactylus irregularis* group was originally considered to comprise only one taxon, namely *Gymnodactylus peguensis* var. *irregularis* Smith, 1921. However, it is currently recognized as the most speciose group within

Accepted by A. Bauer: 22 Mar. 2024; published: 29 Apr. 2024

the megadiverse genus *Cyrtodactylus* with at least 29 known species, including *C. badenensis* Nguyen, Orlov & Darevsky, 2006 and *C. buchardi* David, Teynié & Ohler, 2004 (Grismer *et al.* 2021, Ngo *et al.* 2022). Phylogenetically, the *C. irregularis* group is shown to be a sister to the *C. condorensis* group (which comprises *C. condorensis* Smith, 1921; *C. eisenmanae* Ngo, 2008; *C. grismeri* Ngo, 2008 and *C. leegrismeri* Chan & Norhayati, 2010) based on mitochondrial genes (ND2 or COI) or a combination of mitochondrial and nuclear markers (ND2, Rag1, Mxra5 and PDC) (Grismer *et al.* 2021, Ngo *et al.* 2022). Members of the *C. condorensis* group are distributed from Vietnam's Mekong region towards the Con Dao Islands in the East and across several islands in the Gulf of Thailand, Thailand, to Peninsular Malaysia in the South, whereas species of the *C. irregularis* group are found in central and southern Vietnam, eastern Cambodia, and southeastern Laos (Chan & Norhayati 2010; Nazarov *et al.* 2012; Nazarov *et al*. 2014; Nguyen *et al.* 2013; Grismer & Grismer 2017; Nurngsomsri *et al.* 2019; Neang *et al.* 2020; Ostrowski *et al.* 2020; Grismer *et al.* 2021; Ngo *et al*. 2022). Since 2013, several poorly studied areas in Laos and Vietnam where the *C. irregularis* group is recorded have been surveyed, resulting in the discovery of more than 21 new forms (Grismer *et al.* 2021; Ngo *et al.* 2022). However, there remain several little-known areas in Vietnam and Laos.

During our field research in Phu Cat District, Binh Dinh Province, a new *Cyrtodactylus* population was detected. It was provisionally assigned to *C. irregularis* because of the similar dorsal pattern (Nguyen *et al.* 2017; Grismer *et al.* 2021). More detailed investigation revealed that members of the population were distinct from this and other species in the genus genetically and morphologically. It is therefore described as a new species in the following.

Materials and methods

Sampling. Field surveys were conducted in Phu Cat District, Binh Dinh Province, in August 2016 as well as in May and June 2022 in Ninh Thuan and Binh Thuan provinces, Vietnam. Specimens were anesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmon 2002), fixed in 85% ethanol for six hours and subsequently stored in 70% ethanol for long-term preservation. Specimens were subsequently deposited in the collections of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Molecular data and phylogenetic analyses. DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer's instructions. Extracted DNA was amplified by PCR mastermix (Qiagen, Germany) with 21 µl volume (including 10 µl of mastermix, 5 µl of water, 2 µl of each primer at 10pmol/ml, and 2 µl of extracted DNA). PCR condition was: 95°C for 15 minutes to activate the taq; with 35 cycles at 95°C for 30 seconds, 45°C for 45 seconds, 72°C for 60 seconds; and the final extension at 72°C for 6 minutes. A fragment of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), was amplified using the primer pair VF1d (5'-TTCTCAACCAACCACAARGAYATYGG-3') and VR1d (5'-TAGACTTCTGGGTGGCCRAARAAYCA-3') (Ivanova *et al.* 2006). PCR products were visualized using electrophoresis through a 2% agarose gel stained with ethidium bromide. Successful amplifications were purified to eliminate PCR components using GeneJet™ PCR Purification kit (ThermoFisher Scientific, Lithuania). Purified PCR products were sent to FirstBase (Malaysia) for sequencing in both directions.

After sequences were aligned by Clustal X v2 (Thompson *et al.* 1997), data were analyzed using maximum likelihood as implemented in IQ-TREE v1.6.12 (Nguyen *et al.* 2015), maximum parsimony (MP) as implemented in PAUP*4.0b10 (Swofford 2001) and Bayesian inference (BI) as implemented in MrBayes v3.2.7 (Ronquist *et al.* 2012). For ML analysis, we used IQ-TREE v1.6.12 using a single model and 10,000 ultrafast bootstrap replications (UFB). The optimal model for nucleotide evolution was determined using jModeltest v2.1.10 (Darriba *et al.* 2012). For MP analysis, heuristic analysis was conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch-swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) was calculated using 1000 pseudo-replicates and 100 random taxon addition replicates. All characters were equally weighted and unordered.

For Bayesian analyses, we used the optimal model determined by jModelTest with parameters estimated by MrBayes 3.2.7. Two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10 million generations with a random starting tree and sampled every 1000 generations. Log-likelihood scores of sample points were plotted against generation time to determine stationarity of Markov chains. Trees generated before log-likelihood scores reached stationarity were discarded from the final analyses using the burn-in function. The posterior probability values (PP) for all nodes in the final majority rule consensus tree

were provided. We regard BP ≥ 70% and UFB and PP of ≥ 95% as strong support and values of <70% and < 95%, respectively, as weak support (Hillis & Bull 1993; Minh *et al.* 2013; Ronquist *et al.* 2012). The optimal model for nucleotide evolution was set to GTR+I+G for ML and BI analyses as selected by jModelTest. The cut-off point for the bur-in function was set to 60 in the Bayesian analysis, as -lnL scores reached stationarity after 60,000 generations in both runs. Uncorrected pairwise divergences were calculated in PAUP*4.0b10.

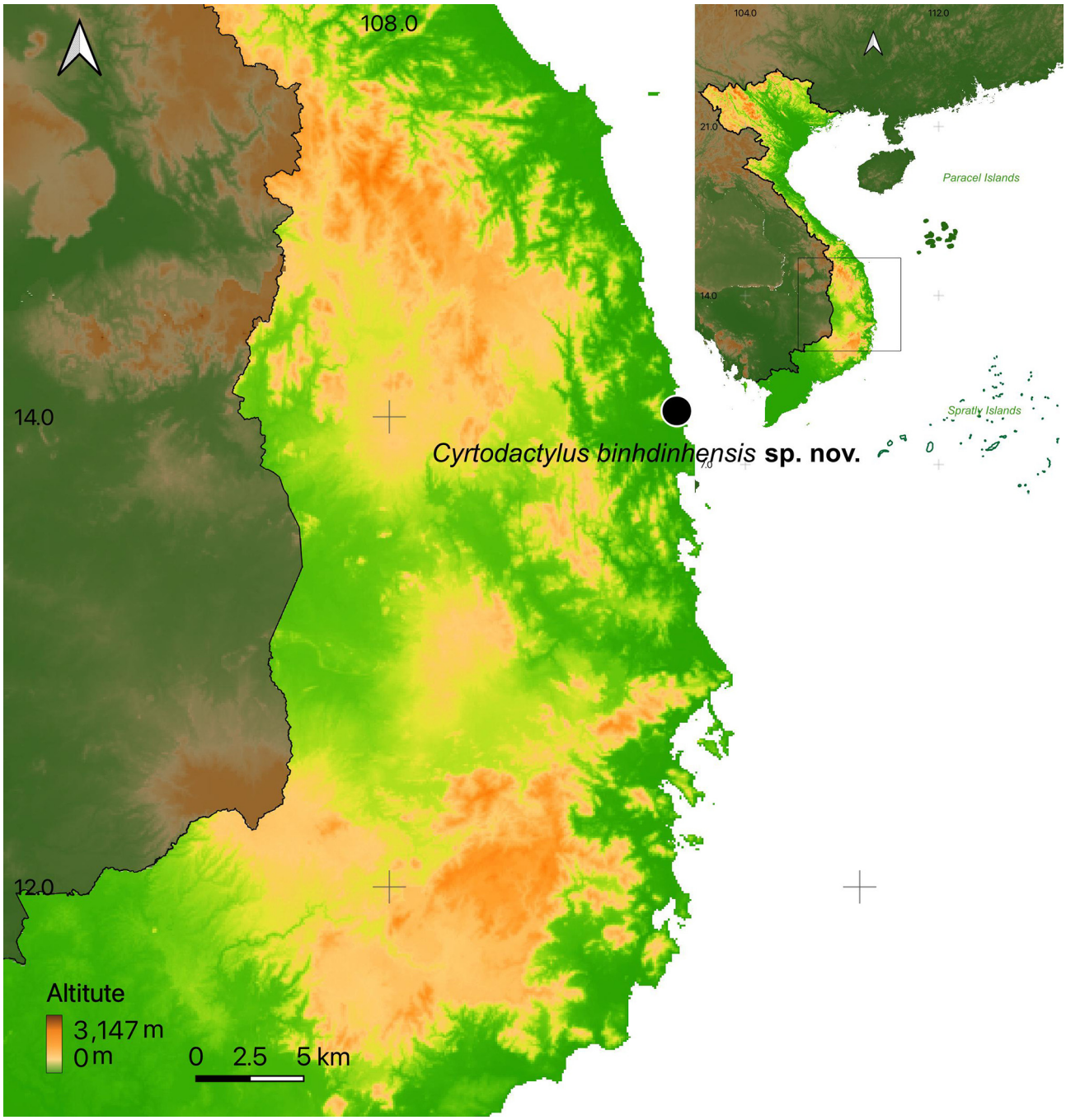

FIGURE 1. Type locality of *Cyrtodactylus binhdinhensis* **sp. nov.** in Binh Dinh Province, Vietnam.

Morphological characteristics. Measurements were taken with a digital caliper to the nearest 0.1 mm. Morphological characters were followed Nguyen *et al.* (2017) and Do *et al.* (2021). Abbreviations are as follows: snout-vent length (SVL), from tip of snout to vent; tail length (TaL), from vent to tip of tail (* regenerated); head length (HL), from tip of snout to retroarticular process of jaw; head width (HW), maximum width of head; head height (HH), from occiput to underside of jaws; orbital diameter (OrbD), greatest diameter of orbit; snout to eye distance (SnE), from tip of snout to anterior-most point of eye; eye to ear distance (EE), from anterior edge of ear

opening to posterior corner of eye; nares to eye distance (NarEye), from anterior-most point of eye to posterior-most point of nostril; ear length (EarL), longest dimension of ear; forearm length (ForeaL), from base of palm to tip of elbow; crus length (CrusL), from base of heel to knee; axilla-groin distance (AG), from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; body width (BW), the widest distance of body; internarial distance (Internar), distance between nares; Interorbital distance (Interorb), shortest distance between left and right supraciliary scale rows; maximum rostral width (RW); maximum rostral height (RH); maximum mental width (MW); maximum mental length (ML).

Scale counts were taken as follows: supralabials (SL), counted from the first labial scale to corner of mouth; infralabials (IL), counted from the first labial scale to corner of mouth; nasal scales surrounding nare (N); postrostrals or internasals (IN); postmentals (PM); granular scales surrounding dorsal tubercles (GST); ventral scales in longitudinal rows at midbody (V); femoral pores (FP), number of total femoral pores on both thigh; precloacal pores (PP); postcloacal tubercles (PAT); the number of dorsal longitudinal rows of tubercles at midbody between the lateral folds (TubR); enlarged femoral scales (EFS), number of enlarged femoral scale beneath each thigh; number of subdigital lamellae on I fingers (LD1); number of subdigital lamellae on IV fingers (LD4); number of subdigital lamellae on I toes (LT1); number of subdigital lamellae on IV toes (LT4). Bilateral scale counts were given as left/right.

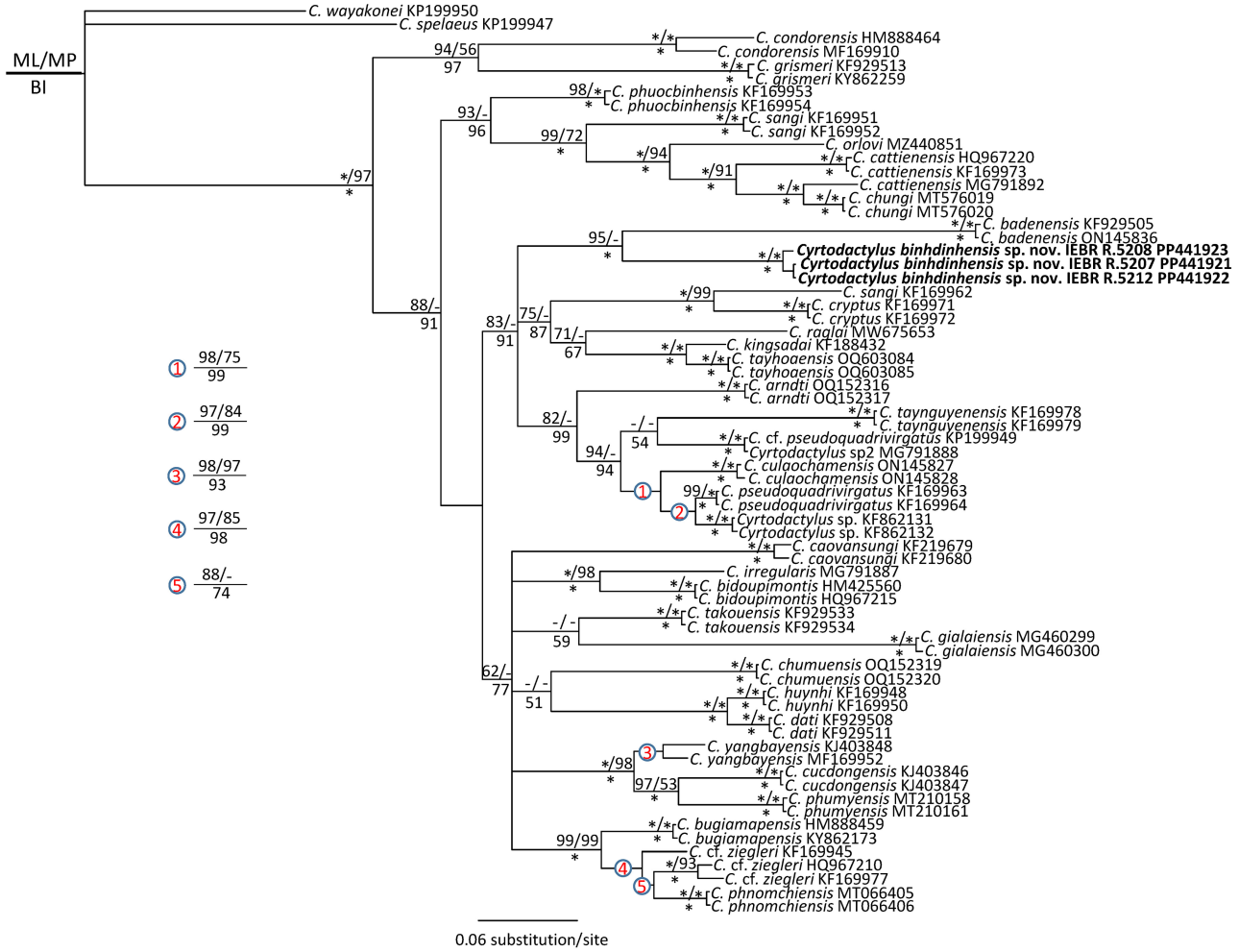

FIGURE 2. Phylogram based on the Bayesian analysis. Number above and below branches are ML/MP bootstrap and ultrafast bootstrap values and Bayesian posterior probabilities, respectively. Dashes represent bootstrap values < 50% and asterisk denotes 100% value.

Results

Phylogenetic analyses. The final matrix contained 652 aligned characters with 259 parsimony informative. MP analysis of the dataset recovered two most parsimonious trees with 1938 steps (Consistency index = 0.25; Retention

index = 0.62). Although the taxon from Phu Cat District, Binh Dinh Province showed a minimal genetic distance of 12.17–12.36% from *C. takouensis* Ngo & Bauer, 2008, this new population was well corroborated as a sister taxon to *C. badenensis* with strong support values from ML and BI analyses (UFB = 95%; PP = 100%) (Fig. 2). Genetically, the unnamed taxon was diverged from *C. badenensis* by approximately 15.34–16.15% based on a fragment of COI (Supplementary data). We, therefore, hypothesize that the new population from Phu Cat District, Binh Dinh Province constitutes a new species and describe it below.

***Cyrtodactylus binhdinhensis* sp. nov.**
(Figs. 3–5)

Holotype. IEBR R.5207 (Field number BD2016.33), adult male, collected by Dang Trong Do and Tan Van Nguyen on 9 August 2016 (14°01.495′ N, 109°13.353′ E; 150 m a.s.l.), Cat Hai Commune, Phu Cat District, Binh Dinh Province.

Paratypes. IEBR R.5208 (Field number BD2016.34), adult male and IEBR R.5209–5212 (Field number BD.2016.35-2016.37, BD.2016.39), adult females, the same collection data as the holotype.

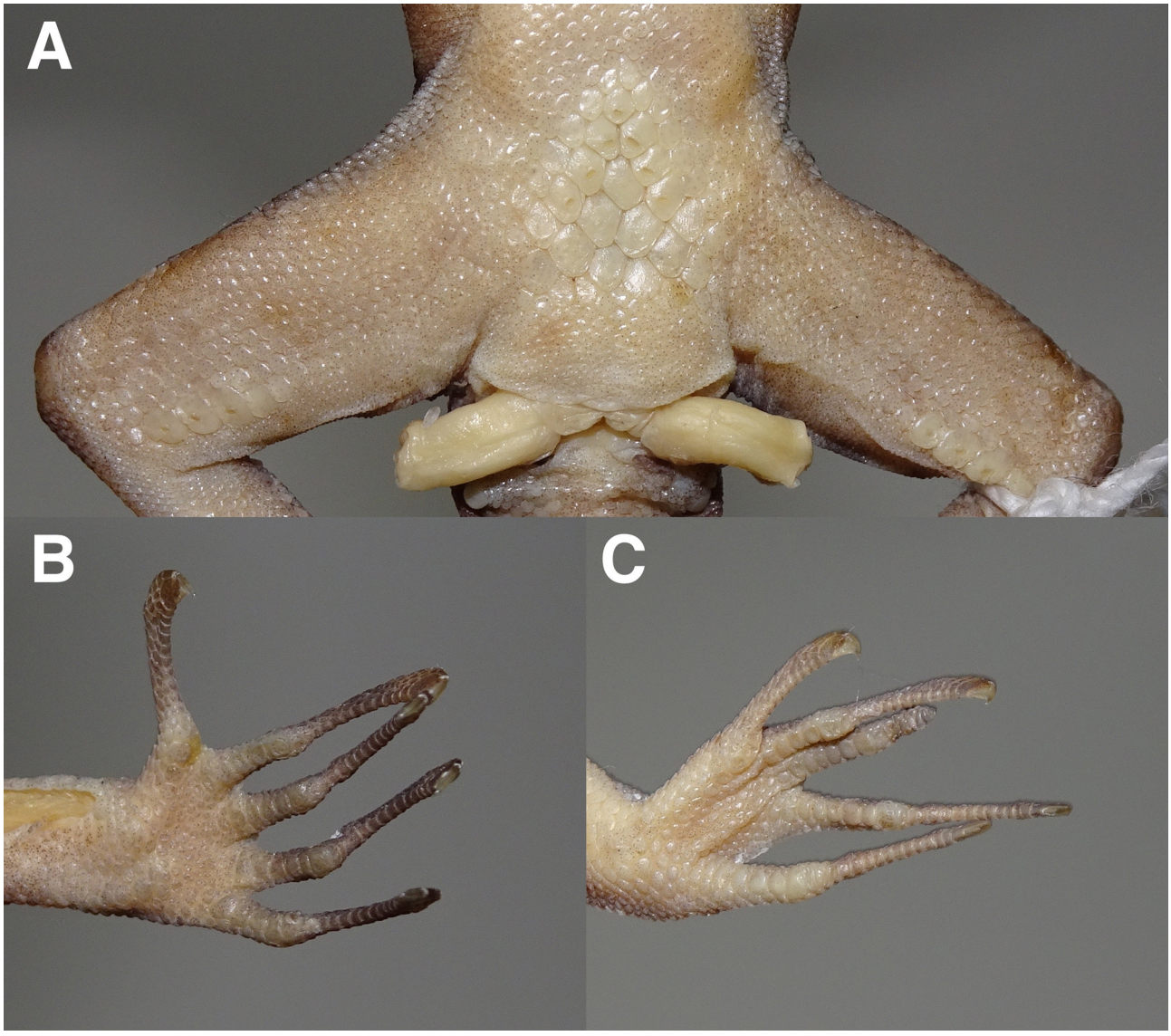

FIGURE 3. The male holotype of *Cyrtodactylus binhdinhensis* **sp. nov.** (IEBR R.5207) in preservative. A) Precloacal region with precloacal pores, B) Subdigital view of left hand, and C) Subdigital view of left foot. Photos: T. Ziegler

Diagnosis. The new species can be distinguished from other members of the genus *Cyrtodactylus* by a combination of the following characteristics: Size medium (SVL up to 80.4 mm); nasal scales 4; internasal single; ventral scale in 39–42 longitudinal rows at midbody; ventrolateral folds present or absent without interspersed tubercles; precloacal pores 6 or 7 in males; 5 or 6 enlarged femoral scales on each thigh; femoral pores 10 in males; postcloacal tubercles 2–4; lamellae under toe IV 18–21; dorsal pattern consisting of slightly clear transverse banding formed by shaped dark brown bands, a continuous neckband with U-shape or triangle shape in the middle, dorsal head surface with small dark brown blotches; tail with ½ or 1/3 scales subcaudals distinctly enlarged in the middle.

Description of holotype. Adult male, snout-vent length (SVL) 80.0 mm; body elongate (AG/SVL 0.46); head distinct from neck, elongate, depressed (HL/SVL 0.29, HW/HL 0.65, HH/HL 0.40); eye medium (OrbD/HL 0.23), pupils large and vertical; upper eyelid fringe with spinous scales; ear opening below the postocular stripes, obliquely directed and oval, small in size (EarL/HL 0.1); nares oval, surrounded by supranasal, rostral, fist supralabial, nasorostral and three postnasals; supranasals separated from each other by one internasal; loreal region and frontal concave; snout medium (SnE/HL 0.40), round, longer than diameter of orbit (OrbD/SnE 0.58); supralabials 12/10; infralabials 9/9.

Dorsal scales granular; dorsal tubercles round, four or five times larger than the size of adjoining scales, each surrounded by 10 or 11 granular scales; tubercles forming 18 irregular longitudinal rows at midbody; ventral scales smooth, medial scales three or four times larger than dorsal granules, in 41 longitudinal rows at midbody, lateral folds present in the right side and unclear in the left side, without interspersed tubercles; seven precloacal pores arranged in a chevron; 6/5 enlarged femoral scales beneath thigh, bearing 10 pores in total (5 on each thigh, three pores and two pitted femoral scales in the right side).

Fore and hind limbs moderately slender (ForeaL/SVL 0.14, CrusL/SVL 0.17); dorsal surface of forelimbs covered by few slightly developed tubercles with round shape, keeled, two times larger than the size of adjoining scales; dorsal surface of hind limbs covered by slightly developed tubercles with round shape, three or four times larger than the size of adjoining scales; fingers and toes lacking distinct webbing; subdigital lamellae: finger I 12/12, finger IV 19/17, toe I 12/13, toe IV 20/19 (left/right).

Tail regenerated, 59.4 mm in length; postcloacal tubercles 3/3; original part of the tail with 1/3 scales subcaudals distinctly enlarged in the end.

Coloration in life. Ground color brown; dorsal surface of head light brown with dark brown blotches, circle, oval and arched shape; eyelids yellow; nuchal loop dark brown, extending from posterior corner of eye to the neck, continuous with U-shape in the middle; dorsum with three or four dark brown irregular transverse bands between limb insertions; tubercles on limbs, dorsum and tail light to yellowish brown; dorsal surface of fore- and hind- limbs with dark brown transverse bands; original part of tail with eight transverse dark light brown bands, dark brown bands wider than light brown interspaces, light brown marbling with dark brown spots, regenerated part dark brown; chin, throat, chest, belly and ventral side of limbs cream (Fig. 4).

Coloration in preservative. Color faded slightly in alcohol, yellow color disappeared in preservation (especially the bright yellow of the eyelids). Ground color of dorsal head, neck, body, limbs and tail greyish brown; skin above the eyes greyish; chin, throat, chest, belly and ventral surface of limbs did not change noticeably in preservation.

Sexual dimorphism and variation. The females differ from male specimens in the absence of hemipenial swelling at the tail base. For other morphological characteristics see Table 1 and Fig. 3.

Distribution. *Cyrtodactylus binhdinhensis* **sp. nov.** is currently known only from the type locality in Phu Cat District, Binh Dinh Province, Vietnam (Fig. 1).

Etymology. Specific epithet *binhdinhensis* is a toponym in reference to the type locality of the new species, Binh Dinh Province of Vietnam. For the common names, we suggest Binh Dinh Bent-toed Gecko (English) and Thạch sùng ngón bình định (Vietnamese).

Natural history. Specimens were encountered at night between 19:00 and 22:00, on granite rocks, along a small rocky stream, approximately 0.5–1.5 m above the ground, at elevations between 140 and 160 m a.s.l. The surrounding habitat was evergreen forest of medium and small hardwoods mixed with shrubs and vines (Fig. 5). The humidity was approximately 50–66% and the air temperature ranged from 28.3 to 32.5°C. Other reptile species found at the sites included *Gekko* sp.; *Scincella rufocaudata* (Darevsky & Nguyen, 1983); *Psammodynastes pulverulentus* (Boie, 1827); and *Trimeresurus albolabris* (Gray, 1842).

FIGURE 4. The female paratype of *Cyrtodactylus binhdinhensis* **sp. nov.** (IEBR R.5209) in life. Photos: D.T. Do

FIGURE 5. Micro-habitat of *Cyrtodactylus binhdinhensis* **sp. nov.** in Phu Cat District, Binh Dinh Province. Photo: D.T. Do

Comparisons. We compared the new species with 29 congeners from the *Cyrtodactylus irregularis* complex based on examination of specimens and data obtained from the literature (Smith 1921; Heidrich *et al.* 2007; Orlov *et al.* 2007; Nazarov *et al.* 2008; Ngo & Bauer 2008; Rösler *et al.* 2008; Geissler *et al.* 2009; Ngo & Chan 2010; Nazarov *et al.* 2012; Ngo 2013; Nguyen *et al.* 2013; Ziegler *et al.* 2013; Schneider *et al.* 2014; Luu *et al.* 2017; Pauwels *et al.* 2018; Neang *et al.* 2020; Ostrowski *et al.* 2020; Ostrowski *et al.* 2021; Nguyen *et al.* 2021; Do *et al.* 2021, 2023; Ngo *et al.* 2023).

TABLE 1. Measurements (in mm) and morphological characters of the type series of *Cyrtodactylus binhdinhensis* **sp. nov.** Bilateral meristic characters are given as left/right. Abbreviations: * = regenerated tail; max = maximum; min = minimum; other abbreviations defined in the text.

Cat No	**IEBR R.5207**	**IEBR R.5208**	**Min–Max**	**IEBR R.5209**	**IEBR R.5210**	**IEBR R.5211**	**IEBR R.5212**	**Min–Max**
Sex	Male	Male	(n = 2)	Female	Female	Female	Female	(n = 4)
SVL	80.0	58.9	58.9–80.0	80.4	73.1	71.0	67.2	67.2–80.4
TaL	59.4*	75.4	Max 75.4	*	84.7	78.0	82.5*	Max 84.7
HL	23.4	18.0	18.0–23.4	23.2	21.6	20.2	19.3	19.3–23.2
HW	15.2	11.5	11.5–15.2	16.1	14.1	13.7	12.9	12.9–16.1
HH	9.3	6.9	6.9–9.3	12.4	8.1	8.0	6.9	6.9–12.4
OrbD	5.3	4.7	4.7–5.3	5.3	5.1	4.9	5.0	4.9–5.3
SnE	9.2	7.5	7.5–9.2	9.3	9.1	8.8	8.3	8.3–9.3
EE	6.1	4.4	4.4–6.1	6.1	5.8	5.7	5.1	5.1–6.1
NarEye	7.1	6.0	6.0–7.1	7.1	7.1	6.6	6.2	6.2–7.1
EarL	2.3	1.9	1.9–2.3	2.1	1.9	1.9	1.9	1.9–2.1
ForeaL	11.4	9.3	9.3–11.4	11.3	10.7	10.6	9.8	9.8–11.3
CrusL	13.3	10.3	10.3–13.3	14.1	12.1	12.3	11.0	11.0–14.1
AG	36.4	23.2	23.2–36.4	32.3	27.1	29.9	28.0	27.1–32.3
BW	16.2	11.0	11.0–16.2	17.3	13.5	14.1	12.0	12.0–17.3
Internar	2.3	2.0	2.0–2.3	2.2	2.5	2.2	2.0	2.0–2.5
Interorb	6.2	5.9	5.9–6.2	6.3	6.9	6.5	5.5	5.5–6.9
RW	3.1	2.9	2.9–3.1	-	3.3	3.1	3.1	3.1–3.3
RH	2.4	1.3	1.3–2.4	2.4	1.7	1.6	1.6	1.6–2.4
MW	3.1	2.6	2.6–3.1	3.1	2.6	2.4	2.4	2.4–3.1
ML	2.1	2.0	2.0–2.1	2.3	2.0	1.6	1.9	1.6–2.3
SL	12/10	12/12	10–12	10/11	12/11	12/12	11/10	10–12
IL	9/9	9/9	9	10/10	10/10	10/9	9/9	9–10
N	4/4	4/4	4	4/4	4/4	4/4	4/4	4
IN	1	1	1	1	1	1	1	1
PM	2	2	2	2	2	2	2	2
GST	10/11/10	9/10/9	9–11	9/11/10	11/11/10	9/9/10	9/9/10	9–11
V	41	42	41–42	42	41	40	39	39–42
FP	5/5 (2 pitted)	5/5	5 each thigh	3/3	3/3	0/3	0/0	0–3 each thigh
PP	7	6	6–7	6	5	6	5	5–6
PAT	3/3	4/3	3–4	3/3	2/2	3/2	3/3	2–3
TubR	18	21	18–21	21	21	20	19	19–21
EFS	6/5	6/6	5–6	5/5	5/5	5/5	5/5	5
LD1	12/12	12/12	12	11/12	12/12	12/12	11/10	10–12
LD4	19/17	19/19	17–19	17/17	18/18	17/18	17/17	17–18
LT1	12/13	12/13	12–13	12/12	12/11	12/12	11/11	11–12
LT4	20/19	20/21	19–21	20/19	21/21	18/19	19/18	18–21

TABLE 2. Morphological comparisons between *Cyrtodactylus binhdinhensis* **sp. nov.** and 29 taxa of the *Cyrtodactylus irregualris* complex based on examination of specimens and data obtained from the literature (Smith 1921a; Heidrich *et al.* 2007; Orlov *et al.* 2007; Nazarov *et al.* 2008; Ngo & Bauer 2008; Rösler *et al.* 2008; Geissler *et al.* 2009; Ngo & Chan 2010; Nazarov *et al.* 2012; Ngo 2013; Nguyen *et al.* 2013; Ziegler *et al.* 2013; Schneider *et al.* 2014; Luu *et al.* 2017; Pauwels *et al.* 2018; Neang *et al.* 2020; Ostrowski *et al.* 2020; Ostrowski *et al.* 2021; Nguyen *et al.* 2021; Do *et al.* 2021; Ngo *et al.* 2023) (measurements in mm, * = regenerated or broken tail, Max = maximum, other abbreviations defined in the text).

No.	*Taxa*	SVL	TaL	V	EFS	FP (M)	FP (F)	PP (M)	PP (F)	LD4	LT4	Color pattern of dorsum	Enlarged subcaudals
1	***Cyrtodactylus binhdinhensis* sp. nov.**	**58.5–80.4**	**max 84.7**	**39–42**	**5–6**	**10**	**0–6**	**6–7**	**5–6**	**17–19**	**18–21**	**banded**	**present**
2	*C. arndti*	73.4–80.9	50.1*–91.51	26–38	5–11	0–2	0	6	6	15–20	17–22	banded	present
3	*C. badenensis*	59.3–74.1	58.6–82.4	25–28	absent	absent	absent	0	0	–	18–22	banded	present
4	*C. bidoupimontis*	74.0–86.3	75.0–86	38–43	8//10	absent	absent	4//6	0	15–20	18–23	banded	absent
5	*C. bugiamapensis*	58.6–76.8	65.3–83.0	36–46	6–10	absent	absent	7–11	0–7	15–17	17–20	blotched	absent
6	*C. buchardi*	60.0–65.0	46.0–54.0	30	absent	absent	?	9	?	14	12	blotched	absent
7	*C. caovansungi*	90.4–94.0	120.0	38–44	8	6	absent	9	0	22	23–25	banded	present
8	*C. cattienensis*	43.5–69.0	51.0–64.7	28–42	3//8	absent	absent	6//8	0	12–16	14–19	banded	absent
9	*C. chumuensis*	67.5	51.4*	43–45	4–5	0–2	?	6–7	?	16–19	17–21	banded	absent
10	*C. chungi*	66.6–68.5	62.75*–82.15	30–31	4//6	absent	absent	7	6		17–20	banded	absent
11	*C. cryptus*	62.5–90.8	63.5–88.4	47–50	absent	absent	absent	9–11	0.	18–19	20–23	banded	absent
12	*C. cucdongensis*	55.8–65.9	max. 81.3	35–44	5–9	absent	absent	5–6	4–6	13–18	15–20	banded	absent
13	*C. culaochamensis*	69.8–79.8	89.7–91.2	45–50	absent	absent	absent	7–8	absent	18–19	20–23	banded	absent
14	*C. dati*	max 70.1	max 57.3	42–48	4//7	3//4 each	?	5//6	?	?	18–19	blotched	absent
15	*C. gialaiensis*	50.1–62.8	?	38–45	Absent	absent	absent	9–10	0–8	14–15	15–17	banded	absent

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TABLE 2. (Continued)

No.	*Taxa*	SVL	TaL	V	EFS	FP (M)	FP (F)	PP (M)	PP (F)	LD4	LT4	Color pattern of dorsum	Enlarged subcaudals
16	*C. huynhi*	67.2–79.8	61.5–78.6	43–46	3–5	3–8	0–6	7–9	0–8 (pitted)	14–17	17–21	banded	absent
17	*C. irregularis*	72.0–86.0	66.0–74	38–45	7–8	absent	absent	5–7	0–6	15–16	18–19	blotched	absent
18	*C. kingsadai*	83.0–94.0	max 117	39–46	9//12	3–7	0	7//9	4//8	19–21	21–25	banded	present
19	*C. orlovi*	61.0–77.7	Max 71.2	36–39	3//8	absent	absent	5//6	0	15–17	16–19	banded	absent
20	*C. phnomchiensis*	76.1–80.7	56.9–79.1	45–54	0–8	absent	0–6	4–5	1–7 pitted	18–20	20–23	banded	absent
21	*C. phumyensis*	63.6–66.8	?	33–41	5–7	absent	absent	5–7	6 pitted	18–19	18–21	banded	absent
22	*C. phuocbinhensis*	46.0–60.4	76.1	43–47	5	absent	absent	7	0	16–21	17–19	striped/blotched	absent
23	*C. pseudoquadrivirgatus*	48.6–83.3	55.7–82.3	41–57	absent	absent	absent	5–9	5–10	15–21	16–25	blotched	absent
24	*C. raglai*	95.0–111.7	113.4–135	36–39	9–Oct	absent	absent	5	0	?	21–22	banded	present
25	*C. sangi*	49.9–56.3	47.9*	37	4	absent	absent	7	4 (Pitted)	?	?	banded	Absent
26	*C. tayhoaensis*	82.9–94.2	max 104.3	37–41	10–11	3–7	0	4–5	0	20–22	22–24	banded	present
27	*C. takouensis*	74.7–81.1	77.7–91	39–40	3//5	0–2	0	3//4	0	16–17	18–20	banded	present
28	*C. taynguyenensis*	60.0–85.0	66.0–94.0	42–49	absent	absent	absent	6	0	13–18	17–21	blotched	absent
29	*C. yangbayensis*	78.5–92.3	91.3–109.1	39–46	5//16	0–2	0	6//8	0	16–19	15–17	banded	present
30	*C. ziegleri*	84.6–93.0	95.0–107.0	33–39	8–10	0–4	0–6	5–8	0–8	16–19	18–21	banded	absent

Among the species of the *Cyrtodactylus irregularis* group, *Cyrtodactylus binhdinhensis* **sp. nov.** differs from *C. arndti* Ngo, Hormann, Le, Pham, Phung, Do, Ostrowski, Nguyen & Ziegler, 2023 by having more ventral scale rows (39–42 *vs*. 26–38 in *C. arndti*), more femoral pores in males (10 *vs.* 0–2 in *C. arndti*), and dark brown transverse bands of the tail wider than light brown interspaces (*vs.* narrower transverse bands than light interspaces in *C. arndti*); from *C. badenensis* by having more ventral scale rows (39–42 *vs*. 25–28 in *C. badenensis*), the presence of enlarged femoral scales (5–6 *vs*. absent in *C. badenensis*), the presence of femoral pores in males (10 *vs*. absent in *C. badenensis*), and the presence of precloacal pores in males (6 or 7 *vs*. absent in *C. badenensis*); from *C. bidoupimontis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler, 2012 by having fewer enlarged femoral scales (5 or 6 *vs*. 8–10 in *C. bidoupimontis*), the presence of femoral pores in males (10 *vs*. absent in *C. bidoupimontis*), and the presence of transversely enlarged subcaudal plates (*vs*. absent in *C. bidoupimontis*); from *C. bugiamapensis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler, 2012 by having the presence of femoral pores in males (10 *vs*. absent in *C. bugiamapensis*), the presence of transversely enlarged subcaudal plates (*vs*. absent in *C. bugiamapensis*), and the difference of dorsal color pattern (banded *vs*. blotched in *C. bugiamapensis*); from *C. buchardi* by having more ventral scale rows (39–42 *vs.* 30 in *C. buchardi*), the presence of enlarged femoral scales (5 or 6 *vs.* absent in *C. buchardi*), the presence of femoral pores in males (10 *vs.* absent in *C. buchardi*), fewer precloacal pores in males (6 or 7 *vs.* 9 in *C. buchardi*), more lamellae under finger IV (17–19 *vs.* 14 in *C. buchardi*), under toe IV (18–21 *vs*. 12 in *C. buchardi*), and the difference of dorsal color pattern (banded *vs*. blotched in *C. buchardi*); from *C. caovansungi* Orlov, Nguyen, Nazarov, Ananjeva & Nguyen, 2007 by having a smaller size (58.5–80.4 mm *vs.* 90.4–94 mm in *C. caovansungi*), fewer enlarged femoral scales (5 or 6 *vs*. 8 in *C. caovansungi*), more femoral pores in males (10 *vs*. 6 in *C. caovansungi*), fewer precloacal pores in males (6 or 7 *vs.* 9 in *C. caovansungi*), and fewer lamellae under finger IV (17–19 *vs.* 22 in *C. caovansungi*) and toe IV (18–21 *vs*. 23–25 in *C. caovansungi*); from *C. cattienensis* Geissler, Nazarov, Orlov, Böhme, Phung, Nguyen & Ziegler, 2009 by the presence of femoral pores in males (10 *vs.* absent in *C. cattienensis*), having more lamellae under finger IV (17–19 *vs.* 12–16 in *C. cattienensis*), and the presence of transversely enlarged subcaudal plates (*vs*. absent in *C. cattienensis*); from *C. chumuensis* Ngo, Hormann, Le, Pham, Phung, Do, Ostrowski, Nguyen & Ziegler, 2023 by having fewer ventral scale rows (39–42 *vs*. 43–45 in *C. chumuensis*), more femoral pores in males (10 *vs.* 0–2 in *C. chumuensis*), the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. chumuensis*), and dark brown transverse bands of the tail wider than light brown interspaces (*vs.* wider transverse bands than light interspaces in *C. chumuensis*); from *C. chungi* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler, 2021 by having more ventral scale rows (39–42 *vs.* 30 or 31 in *C. chungi*), the presence of femoral pores in males (10 *vs.* absent in *C. chungi*), the enlarged subcaudal scales (*vs.* slightly enlarged in *C. chungi*), and dark brown transverse bands of the tail wider than light brown interspaces (*vs.* wider transverse bands than light interspaces in *C. chungi*); from *C. cryptus* Heidrich, Rösler, Vu, Böhme & Ziegler, 2007 by having fewer ventral scale rows (39–42 *vs.* 47–50 in *C. cryptus*), the presence of enlarged femoral scales (5 or 6 *vs.* absent in *C. cryptus*), the presence of femoral pores in males (10 *vs.* absent in *C. cryptus*), fewer precloacal pores in males (6 or 7 *vs.* 9–11 in *C. cryptus*), and the enlarged subcaudal scales (*vs.* subcaudal 2 or 3 times larger than dorsal tail scales in *C. cryptus*); from *C. cucdongensis* Schneider, Phung, Le, Nguyen & Ziegler, 2014 by the presence of femoral pores in males (10 *vs.* absent in *C. cucdongensis*), the presence of transversely enlarged subcaudal plates (*vs*. absent in *C. cucdongensis*), and having dark brown transverse bands of the tail wider than light brown interspaces (*vs.* wider transverse bands than light interspaces in *C. cucdongensis*); from *C. culaochamensis* Ngo, Grismer, Pham & Wood, 2020 by having fewer ventral scale rows (39–42 *vs.* 45–50 in *C. culaochamensis*), the presence of enlarged femoral scales (5 or 6 *vs.* absent in *C. culaochamensis*), the presence of femoral pores in males (10 *vs.* absent in *C. culaochamensis*), and the enlarged subcaudal scales (*vs.* slightly enlarged subcaudal scales in *C. culaochamensis*); from *C. dati* Ngo, 2013 by having fewer ventral scale rows (39–42 *vs.* 42–48 in *C. dati*), more femoral pores in males (10 *vs*. 3 or 4 each thigh in *C. dati*), the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. dati*), and the difference of dorsal color pattern (banded *vs.* blotched in *C. dati*); from *C. gialaiensis* by the presence of enlarged femoral scales (5 or 6 *vs.* absent in *C. gialaiensis*), the presence of femoral pores in males (10 *vs.* absent in *C. gialaiensis*), having fewer precloacal pores in males (6 or 7 *vs.* 9 or 10 in *C. gialaiensis*), more lamellae under finger IV (17–19 *vs.* 14 or 15 in *C. gialaiensis*) and toe IV (18–21 *vs.* 15–17 in *C. gialaiensis*), and the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. gialaiensis*); from *C. huynhi* Ngo & Bauer, 2008 by having fewer ventral scale rows (39–42 *vs.* 43–46 in *C. huynhi*), more femoral pores in males (10 *vs.* 3–8 in *C. huynhi),* the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. huynhi*), more dorsal longitudinal rows of tubercles at midbody between the lateral folds (19–21 *vs.* 16–18 in

C. huynhi); from *C. irregularis sensu stricto* by having fewer enlarged femoral scales (5 or 6 *vs.* 7 or 8 in *C. irregularis*), the presence of femoral pores in males (10 *vs.* absent in *C. irregularis*), more lamellae under finger IV (17–19 *vs.* 15 or 16 in *C. irregularis*), the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. irregularis*), and the difference of dorsal color pattern (banded *vs.* blotched in *C. irregularis*); from *C. kingsadai* Ziegler, Phung, Le & Nguyen, 2013 by having a smaller size (58.5–80.4 mm *vs.* 83–94 mm in *C. kingsadai*), fewer enlarged femoral scales (5 or 6 *vs.* 9–12 in *C. kingsadai*), more femoral pores in males (10 *vs.* 3–7 in *C. kingsadai*) and toe IV (28–21 *vs.* 21–25 in *C. kingsadai*); from *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen, 2021 by having the presence of femoral pores in males (10 *vs.* absent in *C. orlovi*), the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. orlovi*), and dark brown transverse bands of the tail wider than light brown interspaces (*vs.* wider transverse bands than light interspaces in *C. orlovi*); from *C. phnomchiensis* Neang, Henson & Stuart, 2020 by having fewer ventral scale rows (39–42 *vs.* 45–54 in *C. phnomchiensis*), the presence of femoral pores in males (10 *vs.* absent in *C. phnomchiensis*), more precloacal pores in males (6 or 7 *vs.* 4 or 5 in *C. phnomchiensis*), and the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. phnomchiensis*); from *C. phumyensis* Ostrowski, Do, Le, Ngo, Pham, Nguyen, Nguyen & Ziegler, 2020 by the presence of femoral pores in males (10 *vs.* absent in *C. phumyensis*), the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. phumyensis*), and the absence of two irregular dark longitudinal stripes in the shoulder region (*vs.* presence in *C. phumyensis*); from *C. phuocbinhensis* Nguyen, Le, Tran, Orlov, Lathrop, Macculloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang, 2013 by having fewer ventral scale rows (39–42 *vs.* 43–47 in *C. phuocbinhensis*), the presence of femoral pores in males (10 *vs.* absent in *C. phuocbinhensis*), the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. phuocbinhensis*), and the difference of dorsal color pattern (banded *vs.* striped/blotched in *C. phuocbinhensis*); from *C. pseudoquadrivirgatus* Rösler, Nguyen, Vu, Ngo & Ziegler, 2008 by having the presence of enlarged femoral scales (5 or 6 *vs.* absent in *C. pseudoquadrivirgatus*), the presence of femoral pores in males (10 *vs.* absent in *C. pseudoquadrivirgatus*), the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. pseudoquadrivirgatus*), and the difference of dorsal color pattern (banded *vs.* blotched in *C. pseudoquadrivirgatus*); from *C. raglai* Nguyen, Duong, Grismer & Poyarkov, 2021 by having a smaller size (58.5–80.4 mm *vs*. 95–111.7 mm in *C. raglai*), fewer enlarged femoral scales (5 or 6 *vs.* 9–10 in *C. raglai*), the presence of femoral pores in males (10 *vs.* absent in *C. raglai*), and more precloacal pores in males (6 or 7 *vs.* 5 in *C. raglai*); from *C. sangi* Pauwels, Nazarov, Bobrov & Poyarkov, 2018 by having a larger size (58.5–80.4 mm *vs.* 49.9–56.3 mm in *C. sangi*), more ventral scale rows (39–42 *vs*. 37 in *C. sangi*), more enlarged femoral scales (5 or 6 *vs.* 4 in *C. sangi*), the presence of femoral pores in males (10 *vs.* absent in *C. sangi*), and the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. sangi*); from *C. takouensis* by having more femoral pores in males (10 *vs.* 0–2 in *C. takouensis),* more precloacal pores in males (6 or 7 *vs.* 3 or 4 in *C. takouensis*), and a different dorsal color pattern (slightly clear transverse bands formed by shaped dark brown banded *vs.* five pale yellow clear bands, alternating with dark brown bands in *C. takouensis*); from *C. tayhoaensis* Do, Do, Le, Ngo, Ziegler, Nguyen & Pham, 2023 by having a smaller size (58.5–73.7 mm *vs.* 82.9–94.2 mm in *C. tayhoaensis*), fewer enlarged femoral scales (5 or 6 *vs.* 10 or 11 in *C. tayhoaensis*), more femoral pores in males (10 *vs.* 3–7 in *C. tayhoaensis),* more precloacal pores in males (6–7 *vs.* 4–5 in *C. tayhoaensis*), fewer lamellae under finger IV (17–19 *vs.* 20–22 in *C. tayhoaensis*) and toe IV (18–21 *vs.* 22–24 in *C. tayhoaensis*); from *C. taynguyenensis* Nguyen, Le, Tran, Orlov, Lathrop, Macculoch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang, 2013 by the presence of enlarged femoral scales (5 or 6 *vs.* absent in *C. taynguyenensis*), the presence of femoral pores in males (10 *vs.* absent in *C. taynguyenensis*), the presence of transversely enlarged subcaudal plates (*vs*. absent in *C. taynguyenensis*), and the difference of dorsal color pattern (banded *vs.* blotched in *C. taynguyenensis*); from *C. yangbayensis* Ngo & Chan, 2010 by having more femoral pores in males (10 *vs.* 0–2 in *C. yangbayensis*), the presence of pitted precloacal pores in females (5 or 6 *vs.* absent in *C. yangbayensis*), more lamellae under toe IV (18–21 *vs.* 15–17 in *C. yangbayensis*), and the difference of nuchal loop color pattern (continuous with U-shape or triangle shape *vs*. broken into two dark fragments or V-shape); from *C. ziegleri* Nazarov, Orlov, Nguyen & Ho, 2008 by having a smaller size (58.5–80.4 mm *vs.* 84.6–93.0 mm in *C. ziegleri*), fewer enlarged femoral scales (5 or 6 *vs.* 8–10 in *C. ziegleri*), more femoral pores in males (10 *vs.* 0–4 in *C. ziegleri),* and the presence of transversely enlarged subcaudal plates (*vs.* absent in *C. ziegleri*).

Discussion

Cyrtodactylus irregularis is the most diverse group of the genus *Cyrtodactylus* with 30 currently recognized species (Do *et al*. 2023, Ngo *et al*. 2023, this study). The number of taxa within the species complex has dramatically increased thirty times over the last twenty years. They have been found to occupy many different microhabitat types, e.g., coffee plantations (*C. gialaiensis*), granite formations (e.g. *C. arndti, C. badenensis, C. chumuensis, C. cucdongensis, C. phuocbinhensis, C. takouensis, C. nigriocularis, C. yangbayensis*), limestone evergreen forests (e.g., *C. cryptus*), lowland dipterocarp forest (e.g., *C. cattienensis; C. dati*; *C. huynhi; C. phnomchiensis*), small offshore island (e.g., *C. culaochamensis*) or montane evergreen forest (e.g. *C. ziegleri*) and can be active both during day time (e.g. *C. badenensis, C. bidoupimontis, C. huynhi*) and night time (e.g. *C. bugiamapensis, C. caovansungi*) (Grismeri *et al.* 2021; Luu *et al*. 2017; Nazarov *et al.* 2012; Ngo & Bauer 2008; Ngo *et al*. 2022, 2023; Nguyen *et al.* 2006; Orlov *et al.* 2007; Schneider *et al.* 2014). Interestingly, species in this group have been observed in an extended elevation range, from 50–100 m a.s.l (*C. culaochamensis, C. dati, C. kingsadai*) to 1,550–1,920 m a.s.l (*C. bidoupimontis*) and many species within this group have been observed to occur sympatrically (Nazarov *et al.* 2012, Ngo 2013, Ngo *et al.* 2022, Ziegler *et al.* 2013). For example, *C. badenensis* and *C. dati* can be found on Ba Den Mountain (Tay Ninh Province) or *C. chungi* and *C. takouensis* on Ta Kou Mountain (Binh Thuan Province), Vietnam. Remarkably, at least two species subcomplexes have been recognized within the *C. irregularis* group, namely the *C. pseudoquadrivirgatus* and *C. ziegleri* groups, which warrant further taxonomic revisions (Ngo *et al*. 2022). It is clear from this and previous studies that species diversity within the *C. irregularis* group remains underestimated either due to taxonomic confusion or substantial gaps in survey efforts.

The new species represents the third species of *Cyrtodactylus* found in the lowland areas of Binh Dinh Province (Ngo *et al*. 2023). However, the newly described species is not phylogenetically closely related to the two previously known species, *C. arndti* and *C. phumyensis*, from Binh Dinh Province. Genetically, they are separated from each other by 15.18–15.49% based on a fragment of the mitochondrial COI gene (Supplementary 1). The distance between the type locality of *C. binhdinhensis* in Cat Hai Commune, Phu Cat District and the type localities of *C. phumyensis* in My Tho Commune, Phu My District and *C. arndti* in Canh Hiep Commune, Van Canh District are approximately 29 km and 90 km, respectively. In terms of altitudinal gradients, *C. arndti* and *C. phumyensis* are found at slightly higher elevations, i.e., 150–300 m asl. (*C. arndti*) and 150–200 m asl. (*C. phumyensis*) (Ngo *et al.* 2023; Ostrowski *et al.* 2020), whereas *Cyrtodactylus binhdinhensis* was recorded at elevations from 140–160 m asl. Our discovery in this study brings the species number of the *Cyrtodactylus irregularis* species group to 30, including *C. buchardi,* which is still being investigated genetically (Uetz *et al.* 2023, Ngo *et al.* 2023, Do *et al*. 2023).

While the species richness of the *C. irregularis* group is substantially underestimated (Ngo *et al.* 2022), to date, five species are under high risks of extinction, comprising two species classified as Critically Endangered (CR), one as Endangered, and two as Vulnerable (IUCN 2023). Further conservation activities should be undertaken in Vietnam to protect natural habitat and populations of the threatened and micro-endemic bent-toed geckos.

Acknowledgments

We are grateful to the directorates of the Forest Protection Department of Binh Dinh Province for their support of our work and issuing relevant permits. This research is funded by the National Foundation for Science and Technology Development (NAFOSTED) under Grant Number 106.05–2020.24. Doctoral research of H.T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD). Comments from Roman Nazarov, an anonymous reviewer and the editor greatly improved the paper.

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Chapter 2. The discovery of two new species in the Cyrtodactylus irregularis group highlights that hidden diversity remains in the largest clade of the mega-diverse genus Cyrtodactylus

European Journal of Taxonomy 875: 70–100
https://doi.org/10.5852/ejt.2023.875.2141
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2023 · Ngo H.T. *et al.*

Research article

urn:lsid:zoobank.org:pub:CC306FFC-76D4-4D8F-8E87-01E92594483A

The discovery of two new species in the *Cyrtodactylus irregularis* group highlights that hidden diversity remains in the largest clade of the mega-diverse genus *Cyrtodactylus*

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Abstract. The *Cyrtodactylus irregularis* group, originally considered to consist of only one taxon, has been split into 26 species. We herein present the distribution of all species within the group in Cambodia, Laos and Vietnam and describe two new species based on integrative analyses. *Cyrtodactylus chumuensis* sp. nov. is discovered from Dak Lak Province and distinguished from the remaining taxa by more than 11.86% genetic divergence and by the following distinct morphological characters: size medium (SVL 67.5 mm); enlarged femoral scales on each thigh 4–5, femoral pores 0–2 in males; precloacal pores 6–7 in males; ventral scale rows 43–45; lamellae under toe IV 17–21. *Cyrtodactylus arndti* sp. nov. is described from Binh Dinh Province and genetically differentiated from its congeners by a minimum of 11.42% and by the following characters: adult size medium (SVL 73.4–80.8 mm); enlarged femoral scales on each thigh 5–11; femoral pores 0–2 in males; 6 precloacal pores in males, females with 6 pitted precloacal pores; ventral scale rows 26–38; lamellae under toe IV 17–22; subcaudal scales transversely enlarged. Additionally, we highlight the potential cryptic diversity with the taxon currently regarded as *C. pseudoquadrivirgatus* and understudied areas in Vietnam where new species will likely be discovered.

Keywords. *Cyrtodactylus chumuensis* sp. nov., *Cyrtodactylus arndti* sp. nov., molecular phylogeny, taxonomy, Vietnam.

Ngo H.T., Hormann H., Le M.D., Pham C.T., Phung T.M., Do D.T. & Ostrowski S. 2023. The discovery of two new species in the *Cyrtodactylus irregularis* group highlights that hidden diversity remains in the largest clade of the mega-diverse genus *Cyrtodactylus*. *European Journal of Taxonomy* 875: 70–100.
https://doi.org/10.5852/ejt.2023.875.2141

Introduction

The genus *Cyrtodactylus* Gray, 1827 is the most diverse gecko genus with 324 currently recognized species (Uetz *et al.* 2022). The members of *Cyrtodactylus* are known from tropical South Asia, southern China, Indochina, the Philippines, the Indo-Australian Archipelago and the Solomon Islands (Wood *et al.* 2012; Grismer *et al.* 2015, 2021). Species in the genus can adapt to different habitat types, including limestone karst, granitic montane, and lowland evergreen forests, caves, and swamps (Grismer *et al.* 2021). As a result of increased scrutiny in recent years, new taxa have been described in several morphologically conservative species complexes of *Cyrtodactylus*. A special case is the *Cyrtodactylus irregularis* group, which was originally considered to consist of only one taxon, *Gymnodactylus peguensis* var. *irregularis* Smith, 1921. Remarkably, a total of 25 species have been discovered within this group over the last 20 years (Ngo *et al.* 2022; Uetz *et al.* 2022). Of these, only *C. phnomchiensis* Neang, Henson & Stuart, 2020 is found in Cambodia, *C. buchardi* David, Teynié & Ohler, 2004 in Laos, and *C. cryptus* Heidrich, Rösler, Thanh, Böhme & Ziegler, 2007 in both Laos and Vietnam whereas the remaining 23 species are known only from Vietnam: *C. badenensis* Sang, Orlov & Darevsky, 2006; *C. bidoupimontis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler, 2012; *C. bugiamapensis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler, 2012; *C. caovansungi* Orlov, Nguyen, Nazarov, Ananjeva & Nguyen, 2007; *C. cattienensis* Geissler, Nazarov, Orlov, Böhme, Phung, Nguyen & Ziegler, 2009; *C. chungi* Ostrowski, Do, Le, Ngo, Pham, Nguyen, Nguyen & Ziegler, 2021; *C. cucdongensis* Schneider, Phung, Le, Nguyen & Ziegler, 2014; *C. culaochamensis* Ngo, Grismer, Pham & Wood, 2020; *C. dati* Ngo, 2013; *C. gialaiensis* Luu, Tran, Nguyen, Le & Ziegler, 2017; *C. huynhi* Ngo & Bauer, 2008; *C. irregularis sensu stricto*, *C. kingsadai* Ziegler, Phung, Le & Nguyen, 2013; *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen, 2021; *C. phumyensis* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler, 2020; *C. phuocbinhensis* Nguyen, Le, Tram, Orlov, Lathrop, MacCulloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang, 2013; *C. pseudoquadrivirgatus* Rösler, Vu, Nguyen, Ngo & Ziegler, 2008; *C. raglai* Nguyen, Duong, Grismer & Poyarkov, 2021; *C. sangi* Pauwels, Nazarov, Bobrov & Poyarkov, 2018; *C. takouensis* Ngo & Bauer, 2008; *C. taynguyenensis* Nguyen, Le, Tran, Orlov, Lathrop,

MacCulloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang, 2013; *C. yangbayensis* Ngo & Chan, 2010 and *C. ziegleri* Nazarov, Orlov, Nguyen & Ho, 2008 (Uetz *et al.* 2022).

We herein present data on the distribution of known taxa in the *C. irregularis* group and discuss potential cryptic diversity based on our updated molecular analyses. In addition, we also describe two new species collected during our recent fieldwork in Dak Lak and Binh Dinh provinces in south-central Vietnam based on integrative taxonomy, viz. combination of morphological and genetic evidence.

Material and methods

Sampling

Field surveys were conducted in June 2014 in M'Drak District, Dak Lak Province and in August 2016 in Van Canh District, Binh Dinh Province, Vietnam. Specimens were collected between 19:00 and 22:00 h (Fig. 1). After being photographed in life, specimens were euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 80% ethanol for five hours, and later transferred to 70% ethanol for permanent storage. Tissue samples were preserved separately in 70% ethanol prior to fixation. Voucher specimens referred to in this paper were deposited in the collections of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam and the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany.

Molecular data and phylogenetic analysis

All recognized species of the *Cyrtodactylus irregularis* species complex were included in the study, except *C. buchardi* and true *C. pseudoquadrivirgatus* (Table 1). Sequences of the species were downloaded from GenBank. Five new samples from two distinct populations were incorporated into the analysis, comprising of two from Dak Lak Province IEBR R.4928, IEBR R.4929 (field numbers PMT01 and PMT02) and three from Binh Dinh Province IEBR R.5077, IEBR R.4930, and ZFMK 103910 (field numbers BD.2016.85, BD.2016.141, and BD.2016.142). Four taxa, *C. condorensis* Smith, 1921, *C. grismeri* Ngo, 2008, *C. spelaeus* Nazarov, Poyarkov, Orlov, Nguyen, Milto, Martynov, Konstantinov & Chulisov, 2014 and *C. wayakonei* Nguyen, Kingsada, Rösler, Auer & Ziegler, 2010 were used as an outgroup based on their phylogenetic relationships to the *C. irregularis* species group as reported by Luu *et al.* (2016a) and Grismer *et al.* (2021).

DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer's instruction. Extracted DNA was amplified by HotStarTaq PCR mastermix (Qiagen, Germany) with 21 µl volume (10 µl of mastermix, 5 µl of water, 2 µl of each primer at 10 pmol·ml^{-1} and 2 µl of DNA). PCR condition's were 95°C for 15 minutes to activate the taq; with 40 cycles at 95°C for 30 s, 45°C for 45 s, 72°C for 60 s; and a final extension at 72°C for 6 minutes. A fragment of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), was amplified using the primer pair VF1–d (5'–TTCTCAACCAACCACAARGAYATYGG–3') and VR1–d (5'–TAGACTTCTGGGTGGCCRAARAAYCA–3') (Ivanova *et al.* 2006). PCR products were visualized using electrophoresis through a 2 % agarose gel stained with ethidium bromide. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purification kit (ThermoFischer Scientific, Lithuania). Purified PCR products were sent to FirstBase (Malaysia) for sequencing in both directions.

Afterwards, sequences were aligned by ClustalX ver. 2.1 (Thompson *et al.* 1997) with default settings. Data were analyzed using Bayesian inference (BI) as implemented in MrBayes ver. 3.2.7 (Ronquist *et al.* 2012), maximum likelihood as implemented in IQ-TREE ver. 1.6.8 (Nguyen *et al.* 2015), and Maximum Parsimony (MP) implemented in PAUP*4.0b10 (Swofford 2001). For MP analysis, a heuristic analysis was conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR)

branch swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support was calculated using 1000 pseudo-replicates (BP) and 100 random taxon addition replicates. All characters were equally weighted and unordered. For the ML analysis, we employed a single model and 10 000 ultrafast bootstrap replications (UFB). The optimal model for nucleotide evolution was determined using jmodeltest ver. 1.2.4 (Darriba *et al.* 2012).

For the Bayesian analyses, we used the optimal model determined by jmodeltest with parameters estimated by MrBayes ver. 3.2.7. Two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10^7 generations with a random starting tree and sampled every 1000 generations. Loglikelihood scores of sample points were plotted against generation time

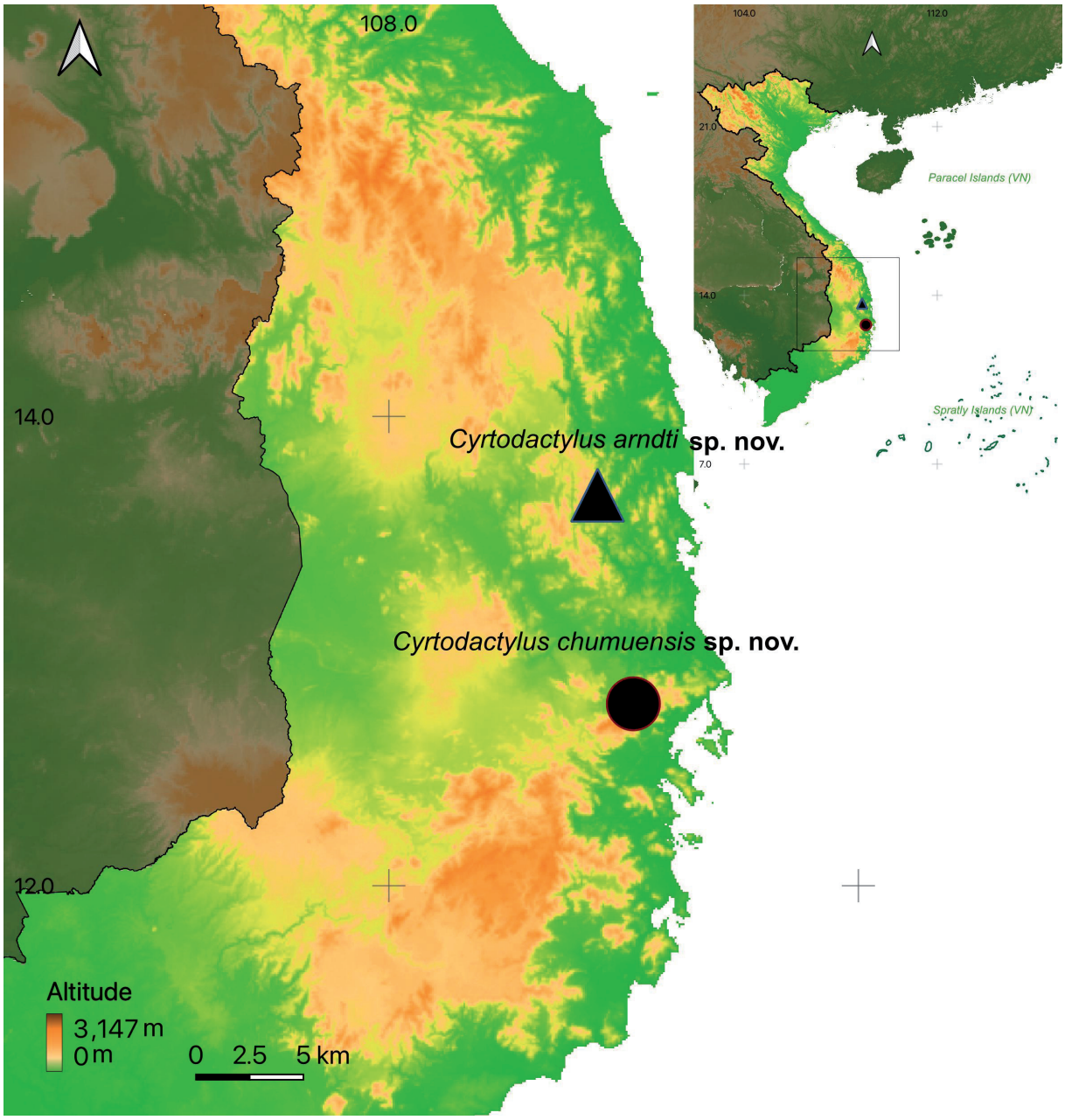

Fig. 1. Type locality of *Cyrtodactylus chumuensis* sp. nov. in Dak Lak Province (black circle) and *Cyrtodactylus arndti* sp. nov. in Binh Dinh Province (black triangle), Vietnam.

Table 1 (continued on next page). Samples of *Cyrtodactylus* Gray, 1827 used in the molecular analyses. Abbreviations: CBC = Royal University of Phnom Penh, Cambodia; IEBR = Institute of Ecology and Biological Resources, Vietnam; ITBCZ = Institute of Tropical Biology Collection and Zoology, Vietnam; KIZ = Kunning Institute of Zoology, Germany; NAP and ZMMU = Zoological Museum of Moscow University, Russia; PNKB = Phong Nha-Ke Bang, Vietnam; ROM = Royal Ontario Museum, Canada; UNS = United States National Museum, USA; VNMN = Vietnam National Museum of Nature, Vietnam; VNUF = Vietnam National University of Forestry, Vietnam; ZFMK = Zoologisches Forschungsmuseum Alexander Koenig, Germany.

Species	GenBank No.	Locality	Voucher number
Cyrtodactylus spelaeus	KP199947	Laos: Kasi	ZMMU R-13980-3
C. wayakonei	KP199950	Laos: Luang Nam Tha	ZMMU R-13981-1
C. badenensis	KF929505	Vietnam: Tay Ninh Prov.	KIZ13689
C. bidoupimontis	HM425560	Vietnam	NAP-01121
C. bidoupimontis	HQ967215	Vietnam: Lam Dong Prov.	ZMMU NAP-00080
C. bugiamapensis	HM888459	Vietnam: Binh Phuoc Prov.	ZMMU R-13093-2
C. bugiamapensis	KY862173	Vietnam: Binh Phuoc Prov.	KIZ33
C. caovansungi	KF219679	Vietnam: Ninh Thuan Prov.	ITBCZ 932
C. caovansungi	KF219680	Vietnam: Ninh Thuan Prov.	ITBCZ 1113
C. cf. *cattienensis*	HQ967220	Vietnam: Dong Nai Prov.	ZMMU NAP-00117.1
C. cf. *cattienensis*	KF169973	Vietnam: Dong Nai Prov.	ROM37887
C. cf. *cattienensis*	MG791892	Vietnam: Ba Ria – Vung Tau Prov.	ZMMU R-14509
C. condorensis	HM888464	Cambodia: Kong Tang Island	ZMMU RAN 1987
C. condorensis	MF169910	Vietnam: Ba Ria – Vung Tau Prov.	UNS 0431
Cyrtodactylus chumuensis sp. nov.	OQ152319	Vietnam: Dak Lak Prov.	IEBR R. 4928
Cyrtodactylus chumuensis sp. nov.	OQ152320	Vietnam: Dak Lak Prov.	IEBR R. 4929
C. chungi	MT576019	Vietnam: Binh Thuan Prov.	IEBR 4581
C. chungi	MT576020	Vietnam: Binh Thuan Prov.	IEBR 4582
C. cryptus	KF169971	Vietnam: Quang Binh Prov.	PNKB3
C. cryptus	KF169972	Vietnam: Quang Binh Prov.	PNKB4
C. cucdongensis	KJ403846	Vietnam: Khanh Hoa Prov.	IEBR A.2013.104
C. cucdongensis	KJ403847	Vietnam: Khanh Hoa Prov.	ZFMK 95513
C. dati	KF929508	Vietnam: Binh Phuoc Prov.	ITBCZ2537
C. dati	KF929511	Vietnam: Binh Phuoc Prov.	ITBCZ2540
C. gialaiensis	MG460299	Vietnam: Gia Lai Prov.	VNUF R.2017.1
C. gialaiensis	MG460300	Vietnam: Gia Lai Prov.	VNUF R.2017.4
C. grismeri	KF929513	Vietnam: An Giang Prov.	ITBCZ 684
C. grismeri	KY862259	Vietnam: An Giang Prov.	ITBCZ 685
Cyrtodactylus arndti sp. nov.	OQ152316	Vietnam: Binh Dinh Prov.	IEBR R.5077
Cyrtodactylus arndti sp. nov.	OQ152317	Vietnam: Binh Dinh Prov.	IEBR R.4930

Table 1 (continued).

Species	GenBank No.	Locality	Voucher number
Cyrtodactylus arndti sp. nov.	OQ152318	Vietnam: Binh Dinh Prov.	ZFMK 103910
C. huynhi	KF169948	Vietnam: Dong Nai Prov.	ITBCZ513
C. huynhi	KF169950	Vietnam: Dong Nai Prov.	ITBCZ530
C. irregularis	MG791887	Vietnam: Lam Dong Prov.	ITBCZ-10025
C. kingsadai	KF188432	Vietnam: Phu Yen Prov.	IEBR A.2013.3
C. phnomchiensis	MT066405	Cambodia: Kampong Thom Prov.	CBC 3003
C. phnomchiensis	MT066406	Cambodia: Kampong Thom Prov.	CBC 3004
C. phumyensis	MT210158	Vietnam: Binh Dinh Prov.	ZFMK 103153
C. phumyensis	MT210161	Vietnam: Binh Dinh Prov.	IEBR 4579
C. phuocbinhensis	KF169953	Vietnam: Ninh Thuan Prov.	ITBCZ1518
C. phuocbinhensis	KF169954	Vietnam: Ninh Thuan Prov.	ITBCZ1529
C. pseudoquadrivirgatus	KF169963	Vietnam: Thua Thien Hue Prov.	ITBCZ3001
C. pseudoquadrivirgatus	KF169964	Vietnam: Thua Thien Hue Prov.	ITBCZ3002
C. cf. *pseudoquadrivirgatus*	KP199949	Vietnam: Da Nang Prov.	ZMMU-R-13095-2
C. cf. *pseudoquadrivirgatus*	MG791888	Vietnam: Quang Tri Prov.	ZMMU R130952
C. raglai	MW675653	Vietnam: Khanh Hoa Prov.	ZMMU R16688
C. sangi	KF169951	Vietnam: Ninh Thuan Prov.	ITBCZ1150
C. sangi	KF169952	Vietnam: Ninh Thuan Prov.	ITBCZ965
Cyrtodactylus sp.	KF169962	Vietnam: Da Nang Prov.	ITBCZ2532
C. takouensis	KF929533	Vietnam: Binh Thuan Prov.	ITBCZ2527
C. takouensis	KF929534	Vietnam: Binh Thuan Prov.	ITBCZ2528
C. taynguyenensis	KF169978	Vietnam: Gia Lai Prov.	ROM32119
C. taynguyenensis	KF169979	Vietnam: Gia Lai Prov.	ROM32120
C. yangbayensis	KJ403848	Vietnam: Khanh Hoa Prov.	VNMN03373
C. yangbayensis	MF169952	Vietnam: Khanh Hoa Prov.	UNS 0476
C. cf. *ziegleri*	HQ967210	Vietnam: Dak Lak Prov.	ZMMU R-13116-3
C. cf. *ziegleri*	KF169945	Vietnam: Dak Lak Prov.	UNS5007
C. cf. *ziegleri*	KF169977	Vietnam: Dak Nong Prov.	VNMN2016

to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function. The posterior probability values (PP) for all clades in the final majority rule consensus tree were provided. Nodal support was evaluated using BP as estimated in PAUP, UFB in IQ-TREE ver. 1.6.7.1, and PP in MrBayes ver. 3.2. UFB and PP $\geq$ 95% and BP $\geq$ 70% are regarded as strong support for a clade (Hillis & Bull 1993; Ronquist *et al.* 2012; Nguyen *et al.* 2015). The optimal model for nucleotide evolution was set to GTR+I+G for ML and Bayesian analyses. The cut-off point for the burn-in function was set to 47 in the Bayesian analysis, as -ln*L* scores reached stationarity after 47 000 generations in both runs. Uncorrected pairwise divergences were calculated in PAUP*4.0b10.

Morphological characters

Measurements followed Ziegler *et al.* (2002) and Luu *et al.* (2015) with slight exceptions and were taken with a slide-caliper to the nearest 0.1 mm. Measurements were taken on the right side of the specimens unless otherwise indicated. Scale counts were taken using a stereo microscope (Euromex NexiusZoom). Bilateral scale counts were given as left/right or as one value (on each side), unless otherwise indicated.

Abbreviations

AG = axilla-groin length, from insertion of posterior margin of front limbs to insertion of anterior margin of hindlimbs
BW = maximum width of body
CrusL = crus length, from heel to flexed knee
ED = greatest diameter of ear opening
EyeEar = distance between posterior margin of orbit and anterior margin of ear opening
FemurL = femur length, from limb insertion to knee
ForeaL = forearm length, from elbow flexed to base of palm
HH = maximum height of head posterior to orbits
HL = head length, from posterior margin of retroarticular process of jaw to tip of snout
HW = maximum width of head posterior to orbits
IND = internarial distance
ML = maximum length of mental
MW = maximum width of mental
OD = orbital diameter, greatest diameter or bony orbit
RW = maximum width of rostral
RH = maximum height of rostral
SE = distance between tip of snout and anterior margin of orbit
SVL = snout-vent length, from tip of snout to anterior margin of cloaca
TaL = tail length, from cloaca to end of tail

Scale counts

DTR = dorsal tubercle rows counted transversely across the midbody between ventrolateral folds
FP = femoral pores in males
EFS = enlarged femoral scales
EPS = distinctly enlarged precloacal scales, at the least twice as large, as the surrounding scales
GST = granular scales surrounding dorsal tubercles
IL = infralabials were counted from the first labial scale to posterior corner of mouth (except for granular scales)
IN = internasals
LD1 = number of subdigital lamellae on first finger
LD4 = number of subdigital lamellae on fourth finger
LT1 = number of subdigital lamellae on first toe
LT4 = number of subdigital lamellae on fourth toe
N = nasal scales, surrounding naris, from rostral to labial, except rostral and labial
PAT = postcloacal tubercles
PM = postmentals
PP = precloacal pores in males or pitted precloacal pores in females
SL = supralabials were counted from the first labial scale to corner of the mouth
V = ventral scales at midbody, counted from one ventrolateral fold to the other

Results

Phylogenetic analyses

The final matrix consisted of 61 terminals, consisting of five from this study and 56 from previous works. The ingroup taxa contained described species (except *C. buchardi* and true *C. pseudoquadrivirgatus*) and two undescribed populations of the *Cyrtodactylus irregularis* group. Both ML and BI produced very similar topologies based on a total of 652 aligned characters with no internal gaps and using a single model of molecular evolution (Fig. 2). In the MP analysis, 254 characters were parsimony informative. A single most parsimonious tree with 1649 steps was recovered (Consistency index = 0.25; Retention index = 0.59). The results show that *Cyrtodactylus condorensis* and *Cyrtodactylus grismeri* are nested within the *Cyrtodactylus irregularis* group in the MP analysis but placed in a separate clade in ML and BI (Fig. 2).

In our analyses, *Cyrtodactylus badenensis* is considered a member of the *Cyrtodactylus irregularis* group in both ML and BI analyses. Similar to the results reported in Ngo *et al.* (2022), *Cyrtodactylus* cf. *pseudoquadrivirgatus* was recovered in three distinct places of the tree, two closely related to *C. taynguyenensis* with 8.2 % and 10.6% genetic divergence from the latter and one as a sister taxon to *C. cryptus* with 9.3 % divergence between the two.

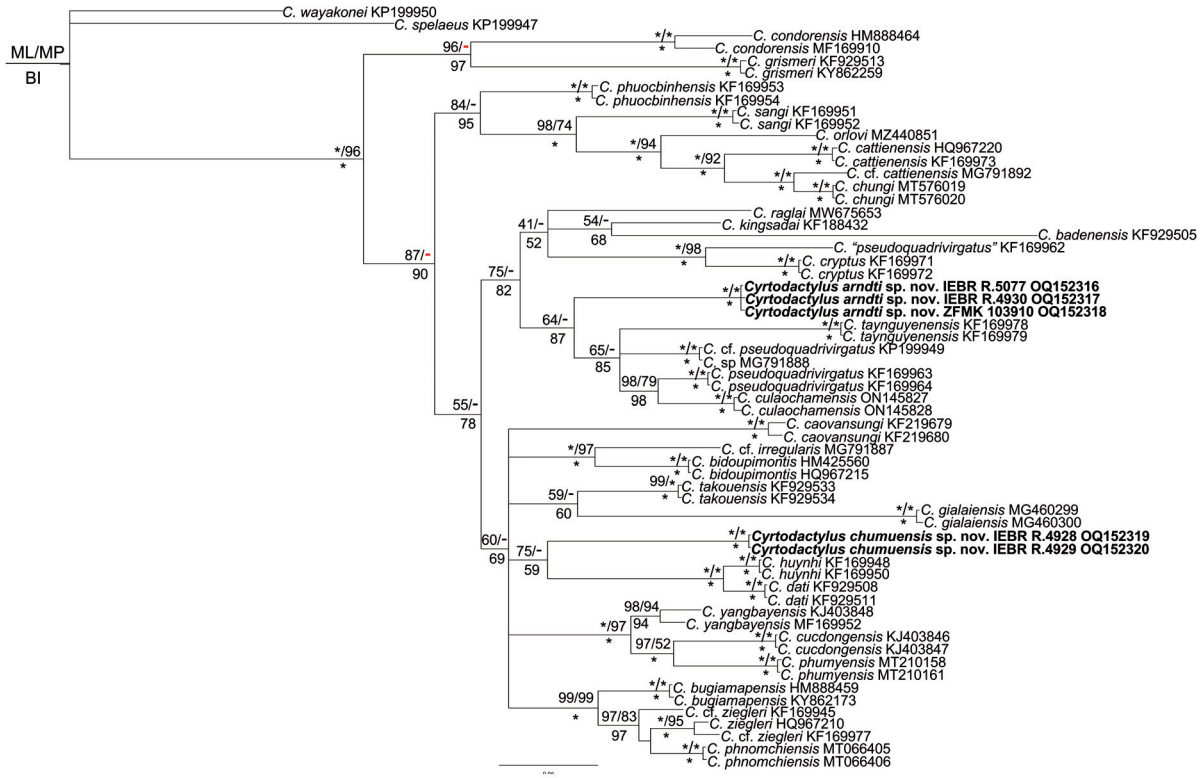

Fig. 2. Phylogram based on the Bayesian analysis. Number above and below branches are ML untrafast bootstrap/MP bootstrap values and Bayesian posterior probabilities, respectively. Dashes denote bootstrap values < 50%. Asterisk denotes 100% value. Red dashes show that *C. condorensis* (Smith, 1921) and *C. grismeri* Ngo, 2008 are nested within the *Cyrtodactylus irregularis* group in the MP analysis.

Moreover, two unnamed taxa from M'Drak District, Dak Lak Province and Van Canh District, Binh Dinh Province, Vietnam are nested within the *Cyrtodactylus irregularis* complex without any clear sister . *Cyrtodactylus arndti* sp. nov. is placed as a member in a clade consisting of *C. badenensis, C. culaochamensis, C. cryptus, C. kingsadai, C. pseudoquadrivirgatus*, *C. taynguyenensis*, *C.* cf. *pseudoquadrivirgatus*, *Cyrtodactylus* sp. (MG791888), and *C. raglai* without nodal support (UFB = 75, BP < 50, and PP=82) and also without any clear sister species. Genetically, *Cyrtodactylus chumuensis* sp. nov. is divergent from other members in *Cyrtodactylus irregularis* group by at least approximately 11.86 %. *Cyrtodactylus arndti* shows a minimum genetic distance of 11.42–11.42% to other members in *Cyrtodactylus irregularis* complex (Supp. file 1).

Taxonomy

Class Reptilia Laurenti, 1768
Order Squamata Oppel, 1811
Family Gekkonidae Gray, 1825
Subfamily Gekkoninae Gray, 1825
Genus *Cyrtodactylus* Gray, 1827

Cyrtodactylus chumuensis sp. nov.
urn:lsid:zoobank.org:act:F6A019B5-B1ED-4976-8C1D-803CC0D91DD9
Figs 3–5; Table 2

Diagnosis

The new species can be distinguished from remaining congeners of the irregularis species group by a combination of the following characters: maximum SVL 67.5 mm; dorsal pattern with 6 irregularly shaped and short longitudinal stripes on the neck; nuchal band thin, interrupted, reaching the posterior margin of the orbits; the absence of transversely enlarged median subcaudal scales; 4 or 5 enlarged femoral scales on each thigh, 17–19 distinctly enlarged precloacal scales; males with 0 or 1 femoral pore on each thigh, 6 or 7 precloacal pores in a continuous series, ^-shaped; ventral scales 43–45; dorsal tubercles in 20 irregular longitudinal rows; precloacal groove absent; internasal scales 2; supralabials 8–14; infralabials 9–11; number of subdigital lamellae on fourth finger 16–19 and on fourth toe 19–21.

Etymology

The new species is named after its type locality, Chu Mu Mountain in Dak Lak Province. We propose the following common names: Chu Mu Bent-toed Gecko (English), Thạch sùng ngón chư mư (Vietnamese).

Type material (Figs 3–5)

Holotype

VIETNAM • ♂; Dak Lak Province, M'Drak District, Ea M'Doal Commune, Chu Mu Mountain; 12°41.330′ N, 108°55.450′ E; 500 m a.s.l.; 20 Jun. 2014; T.M. Phung leg.; Field No. PMT01; IEBR R.4928.

Paratypes

VIETNAM • 1 ♂ (subadult); Dak Lak Province, M'Drak District, Ea M'Doal Commune, Chu Mu Mountain; 12°41.321′ N, 108°55.382′ E; 400 m a.s.l.; 20 Jun. 2014; T.M. Phung leg.; Field No. PMT02; IEBR R.4929.

Description of holotype

Adult male; snout-vent length SVL 67.5 mm; tail regenerated 51.4 mm in length (regenerated portion 43.7 mm); body slender, elongate (AG/SVL 0.4); head distinct from neck, elongate (HL/SVL 0.26), relatively wide (HW/HL 0.71) and depressed (HH/HL 0.36); loreal region concave; snout long, blunt

in dorsal profile (SE/HL 0.44), longer than diameter of orbit (OD/SE 0.56); scales on snout small, round or oval, granular, larger than scales on occiput; orbit large (OD/HL 0.24), pupils vertical; ear opening small, oval (ED/HL 0.06); rostral wider than high, indented medially the top, in contact with first supralabial, naris and nasorostral on each side, two internasal scales; nostril opening small, oval, surrounded by rostral, nasorostral, two supranasals and one or two postnasals; mental scale triangular, wider than high (ML/MW 0.74); two enlarged, triangular postmentals; supralabials 8/9; infralabials 9/9.

Dorsal scales granular, dorsal tubercles round, keeled, conical, in 20 irregular rows at midbody; tubercles on occiput small; tubercles surrounded by 10–12 granular scales; ventral scales smooth, round, midventral scales three times as large as dorsal granular scales, in 45 longitudinal rows at midbody between ventrolateral folds; precloacal groove absent; enlarged femoral scales 4 or 5 on each thigh, the third bearing a femoral pore (Fig. 4C); enlarged precloacal scales 17, arranged in a rhombus; precloacal pores 6, arranged in a ∧-shaped series.

Fore and hindlimbs moderately slender (ForeaL/SVL 0.14 mm, CrusL/SVL 0.17); forelimbs dorsally covered by several slightly enlarged tubercles; dorsal hindlimbs with well-developed tubercles; two postcloacal tubercles on each side on the hemipenal swellings; phalanges without webbing; each claw sheathed by two scales, the ventral sheath larger than the upper; number of subdigital lamellae on first finger 11/11, on first toe 10/11, on fourth finger 17/17, on fourth toe 19/19.

Coloration in preservative

Dorsal surface of head, body and limbs light-brown with some dark-brown bands, pattern without light bordering; occiput marbled with small, irregular dark-brown blotches; rostral, mental and infralabials creamy white, supralabials dark-beige with short greyish brown vertical stripes; neck bands dark-brown,

Fig. 3. *Cyrtodactylus chumuensis* sp. nov., holotype, ♂ (IEBR R.4928), in life.

extending in two thin stripes along lateral sides of the snout to the orbits, broader on the neck, interrupted on the left side; two dark-brown longitudinal stripes, disconnected from the neck band extending to shoulders, one dark-brown blotch next to each stripe; dorsal pattern consisting of 6 irregular bands, each formed by two triangularly shaped blotches, shifted medially along the body axis; dorsolateral region covered with small, irregular dark-brown blotches arranged in a longitudinal row from neck to groin; a blurry dark-brown transverse band on dorsal surface of original part of the tail, regenerated part greyish beige and speckled with very small light-greyish brown blotches; dorsal surface of limbs with 3 or 4 dark-brown, blurry bands; phalanges brown with creamy white knuckles; tubercles white or dark-brown depending on position on pattern or background; venter creamy white; ventral tail greyish beige without bands. For coloration of the paratype in life that closely resembles the holotype in life see Fig. 3.

Variation

The paratype is a subadult and therefore differs greatly in size. Its original tail showed some dark-brown irregular bands, although broken at the base. The number of precloacal pores is 7 and it lacks femoral pores. For more morphological characters see Table 2.

Comparisons

The new species can be distinguished from all other member of *Cyrtodactylus irregularis* group from Vietnam by morphological characteristics (see Table 2).

Cyrtodactylus chumuensis sp. nov. differs from *C. badenensis* by having more ventral scale rows (43–45 vs 25–29 in *C. badenensis*), the presence of enlarged femoral scales (4–5 vs absent in *C. badenensis*), the presence of precloacal pores in males (6–7 vs absent in *C. badenensis*), and the absence of transversely

Fig. 4. *Cyrtodactylus chumuensis* sp. nov. in preservative. **A**. Holotype, ♂ (IEBR R.4928, left) and paratype, ♂ (IEBR R.4929, right). **B**. Paratype, ♂ (IEBR R.4929). **C**. Holotype, ♂ (IEBR R.4928). **A**. Dorsal view. **B**. Precloacal region with precloacal pores. **C**. Precloacal region with precloacal pores and femoral pores.

enlarged subcaudals (vs present in *C. badenensis*); differs from C. *bidoupimontis* by having a smaller size (SVL 67.5 mm vs 74.0–86.3 mm in *C. bidoupimontis*), fewer enlarged femoral scales (4 or 5 vs 8–10 in *C. bidoupimontis*), a different dorsal color pattern (irregularly banded with longitudinal stripes on the neck vs transversal bands with light borders in *C. bidoupimontis*), and a thin discontinuous nuchal band (vs well developed, widened posteriorly in *C. bidoupimontis*); differs from *C. bugiamapensis* by having fewer enlarged femoral scales (4 or 5 vs 6–10 in *C. bugiamapensis*) and the different dorsal color pattern (irregularly banded with longitudinal stripes on the neck vs unclear transversal bands formed by irregular round to oblong, dark-brown spots in *C. bugiamapensis*); differs from *C. buchardi* by having more ventral scale rows (30 vs 43–45 in *C. buchardi*), the presence of enlarged femoral scales (4–5 vs absent in *C. buchardi*), more subdigital lamellae under the fourth finger (16–19 vs 14 in *C. buchardi*), more subdigital lamellae under the fourth toe (17–21 vs 12 in *C. buchardi*); differs from *C. cattienensis* by having more ventral scale rows (43–45 vs 28–42 in *C. cattienensis*), more subdigital lamellae under the fourth finger (16–19 vs 12–16 in *C. cattienensis*), and different dorsal color pattern (irregularly banded with longitudinal stripes on the neck vs irregular dark-brown banded, first band on the shoulder x-shaped *C. cattienensis*); differs from *C. caovansungi* by having a smaller size (SVL 67.5 mm vs 90.4–94 mm in *C. caovansungi*), fewer enlarged femoral scales (4 or 5 vs 8 in *C. caovansungi*), fewer femoral pores on each thigh in males (0–1 vs 6 in *C. caovansungi*), fewer precloacal pores in males (6 or 7 vs 9 in *C. caovansungi*), fewer lamellae under the fourth finger (16–19 vs 22 in *C. caovansungi*), fewer lamellae under the fourth toe (17–21 vs 23–25 in *C. caovansungi*), and the absence of transversely enlarged subcaudal plates (vs present in *C. caovansungi*); differs from *C. chungi* by having more ventral scale rows (43–45 vs 30 or 31 in *C. chungi*), more dorsal tubercle rows (20 vs 18 in *C. chungi*), different dorsal color pattern (irregularly banded with longitudinal stripes on the neck vs irregular transversal bands with a closed nuchal band), and a thin, discontinuous nuchal band (vs continuous nuchal band in *C. chungi*); differs from *C. cryptus* by having fewer ventral scale rows (43–45 vs 47–50 in *C. cryptus*), the presence of enlarged femoral scales (vs absent in *C. cryptus*), fewer precloacal pores in males (6 or 7 vs 9–11 in *C. cryptus*), a thin, discontinuous nuchal band (vs well developed, widened posteriorly in *C. cryptus*), and different dorsal color pattern (irregularly banded with short, longitudinal stripes on the neck vs irregular transverse bands in *C. cryptus*); differs from *C. cucdongensis* by having more dorsal tubercle rows (20 vs 16–19 in *C. cucdongensis*), fewer enlarged femoral scales (4 or 5 vs 5–9 in *C. cucdongensis*), more enlarged precloacal scales (20–21 vs 6–13), and a different dorsal colour pattern (irregularly banded with short, longitudinal stripes on the neck vs irregular dark brown transverse bands); differs from *C. culaochamensis* by having a smaller size (SVL 67.5 mm vs 69.8–79.8 mm in *C. culaochamensis*), the presence of enlarged femoral scales (vs absent in *C. culaochamensis*), fewer lamellae under the first finger (11 vs 13 or 14 in *C. culaochamensis*), and fewer lamellae under the first toe (10 or 11 vs 13–15 in *C. culaochamensis*); differs from *C. dati* by having fewer femoral pores in males (0–2 vs 3 or 4 each side in *C. dati*), fewer lamellae under the first toe (10 or 11 vs 12 or 13 in *C. dati*), the presence of blotches on head (vs absent in *C. dati*), and different dorsal color pattern (irregularly banded with longitudinal stripes on the neck vs irregular dark blotches); differs from *C. gialaiensis* by the presence of enlarged femoral scales (vs absent in *C. gialaiensis*), fewer precloacal pores in males (6 or 7 vs 9 or 10 in *C. gialaiensis*), and more subdigital lamellae under the fourth finger (16–19 vs 14 or 15 in *C. gialaiensis*) as well as under the fourth toe (17–21 vs 15–17 in *C. gialaiensis*); differs from *C. huynhi* by having more dorsal tubercle rows in males (20 vs 16–18 in *C. huynhi*), fewer lamellae under first finger (11 vs 12–15 in *C. huynhi*), fewer lamellae under first toe (10 or 11 vs 13–17 in *C. huynhi*), and a thin discontinuous nuchal band (vs well developed, widened posteriorly in *C. huynhi*); differs from *C. irregularis* by having a smaller size (SVL 67.5 mm vs 72.0–86.0 mm in *C. irregularis*), more ventral scale rows (43–45 vs 37–42 in *C. irregularis*), fewer enlarged femoral scales (4 or 5 vs 7 or 8 in *C. irregularis*), and different dorsal color pattern (irregularly banded with short longitudinal stripes on the neck vs blotched in *C. irregularis*); differs from *C. kingsadai* by having a smaller size (SVL 67.5 mm vs 83.0–94.0 in *C. kingsadai*), fewer enlarged femoral scales (4 or 5 vs 9–12 in *C. kingsadai*), the absence of transversely enlarged subcaudal plates (vs present in *C. kingsadai*), and more internasals (2 vs 1 in *C. kingsadai*); differs from *C. orlovi* by having more ventral scale rows

Table 2. Measurements (in mm) and morphological characters of the type series of *Cyrtodactylus chumuensis* sp. nov. Bilateral meristic characters are given as (left/right). Abbreviations: * = regenerated or broken tail; max = maximum; min = minimum.

Characters	IEBR R.4928 Holotype	IEBR R.4929 Paratype
Sex	♂	♂
SVL	67.5	52.4
TaL	51.4*	41.5*
AG	26.7	21.3
HL	17.6	16.0
HW	12.5	10.3
HH	6.4	5.2
OD	4.25	3.6
SE	7.7	6.8
EyeEar	6.5	4.6
ED	1.0	1.2
IND	2.2	1.6
RW	2.6	2.4
RH	2.1	1.7
MW	2.3	2.1
ML	1.7	1.7
BW	13.7	9.6
ForeaL	9.7	7.6
CrusL	11.2	8.8
FemurL	11.6	7.5
SL	8/9	12/14
IL	9/9	10/11
N	4/5	4/4
IN	2	2
PM	2	2
DTR	20	20
GST	10–12	9–11
V	45	43
EFS	5/4	4/4
FP	1/1	–
EPS	17	19
PP	6	7
PAT	2/2	3/3
LD1	11/11	11/11
LT1	10/11	11/11
LD4	17/17	19/16
LT4	19/17	19/21

(43–45 vs 36–39 in *C. orlovi*); a thin, discontinuous nuchal band (vs continuous nuchal band in *C. orlovi*), and different banded pattern ranges (6 vs 3–5 in *C. orlovi*); differs from *C. phnomchiensis* by having a smaller size (SVL 67.5 vs 76.1–80.7 mm in *C. phnomchiensis*), more precloacal pores in males (6 or 7 vs 5 in *C. phnomchiensis*), and different dorsal color pattern (irregularly banded vs banded in *C. phnomchiensis*); differs from *C. phuocbinhensis* by having a larger size (SVL 67.5 mm vs 46.0–60.4 mm in *C. phuocbinhensis*), different dorsal color pattern (irregularly banded vs stripes or blotches in *C. phuocbinhensis*), and dark-brown transverse banded of the tail than light-brown interspaces (vs dark transverse banded wider than light interspaces in *C. phuocbinhensis*); differs from *C. phumyensis* by having more ventral scale rows (43–45 vs 33–41 in *C. phumyensis*), fewer enlarged femoral scales (4 or 5 vs 5–7 in *C. phumyensis*), more dorsal tubercle row (20 vs 18 or 19 in *C. phumyensis*), fewer enlarged precloacal scales (17–19 vs 21–41 in *C. phumyensis*), and different dorsal color pattern (irregularly banded with short longitudinal stripes on the neck vs anteriorly irregularly spotted and posteriorly banded in *C. phumyensis*); differs *C. pseudoquadrivirgatus* by the presence of enlarged femoral scales on each thigh (vs absent in *C. pseudoquadrivirgatus*), the presence of precloacal pores in males (0–2 vs absent in *C. pseudoquadrivirgatus*), and more enlarged precloacal scales (17–19 vs 1–12 in *C. pseudoquadrivirgatus*); differs *C. raglai* by having a smaller size (SVL 67.5 mm vs 87.5–111.7 mm in *C. raglai*), more ventral scale rows (43–45 vs 36–39 in *C. raglai*), fewer enlarged femoral scales (4 or 5 vs 9 or 10 in *C. raglai*), fewer precloacal pores in males (0–2 vs 5 in *C. raglai*), and the absence of transversely enlarged subcaudal plates (vs present in *C. raglai*); differs from *C. sangi* by having a larger size (SVL 67.5 mm vs 49.9–56.3 mm in *C. sangi*) and more ventral scale rows (43–45 vs 37 in *C. sangi*); differs from *C. takouensis* by having a smaller size (SVL 67.5 mm vs 74.7–81.1 mm in *C. takouensis*), more ventral scale rows (43–45 vs 39–40 in *C. takouensis*), more precloacal pores in males (6 or 7 vs 3 or 4 in *C. takouensis*), the absence of transversely enlarged subcaudal plates (vs present in *C. takouensis*), a thin discontinuous nuchal band (vs well developed, widened posteriorly in *C. takouensis*), and different dorsal color pattern (irregularly banded vs banded in *C. takouensis*); differs from *C. taynguyenensis* by

Fig. 5. Habitat of *Cyrtodactylus chumuensis* sp. nov. in the Chu Mu Mountain, M'Drak District, Dak Lak Province.

the presence of enlarged femoral scales on each thigh (vs absent in *C. taynguyenensis*)*;* irregularly banded of the tail (vs banded in *C. taynguyenensis*), and different dorsal color pattern (irregularly banded vs blotched in *C. taynguyensis*); differs from *C. yangbayensis* by having a smaller size (SVL 67.5 vs 78.5–92.3 mm in *C. yangbayensis*), more subdigital lamellae under the fourth toe (17–21 vs 15–17 in *C. yangbayensis*), fewer subdigital lamellae under the first toe (10–11 vs 18–20 in *C. yangbayensis*), and the absence of transversely enlarged subcaudal plates (vs present in *C. yangbayensis*); differs from *C. ziegleri* by having a smaller size (SVL 67.5 vs 84.6–93.0 mm in *C. ziegleri*), more ventral scale rows (43–45 vs 33–39 in *C. ziegleri*), and fewer enlarged femoral scales (4 or 5 vs 8–10 *C. ziegleri*).

Distribution

Cyrtodactylus chumuensis sp. nov. is currently known only from the Chu Mu Mountain, M'Drak District, Dak Lak Province, Vietnam (Fig. 1).

Natural history

Specimens were found at night between 19:00 and 22:00, on granite rock, along a rocky stream, approximately 0.5–1.0 m above the ground, at elevations between 400 and 500 m a.s.l. The surrounding habitat was evergreen forest of medium and small hardwoods mixed with shrubs and vines (Fig. 5). The humidity was approximately 50–71% and the air temperature ranged from 27.5 to 32.1°C. Other reptile species found at the sites included *Gekko gecko* (Linnaeus, 1758), *Hemidactylus platyurus* (Schneider, 1792), *Ahaetulla prasina* (Boie, 1827), *Lycodon* sp., and *Oligodon* sp.

Cyrtodactylus arndti sp. nov.
urn:lsid:zoobank.org:act:83F60753-8E51-49D9-97E7-BD870AC772F3
Figs 6–9; Table 3

Diagnosis

The new species of *Cyrtodactylus* is distinguished from remaining congeners of the *C. irregularis* species group by a combination of the following characters: SVL: 73.4–80.9 mm; dorsal pattern with 6 or 7 irregularly shaped bands; moderately broad nuchal band; original tail with irregular transverse bands; subcaudals transversely enlarged; 5–11 enlarged femoral scales; males with 0–2 pitted femoral pores, those absence in females; males with 6 precloacal pores, females with 6 pitted precloacal pores, pore-bearing scales arranged in a single ∧-shaped series; ventral scales 26–38; dorsal tubercles in 17–20 irregular longitudinal rows; precloacal groove absent; supralabials 8–13; infralabials 8–12; number of subdigital lamellae on fourth finger 15–20 and on fourth toe 17–22.

Etymology

We name this species in honor of our colleague, Prof. Dr. Hartmut Arndt, Institute of Zoology, University of Cologne, Germany, in recognition of his support for biodiversity research in Vietnam. As common names, we suggest Arndt's Bent-toed Gecko (English) and Thằn lằn ngón arndt (Vietnamese).

Type material (Figs 6–9)

Holotype

VIETNAM • ♂; Binh Dinh Province, Van Canh District, near Hiep Ha Village; 13°39.858′ N, 108°53.355′ E; 270 m a.s.l.; 13 Aug. 2016; D.T. Do and T.V. Nguyen; Field No. BD.2016.141; IEBR R.4930.

Paratypes

VIETNAM • 1 ♂; Binh Dinh Province, Quy Nhon District, Quy Nhon City; 13°41.718′ N, 109°10.277′ E; 140 m a.s.l; 8 Aug. 2016; D.T. Do and T.V. Nguyen; Field No. BD.2016.1; IEBR R.5219 • 2 ♂♂, 1 ♀; Binh Dinh Province, Van Canh District, near Dak Dum Village; 13°38.365′ N, 108°57.863′ E; 150 m a.s.l.; 11 Aug. 2016; D.T. Do and T.V. Nguyen; Field No. BD.2016.86, BD2016.87, BD.2016.88;

IEBR R.4931 to IEBR.4933 • 1 ♀; Binh Dinh Province, Van Canh District, near Hiep Ha Village; 13°39.858′ N, 108°53.355′ E; 270 m a.s.l.; D.T. Do and T.V. Nguyen; Field No. BD.2016.86; IEBR R.5077 • 2 ♂♂; same collection data as for preceding; Field No. BD.2016.142, BD.2016.143; ZFMK 103910, ZFMK 103911.

Description of holotype

Adult male; snout-vent length 74.4 mm; tail regenerated, 98.15 mm in length; body slender, elongate (AG/SVL ratio 0.39); head distinct from neck, elongate, depressed (HL/SVL 0.29, HW/HL 0.67, HH/HL 0.37); loreal region concave; snout long, blunt in dorsal profile (SE/HL 0.37), longer than diameter of orbit (OD/SE 0.61); scales on snout small, round or oval, granular, lager than scales on occiput; orbit large (OD/HL 0.22); pupils vertical; ear opening small, oval (ED/HL 0.06); rostral almost twice as wide as high with an inverse Y-shapted structure, surrounded by first supralabial, naris, nasorostral on each side, and internasal; nostril opening small and oval, surrounding by rostral, nasorostral, 2 supranasals and one postnasal; mental scale triangular, wider than high (ML/MW 0.74); two enlarged, triangular postmentals; supralabials 12/12; infralabials 11/13.

Dorsal scales granular, dorsal tubercles round, keeled, conical, in 20 irregular rows at midbody; tubercles on occiput small; each tubercle surrounded by 9 or 10 granular scales; ventral scales smooth, round, midventral scales approximately 3–4 times as large as dorsal scales, slightly imbricate laterally, in 37 longitudinal rows at midbody between ventrolateral folds; precloacal groove absent; enlarged femoral scales 7 or 8 on each thigh, about twice the size of surrounding scales; enlarged precloacal scales 17, arranged in a rhombus; femoral pores absent; precloacal pores 6, arranged in ∧-shaped series.

Fore and hindlimbs moderately slender (ForeaL/SVL 0.15 mm, CrusL/SVL 0.16); forelimbs dorsally covered by several slightly enlarged tubercles; dorsal surface of hindlimbs bearing well-developed tubercles; two postcloacal tubercles on each side on the hemipenal swellings; phalanges without

Fig. 6. *Cyrtodactylus arndti* sp. nov., holotype, ♂ (IEBR R.4930), in life.

webbing; each claw sheathed by two scales, the ventral sheath larger than the upper scale; number of subdigital lamellae on first finger 12/13, on first toe 13/13, on fourth finger 18/18, on fourth toe 22/22.

Coloration in preservative

Dorsal surface of head, body and limbs light-brown with some dark-brown pattern, without light bordering; occiput marbled with small, irregular dark-brown banded; rostral, mental, first three supralabials and first infralabials greyish brown, remaining infralabials light beige, some with greyish brown speckles or frames; nuchal band discontinuous, consisting of two stripes extending from the orbits to the neck, ending by a dark blotch on each side and a third blotch medially; dorsum with 7 irregular bands, the first two interrupted; dorsolateral region covered with round or elongate dark-brown blotches; tail with 6 dark-brown bands, fade ventrally, some small dark-brown spots arranged in a line along the lateral side of tail, tail tip dark-brown; dorsal surface of limbs with 6 or 7 irregular dark-brown bands; phalanges brown with beige knuckles; dorsal tubercles white or dark-brown depending on position; tubercles on dorsal surface of limbs and tail light-brown; venter greyish brown.

Fig. 7. *Cyrtodactylus arndti* sp. nov., paratypes ♂♂ (IEBR R.4931, IEBR R.4932), in life.

Table 3 (continued on next page). Measurements (in mm) and morphological characters of the type series of *Cyrtodactylus arndti* sp. nov. Bilateral meristic characters are given as (left/right). Abbreviations: * = regenerated or broken tail; max = maximum; min = minimum.

Characters	IEBR R.4930 Holotype	IEBR R.5129 Paratype	IEBR R.5077 Paratype	IEBR R.4931 Paratype	IEBR R.4932 Paratype	IEBR R.4933 Paratype	ZFMK 103910 Paratype	ZFMK 103911 Paratype
Sex	♂	♂	♀	♂	♂ (sub)	♀	♂	♂
SVL	74.4	74.4	75.6	80.9	66.1	74.7	73.4	76.9
TaL	98.2*	67.1*	91.5	50.1*	75.1	85.9*	94*	68.8*
AG	28.7	30.6	31.6	34.7	24.7	27.4	27.2	26.4
HL	21.8	22.4	21.6	23.2	18.8	20.5	21.4	23.2
HW	14.5	14.4	15.1	16.2	13.5	15.1	15.1	15.3
HH	8.1	8.9	9.2	9.8	6.1	8.1	7.8	8.5
OD	4.9	4.8	5.3	6.1	4	5.1	5.2	5.3
SE	8	10.0	9.8	10	8.0	9.9	9.3	9.4
EyeEar	6.4	6.0	6.1	7.1	5.6	6.2	6.6	7.1
ED	1.4	1.8	1.9	1.8	1.4	6.2	1.9	1.1
IND	2.7	2.6	2.5	2.4	2.2	2.3	2.7	2.2
RW	4.5	3.3	4.1	3.8	3.3	3.6	3.2	3.4
RH	2.3	2.2	2.2	2.3	2.0	2.2	2.1	2.2
MW	3.1	3.0	3.0	3.1	3.0	3.2	3.1	3.1
ML	2.3	2.1	2.4	2.6	2.7	2.3	2.4	2.6
BW	14.5	15.6	16.2	17.7	12.5	15.5	15.6	14.5
ForeaL	11.4	11.5	9.9	11.4	9.9	10.3	10.3	10.8
CrusL	12.1	13.3	12.5	13.7	12.4	13.3	12.8	14.1

Table 3 (continued).

Characters	IEBR R.4930 Holotype	IEBR R.5129 Paratype	IEBR R.5077 Paratype	IEBR R.4931 Paratype	IEBR R.4932 Paratype	IEBR R.4933 Paratype	ZFMK 103910 Paratype	ZFMK 103911 Paratype
FemurL	11.7	14.3	14.3	12.9	11.6	12.3	12.5	13.9
SL	11/13	10/9	11/11	10/10	11/11	10/10	11/10	8/12
IL	12/12	10/9	8/8	12/11	9/8	8/9	10/8	8/8
N	4/5	4/4	4/4	4/4	4/4	4/4	4/4	4/4
IN	1	1	1	1	1	2	3	2
PM	2	2	2	2	2	2	2	2
DTR	20	17	17	19	17	18	20	18
GST	9–10	10–11	9–11	10–11	9–11	10–11	9–11	9–12
V	37	26	36	36	38	36	36	36
EFS	8/7	10/10	11/11	7/7	7/7	6/5	8/10	8/7
FP	absent	1/0	0/0	1p/1p	0/1	absent	absent	absent
EPS	17	19	21	19	17	21	23	17
PP	6	6	6	6	6	6	6	6
PAT	2/2	2/2	2/2	2/2	2/2	3/3	3/2	3/3
LD1	12/13	10/10	10/11	11/11	11/11	12/12	11/11	11/10
LT1	13/13	11/11	12/14	11/11	11/12	12/13	12/12	11/11
LD4	18/18	17/16	18/18	18/18	17/15	20/19	19/18	17/17
LT4	22/22	19/19	20/21	21/22	20/19	22/22	20/21	18/17

Sexual dimorphism and variation

The female (IEBR R.4933) differs from the males by the absence of hemipenal swellings. All male specimens have 6 precloacal pores but the females has 6 pitted scales only. Three males (IEBR R.4930, ZFMK 103910, ZFMK 103911) and the female lack femoral pores (IEBR R.5077, IEBR R.4933). For further morphological characters see Table 3.

Comparisons

The new species can be distinguished from all other member of *Cyrtodactylus irregularis* group from Vietnam by morphological characteristics (see Table 4).

Cyrtodactylus arndti sp. nov. differs from *C. badenensis* by having the presence of enlarged femoral scales (5–11 vs absent in *C. badenensis*), the presence of precloacal pores in males (6 vs absent in *C. badenensis*), the presence of pitted precloacal pores in females (6 vs absent in *C. badenensis*), and different dorsal pattern (irregular bands vs banded in *C. badenensis*); differs from *C. bidoupimontis* by having the presence of pitted precloacal pores in females (6 vs absent in *C. bidoupimontis*), having moderately broad nuchal band, continuous or discontinuous band (vs well developed, widened posteriorly in *C. bidoupimontis*), different dorsal pattern (6 or 7 irregular transverse bands, colouration dark-brown on light-brown background vs 4–5 dark irregular transverse dorsal bands, usually with light borders), and the presence of transversely enlarged subcaudals vs absent in *C. bidoupimontis;* differs from *C. bugiamapensis* by having fewer precloacal pores in males (6 vs 7–11 in *C. bugiamapensis*), different dorsal color pattern (irregular, dark-brown transverse bands vs unclear transversal bands formed by irregular roundish to oblong, dark brown spots in *C. bugiamapensis*), moderately broad nuchal band, continuous or discontinuous bands (vs dark nuchal band, which can be medially divided, narrow, U-shape in *C. bugiamapensis*), and the presence of transversely enlarged subcaudals vs absent in *C. bugiamapensis;* differs from *C. buchardi* by having a lagger size (SVL of 73.4–80.9 vs 60–65 in *C. buchardi*), the presence of enlarged femoral scales (5–11 vs absent in *C. buchardi*), more subdigital lamellae on first finger (15–20 vs 14 in *C. buchardi*), more subdigital lamellae on first toe (17–22 vs 12

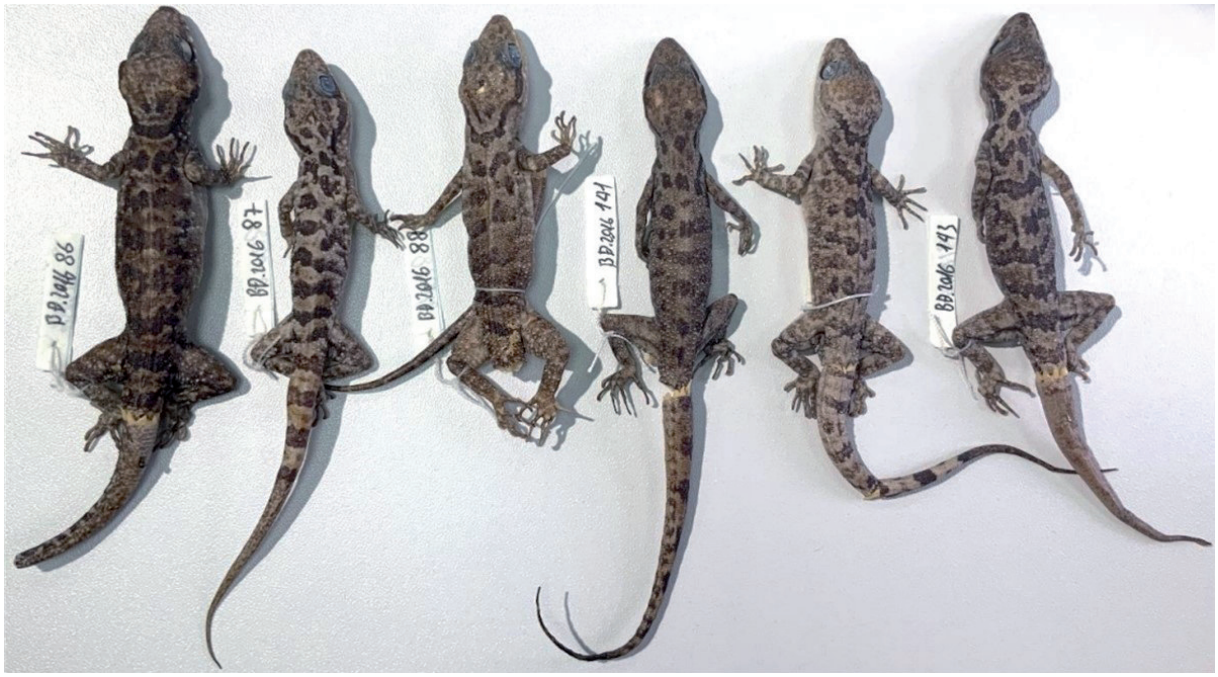

Fig. 8. *Cyrtodactylus arndti* sp. nov., type series in preservative (the holotype is the third from right).

in *C. buchardi*) and the presence of transversely enlarged subcaudals vs absent in *C. buchardi*); differs from *C. caovansungi* by having a smaller size (SVL of 73.4–80.9 vs 90.4–94.0 mm), fewer femoral pores (0–2 vs 6 in *C. caovansungi*), fewer precloacal pores in males (6 vs 9 in *C. caovansungi*), the presence of pitted precloacal pores in females (6 vs absent in *C. caovansungi*), and fewer subdigital lamellae under the fourth toe (17–22 vs 23–25 in *C. caovansungi*); differs from *C. cattienensis* by having a larger size (73.4–80.9 mm vs 43.5–69.0 mm in *C. cattienensis*), the presence of pitted precloacal pores in females (6 vs absent in *C. cattienensis*), and the presence of transversely enlarged subcaudals (vs absent in *C. cattienensis*); differs from *C. chungi* by having a larger size (73.4–80.9 mm vs 66.6–68.5 mm in *C. chungi*), fewer precloacal pores in males (6 vs 7 in *C. chungi*), fewer enlarged precloacal scales (17–23 vs 41–45 in *C. chungi*), and the presence of transversely enlarged subcaudals (vs absent in *C. chungi*); differs from *C. cryptus* by having fewer ventral scale rows (26–38 vs 47–50 in *C. cryptus*), the presence of enlarged femoral scales (5–11 vs absent in *C. cryptus*), fewer precloacal pores in males (6 vs 9–11 in *C. cryptus*), the presence of pitted precloacal pores in females (6 vs absent in *C. cryptus*), and the presence of transversely enlarged subcaudals (vs absent in *C. cryptus*); differs from *C. cucdongensis* by having a larger size (SVL 73.4–80.9 mm vs 55.8–65.9 mm in *C. cucdongensis*), fewer ventral scale rows (26–38 vs 41–44 in *C. cucdongensis*), more subdigital lamellae under the first toe (11–14 vs 8–11 in *C. cucdongensis*), and the presence of transversely enlarged subcaudals (vs absent in *C. cucdongensis*); differs from *C. culaochamensis* by having fewer ventral scale rows (26–38 vs 45–50 in *C. culaochamensis*), the presence of enlarged femoral scales (5–11 vs absent in *C. culaochamensis*), fewer precloacal pores in males (6 vs 7–8 in *C. culaochamensis*), and the presence of pitted precloacal pores in females (6 vs absent in *C. culaochamensis*); differs from *C. dati* by having fewer ventral scale rows (26–38 vs 42–48 in *C. dati*), fewer femoral pores (0–2 vs 3–4 on each side in *C. dati*), different dorsal color pattern (irregular bands vs blotches in *C. dati*), and the presence of transversely enlarged subcaudals (vs absent in *C. dati*); differs from *C. gialaiensis* by having a larger size (73.4–80.9 mm vs 50.1–62.8 mm in *C. gialaiensis*), the presence of enlarged femoral scales (5–11 vs absent in *C. gialaiensis*), fewer precloacal pores in males (6 vs 9–10 in *C. gialaiensis*), and more subdigital lamellae under the fourth finger (15–20 vs 14–15 in *C. gialaiensis*); differs from *C. huynhi* by having fewer ventral scale rows (26–38 vs 43–46 in *C. huynhi*), more enlarged femoral scales (5–11 vs 3–5 in *C. huynhi*), fewer precloacal pores in males (6 vs 7–9 in *C. huynhi*), and the presence of transversely enlarged subcaudals (vs absent in *C. huynhi*); differs from *C. irregularis* by having the presence of precloacal pores in males (1–2 vs absent in *C. irregularis*), different dorsal color pattern (irregular bands vs blotched in *C. irregularis*), and the presence of transversely enlarged subcaudals (vs absent in *C. irregularis*); differs from *C. kingsadai* by having a smaller size (SVL 73.4–80.9 mm vs 83.0–94.0 mm in *C. kingsadai*), fewer ventral scale rows (26–38 vs 39–46 in *C. kingsadai*), and fewer precloacal pores in males (6 vs 7–9 in *C. kingsadai*); differs from *C. orlovi* by having a larger size in males (SVL 73.4–80.9 mm vs 61.0–68.2 mm in *C. orlovi*), the presence of pitted precloacal pores in females (6 vs absent in *C. orlovi*), and the presence of transversely enlarged subcaudals (vs absent in *C. orlovi*); differs from *C. phnomchiensis* by having fewer ventral scale rows (26–38 vs 45–54 in *C. phnomchiensis*), more precloacal pores in males (6 vs 4–5 in *C. phnomchiensis*), fewer AG/SVL ratio (0.34–0.43 vs 0.45–0.48 in *C. phnomchiensis*), and the presence of transversely enlarged subcaudals (vs absent in *C. phnomchiensis*); differs from *C. phumyensis* by having a larger size (SVL 73.4–80.9 mm vs 63.6–66.8 mm in *C. phumyensis*), and the presence of transversely enlarged subcaudals (vs absent in *C. phumyensis*); differs from *C. phuocbinhensis* by having a larger size (SVL 73.4–80.9 mm vs 46.0–60.4 mm in *C. phuocbinhensis*), fewer ventral scale rows (26–38 vs 43–47 in *C. phuocbinhensis*), fewer precloacal pores in males (6 vs 7 in *C. phuocbinhensis*), the presence of pitted precloacal pores in females (6 vs absent in *C. phuocbinhensis*); differs from *C. pseudoquadrivirgatus* by having fewer ventral scale rows (26–38 vs 41–57 in *C. pseudoquadrivirgatus*), the presence of enlarged femoral scales (5–11 vs absent in *C. pseudoquadrivirgatus*), different dorsal color pattern (irregular bands vs blotched in *C. pseudoquadrivirgatus*), and the presence of transversely enlarged subcaudals (vs absent in *C. pseudoquadrivirgatus*); differs from *C. raglai* by having a smaller size (SVL 73.4–80.9 mm vs 95–111.7 mm in *C. raglai*), more precloacal pores in males (6 vs 5 in

Table 4 (continued on next page).Morphological comparisons between *Cyrtodactylus chumuensis* sp. nov., *Cyrtodactylus arndti* sp. nov. and 23 congeners from the *Cyrtodactylus irregularis* complex based on examination of specimens and data obtained from the literature (Ziegler *et al*. 2002; Nguyen *et al*. 2006, 2013, 2014, 2015, 2017a, 2017b, 2021; Heidrich *et al*. 2007; Orlov *et al*. 2007; Nazarov *et al*. 2008, 2012; Ngo 2088, 2013; Ngo & Bauer 2008; Ngo *et al*. 2010; Rösler *et al*. 2008; Ngo & Chan 2010, 2011; Ngo & Grismer 2012; Ziegler *et al*. 2010, 2013; Luu *et al*. 2011, 2017; Phung *et al*. 2014; Le *et al*. 2016; Ostrowski *et al*. 2020, 2021; Do *et al*. 2021). Abbreviations: ? = data not available in literature; – = characteristic not present; + = characteristic present but not uniquely determined; * = regenerated or broken tail.

	Species	SVL (mm)	TaL (mm)	V	EFS	FP	PP (M)	PP (F)	LD4	LT4	Color pattern of dorsum	Subcaudals
1	*Cyrtodactylus chumuensis* sp. nov.	**67.5**	**51.4***	**43–45**	**4–5**	**0–2**	**6–7**	**?**	**16–19**	**17–21**	**banded**	**absent**
2	*Cyrtodactylus arndti* sp. nov.	**73.4–80.9**	**50.1*–91.51**	**26–38**	**5–11**	**0–2**	**6**	**6**	**15–20**	**17–22**	**banded**	**present**
3	*C. badenensis*	59.3–74.1	58.6–82.4	25–29	absent	absent	0	0	?	18–22	banded	present
4	*C. bidoupimontis*	74.0–86.3	75.0–86	38–43	8–10	absent	4–6	0	15–20	18–23	banded	absent
5	*C. bugiamapensis*	58.6–76.8	65.3–83.0	36–46	6–10	absent	7–11	0–7	15–17	17–20	blotched	absent
6	*C. buchardi*	60.0–65.0	46.0–54.0	30	absent	absent	9	0	14	12	blotched	absent
7	*C. caovansungi*	90.4–94.0	120.0	38–44	8	6	9	0	22	23–25	banded	present
8	*C. cattienensis*	43.5–69.0	51–64.7	28–42	3–8	absent	6–8	0–8 pitted scales	12–16	14–19	banded	absent
9	*C. chungi*	66.6–68.5	62.8*–82.2	30–31	4–6	absent	7	6	?	17–20	banded	absent
10	*C. cryptus*	62.5–90.8	63.5–88.4	47–50	absent	absent	9–11	0	18–19	20–23	banded	absent
11	*C. cucdongensis*	55.8–65.9	max. 81.3	41–44	5–9	absent	5–6	4–6	13–18	15–20	banded	absent
12	*C. culaochamensis*	69.8–79.8	89.7–91.2	45–50	absent	absent	7–8	absent	18–19	20–23	banded	absent
13	*C. dati*	max 70.1	max 57.3	42–48	4–7	3–4 each	5–6	?	?	18–19	blotched	absent
14	*C. gialaiensis*	50.1–62.8	?	38–45	absent	absent	9–10	0–8	14–15	15–17	banded	absent
15	*C. huynhi*	67.2–79.8	61.5–78.6	43–46	3–5	3–8	7–9	0–8 pitted scales	14–17	17–21	banded	absent
16	*C. irregularis*	72.0–86.0	66.0–74.0	38–45	7–8	absent	5–7	0–6	15–16	18–19	blotched	absent
17	*C. kingsadai*	83.0–94.0	max 117	39–46	9–12	0–4	7–9	4–8	19–21	21–25	banded	present
18	*C. orlovi*	61–77.7	Max 71.2	36–39	3–8	absent	5–6	0	15–17	16–19	banded	absent

Table 4 (continued).

	Species	SVL (mm)	TaL (mm)	V	EFS	FP	PP (M)	PP (F)	LD4	LT4	Color pattern of dorsum	Subcaudals
19	*C. phnomchiensis*	76.1–80.7	56.9–79.1	45–54	0–8	absent	4–5	1–7 pitted scales	18–20	20–23	banded	absent
20	*C. phumyensis*	63.6–66.8	?	33–41	5–7	absent	5–7	6 pitted scales	18–19	18–21	banded	absent
21	*C. phuocbinhensis*	46.0–60.4	76.1	43–47	5	absent	7	0	16–21	17–19	striped/blotched	absent
22	*C. pseudoquadrivirgatus*	48.6–83.3	55.7–82.3	41–57	absent	absent	5–9	5–10	15–21	16–25	blotched	absent
23	*C. raglai*	95–111.7	113.4–135	36–39	9–10	absent	5	0	?	21–22	banded	present
24	*C. sangi*	49.9–56.3	47.9*	37	4	absent	7	4 pitted scale	?	?	banded	absent
25	*C. takouensis*	74.7–81.1	77.7–91.0	39–40	3–5	0–2	3–4	0	16–17	18–20	banded	present
26	*C. taynguyenensis*	60.0–85.0	66.0–94.0	42–49	absent	absent	6	0	13–18	17–21	blotched	absent
27	*C. yangbayensis*	78.5–92.3	91.3–109.1	39–46	5–16	0–2	6–8	0	16–19	15–17	banded	present
28	*C. ziegleri*	84.6–93.0	95.0–107.0	33–39	8–10	0–6	5–8	0–8	16–19	18–21	banded	absent

C. raglai) and the presence of pitted precloacal pores in females (6 vs absent in *C. raglai*); differs from *C. sangi* by having a larger size (SVL 73.4–80.9 mm vs 49.9–56.3 mm in *C. sangi*), more enlarged femoral scales (5–11 vs 4 in *C. sangi*), fewer precloacal pores in males (6 vs 7 in *C. sangi*), and more pitted precloacal pores in females (6 vs 4 in *C. sangi*); differs from *C. takouensis* by having more enlarged femoral scales (5–11 vs 3–5 in *C. takouensis*), more precloacal pores in males (6 vs 3–4), the presence of pitted precloacal pores in females (6 vs absent in *C. takouensis*), and the different dorsal color pattern (irregular bands vs banded in *C. takouensis*); differs from *C. taynguyenensis* by having more ventral scale rows (26–38 vs 42–49 in *C. taynguyenensis*), the presence of enlarged femoral scales (5–11 vs absent in *C. taynguyenensis*), the different dorsal color pattern (irregular bands vs blotched in *C. taynguyenensis*), and the presence of transversely enlarged subcaudals (vs absent in *C. taynguyenensis*); differs from C. *yangbayensis* by having the presence of pitted precloacal pores in females (6 vs absent in *C. yangbayensis*), more subdigital lamellae under the fourth toe (17–22 vs 15–17), and fewer subdigital lamellae on first toe (11–14 vs 18–20 in *C. yangbayensis*); differs from *C. ziegleri* by having a smaller size (SVL 73.4–80.9 mm vs 84.6–93.0 mm in *C. ziegleri*), the presence of transversely enlarged subcaudals (vs absent in *C. ziegleri*), and dark-brown transverse bands of the tail narrower than the light-brown interspaces (vs dark transverse bands wider than the light interspaces in *C. ziegleri*).

Differs from *C. chumuensis* sp. nov. by having a larger size (SVL 73.4–80.9 mm vs maximum 67.5 mm in *C. chumuensis*), fewer ventral scale rows (26–38 vs 43–45 in *C. chumuensis*), and the presence of transversely enlarged subcaudals (vs absent in *C. chumuensis*).

Distribution

Cyrtodactylus arndti sp. nov. is currently known only from the Van Canh District, Binh Dinh Province, Vietnam (Fig. 1).

Fig. 9. Habitat of *Cyrtodactylus arndti* sp. nov. in Hiep Ha Village, Van Canh District, Binh Dinh Province.

Natural history

Specimens were found at night between 19:00 and 22:00, on trees or on granite rock, along rocky streams, about 0.6–1.5 m above the ground, at elevations between 150 and 300 m a.s.l. The surrounding habitat was evergreen forest of medium and small hardwoods mixed with shrubs and vines (Fig. 9). The humidity was approximately 40–62% and the air temperature ranged from 28.9 to 33.1˚C. Other reptiles species found at the sites included *Acanthosaura coronata* (Günther, 1861) *Dixonius vietnamensis* Das, 2004, *Gekko gecko* (Linnaeus, 1758), *Gekko* sp., *Eutropis multifasciata* (Kuhl, 1820), *Boiga jaspidea* (Duméril, Bibron & Duméril, 1854), *Psammodynastes pulverulentus* (Boie, 1827), and *Trimeresurus* sp.

Discussion

Cyrtodactylus irregularis was described by Smith in 1921 based on specimens collected from the Cam Ly River Valley, Langbian Plateau (now known as Lam Dong Province, Vietnam) (Smith 1921; Grismer *et al.* 2021). After its description, the name *Cyrtodactylus irregularis* was applied to all specimens of *Cyrtodactylus* collected in north-central and south-central Vietnam for more than 80 years. However, as *C. irregularis* is still being investigated, we referred to the specimens of *Cyrtodactylus* from Lac Duong, Lam Dong Province, Vietnam as *C.* cf. *irregularis* in this study. At the moment, the *Cyrtodactylus irregularis* group is split into 28 species (including the two species in the present study) and it is the largest group within the genus *Cyrtodactylus* (Grismer *et al.* 2021; Ngo *et al.* 2022). Most members of the *Cyrtodactylus irregularis* group are distributed in north-central and south-central Vietnam which is also known as the Truong Son Range (Fig. 10) (Grismer *et al.* 2021; Ngo *et al.* 2022). Only a few species

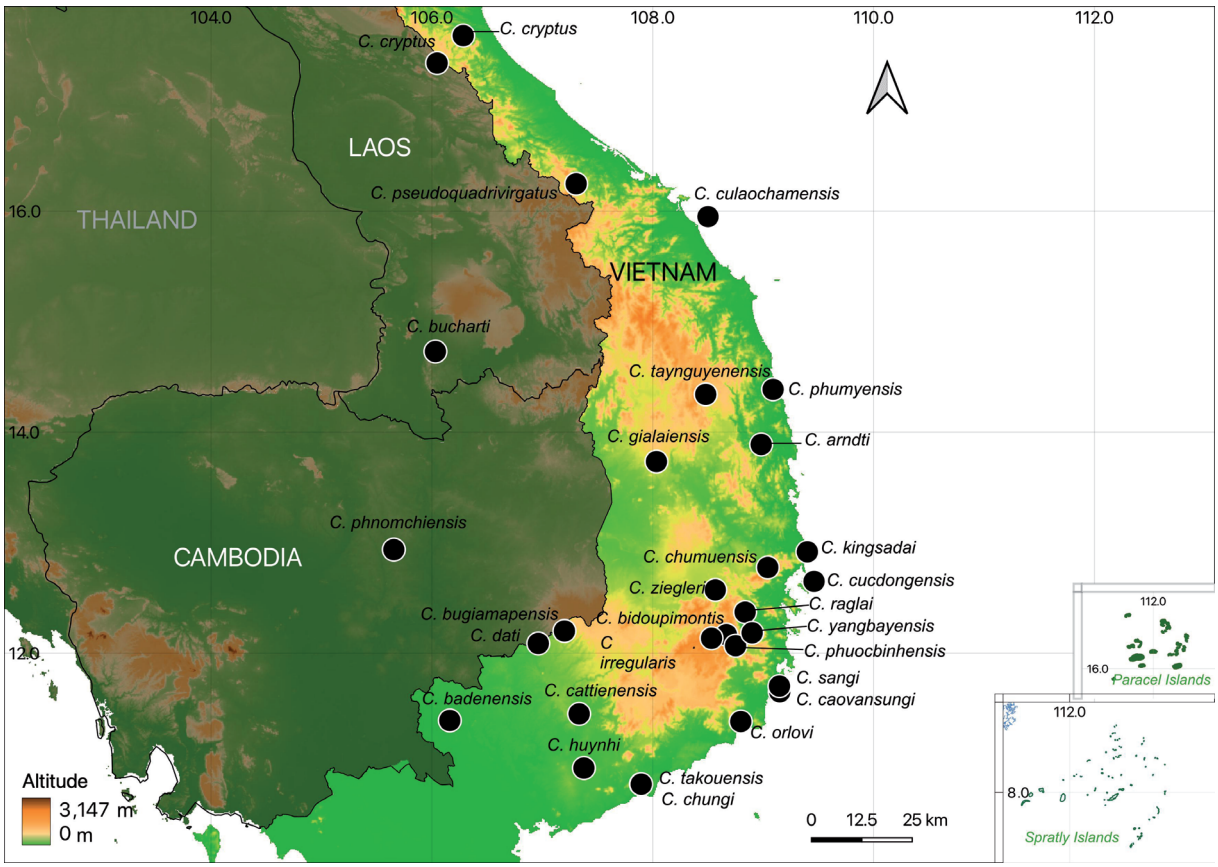

Fig. 10. Type localities of all taxa of *Cyrtodactylus irregularis* group occurring in Cambodia, Laos and Vietnam (the altitude data based on GADM database of Global Administrative Areas, 2022).

inhabit eastern Cambodia and southeast Laos, i.e., *C. buchardi*, *C. cryptus*, and *C. phnomchiensis* (David *et al.* 2004; Luu *et al.* 2016b; Neang *et al.* 2020; Grismer *et al.* 2021).

Species in this group occur in different habitat types, including granitic montane and limestone evergreen forests, and coffee farms. The adaptation ability allows its members to successfully diversify by occupying different ecological niches (Grismer *et al.* 2020; Ngo *et al.* 2022). Many areas in Cambodia, Laos, Vietnam where members of the *C. irregularis* group might occur are still poorly studied, e.g., the Central Highlands in Vietnam (Fig. 10). It is also noted that a number of species complexes and several potentially new species have been reported within the range of these broadly distributed taxa (Grismer *et al.* 2021; Ngo *et al.* 2022). For example, there exist three *C. pseudoquadrivirgatus* clades with high genetic divergence from each other as shown by this and previous studies. *Cyrtodactylus pseudoquadrivirgatus* was described by Rösler *et al.* (2008) from A Luoi, Thua Thien Hue Province; Huong Hoa, Quang Tri Province; Ba Na Nature Reserve, Da Nang Province; Kon Plong, Kon Tum Province. Thus, further studies are required to determine the actual distribution of the *C. irregularis* group by sequencing and examining the type specimens (i.e., *C. pseudoquadrivirgatus*, *C. irregularis*) to show whether it is in fact a species complex, viz. containing multiple taxa.

Similar to those relationships reported by Grismer *et al.* (2021) using ND2, the phylogeny supported by BI and ML in our study showed that *C. grismeri* belongs to the *C. condorensis* group although the former was placed in both the *C. irregularis* and *C. condorensis* groups in the barcoding study by Brennan *et al.* (2017) using a combination of COI and ND2. Moreover, the phylogenetic position of *C. badenensis* is still unclear. Grismer *et al.* (2021) considered *C. badenensis* the sister species to the *C. condorensis* group in their ML analysis but this placement was not supported in the BEAST analysis, using the mitochondrial gene ND2 and tRNAs. However, it was supported as a member of or sister to the *C. irregularis* group in this study and in several previous studies using COI gene or combining mito-nuclear data in both ML and BI analyses (i.e., Brennan *et al.* 2017; Nguyen *et al.* 2017; Grismer *et al.* 2021; Ostrowski *et al.* 2021). In addition, the species was not recognized as representative of the *C. irregularis* group based on morphological characters. It has a different dorsal pattern compared to that of remaining species within the *C. irregularis* group. Instead of an irregular brown dorsal pattern on a beige background and the occiput marbled in the same color, *C. badenensis* shows regular white transverse bands on a black background and a yellow occiput (Nguyen *et al.* 2006). *Cyrtodactylus badenensis* is tentatively placed in the *C. irregularis* group in the present study pending more comprehensive molecular study. Furthermore, *C. buchardi* was considered a member of *C. irregularis* in Grismer *et al.* (2021), but the authors who described this species suggested that *C. buchardi* is more closely related to the *C. angularis* group and especially to *C. papilionoides* based on five morphological characters (David *et al.* 2004; Grismer *et al.* 2021). Unfortunately, its phylogenetic placement has not been determined due to the lack of DNA samples. Thus, the status of *C. buchardi* is still controversial and should be addressed in future studies. *Cyrtodactylus phuocbinhensis* was strongly supported as the sister species to *C. sangi* Pauwels, Nazarov, Bobrov & Zhang and *C. cattienensis* Geissler, Nazarov, Orlov, Böhme, Phung, Nguyen & Ziegler, 2009 and *C. chungi* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler, 2021 and *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen, 2021 with strong nodal support in our BI analysis (Fig. 2), although Pauwels *et al.* (2018) suggested that *C. phuocbinhensis* belongs to a large clade, encompassing all other members of *Cyrtodactylus irregularis* group by high statistical values in both BI and ML analyses. In our study, however, we used different outgroups and incorporated more species than did Pauwels *et al.* (2018), and these changes might have caused the topological differences.

Importantly, knowledge on the distribution of *Cyrtodactylus* is still limited. Almost all species of *Cyrtodactylus* are only known from their type localities. According to the IUCN Red List, several species of this genus are facing severe extinction risks, as there are three species listed as Critically Endangered (CR), three species as Endangered (EN), and six species as Vulnerable (VU). Of these, two species listed as CR, *C. gialaiensis* and *C. takouensis*, one as EN, *C. caovansungi*, and two as VU, *C. badenensis* and *C. huynhi*, belong to the *Cyrtodactylus irregularis* group. Therefore, urgent research is needed not only

for resolving taxonomic issues but also for providing a better understanding of the population status, threats, and distribution of species within the largest group within the mega-diverse *Cyrtodactylus*.

Acknowledgements

We are grateful to the directorate of the Forest Protection Department of Dak Lak and Binh Dinh for support of our field work and issuing relevant permits. T.M Phung is grateful to P.N.T Lam, N.T.T. Mai, D.N.H Anh, (Ho Chi Minh City) for assistance in the field, for the loan of specimens. Thanks to T.V. Nguyen (Ninh Binh) for his assistance in the field. Thanks to T.A. Tran (Hanoi) for providing the map. For the fruitful cooperation within joint research projects, we cordially thank S.V. Nguyen (IEBR, Hanoi), as well as T. Pagel and C. Landsberg (Cologne Zoo). This research was funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under Grant Number 106.05-2020.24. Research of H.T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD). Comments from Aaron Bauer, Lee Grismer, and the editor greatly improved the paper.

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Manuscript received: 11 August 2022
Manuscript accepted: 14 December 2022
Published on: 19 June 2023
Topic editor: Tony Robillard
Section editor: Aurélien Miralles
Desk editor: Pepe Fernández

Supplementary material

Supp. file 1. Uncorrected (p) distance matrix showing percentage pairwise genetic divergence (COI) between *Cyrtodactylus chumuensis* sp. nov., *Cyrtodactylus arndti* sp. nov. and closely related species of *Cyrtodactylus* Gray, 1827. https://doi.org/10.5852/ejt.2023.875.2141.9087

Chapter 3. A new species of the Cyrtodactylus chauquangensis species group (Squamata, Gekkonidae) from Lao Cai Province, Vietnam

ZooKeys 1192: 83–102 (2024)
DOI: 10.3897/zookeys.1192.117135

Research Article

A new species of the *Cyrtodactylus chauquangensis* species group (Squamata, Gekkonidae) from Lao Cai Province, Vietnam

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Abstract

We describe a new species of the genus *Cyrtodactylus* based on five adult specimens from Bac Ha District, Lao Cai Province, northern Vietnam. *Cyrtodactylus luci* **sp. nov.** is distinguished from the remaining Indochinese bent-toed geckos by a combination of the following morphological characteristics: medium size (SVL up to 89.5 mm); dorsal tubercles in 17–19 irregular transverse rows; ventral scales in 32–34 longitudinal rows at midbody; precloacal pores present in both sexes, 9 or 10 in males, 8 or 9 in females; 12–15 enlarged femoral scales on each thigh; femoral pores 9–12 in males, 5–10 in females; postcloacal tubercles 2–4; lamellae under toe IV 21–23; dorsal pattern consisting of 5 or 6 irregular dark bands, a thin neckband without V-shape or triangle shape in the middle, top of head with dark brown blotches; subcaudal scales transversely enlarged. Molecular phylogenetic analyses recovered the new species as the sister taxon to *C. gulinqingensis* from Yunnan Province, China, with strong support from all analyses and the two taxa are separated by approximately 8.87–9.22% genetic divergence based on a fragment of the mitochondrial ND2 gene. This is the first representative of *Cyrtodactylus* known from Lao Cai Province.

Key words: *Cyrtodactylus luci* sp. nov., gecko, molecular phylogeny, morphology, ND2 gene, taxonomy

Academic editor: Anthony Herrel
Received: 10 December 2023
Accepted: 1 February 2024
Published: 19 February 2024

ZooBank: https://zoobank.org/9AE17751-35AF-4665-AA7C-C3906D68808F

Citation: Tran TT, Do QH, Pham CT, Phan TQ, Ngo HT, Le MD, Ziegler T, Nguyen TQ (2024) A new species of the *Cyrtodactylus chauquangensis* species group (Squamata, Gekkonidae) from Lao Cai Province, Vietnam. ZooKeys 1192: 83–102. https://doi.org/10.3897/zookeys.1192.117135

Introduction

The *Cyrtodactylus chauquangensis* species group is broadly distributed in the northern Indochina-Burma region, from northern Thailand and Laos to north central and northwestern Vietnam and to southwestern China (Uetz et al. 2023). Taxa within the group are almost exclusively adapted to karst ecosystems. Le et al. (2016) suggested that the group included at least ten species. Grismer et al. (2021a, 2021b) provided a taxonomic review and analyzed phylogenetic relationships of 17 species and one undescribed form from northern Thailand.

The group currently contains 23 recognized species with several taxa recently discovered from Yunnan Province, southern China (Grismer et al. 2021a, 2021b, 2021c; Liu and Rao 2021, 2022).

Lao Cai Province is located in the border area between Vietnam and China with an international borderline of 203 km (Portal of Lao Cai Province 2023). Although Lao Cai contains an area of limestone forest (Portal of Lao Cai Province 2023), no representative of *Cyrtodactylus* has been known from this province so far. On the other hand, members of the genus have been recorded in several neighboring forests, including six species from Yunnan Province of China (*Cyrtodactylus dianxiensis* Liu & Rao, 2021, *C. gulinqingensis* Liu, Li, Hou, Orlov & Ananjeva, 2021, *C. hekouensis* Zhang, Liu, Bernstein, Wang & Yuan, 2021, *C. menglianensis* Liu & Rao, 2022, *C. wayakonei* Nguyen, Kingsada, Rösler, Auer & Ziegler, 2010, *C. zhenkangensis* Liu & Rao, 2021) and five other species reported from Vietnam: one species from Lai Chau (*C. martini* Ngo, 2011) and four species from Son La (*C. bichnganae* Ngo & Grismer, 2010, *C. otai* Nguyen, Le, Pham, Ngo, Hoang, Pham & Ziegler, 2015, *C. sonlaensis* Nguyen, Pham, Ziegler, Ngo & Le, 2017 and *C. taybacensis* Pham, Le, Ngo, Ziegler & Nguyen, 2019).

During our recent field trip in northern Vietnam, we collected five specimens of an unnamed gekkonid species from Bac Ha District, Lao Cai Province, which can be assigned to the *Cyrtodactylus chauquangensis* group based on molecular data. However, the population from Lao Cai Province can be distinguished from congeners by morphological differences and genetic divergence. Therefore, we describe it as a new species in the following.

Materials and methods

Sampling

Field surveys were conducted in Bac Ha District, Lao Cai Province, Vietnam in June 2022 and October 2023 (Fig. 1). After being photographed in life, specimens were anesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 85% ethanol and subsequently stored in 70% ethanol. Specimens were subsequently deposited in the collections of the Institute of Ecology and Biological Resources (**IEBR**), Hanoi, Vietnam.

Molecular data and phylogenetic analyses

DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Germany) following manufacturer's instructions. Extracted DNA was amplified by HotStar Taq Mastermix (Qiagen, Germany) with 21 µl volume (10 µl of mastermix, 5 µl of water, 2 µl of each primer at 10 pmol and 2 µl of DNA). PCR conditions were: 95 °C for 15 min to active the taq; with 40 cycles at 95 °C for 30 s, 52 °C for 45 s, 72 °C for 60 s; and the final extension at 72 °C for 6 min. A fragment of the mitochondrial gene, NADH dehydrogenase subunit 2 (ND2), was amplified using the primer pair MetF1 (5'-AAGCTTTCGGGCCCATACC-3') and COIR1 (5'-AGRGTGCCAATGTCTTTGTGRTT-3') (Arevalo et al. 1994; Macey et al. 1997). PCR products were visualized using electrophoresis through a 2% agarose gel stained with ethidium bromide. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purification kit (ThermoFischer

Figure 1. Type locality of *Cyrtodactylus luci* sp. nov. in Lao Cai Province (red circle), Vietnam.

Scientific, Lithuania). Purified PCR products were sent to FirstBase (Malaysia) for sequencing in both directions. We included two samples of the newly discovered population from Lao Cai Province, one of *Cyrtodactylus bichnganae*, one of *C. bobrovi*, one of *C. cucphuongensis,* one of *C. huongsonensis*, one of *C. ngoiensis*, one of *C. sonlaensis*, one of *C. taybacensis*, and one of *C. vilaphongi* along with all available GenBank sequences of these species and other members of the *Cyrtodactylus chauquangensis* group. Two species, *C. hontreensis* and *C. septimontium*, of the *C. intermedius* group, were selected as outgroups (Grismer et al. 2021b). In the end, we were able to incorporate all ingroup taxa (Table 1).

After sequences were aligned by Clustal X v.2.1 (Thompson et al. 1997), data were analyzed using maximum likelihood (ML) as implemented in IQ-TREE (Nguyen et al. 2015), maximum parsimony (MP) implemented in PAUP*4.0b10 (Swofford 2001) and Bayesian inference (BI) as implemented in MrBayes v.3.2.7

Table 1. Species of Cyrtodactylus used in the phylogenetic analysis including localities and GenBank accession numbers of the mitochondrial NADH dehydrogenase subunit 2 (ND2) fragment gene (–: data unavailable).

Species	Locality	Museum number/ Field number	Accession number	Reference
C. auribalteatus	Cambodia: Phnom Aural Wildlife Sanctuary, Kampong Speu Province	–	AP018116	Areesirisuk et al. 2018
Cyrtodactylus luci sp. nov.	Vietnam: Coc Ly Commune, Bac Ha District, Lao Cai Province	IEBR R.5240	PP253960	This study
Cyrtodactylus luci sp. nov.	Vietnam: Coc Ly Commune, Bac Ha District, Lao Cai Province	IEBR R.5241	PP253059	This study
C. bichnganae	Vietnam: Son La City, Son La Province	UNS 0473	MF169953	Brennan et al. 2017
C. bichnganae	Vietnam: Son La City, Son La Province	TBU PAT250	PP253951	This study
C. bobrovi	Vietnam: Ngoc Son – Ngo Luong NR, Lac Son District, Hoa Binh Province	IEBR A.2015.29	MT953471	Grismer et al. 2020
C. bobrovi	Vietnam: Tan Lac, Hoa Binh Province	HB.2015.73	PP253953	This study
C. chauquangensis	Vietnam: Quy Hop District, Nghe An Province	NA 2016.1	MT953475	Grismer et al. 2020
C. cucphuongensis	Vietnam: Cuc Phuong NP, Ninh Binh Province	CP 17.02	MT953477	Grismer et al. 2020
C. cucphuongensis	Vietnam: Cuc Phuong NP, Ninh Binh Province	NHQ.17.71	PP253954	This study
C. doisuthep	Thailand: Doi Phrabart abbey, Chiang Dao District, Chiang Mai Province	AUP–00777	MT497801	Chomdej et al. 2021
C. doisuthep	Thailand: Doi Suthep Mt., Chiang Mai Province	AUP–00774	MT550626	Chomdej et al. 2020
C. dumnuii	Thailand: Chiang Dao, Chiang Mai Province	AUP 00768	MW713972	Grismer et al. 2021
C. erythrops	Thailand: Coral Cave, Pang Mapha District, Mae Hong Son Province	AUP–00771	MT497806	Chomdej et al. 2021
C. erythrops	Thailand: Moe Cham Pae, Mae Hong Son	AUP 00772	MW713958	Grismer et al. 2021b
C. gulinqingensis	China: Gulinqing NR, Maguan County, Wenshan Prefecture, Yunnan Province	KIZ 061813	MZ782150	Liu et al. 2021
C. gulinqingensis	China: Gulinqing NR, Maguan County, Wenshan Prefecture, Yunnan Province	KIZ 061816	MZ782152	Liu et al. 2021
C. gulinqingensis	China: Gulinqing NR, Maguan County, Wenshan Prefecture, Yunnan Province	KIZ 061817	MZ782153	Liu et al. 2021
C. houaphanensis	Laos: near Viengxai, Houaphan Province	IEBR A.2013.109	MW792067	Grismer et al. 2021b
C. huongsonensis	Vietnam: Huong Son, My Duc District, Hanoi City	IEBR A.2011.3A	MT953481	Grismer et al. 2020
C. huongsonensis	Vietnam: Lac Thuy, Hoa Binh Province	HB.2016.44	PP253957	This study
C. hontreensis	Vietnam: Hon Tre Island, Kien Hai District, Kien Giang Province	LSUHC8583	JX440539	Wood et al. 2012
C. martini	Vietnam: Lai Chau Town, Lai Chau Province	UNS 0471	MF169968	Brennan et al. 2017
C. menglianensis	China: Menglian County, Puer City, Yunnan Province	KIZ20210714	OM296043	Liu and Rao 2022
C. menglianensis	China: Menglian County, Puer City, Yunnan Province	KIZ20210716	OM296044	Liu and Rao 2022
C. ngoiensis	Laos: Ngoi District, Luang Prabang Province	IEBR A.20213.100	MW792066	Grismer et al. 2021b
C. ngoiensis	Laos: Ngoi District, Luang Prabang Province	AT2012.1	PP253956	This study
C. otai	Vietnam: Xuan Nha NR, Van Ho District, Son La Province	TBU 2017.2	MT953486	Grismer et al. 2020
C. puhuensis	Vietnam: Pu Hu Nature Reserve, Thanh Hoa Province	ND 01.15	MT953489	Grismer et al. 2020
C. septimontium	Vietnam: Co To Mountain, An Giang Province	NAP 05321	MH940237	Murdoch et al. 2019
C. sonlaensis	Vietnam: Muong Bang Commune, Phu Yen District, Son La Province	IEBR A.2017.1	MT953492	Grismer et al. 2020
C. sonlaensis	Vietnam: Muong Bang Commune, Phu Yen District, Son La Province	IEBR A.2017.2	PP253958	This study
C. soni	Vietnam: Van Long Wetland NR, Gia Vien District, Ninh Binh Province	IEBR R.2016.4	MT953491	Grismer et al. 2020
C. spelaeus	Laos: Kasi District, Vientiane Province	HLM 0315	MW713962	Grismer et al. 2021b
C. taybacensis	Vietnam: Ca Nang Commune, Quynh Nhai District, Son La Province	IEBR 4379	MT953495	Grismer et al. 2020
C. taybacensis	Vietnam: Ta Ma Commune, Tuan Giao District, Dien Bien Province	DB2021.1	PP253952	This study
C. vilaphongi	Laos: Luang Prabang District, Luang Prabang Province	NUOL R–2013.5	PP253955	This study
C. vilaphongi	Laos: Luang Prabang District, Luang Prabang Province	IEBR A.2013.13	MT953497	Grismer et al. 2021b
C. wayakonei	Laos: Ban Nam Eng, Vieng Phoukha District, Luang Nam Tha Province	ZFMK 91016	MT953498	Grismer et al. 2020
C. zhenkangensis	China: Zhenkang County, Lincang City, Yunnan Province	KIZL2020047	MW792062	Grismer et al. 2021b

(Ronquist et al. 2012). For the MP analysis, heuristic analysis was conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch-swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) was calculated using 1000 pseudo-replicates and 100 random taxon addition replicates. All characters

were equally weighted and unordered. For the ML analysis, we used IQ-TREE v.1.6.8 (Nguyen et al. 2015) with a single model and 10000 ultrafast bootstrap replications (UFB). The optimal model for nucleotide evolution was determined using jModelTest v.1.2.4 (Darriba et al. 2012).

For the BI analysis, we used the optimal model determined by jModelTest with parameters estimated by MrBayes v.3.2.7. Two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10^7 generations with a random starting tree and sampled every 1000 generations. Loglikelihood scores of sample points were plotted against generation time to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function. The posterior probability values (PP) for all nodes in the final majority rule consensus tree were provided. We regard BP ≥ 70% and UFB and PP of ≥ 95% as strong support and values of < 70% and < 95%, respectively, as weak support (Hillis and Bull 1993; Ronquist et al. 2012; Minh et al. 2013).

The optimal model for nucleotide evolution was set to GTR+I+G for ML and BI analysis. The cut-off point for the burn-in function was set to 60, or 0.6% of the total number of trees generated, in the Bayesian analysis, as -lnL scores reached stationarity after 60,000 generations in both runs. Uncorrected pairwise divergences were calculated in PAUP*4.0b10.

Morphological characters

Measurements were taken with a digital calliper to the nearest 0.1 mm. Abbreviations are as follows: **SVL:** snout-vent length, measured from tip of snout to vent; **TaL:** tail length, measured from vent to tip of tail (* = regenerated); **HL:** head length, measured from tip of snout to retroarticular process of jaw; **HW:** head width, maximum width of head; **HH:** head height, from occiput to underside of jaws; **OrbD:** orbital diameter, greatest diameter of orbit; **SE:** snout to eye distance, from tip of snout to anterior-most point of eye; **EE:** eye to ear distance, from anterior edge of ear opening to posterior corner of eye; **NE:** nares to eye distance, from anterior-most point of eye to posterior-most point of nostril; **ED:** ear length, longest dimension of ear; **ForeaL:** forearm length, from base of palm to tip of elbow; **CrusL:** crus length, from base of heel to knee; **TrunkL:** trunk length, distance from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; **BW:** body width, the widest distance of body; **Internar:** internarial distance, distance between nares; **Interorb:** interorbital distance, shortest distance between left and right supraciliary scale rows.

Scale counts were taken as follows: **SL:** supralabials, counted from the first labial scale to corner of mouth; **IL:** infralabials, counted from the first labial scale to corner of mouth; **N:** nasal scales surrounding nare; **IN:** postrostrals or internasals; **PM:** postmentals; **GST:** granular scales surrounding dorsal tubercles; **V:** ventral scales in longitudinal rows at midbody; **SLB:** number of scales along the midbody from mental to anterior edge of cloaca; **FP:** femoral pores; **PP:** precloacal pores; **PAT:** postcloacal tubercles; **TubR:** tubercle, number of dorsal longitudinal rows of tubercles at midbody between the lateral folds; **EFS:** enlarged femoral scales, number of enlarged femoral scale beneath each thigh; **NSF IV:** number of subdigital lamellae on the fourth finger; **NST IV:** number of subdigital lamellae on the fourth toe. Bilateral scale counts were given as left/right; above sea level (asl).

Multiple Factor Analysis (MFA)

The MFA was also applied in this study using morphometric and meristic characteristics, including SVL, HL, HW, HH, OrbD, SE, EE, ED, ForeaL, CrusL, TrunkL, Internar, Interob and SL, IL, GST, V, TubR, EFS, FP, PP, PAT, NSF IV, NST IV. Other morphological characteristics were not used due to the limitation of available morphometric and meristic data or incomplete sampling (regenerated tail). All statistical analyses were performed using R Core Team (2023). The MFA used six quantitative groups – "SVL", "Head" (including HL, HW, HH), "Eye" (consist of OrbD, SE, EE, ED), "FT" (including ForeaL and CrusL), "TrunkL", "Inter" (consist of Internar and Interorb) and eight qualitative groups – "SpeciesInfor" (including Name of species and ID), "SL-IL" (consist of SL and IL in both sides), "GST_PAT_TubR" (including GST, PAT in both sides and TubR), "V", "EFS" in both sides, "FP" in both sides, "PP", "LIV" (consist of NSF IV and NST IV in left side). To remove the effects of allometry, morphometric data were also normalized to adjust raw data of morphometrics through the allom() function in R package GroupStruct (available at heep://github.com/chankinonn/GroupStruct). Accordingly, the allometric formula is $X_{adj} = \log_{10}(X) - ß[\log_{10}(SVL)-\log_{10}(SVL_{mean})]$, where X_{adj} = adjusted value; X = measured value; ß = unstandardized regression coefficient for each population and SVL_{mean} = overall average SVL of two populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000; Grismer et al. 2021a; Chan and Grismer 2022). The ordination test was performed using packages Factoextra (Kassambara and Mundt 2017) and FactoMineR (Le et al. 2008) in the software R. The approach was applied to identify active groups and to explain phenotypic variance by estimating the first two Dim values-eigenvalue proportions. Similar coded colors in the MFA scatter plot, surrounded with convex hulls, were presented to visualize the phenotypic spaces of the new species and the most closely related species from China, namely *Cyrtodactylus gulinqingensis* Liu, Li, Hou, Orlov & Ananjeva, 2021; spaces were shown within a spatial coordinate of dimension axes (Dim1 and Dim2). To evaluate the overlap, the loadings of Dim1 and Dim2 of each *Cyrtodactylus* individual were extracted to identify the difference between the two species using the T-test. For all the tests, we applied a significance level of $p < 0.05$.

Results

Phylogenetic analysis

The matrix of molecular data contained 1300 aligned characters, of which 580 were parsimony informative. The MP analysis produced a single most parsimonious tree (tree length = 2359, consistency index = 0.49, retention index = 0.66). Tree topologies from three analyses, ML, MP, and BI were similar and the *Cyrtodactylus* from Bac Ha District, Lao Cai Province was recovered with strong statistical support in all analyses as the sister taxon to *C. gulinqingensis* (BP = 94%; UBP = 100%; PP = 1.00) (Fig. 2). In terms of genetic divergences, the new species is separated from *C. gulinqingensis* by 8.87–9.22% based on a fragment of the mitochondrial ND2 gene. Genetically, it is also significantly divergent from other species within the *C. chauquangensis* group with a pairwise divergence of 12.32–23.85% (Suppl. material 1).

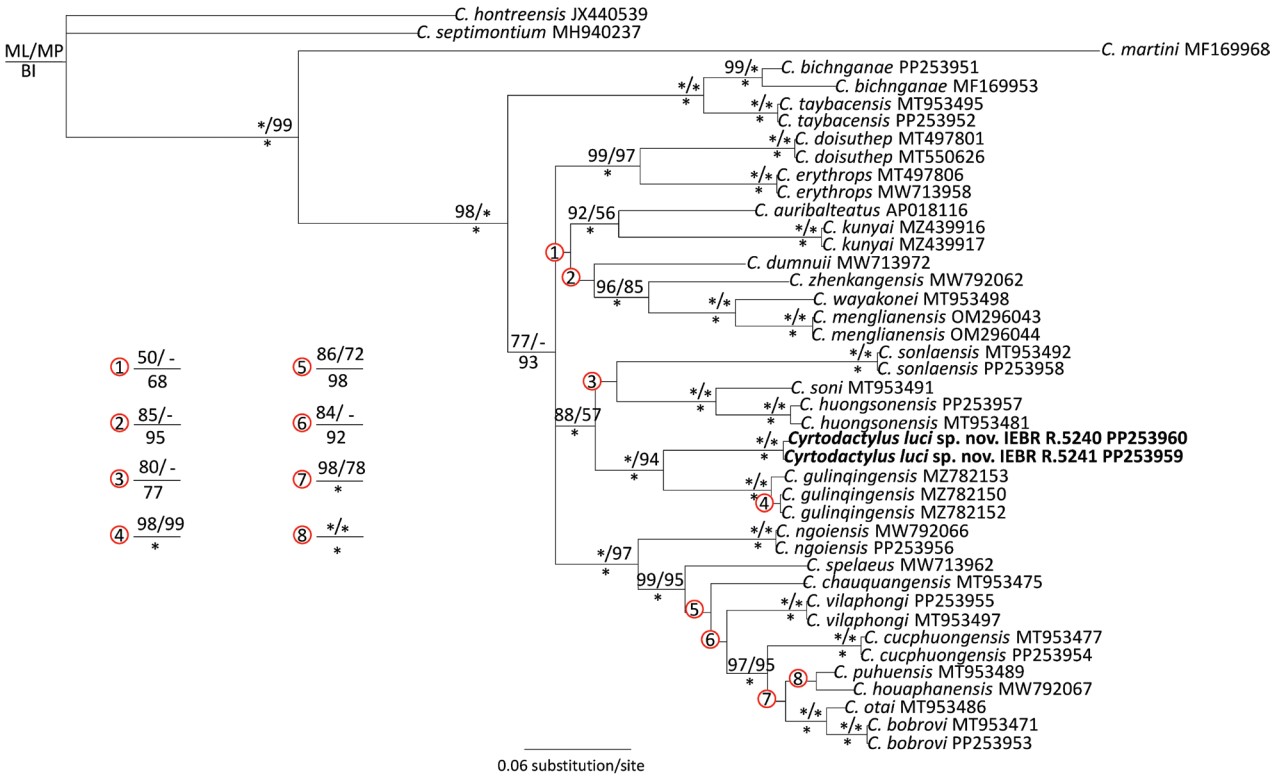

Figure 2. Phylogram based on the Bayesian analysis. Number above and below branches are ML/MP bootstrap and ultrafast bootstrap values and Bayesian posterior probabilities (≥ 50%), respectively. Asterisk and hyphen denote 100% and > 50% values, respectively.

Morphological analysis

Morphologically, the new species from Bac Ha District, Lao Cai Province is closely similar to *C. gulinqingensis* from Yunnan Province, China, however, they plotted separately from each other in MFA (Fig. 3A) and there was a significant difference between two species ($p < 0.05$). The MFA also identified the data set of SVL, Head, Eye, FT, TrunkL, Inter, SL-IL, GST_PAT_TubR, V, EFS, FP, PP as active groups (Fig. 3B). The Eye, FT, Head, Inter, SVL and Trunk groups were the most important in both the first and second multi-factorial dimensions (Fig. 3C, D).

Taxonomy

***Cyrtodactylus luci* sp. nov.**

https://zoobank.org/B03559F4-9C45-4991-8A74-5C346FCD6C37

Figs 4, 5

Type material. *Holotype*. IEBR R.5237 (Field number BH-LC 2022.5), adult male, collected by T.T. Tran, T.Q. Phan and N.H. Nguyen on 30 June 2022, in limestone karst forest near Tham Phuc Village (22°29.514'N, 104°12.416'E, at an elevation of 677 m a.s.l), Coc Ly Commune, Bac Ha District, Lao Cai Province, Vietnam. ***Paratypes*.** IEBR R.5238 (Field number BH-LC 2022.1), IEBR R.5239 (Field number BH-LC 2022.3), adult males and IEBR R.5240, R.5241 (Field numbers BH-LC 2022.2, 2022.4), adult females, bear the same collection data as the holotype.

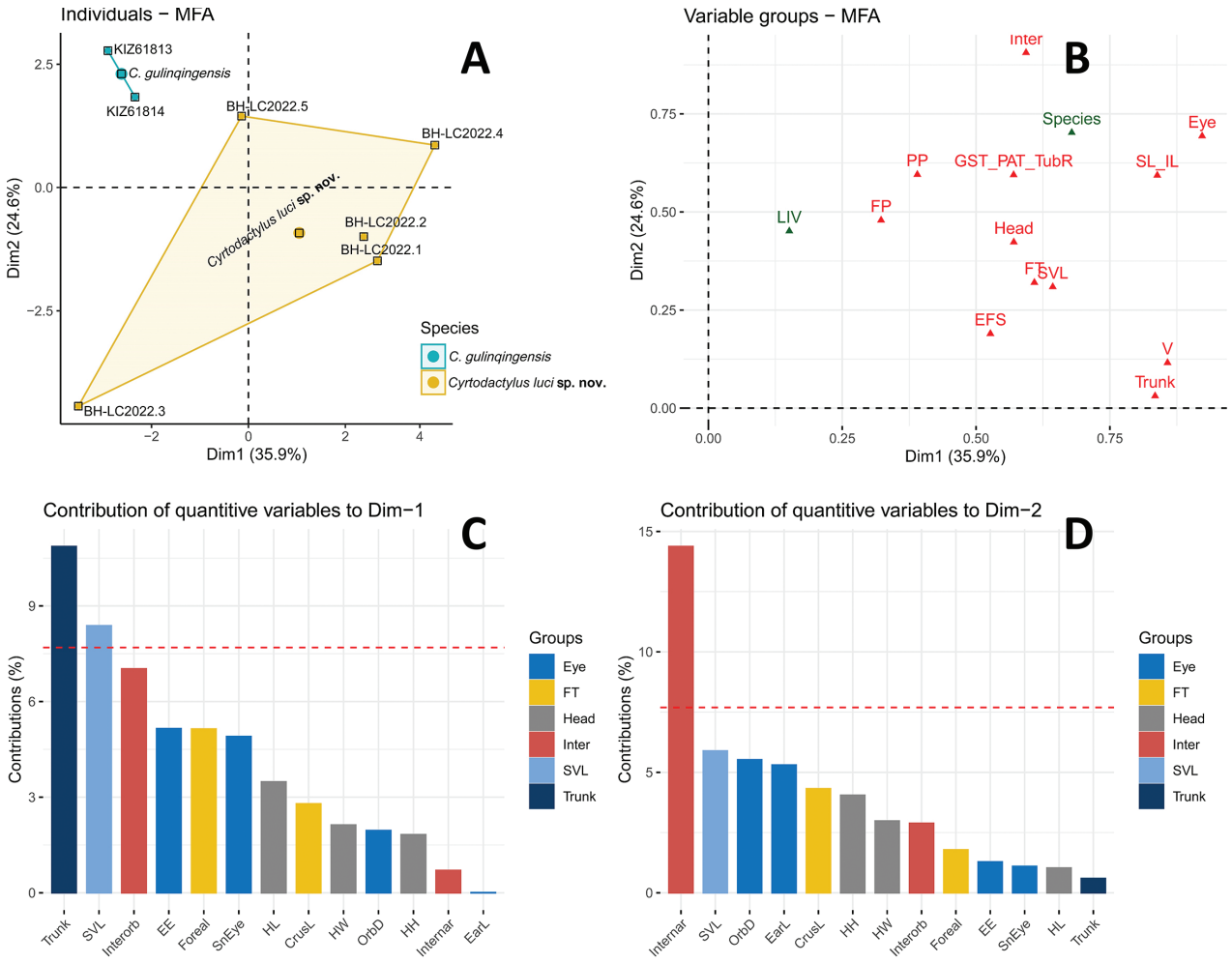

Figure 3. A MFA of *Cyrtodactylus luci* sp. nov. from Vietnam and *C. gulinqingensis* from China **B** scatterplot the groups of all variables for Dim1 and Dim2 axes in the MFA, green triangles as inactive groups of variables, red triangles as active groups of variables **C** bar plot of groups' contribution to the first axes (Dim1) in the MFA **D** bar plot of groups' contribution to the second axes (Dim2) in the MFA.

Diagnosis. The new species can be distinguished from other members of the genus *Cyrtodactylus* by a combination of the following characteristics: Size medium (SVL up to 89.5 mm); dorsal tubercles in 17–19 irregular transverse rows; ventral scales in 32–34 longitudinal rows at midbody; precloacal pores present in both sexual, 9 or 10 in males, 8 or 9 in females; 12–15 enlarged femoral scales on each thigh; femoral pores 9–12 in males, 5–10 in females; postcloacal tubercles 2–4; lamellae under toe IV 21–23; dorsal pattern consisting of 5 or 6 irregular dark bands, a discontinuous thin neckband without V-shape or triangle shape in the middle, dorsal head surface with dark brown blotches; subcaudal scales transversely enlarged.

Description of holotype. Adult male, snout-vent length (SVL) 86.3 mm; body relatively short (TrunkL/SVL 0.4); head distinct from neck, moderately long (HL/SVL 0.28), relatively wide (HW/HL 0.69), slightly depressed (HH/HL 0.41); eye slightly large (OrbD/HL 0.24), pupils vertical; upper eyelid fringe with spinous scales; ear opening below the postocular stripes, obliquely directed and oval, small in size (ED/HL 0.06); two enlarged supranasals, separated from each other anteriorly by one internasal; nares oval, surrounded by supranasal, ros-

tral, first supralabial and three postnasals; loreal region and frontal concave; snout long (SE/HL 0.41), round anteriorly, longer than diameter of orbit (OrbD/SE 0.58); snout scales small, round, granular, larger than those in frontal and parietal regions; rostral wider than high with a medial suture, bordered by first supralabial on each side, nostrils, two supranasals and one internasal; mental triangular, wider than high; postmentals two, enlarged, in contact posteriorly, bordered by mental anteriorly, first infralabial laterally, and an enlarged chin scale posteriorly; supralabials 11/10; infralabials 11/10.

Dorsal scales granular; dorsal tubercles round, keeled, conical, four or five times larger than the size of adjoining scales, each surrounded by 10 granular scales, tubercles forming 17 irregular longitudinal rows at midbody; ventral scales smooth, medial scales 2–3 times larger than dorsal granules, round, subimbricate, largest posteriorly, in 32 longitudinal rows at midbody; lateral folds present, without interspersed tubercles; gular region with homogeneous smooth scales; ventral scales between mental and cloacal slit 170; precloacal groove absent; three rows of enlarged scales present in posterior region of pore-bearing scales; ten precloacal pores arranged in a chevron; 12 or 13 enlarged femoral scales beneath thighs continuous with pore-bearing precloacal scales; femoral pores present on each enlarged femoral scales (except one on right thigh), 24 in total; precloacal pores large, horizontal elongated, positioned in posterior margin of scales; femoral pores small, round, positioned in the center of scales.

Fore and hind limbs moderately slender (ForeaL/SVL 0.16, CrusL/SVL 0.19); dorsal surface of forelimbs covered by few slightly developed tubercles; fingers and toes lacking distinct webbing; subdigital lamellae: finger I 12, finger II 16, finger III 17, finger IV 20, finger V 18, toe I 12, toe II 17, toe III 20, toe IV 21, toe V 20.

Tail regenerated, 104.5 mm in length (generated part 19.5 mm); longer than snout-vent length (TaL/SVL: 1.21); postcloacal tubercles 4/4; subcaudals on original part of tail distinctly transversely enlarged, flat, smooth.

Coloration in life. Ground color of dorsal surface of head, neck, body, limbs and tail light brown. Dorsal surface of head with some dark brown blotches; labial region brown with yellowish cream stripes; skin above the eye gray; eyelid with light yellow color; iris yellow copper with black marking; pupil vertical, elliptical, black; nuchal loop dark brown, discontinous, extending from posterior corner of eye to the neck; tubercles on head, limbs, dorsum light brown to yellow; dorsum with five irregularly-shaped transversal bands and additional irregular smaller blotches; upper surface of limbs with irregular brown marks; six dark brown irregular bands on original part of tail while regenerated part of tail dark gray; chin, throat, chest, belly, lower limbs and ventral surface of tail cream.

Coloration in preservative. The overall color scheme slightly fades in 70% alcohol; yellow color disappeared in preservation while main characteristics are still clearly discernible; dorsal ground color of head, neck, body, limbs and tail grayish brown; color of chin, throat, chest, belly and lower limbs did not change noticeably in preservation.

Sexual dimorphism and variation. The males differ from females in the shape of precloacal pores (larger in males), and the presence of hemipenial swellings at the tail base. For other morphological characteristics see Table 2, Figs 4, 5.

Distribution. *Cyrtodactylus luci* sp. nov. is currently known only from the type locality in Bac Ha District, Lao Cai Province, Vietnam (Fig. 1).

Etymology. The species was named after the zoologist from the Vietnam National Museum of Nature, Vietnam Academy of Science and Technology, late Associate Professor Doctor Luc Van Pham, who contributed greatly to the biodiversity study in Vietnam. For the common names, we suggest Luc's Bent-toed Gecko (English) and Thạch sùng ngón lực (Vietnamese).

Figure 4. Male holotype of *Cyrtodactylus luci* sp. nov. (IEBR R.5237) in life. Photo: T.Q. Phan.

Figure 5. Female paratype of *Cyrtodactylus luci* sp. nov. (IEBR R.5241) in life. Photo: T.Q. Phan.

Natural history. The bent-toed geckos were collected between 19:00 and 22:00, both on limestone cliffs and on trees, about 1.0–1.8 m above the ground. The surrounding habitat was secondary karst forest of medium and small hardwoods mixed with shrubs and vines (Fig. 6). Air temperature was 25.9 °C and relative humidity was 92%.

Comparisons. *Cyrtodactylus luci* sp. nov. is distinguishable from all other members of the *C. chauquangensis* species group by a unique combination of morphological characteristics.

Cyrtodactylus luci sp. nov. differs from *C. auribalteatus* Sumontha, Panitvong & Deein, 2010 by having fewer ventral scale rows (32–34 vs. 38–40 in *C. auribalteatus*), more enlarged femoral scales on each side (12–15 vs. 5–7 in *C. auribalteatus*), more femoral pores on each side in males (9–12 vs. 4 or 5 in *C. auribalteatus*), the presence of femoral pores on each side in females (5–10 vs.

Table 2. Measurements (in mm) and morphological characteristics (abbreviations as in Material and methods) of the type series of *Cyrtodactylus luci* sp. nov. (* = regenerated or broken tail); bilateral meristic characteristics are given as (left/right).

Characters	IEBR R.5237 (Holotype)	IEBR R.5238 (Paratype)	IEBR R.5239 (Paratype)	IEBR R.5240 (Paratype)	IEBR R.5241 (Paratype)	Min–Max
Sex	M	M	M	F	F	
SVL	86.3	88.7	71.7	87.1	89.5	71.7–89.5
TaL	104.5*	107.7	86.2	84.2*	84.1*	86.2–107.7
HL	24.5	24.0	20.3	24.6	25.2	20.3–25.2
HW	16.9	16.6	12.8	17.4	17.4	12.8–17.4
HH	10.1	9.8	7.1	9.7	10.6	7.1–10.6
OrbD	5.9	4.9	4.7	5.1	4.8	4.7–5.9
SE	10.2	10.0	8.4	10.6	10.8	8.4–10.8
EE	6.5	6.6	5.5	6.6	7.2	5.5–7.2
NE	7.5	7.9	6.0	7.7	8.7	6.0–8.7
ED	1.4	1.6	1.9	1.8	1.3	1.4–1.9
ForeaL	14.2	14.2	11.5	14.1	14.4	11.5–14.4
CrusL	16.3	17.2	13.5	16.7	16.8	13.5–17.2
TrunkL	34.4	39.7	31.5	39.7	42.1	31.5–42.1
BW	13.8	14.0	9.4	17.6	19.2	9.4–19.2
Internar	2.8	2.5	2.0	2.7	3.0	2.0–3.0
Interorb	6.9	7.3	5.2	7.6	7.8	5.2–7.8
SL	11/10	11/11	10//10	11/10	11/9	9–11
IL	11/10	12/12	11/13	11/10	9/12	9–13
N	4/4	4/4	4/4	4/4	4/5	4–5
IN	1	1	1	1	1	1
PM	2	3	2	2	2	2
GST	10/10/10	10/10/10	10/9/10	10/10/10	10/10/10	9–10
V	32	34	32	34	34	32–34
SLB	170	171	169	171	166	166–171
FP	12/12	10/9	11/12	10/10	7/5	9–12 in males 5–10 in females
PP	10	9	9	8	9	9–10 in males 8–9 in females
PAT	3/3	4/2	3/3	4/3	3/3	2–4
TubR	17	17	17	19	18	17–19
EFS	13/12	14/15	14/14	13/13	17/15	12–15
NSF IV	18	21	20	19	20	18–21
NST IV	21	23	23	21	23	21–23

absent in *C. auribalteatus*), more precloacal pores in males (9 or 10 vs. 6 in *C. auribalteatus*), the presence of precloacal pores in females (8 or 9 vs. absent in *C. auribalteatus*) and fewer dorsal tubercle rows (17–19 vs. 22–24 in *C. auribalteatus*); from *C. bichnganae* Ngo & Grismer, 2010 by having a smaller size (SVL 71.7–89.5 mm vs. 95.3–99.9 mm in *C. bichnganae*), more ventral scale rows (32–34 vs. 30 or 31 in *C. bichnganae*), more femoral pores on each side in females (5–10 vs. 1 in *C. bichnganae*), and more lamellae under toe IV (21–23 vs. 16–20 in *C. bichnganae*); from *C. bobrovi* Nguyen, Le, Pham, Ngo, Hoang, Pham & Ziegler, 2015 by having fewer ventral scale rows (32–34 vs. 40–45 in *C. bobrovi*), the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. bobrovi*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. bobrovi*) and in females (5–10 vs. absent in *C. bobrovi*), more precloacal pores in males (9 or 10 vs. 5 in *C. bobrovi*), the presence of precloacal pores in females (8 or 9 vs. absent in *C. bobrovi*), and the presence of transversely enlarged subcaudal plates (vs. absent in *C. bobrovi*); from *C. chauquangensis* Hoang, Orlov, Ananjeva, Johns, Hoang & Dau, 2007 by having a smaller size (SVL 71.7–89.5 mm vs. 91.0–99.3 mm in *C. chauquangensis*), fewer ventral scale rows (32–34 vs. 36–38 in *C. chauquangensis*), the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. chauquangensis*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. chauquangensis*) and also in females (5–10 vs. absent in *C. chauquangensis*), more precloacal pores in males (9 or 10 vs. 6 or 7 in *C. chauquangensis*) and also in females (8 or 9 vs. 6 or 7 in *C. chauquangensis*); from *C. cucphuongensis* Ngo & Chan, 2011 by having fewer ventral scale rows (32–34 vs. 42 in *C. cucphuongensis*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. cucphuongensis*) and in females (5–10 vs. absent in *C. cucphuongensis*) and the presence of precloacal pores in males (9–10 vs. absent in *C. cucphuongensis*); from *C. doisuthep* Kunya, Panmongkol, Pauwels, Sumontha, Meewasana, Bunkhwamdi & Dangsri, 2015 by the presence of femoral pores on each side in males (9–12 vs. absent in *C. doisuthep*) and in females (5–10 vs. absent in *C. doisuthep*), more precloacal pores in males (9 or 10 vs. 5 or 6 in *C. doisuthep*) and also in females (8 or 9 vs. absent in *C. doisuthep*); from *C. dumnuii* Bauer, Kunya, Sumontha, Niyomwan, Pauwels, Chanhome & Kunya, 2010 by having fewer ventral scale rows (32–34 vs. 40 in *C. dumnuii*), more femoral pores on each side in males (9–12 vs. 6–7 in *C. dumnuii*) and in females (5–10 vs. absent in *C. dumnuii*), more precloacal pores in males (9 or 10 vs. 5 or 6 in *C. dumnuii*) and also in females (8 or 9 vs. 0–7 in *C. dumnuii*) and more lamellae under toe IV (21–23 vs. 19 in *C. dumnuii*); from *C. erythrops* Bauer, Kunya, Sumontha, Niyomwan, Panitvong, Pauwels, Chanhome & Kunya, 2009 by having more ventral scale rows (32–34 vs. 28 in *C. erythrops*), more lamellae under finger IV (18–21 vs. 16 in *C. erythrops*), more lamellae under toe IV (21–23 vs. 20 in *C. erythrops*) and differences in dorsal color pattern (banded vs. blotched in *C. erythrops*); from *C. gulinqingensis* Liu, Li, Hou, Orlov & Ananjeva, 2021 by having more dorsal tubercle rows (17–19 vs. 14–16 in *C. gulinqingensis*), fewer femoral pores on each side in males (9–12 vs. 13–15 in *C. gulinqingensis*) and in females (5–10 vs. 1–3 in *C. gulinqingensis*) and fewer precloacal pores in females (8 or 9 vs. 7 in *C. gulinqingensis*); from *C. houaphanensis* Schneider, Luu, Sitthivong, Teynié, Le, Nguyen & Ziegler, 2020 by having fewer ventral scale rows (32–34 vs. 35 in *C. houaphanensis*), the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. houaphanensis*), the presence of femoral pores on each

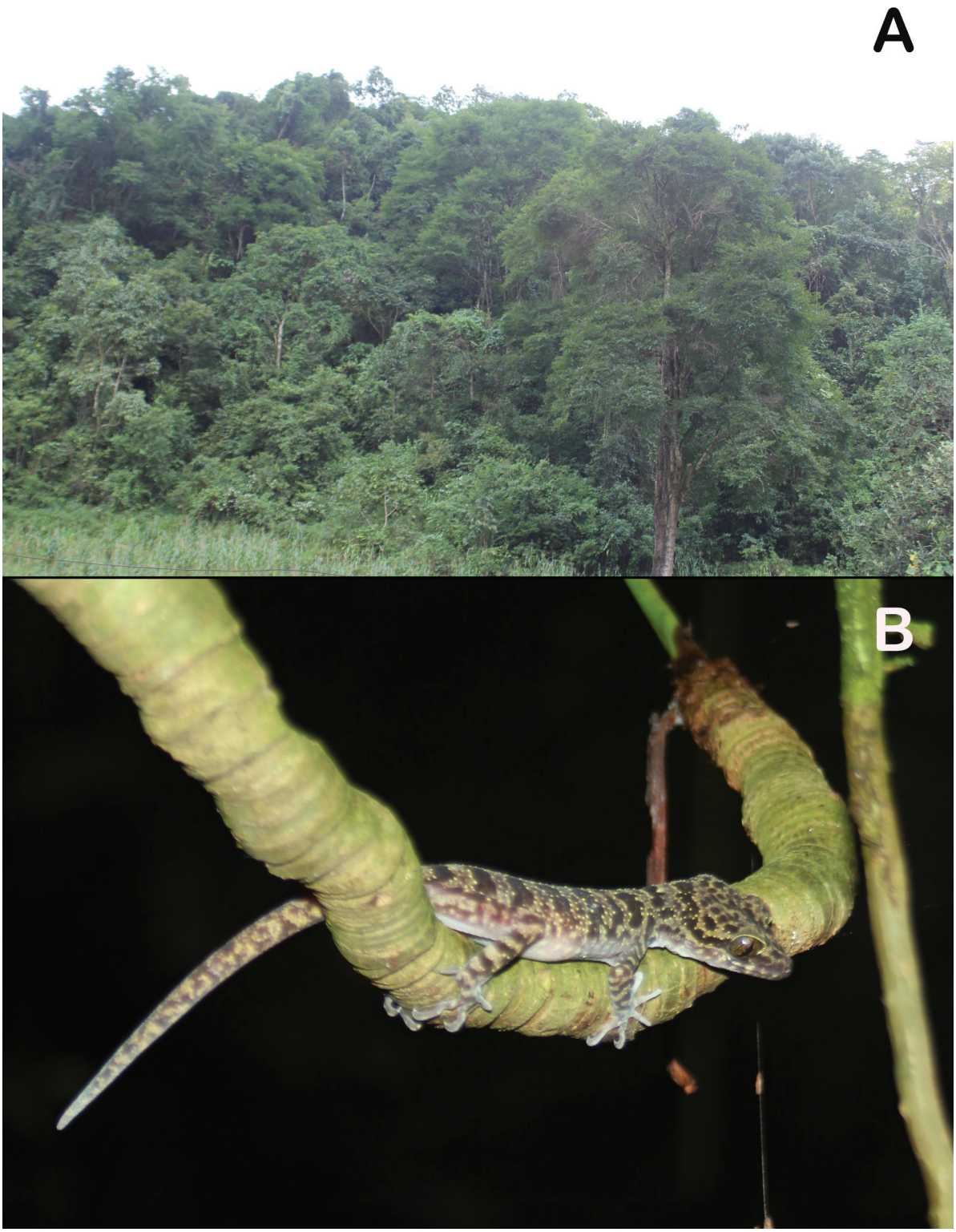

Figure 6. A macrohabitat **B** microhabitat of *Cyrtodactylus luci* sp. nov. Coc Ly Commune, Bac Ha District, Lao Cai Province, Vietnam. Photo: T.Q. Phan.

side in males (9–12 vs. absent in *C. houaphanensis*) and in females (5–10 vs. absent in *C. houaphanensis*) and more precloacal pores in males (9 or 10 vs. 6 in *C. houaphanensis*); from *C. huongsonensis* Luu, Nguyen, Do & Ziegler, 2011 by having fewer ventral scale rows (32–34 vs. 41–48 in *C. huongsonensis*), more

enlarged femoral scales on each side (12–15 vs. 7–9 in *C. huongsonensis*) and more precloacal pores in males (9 or 10 vs. 6 in *C. huongsonensis*); from *C. martini* Ngo, 2011 by having fewer ventral scale rows (32–34 vs. 39–43 in *C. martini*), more precloacal pores in males (9 or 10 vs. 4 in *C. martini*), the presence of precloacal pores in females (8 or 9 vs. absent in *C. martini*) and the presence of transversely enlarged subcaudal plates (vs. absent in *C. martini*); from *C. menglianensis* Liu & Rao, 2022 by having more ventral scale rows (32–34 vs. 26–29 in *C. menglianensis*), the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. menglianensis*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. menglianensis*) and in females (5–10 vs. absent in *C. menglianensis*), more precloacal pores in males (9 or 10 vs. 7 in *C. menglianensis*) and the presence of precloacal pores in females (8 or 9 vs. absent in *C. menglianensis*); from *C. ngoiensis* Schneider, Luu, Sitthivong, Teynié, Le, Nguyen & Ziegler, 2020 by having fewer ventral scale rows (32–34 vs. 38–43 in *C. ngoiensis*), more enlarged femoral scales on each side (12–15 vs. 7–10 in *C. ngoiensis*), more femoral pores on each side in males (9–12 vs. 7 in *C. ngoiensis*) and in females (5–10 vs. absent in *C. ngoiensis*), more precloacal pores in males (9 or 10 vs. 7 in *C. ngoiensis*) and in females (8 or 9 vs. 7 in *C. ngoiensis*) and more lamellae under toe IV (21–23 vs. 19–20 in *C. ngoiensis*); from *C. otai* Nguyen, Le, Pham, Ngo, Hoang, Pham & Ziegler, 2015 by having fewer ventral scale rows (32–34 vs. 38–43 in *C. otai*), the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. otai*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. otai*) and in females (5–10 vs. absent in *C. otai*), more precloacal pores in males (9 or 10 vs. 7 or 8 in *C. otai*), the presence of precloacal pores in females (8 or 9 vs. absent in *C. otai*), and the presence of transversely enlarged subcaudal plates (vs. absent in *C. otai*); from *C. puhuensis* Nguyen, Yang, Le, Nguyen, Orlov, Hoang, Nguyen, Jin, Rao, Hoang, Che, Murphy & Zhang, 2014 by having fewer ventral scale rows (32–34 vs. 36 in *C. puhuensis*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. puhuensis*) and in females (5–10 vs. absent in *C. puhuensis*), and more precloacal pores in males (9 or 10 vs. 5 in *C. puhuensis*); from *C. soni* Le, Nguyen, Le & Ziegler, 2016 by having fewer ventral scale rows (32–34 vs. 41–45 in *C. soni*), more dorsal tubercle rows (17–19 vs. 10–13 in *C. soni*), more enlarged femoral scales on each side (12–15 vs. 8–11 in *C. soni*), more femoral pores on each side in males (9–12 vs. 6–8 in *C. soni*), and more precloacal pores in males (9 or 10 vs. 6 or 7 in *C. soni*); from *C. sonlaensis* Nguyen, Pham, Ziegler, Ngo & Le, 2017 by having more dorsal tubercle rows (17–19 vs. 13–15 in *C. sonlaensis*), fewer femoral pores on each side in males (9–12 vs. 14–15 in *C. sonlaensis*), the presence of femoral pores on each side in females (5–10 vs. absent in *C. sonlaensis*), more precloacal pores in males (9 or 10 vs. 8 in *C. sonlaensis*) and the presence of precloacal pores in females (8 or 9 vs. absent in *C. sonlaensis*); from *C. spelaeus* Nazarov, Poyakov, Orlov, Nguyen, Milto, Martynov, Konstantinov & Chulisov, 2014 by having fewer ventral scale rows (32–34 vs. 36–39 in *C. spelaeus*), the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. spelaeus*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. spelaeus*) and in females (5–10 vs. absent in *C. spelaeus*) and differences in dorsal color pattern (banded vs. blotched in *C. spelaeus*); from *C. taybacensis* Pham, Le, Ngo, Ziegler & Nguyen, 2019 by having more dorsal tubercle rows (17–19 vs. 13–16 in *C. taybacensis*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. taybacensis*)

and in females (5–10 vs. absent in *C. taybacensis*), fewer precloacal pores in males (9 or 10 vs. 11–13 in *C. taybacensis*) and more lamellae under toe IV (21–23 vs. 16–20 in *C. taybacensis*); from *C. vilaphongi* Schneider, Nguyen, Le, Nophaseud, Bonkowski & Ziegler, 2014 by having more dorsal tubercle rows (17–19 vs. 15–16 in *C. vilaphongi*), the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. vilaphongi*), the presence of femoral pores on each side in females (5–10 vs. absent in *C. vilaphongi*) and in females (8 or 9 vs. absent in *C. vilaphongi*), more lamellae under toe IV (21–23 vs. 18–20 in *C. vilaphongi*), and the presence of transversely enlarged subcaudal plates (vs. absent in *C. vilaphongi*); from *C. wayakonei* Nguyen, Kingsada, Rosler, Auer & Ziegler, 2010 by the presence of enlarged femoral scales on each side (12–15 vs. absent in *C. wayakonei*), the presence of femoral pores on each side in males (9–12 vs. absent in *C. wayakonei*) and in females (5–10 vs. absent in *C. wayakonei*), more precloacal pores in males (9 or 10 vs. 6–8 in *C. wayakonei*) and in females (8 or 9 vs. 7 in *C. wayakonei*), and more lamellae under toe IV (21–23 vs. 19–20 in *C. wayakonei*); from *C. zhenkangensis* Liu & Rao, 2021 by having fewer dorsal tubercle rows (17–19 vs. 20–24 in *C. zhenkangensis*), more femoral pores on each side in males (9–12 vs. 2–5 in *C. zhenkangensis*) and in females (5–10 vs. 0–3 in *C. zhenkangensis*) and the presence of dark-colored nuchal loop (vs. absent in *C. zhenkangensis*).

Discussion

The new species from Bac Ha District, Lao Cai Province, is most similar to *Cyrtodactylus gulinqingensis*, a recently described species from Muguan County, Wenshan Prefecture, Yunnan Province of China (Liu et al. 2021). In terms of geographic distribution, the type locality of *C. luci* is approximately 40 km distant from that of its sister species in China. However, they are distinguished from each other by morphological differences as well as a genetic divergence of 8.87–9.22% (ND2 gene).

Our tree topology (Fig. 2) is similar to that reported in Grismer et al. (2021b). However, while *C. auribalteatus* is recovered as a member of the clade including *C. dumnuii*, *C. wayakonei* and other taxa in this study, it is grouped with the lineage consisting of *C. sonlaensis*, *C. huongsonensis* and *C. soni* in Grismer et al. (2021b). According to our phylogenetic analyses, the new species and *C. gulinqingensis* from Yunnan cluster with the latter clade with strong nodal support provided only by BI (Fig. 2). In addition to *C. luci* and *C. gulinqingensis*, the other species in the group occur in Son La (*C. sonlaensis*) and Ninh Binh (*C. soni*) provinces and the suburb of Ha Noi City (*C. huongsonensis*), northwestern Vietnam.

In the *Cyrtodactylus chauquangensis* group, except for *C. doisuthep*, a species known from dry evergreen and deciduous dipterocarp forests in Thailand (Kunya et al. 2014), all 23 remaining species are karst dwellers, comprising three species from Yunnan Province of China, five species from northern Laos, four species from northern Thailand, and 12 species from northern Vietnam (Uetz et al. 2023, this study). In terms of altitudinal distribution range, the members of this species group are found at elevations from 17 m (*C. soni*) to 1660 m (*C. doisuthep*) but most of them occur at elevations between 300 and 800 m a.s.l (Kunya et al. 2015; Le et al. 2016). The new species is the 24th species of the *C. chauquangensis* group, the first species from Lao Cai Province and the eastern side of the Red River in Vietnam, and the 53rd species of *Cyrtodactylus* known from Vietnam (Ngo et al. 2022; Uetz et al. 2023).

Acknowledgements

We are grateful to the directorate of the Forest Protection Department of Lao Cai Province for supporting our field work. We thank N.H. Nguyen (Hanoi) for his assistance in the field and T.A. Tran (Hanoi) for providing the map. For the fruitful collaboration within joint research projects, we cordially thank A.H. Le (IEBR, Hanoi), as well as T. Pagel and C. Landsberg (Cologne Zoo). Many thanks to L.L. Grismer (La Sierra) and V.Q. Luu (Hanoi) for their helpful comments on our manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research is funded by the National Foundation for Science and Technology Development (NAFOSTED, Grant No. 106.05-2021.19). Doctoral research of HT Ngo in Germany is funded by the German Academic Exchange Service (DAAD).

Author contributions

Conceptualization: TQN. Data curation: TQP, HTN, QHD, CTP, TTT. Formal analysis: HTN, CTP, MDL, QHD. Funding acquisition: TQN. Investigation: TQP, TTT. Methodology: MDL, TZ, TQN. Supervision: TQN, TZ. Writing - original draft: HTN, TQN, QHD. Writing - review and editing: MDL, HTN, TZ, CTP, TQP, TTT, TQN.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Pair-wise genetic distance between samples used in this study

Authors: Tung Thanh Tran, Quyen Hanh Do, Cuong The Pham, Tien Quang Phan, Hanh Thi Ngo, Minh Duc Le, Thomas Ziegler, Truong Quang Nguyen

Data type: xlsx

Link: https://doi.org/10.3897/zookeys.1192.117135.suppl1

Chapter 4. A new species of Cyrtodactylus (Squamata: Gekkonidae) from Phu Yen Province, Vietnam

Zootaxa 5271 (3): 503–524
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5271.3.4
http://zoobank.org/urn:lsid:zoobank.org:pub:790F14F6-A9F1-44FE-8B8F-CF241DF189AF

A new species of *Cyrtodactylus* (Squamata: Gekkonidae) from Phu Yen Province, Vietnam

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Abstract

We describe a new species of the genus *Cyrtodactylus* based on six adult specimens from Lac Dao forests, Phu Yen Province, southern Vietnam. *Cyrtodactylus tayhoaensis* **sp. nov.** is distinguished from the remaining Indochinese bent-toed geckos by a combination of the following characters: medium size (SVL up to 94.2 mm); nasal scales 5–6; internasal single or double; ciliaria 29–34; dorsal tubercles in 20–22 irregular transverse rows; ventral scale in 37–41 longitudinal rows at midbody; ventrolateral folds present without interspersed tubercles; precloacal pores absent in females, precloacal pores 4 or 5 in males; 10 or 11 enlarged femoral scales on each thigh; femoral pores 3–7 in males, absent in females; postcloacal tubercles 3 or 4; lamellae under toe IV 22–24; dorsal pattern consisting of unclear transverse bands formed by irregularly shaped dark-brown blotches, a discontinuous neckband with V-shape or triangle shape in the middle, dorsal head surface with dark-brown blotches; subcaudal scales transversely enlarged. In the phylogenetic analyses, the new species is recovered as a sister taxon to *C. kingsadai* with approximately 4% genetic divergence between the two species based on a fragment of the COI gene. This is the second species of *Cyrtodactylus* known from Phu Yen Province located in southern Vietnam.

Key words: *Cyrtodactylus tayhoaensis* **sp. nov.**, molecular phylogeny, taxonomy, Phu Yen Province

Introduction

The *Cyrtodactylus irregularis* species group currently contains 24 species (except *C. badenensis*) known from southern Indochina, in particular southern Vietnam (Nazarov *et al.* 2012; Pauwels *et al.* 2018; Do *et al.* 2021; Grismer *et al*. 2021a,b; Ngo *et al*. 2022; Uetz *et al.* 2022). In the last five years, eight new species in this group have been described, namely *C. chungi* Ostrowski, Do, Le, Ngo, Pham, Nguyen, Nguyen & Ziegler; *C. culaochamensis* Ngo, Grismer, Pham & Wood; *C. gialaiensis* Luu, Tran, Nguyen, Le & Ziegler; *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen; *C. phnomchiensis* Neang, Henson & Stuart; *C. phumyensis* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler; *C. raglai* Nguyen, Duong, Grismer & Poyarkov; and *C. sangi* Pauwels, Nazarov, Bobrov & Poyarkov (Do *et al*. 2021; Luu *et al*. 2017; Neang *et al*. 2020; Ngo *et al*. 2020; Nguyen *et al*. 2021; Ostrowski *et al*. 2020, 2021; Pauwels *et al*. 2018).

Accepted by A. Bauer: 20 Feb. 2023; published: 27 Apr. 2023

Recent field research in Vietnam's Phu Yen Province has led to the discovery of a so far unknown *Cyrtodactylus* population. Phu Yen Province is located in the southern Vietnam and harbors 116.819 ha of evergreen forest (Statistical Office of Phu Yen Province 2016). However, the biodiversity of this province is poorly studied, in particular the herpetofauna. Five new species were recently described from Phu Yen Province, namely *Cyrtodactylus kingsadai* Ziegler, Phung, Le & Nguyen, *Leptolalax macrops* Duong, Do, Ngo, Nguyen, & Poyarkov, *Acanthosaura murphyi* Nguyen, Do, Hoang, Nguyen, McCormack, Nguyen, Orlov, Nguyen & Nguyen, *Limnonectes phuyenensis* Pham, Do, Le, Ngo, Nguyen, Ziegler & Nguyen, and *Gekko phuyenensis* Nguyen, Nguyen, Orlov, Murphy & Nguyen (Ziegler *et al.* 2013; Duong *et al.* 2018; Nguyen *et al.* 2018; Pham *et al.* 2020; Nguyen *et al.* 2021).

During field research in Lac Dao Village, Tay Hoa District, a *Cyrtodactylus* population was found, that differs from other species of *Cyrtodactylus* in morphological characteristics and genetic divergence. It is therefore described as a new species in the following.

Material and methods

Sampling. Field surveys were conducted in Lac Dao Forest, Son Thanh Tay Commune, Tay Hoa District, Phu Yen Province, Vietnam in September 2015 and September 2019 (Fig. 1). Specimens were anaesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 85% ethanol and subsequently stored in 70% ethanol. Specimens were subsequently deposited in the collections of the Phu Yen University (PYU), Phu Yen Province and the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Molecular data and phylogenetic analyses. As the new population possesses morphological characters representing the *Cyrtodactylus irregularis* complex, we incorporated all taxa of the group (sensu Grismer *et al*. 2021) in the analyses. Four species, *C. condorensis* Smith, *C. grismeri* Ngo, *C. spelaeus* Nazarov, Poyarkov, Orlov, Nguyen, Milto, Martynov, Konstantinov & Chulisov, and *C. wayakonei* Nguyen, Kingsada, Rösler, Auer & Ziegler, were used as outgroups based on the results of Grismer *et al*. (2021).

DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer's instruction. Extracted DNA was amplified by PCR mastermix (Qiagen, Germany) with 21 µl volume (10 µl of mastermix, 5 µl of water, 2 µl of each primer at 10 pmol/ml and 2 µl of DNA). PCR condition was: 95°C for 15 minutes to active the taq; with 40 cycles at 95°C for 30 seconds, 45°C for 45 seconds, 72°C for 60 seconds; and the final extension at 72°C for 6 minutes. A fragment of the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), was amplified using the primer pair VF1-d (5'-TTCTCAACCAACCACAARGAYATYGG-3') and VR1-d (5'-TAGACTTCTGGGTGGCCRAARAAYCA-3)' (Ivanova *et al.* 2006). PCR products were visualized using electrophoresis through a 2% low melting-point agarose gel stained with ethidium bromide. Successful amplifications were purified to eliminate PCR components using GeneJETTM PCR Purification kit (ThermoFisher Scientific, Lithuania). Purified PCR products were sent to FirstBase (Malaysia) for sequencing in both directions.

After sequences were aligned by Clustal X v2 (Thompson *et al*. 1997), data were analyzed using maximum parsimony (MP) implemented in PAUP*4.0b10 (Swofford 2001), maximum likelihood as implemented in IQ-TREE (Nguyen *et al*. 2015) and Bayesian inference (BI) as implemented in MrBayes v3.2 (Ronquist *et al*. 2012). For MP analysis, heuristic analysis was conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) was calculated using 1000 pseudo-replicates and 100 random taxon addition replicates. All characters were equally weighted and unordered. For the maximum likelihood (ML) analysis, we used IQ-TREE v.1.6.7.1 (Nguyen *et al.* 2015) with a single model and 10,000 ultrafast bootstrap replications (UFB). The optimal model for nucleotide evolution was determined using jModelTest v2.1.4 (Darriba *et al*. 2012).

For Bayesian analyses, we used the optimal model determined by jModeltest with parameters estimated by MrBayes 3.2.7. Two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10 million generations with a random starting tree and sampled every 1000 generations. Log-likelihood scores of sample points were plotted against generation time to determine stationarity of Markov chains. Trees generated before log-likelihood scores reached stationarity were discarded from the final analyses using the burn-in function. The posterior probability values (PP) for all nodes in the final majority rule consensus tree were provided. We regard BP ≥ 70% and UFB and PP of ≥ 95% as strong support and values of <70% and < 95%, respectively, as weak support (Hillis & Bull 1993; Minh *et al*. 2013; Ronquist *et al*. 2012).

FIGURE 1. Type locality of *Cyrtodactylus tayhoaensis* **sp. nov.** in Phu Yen Province (purple circle), Vietnam.

The optimal model for nucleotide evolution was set to TIM2+I+G for ML analysis and GTR+I+G for Bayesian analysis as selected by jModelTest. For Bayesian analysis, the cutoff point for the burn-in function was set to 56 as -ln*L* scores reached stationarity after 56,000 generations. Uncorrected pairwise divergences were calculated in PAUP*4.0b10.

Morphological characters. Measurements were taken with a digital calliper to the nearest 0.1 mm. Morphological characters followed Nguyen *et al.* (2017). Abbreviations are as follows: snout-vent length (SVL), from tip of snout to vent; tail length (TaL), from vent to tip of tail (* regenerated); head length (HL), from tip of snout to retroarticular process of jaw; head width (HW), maximum width of head; head height (HH), from occiput to underside of jaws; orbital diameter (OD), greatest diameter of orbit; snout to eye distance (SE), from tip of snout to anterior-most point of eye; eye to ear distance (EE), from anterior edge of ear opening to posterior corner of eye; nares to eye distance (NE), from anterior-most point of eye to posterior-most point of nostril; ear length (ED), longest dimension of ear; forearm length (ForeaL), from base of palm to tip of elbow; crus length (CrusL), from base of heel to knee; axilla-groin distance (AG), from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; body width (BW), the widest distance of body; internarial distance (IND), distance between nares; Interorbital distance (IOD), shortest distance between left and right supraciliary scale rows; maximum rostral width (RW); maximum rostral height (RH); maximum mental width (MW); maximum mental length (MH).

Scale counts were taken as follows: supralabials (SL), counted from the first labial scale to corner of mouth; infralabials (IL), counted from the first labial scale to corner of mouth; nasal scales surrounding nare (N); postrostrals or internasals (IN); ciliaria (CIL), scales on eyelid fringe; postmentals (PM); granular scales surrounding dorsal tubercles (GST); ventral scales in longitudinal rows at midbody (V); number of scales along the midbody from mental to anterior edge of cloaca (SLB); enlarged femoral scales (EFS), number of enlarged femoral scale beneath each thigh; femoral pores (FP); precloacal pores (PP); postcloacal tubercles (PAT); the number of dorsal longitudinal rows of tubercles at midbody between the lateral folds (TubR), number of subdigital lamellae on all fingers (NSFI, NSFII, NSFIII, NSFIV, NSFIV); number of subdigital lamellae on all toes (NSTI, NSTII, NSTIII, NSTIV, NSTV). Bilateral scale counts were given as left/right.

Multiple Factor analysis (MFA). We further applied a multiple factor analysis using morphometric and meristic characters in MFA were SVL, HL, HW, HH, OD, SE, SL, IL, N, V, SLB, FP, PP, DTR, EFS, NSFV and NSTIV. Other morphological characters were not used due to the limitation of available morphometric and meristic data or incomplete sampling (regenerated tail). All statistical analyses were performed using R Core Team (2018). The MFA were clustered into different groups comprising three quantitative groups - "SVL, "Head (including HL, HW, HH), "Eye and XX qualitative groups—"Species, "SL.IL (consist of SL and IL), "Nasal, "V (including V and SLB), "FP, "PP, "DTR, "EFS, "LIV (consist of NSFIV and NSTIV). To remove the effects of allometry, morphometric data were also normalized to adjust raw data of morphometrics through the allom() function in R package *GroupStruct* (available at http://github.com/chankinonn/GroupStruct). Accordingly, the allometric formula is $X_{adj} = \log_{10}(X) - \beta[\log_{10}(SVL)-\log_{10}(SVL_{mean})]$, where X_{adj} = adjusted value; X = measured value; β = unstandardized regression coefficient for each population and SVL_{mean} = overall average SVL of two populations (Thorpe 1975, 1983; Turan 1999; Lleonart *et al*. 2000; Chan *et al*. 2021, Grismer *et al*. 2022). The ordination test was performed using the package *Factoextra* (Kassambara and Mundt 2017) and *FactoMinerR* (Le *et al*. 2008) in the software R. The approach was applied to identify active groups and to explain phenotypic variance by estimating the first two Dim values—eigenvalue proportions. Similar coded colors in the MFA scatter plot, surrounded with convex hulls, were presented to visualize the phenotypic spaces of *Cyrtodactylus tayhoaensis* **sp. nov.** and *C. kingsadai*. Their spaces were shown within a spatial coordinate of dimension axes (Dim1 and Dim2). To evaluate the overlap, the Dim1 and Dim2 values of each *Cyrtodactylus* individual were extracted to identify the difference between the two species using the T-test. For all of the tests, we applied a significance level of $p < 0.05$.

Results

Phylogenetic analyses. The final matrix contained 652 aligned characters with 250 parsimony informative. MP analysis of the dataset recovered five most parsimonious trees with 1547 steps (Consistency index = 0.3; Retention index = 0.65). The topology recovered by the BI analysis was similar to that reported in Ngo *et al*. (2022). The new population from Phu Yen Province was well corroborated as a sister taxon to *C. kingsadai* also from the same province with perfect support values from all analyses (Fig. 2). The two taxa were diverged by approximately 4%

in pairwise genetic distance based on a fragment of COI (Supplementary data). They in turn were shown to be most closely related to *C. raglai* from Khanh Hoa Province with strong support found only in the BI analysis (UFB = 91%, BP <50%, PP = 99%). Based on the molecular evidence and the morphological data presented below, we hypothesize that the new population from Phu Yen Province is a new species and describe it below.

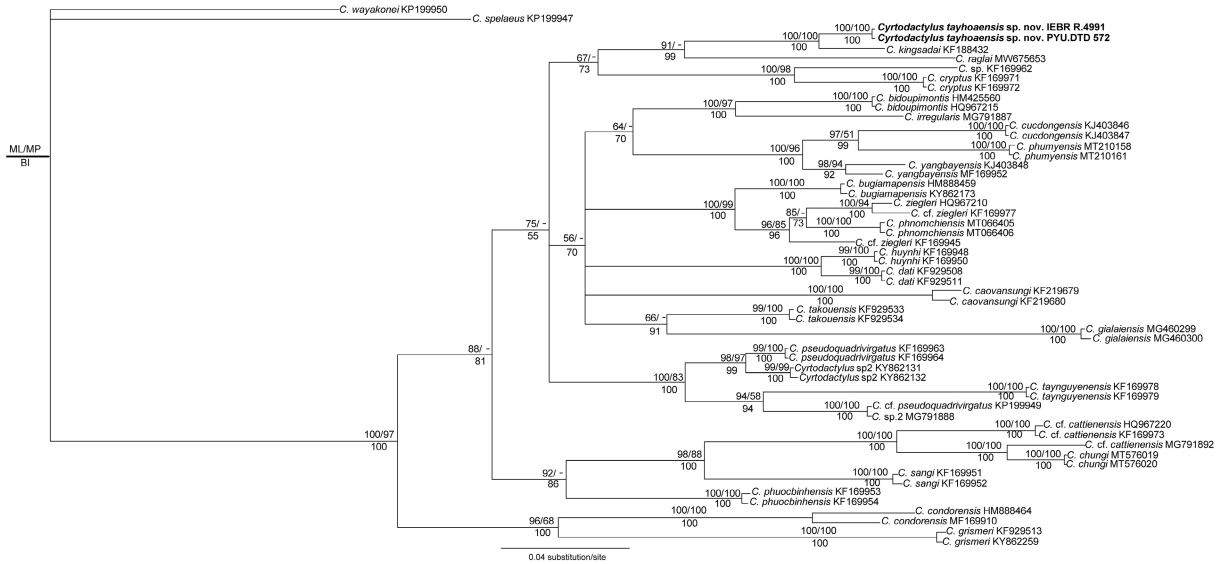

FIGURE 2. Phylogram based on the Bayesian analysis. Number above and below branches are ML/MP bootstrap and ultrafast bootstrap values and Bayesian posterior probabilities (≥ 50%), respectively.

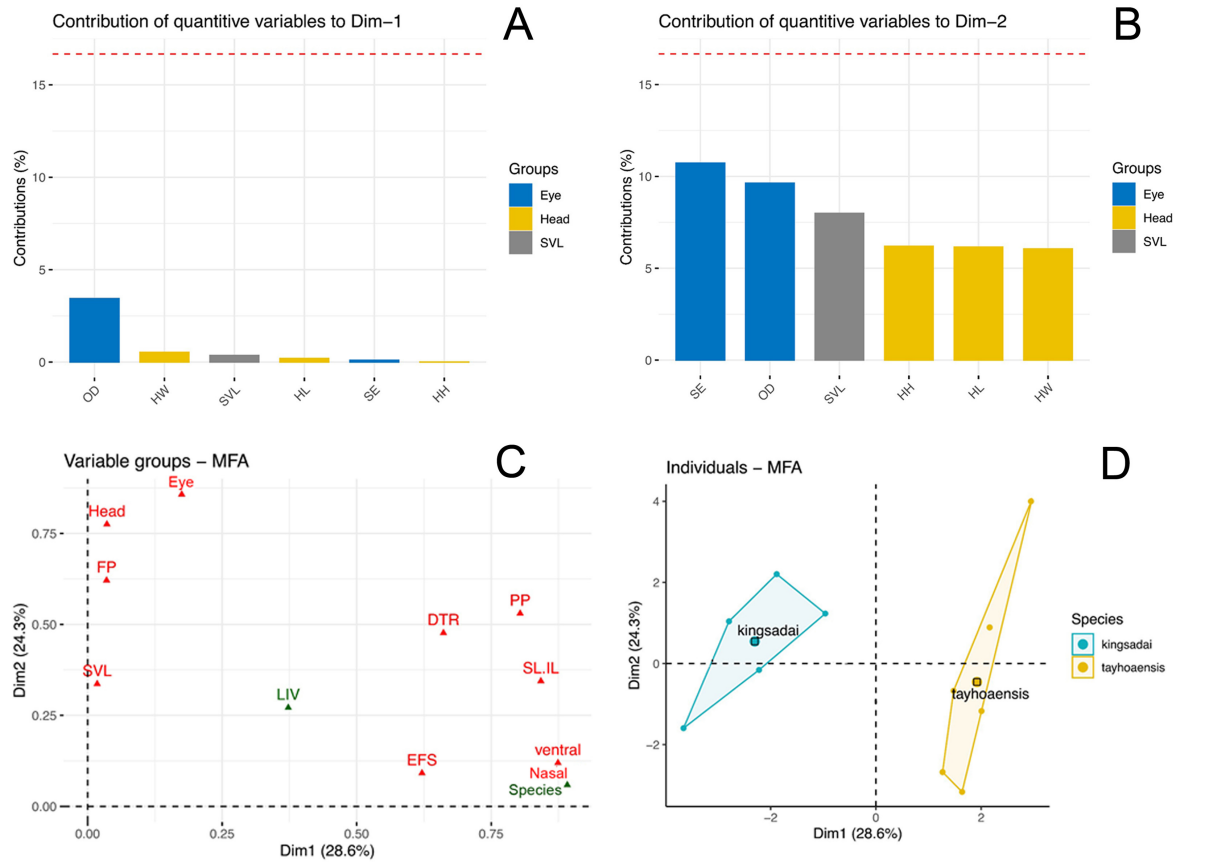

FIGURE 3. (A) The first four important variables of the first axes (Dim1) in the Multiple factor analysis (MFA); (B) The first four important variables of the second axes (Dim2) in the MFA; (C) Scatterplot of all variable groups for Dim1 and Dim2 axes in the MFA, green triangles as inactive groups, red triangles as active groups or variables; (D) A MFA of *Cyrtodactylus tayhoaensis* **sp. nov.** and *C. kingsadai.*

Morphological analysis. Morphologically, the new form from Tay Hoa District, Phu Yen Province is closely similar to *C. kingsadai* from Dai Lanh Cape, Tuy Hoa District, Phu Yen Province, however, they plotted separately from each other in MFA and there was a significant difference between two species ($p < 0.05$). The MFA also identified the data set of SVL, Head, Eye, SL, V, EFS, FP, PP, DTR, SL as active groups (Fig. 3). Morphometrics and meristics that contributed the most to the first multi-factorial dimension were OD, HW, SVL, HL, SE. HH (Fig. 3), while SE, OD, SVL, HH, HL, HW were the most important in the second multi-factorial dimension (Fig. 3).

***Cyrtodactylus tayhoaensis* sp. nov.**
(Figs. 4–6)

Holotype. IEBR R.4991, adult male, collected by Dang Trong Do on 14 September 2019, in a rocky stream, near Lac Dao Village (12°53′50.0″ N, 109°1′28.0″ E, elevation 130 m a.s.l), Son Thanh Tay Commune, Tay Hoa District, Phu Yen Province, southern Vietnam.

Paratypes. IEBR R.5078, R.5079, adult females and IEBR R.4992, adult male, the same collection data as the holotype. PYU.DTD 572, adult female; PYU.DTD 573 adult male, collected by Do Trong Dang on 4 September 2015, in a rocky stream, near Lac Dao Village (1253′13.0 N, 1091′17.3 E, elevation 230 m a.s.l), Son Thanh Tay Commune, Tay Hoa District, Phu Yen Province, southern Vietnam.

Diagnosis. The new species can be distinguished from other members of the genus *Cyrtodactylus* by a combination of the following characters: medium size (SVL up to 94.2 mm); nasal scales 5–6; internasal single or double; ciliaria 29–34; dorsal tubercles in 20–22 irregular transverse rows; ventral scale rows in 37–41 longitudinal rows at midbody; ventrolateral folds present without interspersed tubercles; precloacal pores absent in females, precloacal pores 4 or 5 in males; 10 or 11 enlarged femoral scales on each thigh; femoral pores 3–7 in males, absent in females; postcloacal tubercles 3 or 4; lamellae under toe IV 22–24; dorsal pattern consisting of unclear transverse bands formed by irregularly shaped dark-brown blotches, a discontinuous neckband with V-shape or triangle shape in the middle, dorsal head surface with dark-brown blotches; subcaudal scales transversely enlarged.

Description of holotype. Adult male, snout-vent length (SVL) 85.5 mm; body elongate (AG/SVL 0.44); head distinct from neck, elongate, depressed (HL/SVL 0.30, HW/HL 0.66, HH/HL 0.39); eye large (OD/HL 0.27), pupils vertical; upper eyelid fringe with spinous scales; ear opening below the postocular stripes, obliquely directed and oval, small in size (ED/HL 0.08); nares oval, surrounded by supranasal, rostral, first supralabial and three postnasals; supranasals separated from each other by two nasorostrals and two pentagonal internasals; loreal region and frontal concave; snout long (SE/HL 0.44), round anteriorly, longer than diameter of orbit (OD/SE 0.61); snout scales small, round, granular, larger than those in frontal and parietal regions; rostral rectangular, wider than high (RH/RW 0.61) with a medial suture, bordered by first supralabial, nostril, internasals and supranasal on each side; mental triangular, wider than high (MH/MW 0.58); postmentals two, enlarged, in contact posteriorly, bordered by mental anteriorly, first infralabial laterally, and an enlarged chin scale posteriorly; supralabials 10/11; infralabials 9/9.

Dorsal scales granular; dorsal tubercles round, keeled, conical, four or five times larger than the size of adjoining scales, each surrounded by 9 or 10 granular scales, tubercles forming 22 irregular longitudinal rows at midbody; ventral scales smooth, medial scales 2–3 times larger than dorsal granules, round, subimbricate, largest posteriorly, in 38 longitudinal rows at midbody; lateral folds present, without interspersed tubercles; gular region with homogeneous smooth scales; ventral scales between mental and cloacal slit 143; precloacal groove absent; three rows of enlarged scales present in posterior region of pore-bearing scales; five precloacal pores arranged in a chevron; 11 enlarged femoral scales beneath thigh continuous with enlarged precloacal scales but not continuous with precloacal pores; femoral pores bearing scales enlarged, 7 in total (4 in right thigh, 3 in left thigh), separated from pore-bearing precloacal scales by poreless femoral scales.

Fore and hind limbs moderately slender (ForeaL/SVL 0.17, CrusL/SVL 0.20); dorsal surface of forelimbs covered by few slightly developed tubercles; dorsal surface of hind limbs covered by by slightly developed tubercles; fingers and toes lacking distinct webbing; subdigital lamellae: finger I 15, finger II 18, finger III 18, finger IV 20, finger V 18, toe I 14, toe II 18, toe III 21, toe IV 22, toe V 21.

Tail regenerated, 91.5 mm in length; longer than snout-vent length (TaL/SVL: 1.07); postcloacal tubercles 4/3; subcaudals distinctly enlarged, smooth.

FIGURE 4. The male holotype of *Cyrtodactylus tayhoaensis* **sp. nov.** (IEBR R.4991) in life. Photo: D.T. Do.

FIGURE 5. Cloacal region of the holotype of *Cyrtodactylus tayhoaensis* **sp. nov.** (IEBR R.4991) in life. Photo: D.T. Do.

Coloration in life. Ground color light brownish-yellow; dorsal head surface with dark-brown blotches; labials brown with yellow cream sutures; skin above the eyes yellowish blue; eyelids with light yellow color; iris yellow grey with black marking; pupil vertical, elliptical, black; a dark band crosses from the posterior of the eye to the upper border of the ear to the posterior of the nuchal area, where it runs into the V-shape with the same color on the posterior nape on each side, forming a discontinuous neckband; tubercles on head, limbs, dorsum and tail light to dark-brown; dorsal pattern consisting of six unclear transverse dark-brown bands, formed by irregular dark-brown blotches; dorsal surface of fore and hind limbs with dark-brown blotches and bars; regenerated part of tail brownish cream with dark-brown marking; chin, throat, chest, belly, lower limbs and ventral surface of tail cream.

FIGURE 6. A) Type series of *Cyrtodactylus tayhoaensis* **sp. nov.** in preservative, B) The female paratype (IEBR R.5078) in life. Photos: D.T. Do.

Coloration in preservative: The overall color scheme slightly fades in 70% alcohol; yellow color disappeared in preservation; the bright yellow of the eyelids is no longer visible while main characteristics are still clearly discernible; dorsal ground color of head, neck, body, limbs and tail greyish brown; the color of chin, throat, chest, belly and lower limbs did not change noticeably in preservation.

Sexual dimorphism and variation. The females differ from male specimens in the absence of precloacal pores, femoral pores and hemipenial swellings at the tail base. For other morphological characters see Table 1 and Fig. 6A.

Distribution. *Cyrtodactylus tayhoaensis* **sp. nov.** is currently known only from the type locality in Tay Hoa District, Phu Yen Province, Vietnam (Fig. 1).

Etymology. Specific epithet *tayhoaensis* is a toponym in reference to the type locality of the species. For the common names we suggest Tay Hoa Bent-toed Gecko (English) and Thạch sùng ngón tây hòa (Vietnamese).

Natural history. The geckos were found between 19:00 and 21:00, on granite in water in rocky streams, about 0.5–1.5 m above the ground, at elevations between 130 and 230 m a.s.l. The surrounding habitat was disturbed evergreen forest of medium or small hardwood and shrub (Fig. 7). The humidity was approximately 70–80% and the air temperature ranged from 25 to 30°C.

FIGURE 7. Habitat of *Cyrtodactylus tayhoaensis* **sp. nov.** in Son Thanh Tay Commune, Tay Hoa District, Phu Yen Province, southern Vietnam. Photo: D.T. Do.

Comparisons. We compared the new species with its 24 congeners from the *Cyrtodactylus irregularis* complex based on examination of specimens and data obtained from the literature (Smith 1921a; Heidrich *et al.* 2007; Orlov *et al.* 2007; Nazarov *et al.* 2008; Ngo & Bauer 2008; Rösler *et al.* 2008; Geissler *et al.* 2009; Ngo & Chan 2010; Nazarov *et al.* 2012; Ngo 2013; Nguyen *et al.* 2013; Ziegler *et al.* 2013; Schneider *et al.* 2014; Luu *et al.* 2017; Pauwels *et al.* 2018; Ngo *et al.* 2020; Neang *et al.* 2020; Ostrowski *et al.* 2020; Ostrowski *et al.* 2021; Nguyen *et al.* 2021; Do *et al.* 2021) (see Table 2). The new species can be distinguished from all other *Cyrtodactylus* species from Vietnam by morphological characteristics (see Table 3).

Among the species of the *Cyrtodactylus irregularis* group, *Cyrtodactylus tayhoaensis* **sp. nov.** differs from its sister taxon in the phylogenetic analysis, *C. kingsadai*, by having more nasal scales surrounding nare (5–6 *versus* 4 in *C. kingsadai*), fewer scales along the midbody from mental to anterior edge of cloaca (140–157 *versus* 165–167 in *C. kingsadai*), fewer precloacal pores in males (4–5 *versus* 7–9 in *C. kingsadai*), the absence of precloacal pores in females (*versus* 4–8 in *C. kingsadai*), dark brown transversal bands of the dorsum larger than light brown interspaces (*versus* dark transversal bands as wide as light interspaces in *C. kingsadai*), and the presence of an

TABLE 1. Measurements (in mm) and morphological characters of the type series of *Cyrtodactylus tayhoaensis* **sp. nov.** (* = regenerated or broken tail); bilateral meristic characters are given as (left/right).

Characters	**IEBR R.4991**	**IEBR R.4992**	**PYU.DTD 573**	**IEBR R.5078**	**IEBR R.5079**	**PYU.DTD 572**	Min–Max
	(Holotype)	(Paratype)	(Paratype)	(Paratype)	(Paratype)	(Paratype)	
Sex	M	M	M	F	F	F	
SVL	85.5	94.2	87.7	89.7	83.1	82.9	82.9–94.2
TaL	91.5*	79.6*	86.1*	104.3	77.3*	101.8	max 104.3
HL	25.3	26.9	24.3	25.8	24.2	23.6	23.6–26.9
HW	16.7	17.5	16.4	16.8	15.9	15.7	15.7–17.5
HH	9.9	10.8	10.1	9.9	9.5	9.7	9.5–10.8
OD	6.8	7.1	6.4	6.4	6.2	6	6–7.1
SE	11.1	11.8	10.9	10.8	10.5	10.2	10.2–11.8
EE	6.4	6.9	6.5	6.6	6.1	6.3	6.1–6.9
NE	8.2	8.8	8	8.3	8	8.1	8–8.8
ED	2	2.1	2.2	1.7	2.1	1.8	1.7–2.2
ForeaL	14.8	14.3	13.3	14.6	13.5	13	13–14.8
CrusL	17.3	18.1	16.3	17.1	16.6	15.8	15.8–18.1
AG	37.8	42.6	38.9	39.2	34.4	34.1	34.1–42.6
BW	18.9	19.3	18.5	18.9	17.5	17.6	17.5
IND	2.5	2.6	2.4	2.5	2.3	2.4	2.3–2.6
IOD	3.5	3.6	3.6	3.6	3.5	3.7	3.5–3.7
RW	4.1	4.2	3.8	4.4	3.8	3.7	3.7–3.8
RH	2.5	2.6	2.5	2.6	2.2	2.2	2.2–2.6
MW	4.5	4.4	4.1	4.7	3.8	3.8	3.8–4.7
MH	2.6	2.7	2.7	3.2	2.6	2.6	2.6–3.2
SL	10/11	11/11	10/11	11/11	11/11	12/11	10–12
IL	9/9	9/9	9/10	10/10	10/10	9/9	9–10

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TABLE 1. (Continued)

Characters	**IEBR R.4991** (Holotype)	**IEBR R.4992** (Paratype)	**PYU.DTD 573** (Paratype)	**IEBR R.5078** (Paratype)	**IEBR R.5079** (Paratype)	**PYU.DTD 572** (Paratype)	Min–Max
Sex	M	M	M	F	F	F	
N	6/6	6/6	6/6	6/6	6/5	6/6	5–6
IN	2	1	1	1	2	1	1–2
CIL	31	29	29	32	34	31	29–34
PM	2	2	2	2	2	2	2
GST	9 or 10	10	9	11 or 10	9 or 10	9 or 10	9–11
V	38	38	40	37	41	39	37–41
SLB	143	140	142	156	155	157	140–157
FP	3/4	3/3	2/1	0	0	0	3–7 in males
PP	5	4	5	0	0	0	4–5 in males
PAT	4/3	3/3	3/3	3/3	3/3	3/3	3–4/3
TubR	22	20	21	21	21	21	20–22
EFS	11/11	11/10	10/10	10/11	10/10	10/11	10–11
NSFIV	20	22	21	20	21	20	20–22
NSTIV	22	24	23	23	24	22	22–24

TABLE 2. Morphological comparisons between *Cyrtodactylus tayhoaensis* **sp. nov.** and its 24 congeners from the *Cyrtodactylus irregularis* complex based on examination of specimens and data obtained from the literature (Smith 1921a; Heidrich *et al.* 2007; Orlov *et al.* 2007; Nazarov *et al.* 2008; Ngo & Bauer 2008; Rösler *et al.* 2008; Geissler *et al.* 2009; Ngo & Chan 2010; Nazarov *et al.* 2012; Ngo 2013; Nguyen *et al.* 2013; Ziegler *et al.* 2013; Schneider *et al.* 2014; Luu *et al.* 2017; Pauwels *et al.* 2018; Ngo *et al.* 2020; Neang *et al.* 2020; Ostrowski *et al*. 2020; Ostrowski *et al*. 2021; Nguyen *et al.* 2021; Do *et al.* 2021) (measurements in mm, * = regenerated or broken tail, Max = maximum, other abbreviations defined in the text).

No.	Taxa	SVL	TaL	V	EFS	FP	PP(M)	PP (F)	LD4	LT4	Color patterm of dorsum	Enlarged subcaudals
1	***Cyrtodactylus tayhoaensis* sp. nov.**	**82.9–94.2**	**max 104.3**	**37–41**	**10–11**	**3–7 males 0 females**	**4–5**	**0**	**20–22**	**22–24**	**banded**	**present**
2	*C. bidoupimontis*	74.0–86.3	75.0–86	38–43	8–10	absent	4–6	0	15–20	18–23	banded	absent
3	*C. bugiamapensis*	58.6–76.8	65.3–83.0	36–46	6–10	absent	7–11	0–7	15–17	17–20	blotched	absent
4	*C. caovansungi*	90.4–94	120	38–44	8	6	9	0	22	23–25	banded	present
5	*C. cattienensis*	43.5–69	51–64.7	28–42	3–8	absent	6–8	0	12–16	14–19	banded	absent
6	*C. chungi*	66.6–68.5	62.8*–82.2	30–31	4–6	absent	7	6	15–18	17–20	banded	absent
7	*C. cryptus*	62.5–90.8	63.5–88.4	47–50	absent	absent	9–11	0.	18–19	20–23	banded	absent
8	*C. cucdongensis*	55.8–65.9	max. 81.3	41–44	5–9	absent	5–6	4–6	13–18	15–20	banded	absent
9	*C. culaochamensis*	69.8–79.8	89.7–91.2	45–50	absent	absent	7–8	absent	18–19	20–23	banded	absent
10	*C. dati*	max 70.1	max 57.3	42–48	4–7	3–4 each	5–6	?	?	18–19	blotched	absent
11	*C. gialaiensis*	50.1–62.8	?	38–45	present	absent	9–10	0–8	14–15	15–17	Banded	absent
12	*C. huynhi*	67.2–79.8	61.5–78.6	43–46	3–5	3–8	7–9	0–8 (pitted scales)	14–17	17–21	banded	absent
13	*C. irregularis*	72–86	66.0–74	38–45	7–8	absent	5–7	0–6	15–16	18–19	blotched	absent
14	*C. kingsadai*	83–94	max 117	39–46	9–12	3–7	7–9	4–8	19–21	21–25	banded	present
15	*C. orlovi*	61–77.7	max 71.2	36–39	3–8	absent	5–6	0	15–17	16–19	banded	absent
16	*C. phnomchiensis*	76.1–80.7	56.9–79.1	45–54	0–8	absent	4–5	1–7 pitted	18–20	20–23	banded	Absent
17	*C. phumyensis*	63.6–66.8	?	33–41	5–7	absent	5–7	6 pitted	18–19	18–21	banded	absent
18	*C. phuocbinhensis*	46–60.4	76,1	43–47	5	absent	7	0	16–21	17–19	striped/ blotched	absent

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TABLE 2. (Continued)

No.	Taxa	SVL	TaL	V	EFS	FP	PP(M)	PP (F)	LD4	LT4	Color patterm of dorsum	Enlarged subcaudals
19	*C. pseudoquadrivirgatus*	48.6–83.3	55.7–82.3	41–57	absent	absent	5–9	5–10	15–21	16–25	bande/blotched	absent
20	*C. raglai*	87.5–111.7	113.4–119	36–39	9–10	0	5	0	?	21–22	banded	present
21	*C. sangi*	49.9–56.3	47.9* but just 15.1 mm original	37	4	Absent	7	4 (Pitted)	?	?	banded	absent
22	*C. takouensis*	74.7–81.1	77.7–91	39–40	3–5	0–2	3–4	0	16–17	18–20	banded	present
23	*C. taynguyenensis*	60.0–85.0	66.0–94.0	42–49	absent	absent	6	0	13–18	17–21	blotched	absent
24	*C. yangbayensis*	78.5–92.3	91.3–109.1	39–46	5–16	0–2	6–8	0	16–19	15–17	banded	Present
25	*C. ziegleri*	84.6–93.0	95.0–107.0	33–39	8–10	0–6	5–8	0–8	16–19	18–21	banded	Absent

TABLE 3. Morphological comparisons between *Cyrtodactylus* **sp. nov.** and and its other congeners from Vietnam (after Smith 1921b; Ziegler *et al.* 2002, 2010; Nguyen *et al.* 2006, 2014; Hoang *et al*. 2007; Ngo 2008, 2011; Ngo *et al.* 2008, 2010; Chan & Norhayati 2010; Ngo & Grismer 2010, 2012; Luu *et al.* 2011; Ngo & Chan 2011; Nguyen *et al.* 2015, 2017; Le *et al.* 2016, 2021; Pham *et al.* 2017, 2019; Murdoch *et al.* 2019).

No.	Taxa	SVL	TaL	V	EFS	FP	PP(M)
1	***Cyrtodactylus tayhoaensis* sp. nov.**	**82.9–94.2**	**max 104.3**	**37–41**	**10–11**	**3–7 males 0 females**	**4–5**
2	*C. badenensis*	59.3–74.1	58.6–82.4	25–28	absent	absent	0
3	*C. bichnganae*	95.3–99.9	96.6*–115.6	30–31	11–13	18	10
4	*C. bobrovi*	75.2–96.4	max 95.4	40–45	absent	0	5
5	*C. chauquangensis*	90.95–99.3	97–108.3	36–38	absent	absent	6–7
6	*C. condorensis*	80	100	35–40	present	?	4–7
7	*C. cucphuongensis*	96	79.3*	42	14	absent	0
8	*C. eisenmanae*	76.8–89.2	91–103.8	44–45	4–6	absent	0
9	*C. grismeri*	68.3–95	111.3–115.1	33–38	0–3	0	0
10	*C. hontreensis*	72.4–88.9	84.2–106.5	40–42	2–5	absent	7–8
11	*C. huongsonensis*	73.4–89.8	90.5	41–48	7–9 each	7–8 each	6
12	*C. intermedius*	61.0–85.0	80.0–100.0	40–50	6–10	?	8–10
13	*C. leegrismeri*	80.6–92	58–99	27–35	present	absent	4
14	*C. martini*	64.4–96.2	76–101.2	39–43	14–18	absent	4
15	*C. nigriocularis*	82.7–107.5	70.6–121	42–49	absent	absent	0–2
16	*C. ngati*	66.5–69.3	74.1–83.2	32–38	present	14	13
17	*C. otai*	85.2–90.6	89.7–97.6	38–43	absent	0	7–8
18	*C. phongnhakebangensis*	78.5–96.3	98–110	32–42	present	present	33–42 (PP+FP)
19	*C. phuquocensis*	62.2–85.8	80.5–103.1	38–43	10–11	absent	7–9
20	*C. puhuensis*	79.24	82.59	36	?	absent	5
21	*C. roesleri*	51.1–75.3	63.4–101	34–40	7–10	present	20–28
22	*C. septimontium*	59.5–90.4	85–119	37–46	24–33	?	7–8
23	*C. soni*	88.7–103	70.6–113	41–45	8–11	6–8	6–7
24	*C. sonlaensis*	63.1–83.2	89.8–103	34–42	15–17	14–15	8
25	*C. taybacensis*	77.6–97.5	97.1−104.1	30–38	11–14	Absent	11–13

.....continued on the next page

TABLE 3. (Continued)

No.	Taxa	PP (F)	LD4	LT4	Color patterm of dorsum	Enlarged subcaudals
1	***Cyrtodactylus tayhoaensis* sp. nov.**	**0**	**20–22**	**22–24**	**banded**	**present**
2	*C. badenensis*	0	–	18–22	banded	present
3	*C. bichnganae*	8	18–20	16–20	banded	present
4	*C. bobrovi*	0	19–21	21–22	banded	absent
5	*C. chauquangensis*	6–7	16–18	19–23	banded	present
6	*C. condorensis*	?	?	?	blotched	present
7	*C. cucphuongensis*	?	21	24	banded	present
8	*C. eisenmanae*	0	18–20	17–18	banded	present
9	*C. grismeri*	0	16–18	16–19	banded	present
10	*C. hontreensis*	0	16	17–19	banded	present
11	*C. huongsonensis*	8	17–19	20–23	banded	present
12	*C. intermedius*	?	?	22	banded	present
13	*C. leegrismeri*	0	–	18–20	blotched	present
14	*C. martini*	0	19–23	22–24	banded	absent
15	*C. nigriocularis*	0	–	17–21	Unifomly brown	present
16	*C. ngati*	0	16–17	12–17	banded	?
17	*C. otai*	0	16–19	19–22	banded	absent
18	*C. phongnhakebangensis*	0–41 (PP+FP)	15–20	18–26	banded	present
19	*C. phuquocensis*	0–8 pitted	?	15–18	banded	present
20	*C. puhuensis*	?	18	23	banded	present
21	*C. roesleri*	17–22	17–19	17–21	banded	present
22	*C. septimontium*	6–8	?	17–20	Banded	?
23	*C. soni*	7–8	15–19	18–22	banded	present
24	*C. sonlaensis*	0	17–19	18–21	Banded	Present
25	*C. taybacensis*	5–15 (Pitted)	17–19	16–20	Banded	Present

uncontinuous white vertebral line extending from neck to base of tail (*versus* absent in *C. kingsadai*); from *C. bidoupimontis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler by having a longer tail length (101.8–104.3 mm, mean ratio TL/SVL 1.20 *versus* 75–86 mm, ratio TL/SVL 1.05 in *C. bidoupimontis*), fewer scales along the midbody from mental to anterior edge of cloaca (140–157 *versus* 166–198 in *C. bidoupimontis*), the presence of femoral pores in males (3–7 *versus* absent in *C. bidoupimontis*), differences in dorsal color pattern (thinner nuchal band, divided with V-shape or triangle shape in the middle *versus* posteriorly distinctly widened nuchal band in *C. bidoupimontis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. bidoupimontis*); from *C. bugiamapensis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler by having a larger size (SVL 82.9–94.2 mm *versus* 58.6–76.8 mm in *C. bugiamapensis*), fewer scales along the midbody from mental to anterior edge of cloaca (140–157 *versus* 164–205 in *C. bugiamapensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. bugiamapensis*), fewer precloacal pores in males (4–5 *versus* 7–8 in *C. bugiamapensis*), more lamellae under finger IV (20–22 *versus* 15–17 in *C. bugiamapensis*) and under toe IV (22–24 *versus* 17–20 in *C. bugiamapensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. bugiamapensis*); from *C. caovansungi* Orlov, Nguyen, Roman, Natalia & Nguyen by having fewer scales along the midbody from mental to anterior edge of cloaca (140–157 *versus* 162–187 in *C. caovansungi*), more dorsal tubercle rows (20–22 *versus* 16–18 in *C. caovansungi*), more enlarged femoral scales (10–11 *versus* 8 in *C. caovansungi*), fewer precloacal pores in males (4–5 *versus* 9 in *C. caovansungi*), and differences in dorsal color pattern (nuchal band thinner, divided with V-shape or triangle shape in the middle *versus* well developed, continuous nuchal band in *C. caovansungi*); from *C. cattienensis* Geissler, Nazarov, Orlov, Böhme, Phung, Nguyen & Ziegler by having a larger size (SVL 82.9–94.2 mm *versus* 43.5–69.0 mm in *C. cattienensis*), more enlarged femoral scales (10–11 *versus* 3–8 in *C. cattienensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. cattienensis*), fewer precloacal pores in males (4–5 *versus* 6–8 in *C. cattienensis*), more lamellae under finger IV (20–22 *versus* 12–16 in *C. cattienensis*) and under toe IV (22–24 *versus* 14–19 in *C. cattienensis*), differences in dorsal color pattern (nuchal band divided with V-shape or triangle shape in the middle *versus* continuous nuchal band in *C. cattienensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. cattienensis*); from *C. chungi* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler by having a larger size (SVL 82.9–94.2 mm *versus* 66.6–68.5 mm in *C. chungi*), more ventral scale rows (37–41 *versus* 30–31 in *C. chungi*), more dorsal tubercle rows (20–22 *versus* 17–18 in *C. chungi*), more enlarged femoral scales (10–11 *versus* 4–6 in *C. chungi*), the presence of femoral pores in males (3–7 *versus* absent in *C. chungi*), fewer precloacal pores in males (4–5 *versus* 7 in *C. chungi*), the absence of precloacal pores in females (*versus* 6 in *C. chungi*), more lamellae under finger IV (20–22 *versus* 15–18 in *C. chungi*), and under toe IV (22–24 *versus* 17–20 in *C. chungi*), differences in dorsal color pattern (nuchal band divided with V-shape or triangle shape in the middle *versus* continuous nuchal band in *C. chungi*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. chungi*); from *C. cryptus* Heidrich, Rösler, Vu, Böhme & Ziegler by having fewer ventral scale rows (38–41 *versus* 47–50 in *C. cryptus*), the presence of enlarged femoral scales (10–11 *versus* absent in *C. cryptus*), the presence of femoral pores in males (3–7 *versus* absent in *C. cryptus*), fewer precloacal pores in males (4–5 *versus* 9–11 in *C. cryptus*), more lamellae under finger IV (20–22 *versus* 18–19 in *C. cryptus*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. cryptus*); from *C. cucdongensis* Schneider, Phung, Le, Nguyen & Ziegler by having a larger size (SVL 82.9–94.2 mm *versus* 55.8–65.9 mm in *C. cucdongensis*), higher dorsal tubercle rows (20–22 *versus* 16–19 in *C. cucdongensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. cucdongensis*), the absence of precloacal pores in females (*versus* 4–6 in *C. cucdongensis*), more lamellae under finger IV (20–22 *versus* 13–18 in *C. cucdongensis*) and under toe IV (22–24 *versus* 15–20 in *C. cucdongensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. cucdongensis*); from *C. culaochamensis* Ngo, Grismer, Pham & Wood by having a larger size (SVL 82.9–94.2 mm *versus* 69.8–79.8 mm in *C. culaochamensis*), fewer ventral scale rows (37–41 *versus* 45–50 in *C. culaochamensis*), the presence of enlarged femoral scales (10–11 *versus* absent in *C. culaochamensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. culaochamensis*), fewer precloacal pores in males (4–5 *versus* 7–8 in *C. culaochamensis*), and more lamellae under finger IV (20–22 *versus* 18–19 in *C. culaochamensis*); from *C. dati* Ngo by having a larger size (SVL 82.9–94.2 mm *versus* max 70.1 in *C. dati*), a longer tail length (101.8–104.3 mm, mean ratio TL/SVL 1.20 *versus* Max 57.3 mm, mean ratio TL/SVL: 1.06), fewer ventral scale rows (38–41 *versus* 42–48 in *C. dati*), more enlarged femoral scales (10–11 *versus* 4–7 in *C. dati*), more lamellae under toe IV (22–24 *versus* 18–19 in *C. dati*), and differences in dorsal color pattern (banded *versus* small blotched in *C. dati*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. dati*); from *C. gialaiensis* Luu, Tran, Nguyen, Le & Ziegler by having

a larger size (SVL 82.9–94.2 mm *versus* 50.1–62.8 mm in *C. gialaiensis*), fewer scales along the midbody from mental to anterior edge of cloaca (140–157 *versus* 165–178 in *C. gialaiensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. gialaiensis*), fewer precloacal pores in males (4–5 *versus* 9–10 in *C. gialaiensis*), the absence of precloacal pores in adult females (*versus* 8 pitted scales in *C. gialaiensis*), more lamellae under finger IV (20–22 *versus* 14–15 in *C. gialaiensis*) and under toe IV (22–24 *versus* 15–17 in *C. gialaiensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. gialaiensis*); from *C. huynhi* Ngo & Bauer by having a larger size (SVL 82.9–94.2 mm *versus* 68.5–79.8 mm in *C. huynhi*), fewer ventral scale rows (37–41 *versus* 43–46 in *C. huynhi*), more dorsal tubercle rows (20–22 *versus* 16–18 in *C. huynhi*), more enlarged femoral scales (10–11 *versus* 3–5 in *C. huynhi*), fewer precloacal pores in males (4–5 *versus* 7–9 in *C. huynhi*), more lamellae under finger IV (20–22 *versus* 14–17 in *C. huynhi*) and under toe IV (22–24 *versus* 17–21 in *C. huynhi*), differences in dorsal color pattern (nuchal band divided with V-shape or triangle shape in the middle *versus* continuous nuchal band in *C. huynhi*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. huynhi*); from *C. irregularis* (Smith) by having more enlarged femoral scales (10–11 *versus* 7–8 in *C. irregularis*), the absence of precloacal pores in females (0 *versus* 0–6 in *C. irregularis*), more lamellae under finger IV (20–22 *versus* 15–16 in *C. irregularis*) and under toe IV (22–24 *versus* 18–19 in *C. irregularis*), differences in dorsal color pattern (banded *versus* blotched in *C. irregularis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. irregularis*); from *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen by having a larger size (SVL 82.9–94.2 mm *versus* 61–77.7 mm in *C. orlovi*), more enlarged femoral scales (10–11 *versus* 3–8 in *C. orlovi*), the presence of femoral pores in males (3–7 *versus* absent in *C. orlovi*), more lamellae under finger IV (20–22 *versus* 15–17 in *C. orlovi*) and under toe IV (22–24 *versus* 16–19 in *C. orlovi*), differences in dorsal color pattern (nuchal band divided with V–shape or triangle shape in the middle *versus* continuous nuchal band in *C. orlovi*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. orlovi*); from *C. phnomchiensis* Neang, Henson & Stuart by having a larger size (SVL 82.9–94.2 mm *versus* 76.1–80.7 mm in *C. phnomchiensis*), a longer tail (101.8–104.3 mm, mean ratio TL/SVL 1.20 *versus* 56.9–79.1 mm, mean ratio TL/SVL 0.88 in *C. phnomchiensis*), fewer ventral scale rows (37–41 *versus* 45–54 in *C. phnomchiensis*), more enlarged femoral scales (10–11 *versus* 0–8 in *C. phnomchiensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. phnomchiensis*), the absence of precloacal pores in females (*versus* 1–7 pitted scales in *C. phnomchiensis*), differences in dorsal color pattern (nuchal band divided with V-shape or triangle shape in the middle *versus* continuous nuchal band in *C. phnomchiensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. phnomchiensis*); from *C. phumyensis* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler by having a larger size (SVL 82.9–94.2 mm *versus* 63.6–66.8 mm in *C. phumyensis*), more enlarged femoral scales (10–11 *versus* 5–7 in *C. phumyensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. phumyensis*), the absence of precloacal pores in females (*versus* 6 pitted scales in *C. phumyensis*), more lamellae under finger IV (20–22 *versus* 18–19 in *C. phumyensis*) and under toe IV (22–24 *versus* 18–21 in *C. phumyensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. phumyensis*); from *C. phuocbinhensis* Nguyen, Le, Tran, Orlov, Lathrop, Macculloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang by having a larger size (SVL 82.9–94.2 mm *versus* 46.0–60.4 mm in *C. phuocbinhensis*), fewer ventral scale rows (37–41 *versus* 43–47 in *C. phuocbinhensis*), more enlarged femoral scales (10–11 *versus* 5 in *C. phuocbinhensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. phuocbinhensis*), fewer precloacal pores in males (4–5 *versus* 7 in *C. phuocbinhensis*), more lamellae under toe IV (22–24 *versus* 17–19 in *C. phuocbinhensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. phuocbinhensis*); from *C. pseudoquadrivirgatus* Rösler, Vu, Nguyen, Ngo & Ziegler by having the presence of enlarged femoral scales (10–11 *versus* absent in *C. pseudoquadrivirgatus*), the presence of femoral pores in males (3–7 *versus* absent in *C. pseudoquadrivirgatus*), the absence of precloacal pores in females (*versus* 5–10 in *C. pseudoquadrivirgatus*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. pseudoquadrivirgatus*); from *C. raglai* Nguyen, Duong, Grismer & Poyarkov by having a shorter tail (101.8–104.3 mm, mean ratio TL/SVL 1.20 *versus* 119–135 mm, mean ratio TL/SVL 1.25 in *C. raglai*), the presence of femoral pores in males (3–7 *versus* absent in *C. raglai*), more dorsal tubercle rows (20–22 *versus* 14–15 in *C. raglai*), the presence of raised, moderately to strongly keeled body tubercles (*versus* low, weakly keeled body tubercles in *C. raglai*), and differences in dorsal color pattern (nuchal band divided with V–shape or triangle shape in the middle *versus* continuous nuchal band in *C. raglai*); from *C. sangi* Pauwels, Nazarov, Bobrov & Poyarkov by having a larger size (SVL 82.9–94.2 mm *versus* 49.9–56.3 mm in *C. sangi*), more enlarged femoral scales (10–11 *versus* 4 in *C. sangi*), the presence of femoral pores in males (3–7 *versus* absent in *C. sangi*), fewer precloacal pores in males (4–5 *versus* 7 in *C. sangi*), the

absence of precloacal pores in females (*versus* 4 pitted scales in *C. sangi*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. sangi*); from *C. takouensis* Ngo & Bauer by having a larger size (SVL 82.9–94.2 mm *versus* 74.7–81.1 mm in *C. takouensis*), more enlarged femoral scales (10–11 *versus* 3–5 in *C. takouensis*), more femoral pores in males (3–7 *versus* 0–2 in *C. takouensis*), more lamellae under finger IV (20–22 *versus* 16–17 in *C. takouensis*) and under toe IV (22–24 *versus* 18–20 in *C. takouensis*); from *C. taynguyenensis* Nguyen, Le, Tran, Orlov, Lathrop, Macculloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang by fewer ventral scale rows (38–41 *versus* 42–49 in *C. taynguyenensis*), the presence of enlarged femoral scales (10–11 *versus* absent in *C. taynguyenensis*), the presence of femoral pores in males (3–7 *versus* absent in *C. taynguyenensis*), fewer precloacal pores in males (4–5 *versus* 6 in *C. taynguyenensis*), more lamellae under finger IV (20–22 *versus* 13–18 in *C. taynguyenensis*) and under toe IV (22–24 *versus* 17–21 in *C. taynguyenensis*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. taynguyenensis*); from *C. yangbayensis* Ngo & Chan by having more femoral pores in males (3–7 *versus* 0–2 in *C. yangbayensis*), fewer precloacal pores in males (4–5 *versus* 6–8 in *C. yangbayensis*), more lamellae under finger IV (20–22 *versus* 16–19 in *C. yangbayensis*) and under toe IV (22–24 *versus* 15–27 in *C. yangbayensis*); from *C. ziegleri* Nazarov, Orlov, Nguyen & Ho by having fewer scales along the midbody from mental to anterior edge of cloaca (140–157 *versus* 173–198 in *C. ziegleri*), the absence of precloacal pores in females (*versus* 0–8 in *C. ziegleri*), more lamellae under finger IV (20–22 *versus* 16–19 in *C. ziegleri*) and under toe IV (22–24 *versus* 18–21 in *C. ziegleri*), and the presence of transversely enlarged subcaudal plates (*versus* absent in *C. ziegleri*).

Discussion

Our discovery brings the number of bent-toed geckos reported to occur in Vietnam to 49 species (Ngo *et al*. 2022; Uetz *et al*. 2022). It further represents the 25th species of the *Cyrtodactylus irregularis* species group. In our phylogenetic analyses, the new species is placed as a sister taxon to *C. kingsadai*, the second species of the genus known from Phu Yen Province. Both species inhabit evergreen forest on granite rock. However, *C. kingsadai* has been recorded from big rocks at 50–100 m a.s.l., whereas *Cyrtodactylus tayhoaensis* **sp. nov.** has been found on smaller rocks along streams at 130–230 m a.s.l. The two species thus seem to occupy different microhabitats. Both sister taxa can also be well differentiated based on morphological and molecular distinctiveness. They are most closely related to *C. raglai* from neighboring Khanh Hoa Province (Fig. 2; Nguyen *et al*. 2021). Our field observations suggest that *Cyrtodactylus tayhoaensis* **sp. nov.** is under threat of habitat loss and degradation. Evergreen forests have been converted to agriculture land at the site and logging activities have polluted streams where the species lives. More assessments need to be undertaken to evaluate the population status and the level of threats the species is facing.

Acknowledgments

We are grateful to the directorates of the Forest Protection Department of Phu Yen Province for their support of our work. For the fruitful cooperation within joint research projects, we cordially thank S.V. Nguyen (IEBR, Hanoi), as well as T. Pagel and C. Landsberg (Cologne Zoo). We would like to thank the local people in Son Thanh Tay Commune, Tay Hoa District for their assistance in the field and H. N. Ngo (IGR, Hanoi) for assisted laboratory work. We thank T. A. Tran (IEBR, Hanoi) for providing the map. This research is funded by the National Foundation for Science and Technology Development (NAFOSTED) under Grant Number 106.05–2020.02 and the Rufford Foundation (Grant No. 39056-1) to C.T. Pham.

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Chapter 5. Hidden biodiversity on the highest mountain in southern Vietnam: the fourth species of Cyrtodactylus Gray, 1927 (Squamata: Gekkonidae) from Tay Ninh Province

(Accepted to Zootaxa)

Hidden biodiversity on the highest mountain in southern Vietnam: the fourth species of *Cyrtodactylus* Gray, 1927 (Squamata: Gekkonidae) from Tay Ninh Province

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Number of plates: 6

Number of references: 60

Higher Order Group: Reptilia

New described taxa: 1 species

Accepted by A.M. Bauer; 28 August 2025

Hidden biodiversity on the highest mountain in southern Vietnam: the fourth species of *Cyrtodactylus* Gray, 1927 (Squamata: Gekkonidae) from Tay Ninh Province

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Abstract

We describe the fourth species of the *Cyrtodactylus irregularis* complex from Ba Den Mountain, Tay Ninh Province, southern Vietnam based on molecular divergence and morphological differences. *Cyrtodactylus tayninhensis* **sp. nov.** is distinguished from the remaining congeners of the *C. irregularis* group by having the unique combination of: size medium (SVL 73.4–80 mm); dorsal tubercles in 13 or 14 irregular rows; 36–39 ventral scale rows; enlarged femoral scales absent; precloacal pores absent in females, 4–6 in males, in a continuous row; femoral pores absent; postcloacal spurs two or three on each side; lamellae under toe IV 16–18; a U-shaped continuous neckband, dorsal pattern between limb insertions consisting of two or three irregular yellow-brown bands and tail with 8–10 thin light bands (with two bands near vent light brown, others white); the absence of transversely enlarged median subcaudal scales. In terms of phylogenetic analyses, the new species is recovered as a member of the *Cyrtodactylus irregularis* species group without any clear sister. Genetically, *Cyrtodactylus tayninhensis* **sp. nov.** is separated from the remaining taxa of the *Cyrtodactylus irregularis* group by a minimum of 13.09% and 15.98% based on fragments of the mitochondrial COI and ND2 genes, respectively.

Key words: Ba Den Mountain, *Cyrtodactylus tayninhensis* **sp. nov.**, *C. irregularis*, morphology, phylogenetic relationships

Introduction

The bent-toed geckos of the genus *Cyrtodactylus* are the most diverse genus in the Family Gekkonidae, comprising more than 380 recognized species (Uetz *et al.* 2025). This widely distributed group occurs throughout tropical South Asia, Indochina, the Philippines, the Indo-Australian Archipelago and the Solomon Islands (Wood *et al.* 2012; Grismer *et al.* 2021). Vietnam has long been recognized as a region of global importance with regard to the diversity of *Cyrtodactylus* and it has also been a hotspot of new bent-toed gecko discoveries. Up to 52 new species have been described in Vietnam since 1997, resulting in a total of 55 species for the country (Uetz *et al.* 2025).

The Ba Den Mountain Cultural and Historical Complex (MCHC) is located in southeastern Vietnam and is considered the “roof” of the Mekong Delta, with the highest peak of 986 m a.s.l. (Nguyen *et al*. 2019). This area is well known for its high level of

biodiversity, with many reptiles endemic to the country, e.g., *Gekko badenii* Szczerbak & Nekrasova, 1994; *Cyrtodactylus badenensis* Nguyen, Orlov, Darevsky, 2006; *Cyrtodactylus nigriocularis* Nguyen, Orlov & Darevsky, 2006; *Cyrtodactylus dati* Ngo, 2013; *Scincella badenensis* Nguyen, Nguyen, Nguyen & Murphy, 2019 (Szczerbak *et al.* 1994; Nguyen *et al.* 2006; Ngo 2013; Nguyen *et al.* 2019). During our recent fieldwork in Ba Den MCHC, we recorded a new population of gekkonid lizards, which can be assigned to the *Cyrtodactylus irregularis* group (*fide* Grismer *et al*. 2021) based on morphological features and phylogenetic analyses. However, this population also shows genetic variation and morphological differences from known congeners in this group. Thus, we here describe another new species of *Cyrtodactylus* from Ba Den MCHC, Tay Ninh Province, southern Vietnam.

Material and methods

Sampling. A field survey was conducted in Ba Den MCHC, Tay Ninh Province in September 2022 and August 2024 (Fig. 1). Collected geckos were anaesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 85% ethanol for eight hours, then later transferred to 70% ethanol for permanent storage. Specimens were deposited in the collection of the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Molecular data and phylogenetic analyses. DNA was extracted using GeneJET Genomic DNA Purification kit (ThermoFisher Scientific, Lithuania) following the manufacturer's instructions. Two fragments of the mitochondrial genes, cytochrome c oxidase subunit I (COI) and NADH dehydrogenase subunit 2 (ND2), were amplified using the primer pair VF1d (5'-TTCTCAACCAACCACAARGAYATYGG-3') and VR1d (5'-TAGACTTCTGGGTGGCCRAARAAYCA-3') (Ivanova *et al*. 2006) and MetF1 (5'–AAGCTTTCGGGCCCATACC–3') and COIR1 (5'–AGRGTGCCAATGTCTTTGTGRTT–3') (Arévalo *et al*. 1994; Macey *et al*. 1997). Extracted DNA was amplified by Dream Taq Mastermix (ThermoFisher Scientific, Lithuania) with 21 µl volume (10 µl of mastermix 2X, 5 µl of water, 2 µl of each primer at 10 pmol/µl and 2 µl of DNA). PCR conditions were: 95^{o}C for 5 min to active the taq; with 35 cycles at 95^{o}C for 30s, 48^{o}C for 45s, 72^{o}C for 60s; and the final extension at 72^{o}C for 6 min. PCR products were visualized using electrophoresis through a 2% agarose gel stained with ethidium bromide. Successful amplifications were

purified to eliminate PCR components using GeneJET^{TM} PCR Purification kit (ThermoFisher Scientific, Lithuania). Purified PCR products were sent to FirstBase (Malaysia) for sequencing in both directions with the same primers as amplification. Three new samples of the newly discovered population from Ba Den MCHC and other members of the *Cyrtodactylus irregularis* group were included in this study (Table 1). Two species, *C. spelaeus* (HLM0315) and *C. wayakonei* (ZFMK91016) were selected as outgroups (Grismer *et al*. 2021; Ngo *et al*. 2024).

After sequences were aligned by Clustal X v2.1 (Thompson *et al*. 1997), data were analyzed using maximum likelihood (ML) as implemented in IQ-TREE v2.4.0 (Minh *et al*. 2020), maximum parsimony (MP) as implemented in PAUP*4.0b10 (Swofford 2001) and Bayesian inference (BI) as implemented in MrBayes v3.2.7 (Ronquist *et al*. 2012). For the MP analysis, heuristic analysis was conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch-swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) was calculated using 1,000 pseudo-replicates and 100 random taxon addition replicates. All characters were equally weighted and unordered. For the ML analysis, 10,000 ultrafast bootstrap replications (UFB) were used (Hoang *et al*. 2018) and the best-fit model for nucleotide evolution was TIM+F+I+R4, as determined by ModelFinder implemented in IQ-TREE v2.4.0 (Kalyaanamoorthy *et al*. 2017).

For the BI analysis, we used the optimal model determined by jModeltest (Darriba *et al*. 2012) with parameters estimated by MrBayes v3.2.7. Two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10^7 generations with a random starting tree and sampled every 1,000 generations. Loglikelihood scores of sample points were plotted against generation time to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function in the sumt command. The posterior probability values (PP) for all nodes in the final majority rule consensus tree were provided. We regard BP ≥ 70%, UFB ≥ 95 and PP ≥ 0.95 as strong support, UFB ≥ 90 and PP ≥ 0.90 as well support and values of < 70%, < 95 and < 0.95, respectively, as weak support (Hillis and Bull 1993; Ronquist *et al*. 2012; Hoang *et al*. 2018).

The optimal model for nucleotide evolution was set to GTR+I+G for BI analysis. The cut-off point for the burnin function was set to 25% of the total number of trees generated in the Bayesian analysis. Uncorrected pair-wise divergences were calculated in PAUP*4.0b10.

Morphological characters. Measurements were taken with a digital caliper to the nearest 0.1 mm. Abbreviations are as follows: snout-vent length (SVL), from tip of snout to vent; tail length (TaL), from vent to tip of tail (marking * for regenerated tail); head length (HL), from tip of snout to retroarticular process of jaw; head width (HW), maximum width of head; head height (HH), from occiput to underside of jaws; orbital diameter (OD), greatest diameter of orbit; snout to eye distance (SE), from tip of snout to anterior-most point of eye; eye to ear distance (EE), from anterior edge of ear opening to posterior corner of eye; nares to eye distance (NE), from anterior-most point of eye to posterior-most point of nostril; ear length (ED), longest dimension of ear; forearm length (ForeaL), from base of palm to tip of elbow; crus length (CrusL), from base of heel to knee; trunk length (AG), from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; body width (BW), the widest distance of body; internarial distance (IND), distance between nares; interorbital distance (IOD), shortest distance between left and right supraciliary scale rows.

Scale counts were taken as follows: Supralabials (SL), counted from the first labial scale to corner of mouth; infralabials (IL), counted from the first labial scale to posterior corner of mouth; nasal scales surrounding nare (N); postrostrals or internasals (IN); postmentals (PM); granular scales surrounding dorsal tubercles (GST); ventral scales in longitudinal rows at midbody (V); number of scales along the midbody from mental shield to anterior edge of cloaca (SLB); femoral pores (FP); precloacal pores in males (PP); postcloacal tubercles (PAT); dorsal tubercle rows (TubR), counted transversely across the center of the dorsum from one ventrolateral fold to the other; enlarged femoral scales (EFS), number of enlarged femoral scales; number of subdigital lamellae on fourth finger (NSF IV) and number of subdigital lamellae on fourth toe (NST IV). Bilateral scale counts were given as right/left.

Statistical analyses. All statistical analyses were conducted using R Core Team (2024). The MFA was implemented in this study using the R package *FactorMieR* (Le *et al*. 2008) and visualized it using the *Factoextra* package (Kassambara and Mundt 2020). A concatenated dataset comprised of 15 morphometric (SVL, TaL, HL, HW, HH, OD, SE, EE, NE, ED, ForeaL, CrusL, AG, IND, IOD) and 18 meristic (SL (left/right), IL (left/right), N (left/right), IN, PM, GST, V, SLB, FP (left/right), PP, PAT, NSF IV, NST IV) characters was used as input for the analysis. Other morphometric and meristic features were not incorporated due to limited data availability. To remove the effects of allometry, morphometric data were normalized to adjust raw morphometric data through the allom() function in R package GroupStruct (available at https://github.com/chankinonn/GroupStruct).

The allometric formula is $X_{adj} = \log_{10}(X) – ß[\log_{10}(SVL) – \log_{10}(SVL_{mean})]$, where X_{adj} = adjusted value for character X; X = measured value for character X; ß = unstandardized regression coefficient for log(X) against log(SVL) and SVL_{mean} = grand mean of SVL (Thorpe 1975, 1983; Turan 1999; Lleonart *et al*. 2000; Chan and Grismer 2022).

A permutation–based Multivariate Analysis of Variance (perMANOVA) from pairwiseAdonis package in R (available at https://github.com/pmartinezarbizu/pairwiseAdonis/tree/master/pairwiseAdonis) was used to determine if the centroid locations and group clusters of each species were statistically different from each other based on the MFA load scores of dimensions 1–5 (Anderson *et al*. 2017; Oksanen *et al*. 2020). A Euclidean (dis) similarity matrix was calculated using 50,000 permutations (Grismer *et al*. 2024). A pairwise post hoc test was also applied to estimate the differences between studied species pairs. A p-value of < 0.05 was considered to indicate a significant difference between the studied taxa.

Results

Phylogenetic analyses. The matrix of molecular data contained 1,755 aligned characters (COI: 657 and tRNA+ND2: 1,098), of which 797 were constant, 818 parsimony informative, and 140 variable characters parsimony-uninformative. The MP analysis produced a single most parsimonious tree (tree length = 5,047, consistency index = 0.33, retention index = 0.53). The unnamed taxon from Ba Den MCHC, Tay Ninh Province was recovered as a member of an unresolved clade consisting of *C. bidoupimontis, C. bugiamapensis, C. chumuensis, C. cudongensis, C. dati, C. gialaiensis, C. huynhi, C. irregularis, C. phnomchiensis, C. phumyensis, C. takouensis, C. yangbayensis,*and *C. ziegleri* with moderate to well nodal support in ML and BI analyses (UFB = 93, PP = 0.95) but without any clear sister species (Fig. 2). However, *Cyrtodactylus tayninhensis* **sp. nov.** was divergent from other members in the *Cyrtodactylus irregularis* group by at least 13.13% and 15.98% (*C. takouensis*) based on fragments of the COI and ND2 genes, respectively (Tables 2 and 3).

Morphological analysis. Until now, three species of *Cyrtodactylus* have been recorded in Ba Den Mountain, namely *C. badenensis, C. dati,* and *C. nigriocularis* (Nguyen *et al*. 2006; Ngo 2013; Phung *et al*. 2014; Ngo *et al*. 2022). While *C. nigriocularis* has been identified as part of the *C. angularis* group by all previous phylogenetic analyses (Grismer *et al*. 2021, 2022; Ngo *et al*. 2022), it was still included in the MFA analysis. The holotypes and

paratypes of *C. dati*, described by Ngo (2013), were collected from Lam Dong and Binh Phuoc provinces. However, the original description did not provide details on the morphometric and meristic characters of the type series. On the other hand, *C. thuongae*, whose holotype and paratypes originate from Ba Den Mountain in Tay Ninh Province (Phung *et al*. 2014), has been synonymized with *C. dati* based on morphological and molecular evidence (Ngo *et al.* 2022). Therefore, the morphometric and meristic data of *C. dati* from Ba Den were included in the MFA analysis.

The MFA analysis revealed that the new population, *C. badenensis* and *C. dati* overlap along dimension 1, which accounted for 28.9% of the variation in the data set (Fig. 3). However, they were distinguished from each other along dimension 2 which accounted for an additional 23.1% of the variation (Fig. 3). The perMANOVA analysis indicated that the new population from Ba Den Mountain differs significantly in morphospace from *C. badenensis* and *C. dati* (Table 4).

Taxonomy

***Cyrtodactylus tayninhensis* sp. nov.**
(Figs. 4-5)

Holotype. IEBR R.6331 (Field number CT.TN2022.T04.N14), adult male, collected on 29 September 2022 by H. T. Ngo, Q. H. Do, H.Q. Nguyen from the Ba Den MCHC (11.3891N, 106.16755E; at an elevation of 699 m asl.), Tay Ninh Province, southern Vietnam.

Paratypes. Two adult males: IEBR R.6332 (Field number CT.TN2022.T04.N03), IEBR R.6333 (Field number CT.TN2022.T04.N11); two adult females: IEBR R.6334 (Field number CT.TN2022.T04.N09), IEBR R.6335 (Field number CT.TN2022.T04.N13), identical collection data and collectors as the holotype.

Diagnosis. The new species can be distinguished from other members of the *Cyrtodactylus irregularis* group by a combination of the following characters: size medium (SVL 73.4–80 mm); dorsal tubercles in 13 or 14 irregular rows; 36–39 ventral scale rows; enlarged femoral scales absent; precloacal pores absent in females, 4–6 in males, in a continuous row; femoral pores absent; postcloacal spurs 2 or 3 on each side; lamellae under toe IV 16–18; a U-shaped continuous neckband, dorsal pattern between limb insertions consisting two or three irregular yellow-brown bands and tail with 8–10 thin light bands

(with 2 bands near vent light brown, others white); the absence of transversely enlarged median subcaudal scales.

Description of holotype. Adult male, medium size, snout-vent length (SVL) 76.4 mm. Head wider than body, relatively long (HL 22.9 mm, HL/SVL 0.3) and wide (HW 15.1 mm, HW/HL 0.66), relatively depressed (HH 8.5 mm; HH/HL 0.37; HH/HW 0.56), distinct from neck. Prefrontal and postnasal regions concave. Snout elongate (SE/HL 0.4), round anteriorly, longer than orbit diameter (OD/SE 0.56). Scales on snout small, round to oval, granular to weakly conical, mostly homogeneous, larger than those on crown, interorbital and occipital regions. Orbit of moderate size (OD/HL 0.22), pupils vertical, supraciliaries short, forming conical spines, larger anteriorly. Ear opening vertically oval, small (ED/HL 0.1), eye to ear distance longer than orbit diameter (Eye-Ear/OD 1.25). Rostral much wider than deep with a medial suture, bordered by first supralabial on each side, nostrils, two supranasals and one internasal. Nostrils oval, each surrounded by supranasal, rostral, first supralabial and three postnasals. Two enlarged supranasals separated from each another anteriorly by one internasal. Mental triangular, wider than deep. A single pair of greatly enlarged postmentals in broad contact behind mental, bordered by mental anteriorly, first infralabial laterally, and six enlarged chin scales posteriorly. Supralabials 10/10; infralabials 7/8. Scales of labial area decrease in size towards jaw.

Body moderately slender, relatively long (TrunkL/SVL 0.43) with the presence of non-denticulate, ventrolateral skin folds. Dorsal scales granular; dorsal tubercles round, conical, present on occipital region and back, each surrounded by 9 or 10 granular scales, in 14 irregular longitudinal rows at midbody. Ventral scales larger than dorsal scales, smooth, oval, subimbricate, largest on posterior abdomen and in precloacal region. Midbody scale rows across belly between ventrolateral folds 36. Gular region with homogeneous, smooth, juxtaposed granular scales. Enlarged femoral scales, femoral pores and preanal groove absent. Precloacal scales arranged in a diamond shape, precloacal pores 6, in a continuous row, pore-bearing scales enlarged.

Fore and hind limbs moderately slender and long (ForeaL/SVL 0.16, CrusL/SVL 0.19); tubercles on dorsum of fore and hind limbs weakly developed. Fingers and toes without distinct webbing; subdigital lamellae on finger IV 14 and on toe IV 16.

Tail 79.6 mm, approximately the length of the snout-vent length (TaL/SVL 1.03); postcloacal spurs each bearing three much enlarged conical scales; subcaudals without enlarged plate row, flat, smooth, imbricate, about two times larger than scales on tail dorsum.

Coloration in life. Dorsal surface of head brown with irregular light brown markings; nuchal loop dark-brown, U-shaped, edged in yellow-brown, extending from posterior margin of orbit, crossing the upper edge of ear opening to neck; ground color on back dark brown, dorsal pattern between limb insertions consisting of two irregular yellow brown bands; tail with 8 thin light bands (with 2 bands near vent light brown, others white) and 8 thick dark bands; venter of body cream.

Coloration in preservative. In 70% alcohol, the color pattern is slightly faded. The yellow edges disappear and brown turns to whitish grey but the main characteristics are still clearly visible.

Sexual dimorphism and variation. Females differ from males in the absence of precloacal pores and hemipenial swellings at the tail base. The number of narrow light bands on the tail varies by one to two. For other morphological characters see Table 5.

Distribution. *Cyrtodactylus* **sp. nov.** is currently known only from the type locality in Ba Den MCHC, Tay Ninh Province, Vietnam (Fig. 1).

Etymology. The specific epithet of the new species refers to the type locality of the new species, Tay Ninh, a province in southern Vietnam. Suggested common names: Tay Ninh Bent-toed Gecko (English), Thằn lằn chân ngón tây ninh (Vietnamese).

Natural history. The type series was found between 19:00 and 24:00 in deep rocky caves with low light. More than 50 individuals were observed, but not collected, during our field surveys in September 2022 and August 2024interesting.. The microclimatic conditions of *Cyrtodactylus tayninhensis* **sp. nov.** were characterized by air microtemperatures ranging from 22.0 to 27.5°C and substrate temperatures varying between 19.8 and 30.7°C. The majority of individuals were discovered in habitats with high canopy coverage (≥ 80%) and elevated humidity levels (≥ 80%). The surrounding habitat was secondary forest of medium and small hardwoods mixed with shrubs. We also found *C. badenensis* in the same habitat. However, *C. badenensis* tends to stay near the cave entrance and outside the cave, while *C. tayninhensis* **sp. nov.** stays deep inside the cave (for more details see the discussion below).

Comparisons. We compared the new species with its 30 congeners from the *Cyrtodactylus irregularis* complex based on examination of specimens and data obtained from the literature (Smith 1921; Heidrich *et al.* 2007; Orlov *et al.* 2007; Nazarov *et al.* 2008; Ngo & Bauer 2008; Rösler *et al.* 2008; Geissler *et al.* 2009; Ngo & Chan 2010; Nazarov *et al.* 2012; Ngo 2013; Nguyen *et al.* 2013; Ziegler *et al.* 2013; Schneider *et al.* 2014; Luu *et al.* 2017; Pauwels *et al.* 2018; Ngo *et al.* 2020; Neang *et al.* 2020; Ostrowski *et al.* 2020, 2021; Nguyen *et al.* 2021; Do *et al.* 2021, 2023; Ngo *et al.* 2023, 2024) (Table 6).

Below we compared the new species with the most phenotypically similar species. In particular, *Cyrtodactylus tayninhensis* **sp. nov.** differs from *C. arndti* Ngo, Hormann, Le, Pham, Phung, Do, Ostrowski, Nguyen & Ziegler by the absence of enlarged femoral scales (*versus* 5–11), the absence of precloacal pores in females (*versus* 6), the absence of transversely enlarged subcaudal plates (*versus* present), and different dorsal color pattern (distinct bands with thin light bands; two bands nearest to vent light brown and the others white) *versus* irregular bands with thicker light brown bands; from *C. badenensis* by having more ventral scale rows (36–39 *versus* 25–28), the presence of precloacal pores in males (*versus* absent), the absence of transversely enlarged subcaudal plates (*versus* present), and different dorsal color pattern (thin light bands with two bands nearest to vent light brown and the others white *versus* four white bands across the back, the first at forelimbs, the last at hindlimbs and two others in the middle of the back); from *C. bidoupimontis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler by having fewer dorsal tubercle rows (13 or 14 *versus* 18–24), the absence of enlarged femoral scales (*versus* 8–10), and different tail color pattern (thin light bands with two bands nearest the vent light brown and the others white *versus* thicker light brown bands); from *C. chumuensis* Ngo, Hormann, Le, Pham, Phung, Do, Ostrowski, Nguyen & Ziegler by larger size (SVL 73.4–80.0 mm *versus* 67.5 mm), fewer ventral scale rows (36–39 *versus* 43–45), no enlarged femoral scales (*versus* 4 or 5), and different dorsal color pattern (distinct bands with thin light bands, two bands nearest the vent light brown and others white *versus* irregular bands with thicker light brown bands and short longtitudinal stripes on the neck); from *C. irregularis* (Smith) by lacking enlarged femoral scales (*versus* 7 or 8), difference in color pattern of dorsum (banded *versus* blotched), and different tail color pattern (thin light bands *versus* thicker light brown bands); from *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen by having fewer dorsal tubercle rows (13 or 14 *versus* 16–20), the absence of enlarged femoral scales (*versus* 3–8), and different tail color pattern (dark transverse bands of wider than light brown interspaces *versus* dark brown transverse bands narrower than interspaces); and from *C. taynguyenensis* Nguyen, Le, Tran, Orlov, Lathrop, Macculloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang by having more supralabial scales (10–12 *versus* 8 or 9), fewer ventral scale rows (36–39 *versus* 42–49), and different dorsal color pattern (banded *versus* blotched).

Discussion

The new species represents the fourth species of *Cyrtodactylus* from Ba Den MCHC (Nguyen *et al*. 2006). The newly described species is clearly distinguishable from three previously known species, *C. badenensis, C. dati* and *C. nigriocularis* based on the morphological and molecular analyses presented in this study and Fig. 6. According to our field observations, *C. badenensis*, *C. nigriocularis* and *C. tayninhensis* **sp. nov.** are found only in the granite sites of Ba Den, whereas *C. dati* occupies several different microhabitat types, including on the ground with leaf litter and soil, on dead branches and on granite rocks.

In terms of altitudinal gradients, *C. nigriocularis* was found at elevations lower than 700 m asl., whereas *C. badenensis* and *C. dati* were encountered at elevations both lower and higher than 700 m asl. In contrast, *Cyrtodactylus tayninhensis* was only detected at elevations above 700 m asl. Moreover, although *C. badenensis* and *Cyrtodactylus tayninhensis* were observed in syntopy, they are morphologically divergent and genetically separated from each other. This study once again illustrates a high level of sympatry but not syntopy in bent-toed geckos. Several other localities in Vietnam and other countries harbor more than three co-occurring species of *Cyrtodactylus*, including Phong Nha-Ke Bang, Ta Kou Nature Reserve (Ngo & Bauer 2008; Ostrowski *et al*. 2021; Duong *et al*. 2024). The ability to co-exist in the same habitats might partly explain the stunning diversity of the genus and their habitat preference (Grismer *et al*. 2020, 2021) so they avoid syntopy. Our discovery brings the species number of the *C. irregularis* species group to 31, including *C. buchardi,* which is still being investigated genetically (Grismer et al. 2021; Ngo et al. 2023, 2024; Uetz et al. 2024;).

The population status of the new species and the other three bent-toed geckos, and the anthropogenic threats to their microhabitats in Ba Den MCMC still need to be further investigated. According to Nguyen et al. (2018a,b), Ba Den MCHC has become a popular tourist destination with rapid tourism development. Presently, *C. nigriocularis* is classified as Critically Endangered, *C. badenensis* as Vulnerable, and *C. dati* as Data Deficient in the IUCN Red List (Nguyen et al. 2018a,b; IUCN Red List 2024). Moreover, Ba Den MCHC receives a lower level of protection compared to a nature reserve. Therefore, further studies of the four species, all restricted to a single mountain surrounded by lowlands heavily used for agriculture and other infrastructural developments, should be performed and appropriate conservation measures should be designed to protect their natural habitat and populations.

Acknowledgements

We are grateful to the directorate of Ba Den MCHC for their support of our fieldwork and for issuing relevant permits. We thank L. V. Pham (Tay Ninh) for his assistance in the field. We thank T. T. Nguyen, M. H. Le for their support in the laboratory. For the fruitful cooperation within joint research projects, we cordially thank A. H. Le (IEBR, Hanoi), as well as T. Pagel and C. Landsberg (Cologne Zoo). We would like to thank the editors and reviewers for their valuable comments on the manuscript. Doctoral research of H. T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD). This research is supported by the Zoological Society for the Conservation of Species and Populations (ZGAP, Project code 7.Ba Den Mountain. VNM-2024) and Cologne Zoo. Comments from Aaron Bauer, Lee Grismer and an anonymous reviewer greatly improved the paper.

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Figure 1. Type locality of *Cyrtodactylus tayninhensis* **sp. nov.** in Tay Ninh Province (black circle), Vietnam.

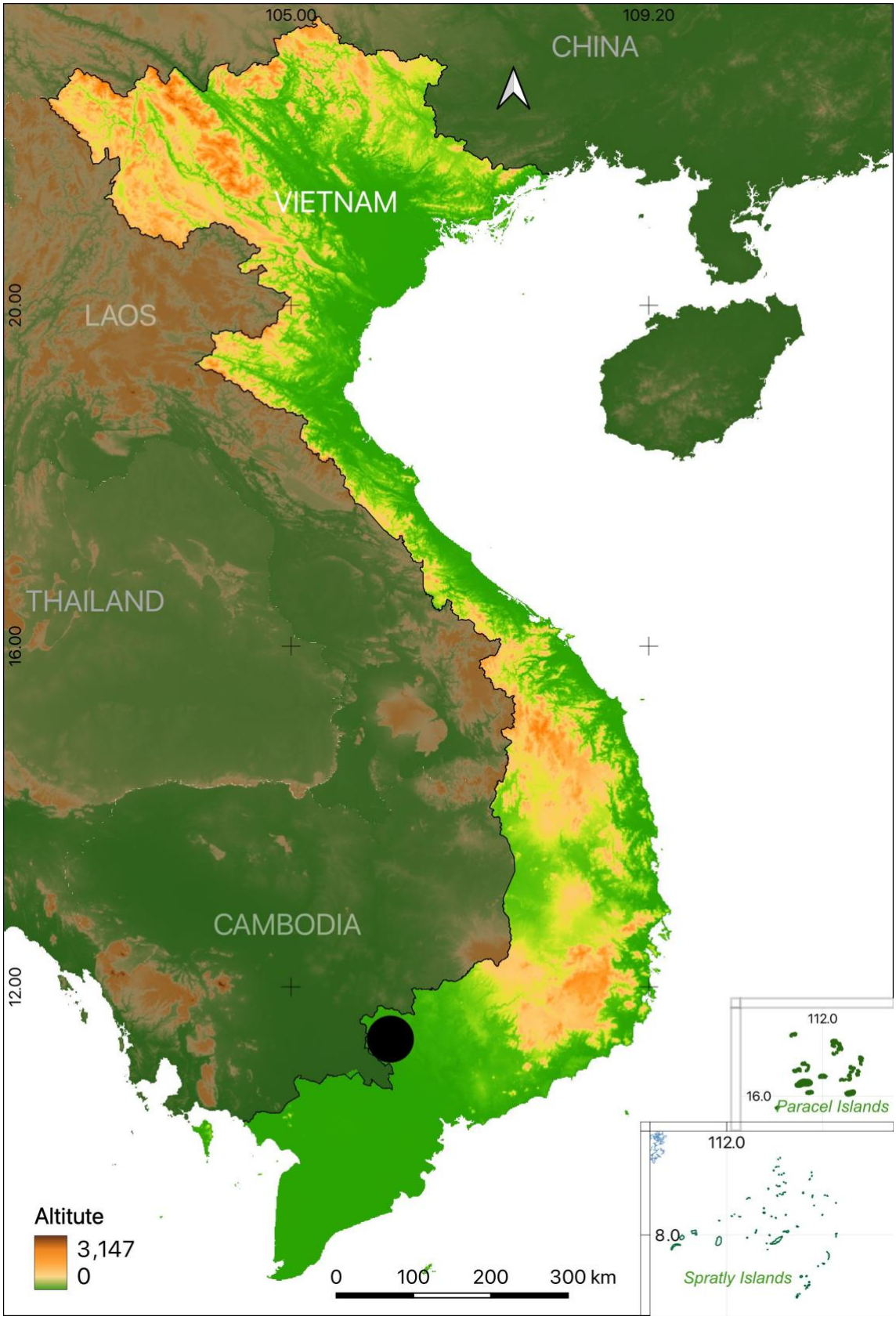

Figure 2. Phylogram based on the Bayesian analysis. Numbers above and below branches are ML/MP bootstrap and ultrafast bootstrap values and Bayesian posterior probabilities (≥ 50%), respectively. Asterisk and hyphen denote 100 or 100% or 1 and < 50 or 50% or 0.5 values, respectively.

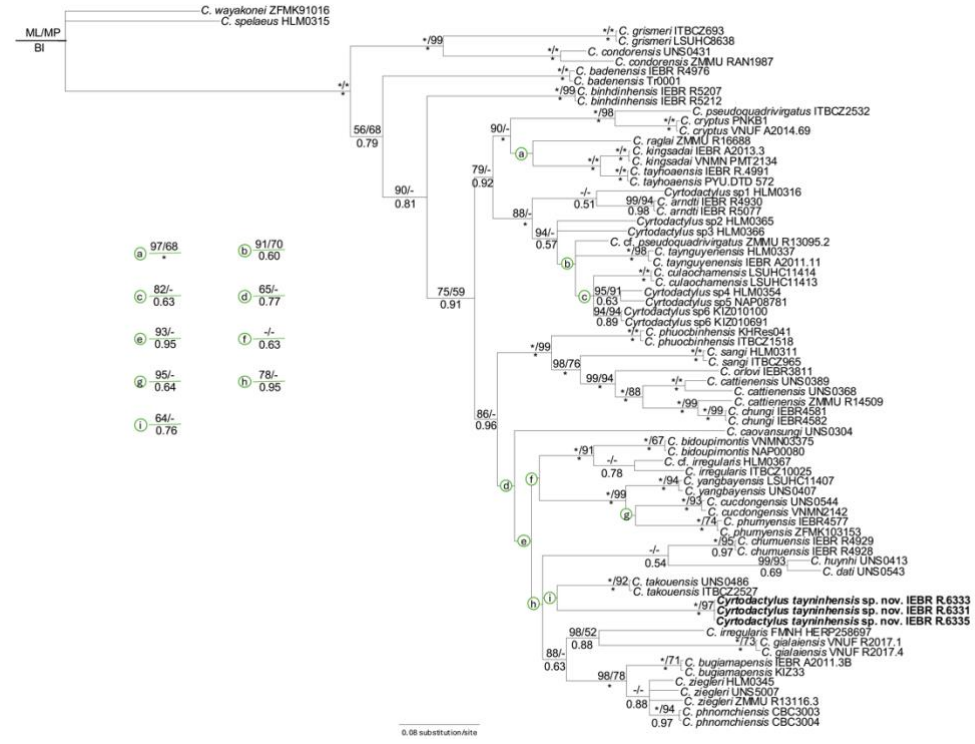

Figure 3. A. MFA of *Cyrtodactylus tayninhensis* **sp. nov.** and *C. badenensis, C. dati,* and *C. nigriocularis* for Dim 1 and Dim 2 axes. B. Percent contribution of the quantitive variables to the dimension 1. C. Percent contribution of the quantitative variables to the dimension 2. Dotted red line is the mean percentage if all values were equal.

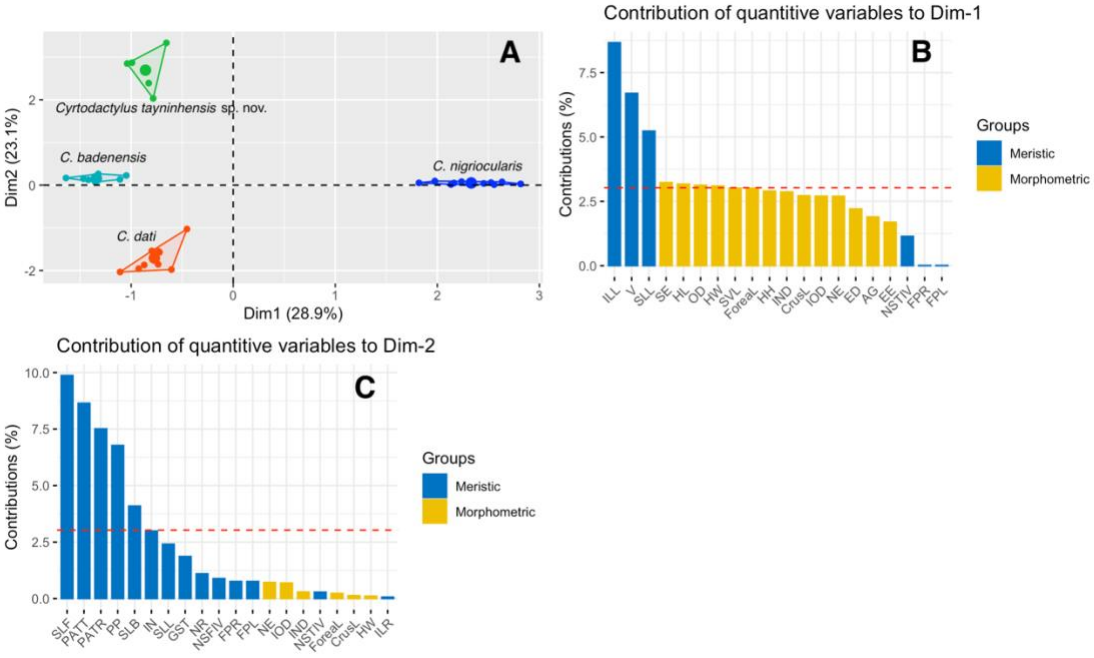

Figure 4. The male holotype of *Cyrtodactylus tayninhensis* **sp. nov.** (IEBR R.6331) in life. A) Dorsal view. B) Ventral view. Photos: H.Q. Nguyen.

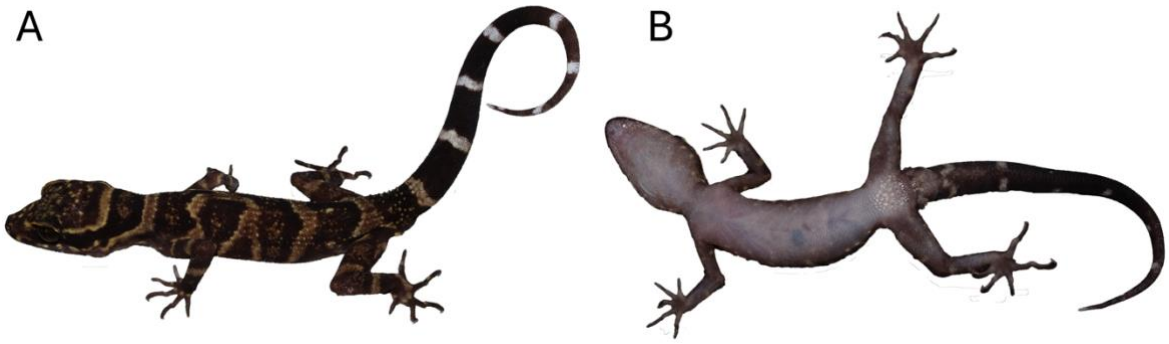

Figure 5. A) Male paratype of *Cyrtodactylus tayninhensis* **sp. nov.** in life (IEBR R.6333). B) Female paratype paratype of *Cyrtodactylus tayninhensis* **sp. nov.** in life (IEBR R.6335). Photos: H.Q. Nguyen.

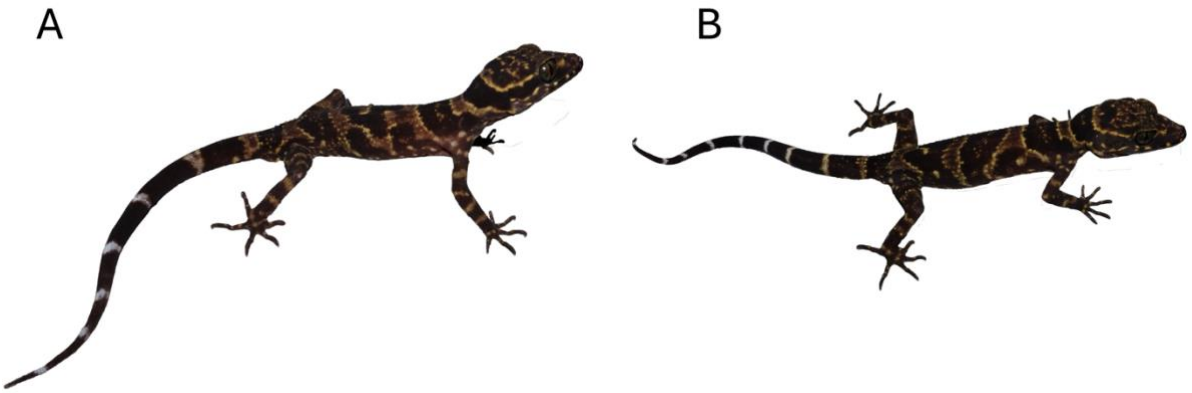

Figure 6. Species comparisons. A) *Cyrtodactylus tayninhensis* **sp. nov.** from Ba Den MCHC, Tay Ninh Province, Vietnam. B) *C. dati* from Ba Den MCHC, Tay Ninh Province, Vietnam. Photos: H.N. Ngo. C) *C. badenensis* from Ba Den MCHC, Tay Ninh Province, Vietnam. D) *C. nigriocularis* from Ba Den MCHC, Tay Ninh Province, Vietnam. Photos: H.Q. Nguyen.

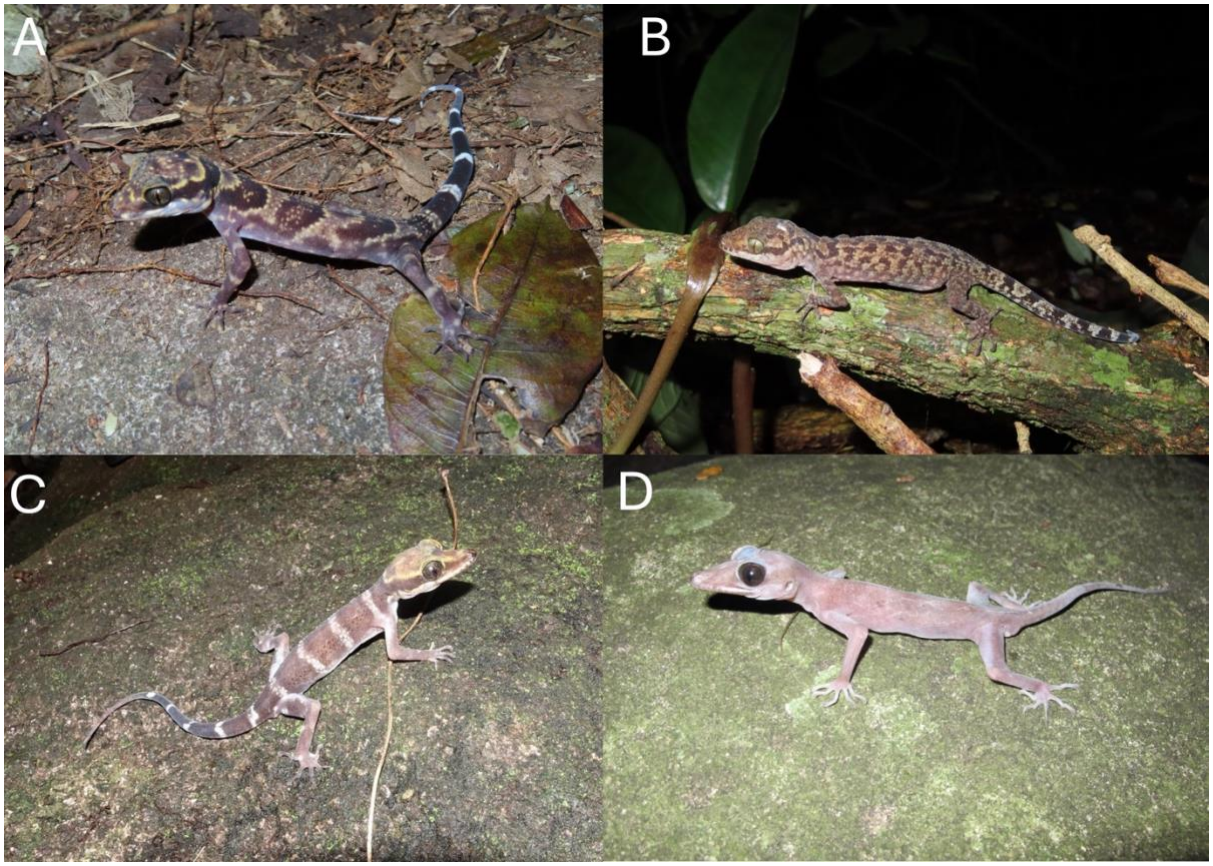

Table 1. Species of *Cyrtodactylus* used in the phylogenetic analysis including localities and GenBank accession numbers of the mitochondrial COI and ND2 fragment genes

Species	Locality	Museum number/Field number	ND2	COI
C. spelaeus	Laos: Vientiane, Kasi	HLM0315	MW713962	-
C. wayakonei	Laos: Luang Nam Tha Prov., Vieng Phoukha District, Ban Nam Eng, Kao Rao Cave	ZFMK91016	MT953498	KJ817438
Cyrtodactylus tayninhensis **sp. nov.**	Vietnam: Tay Ninh Prov., Ba Den Mt	IEBR R.6333	-	XXXXXX
Cyrtodactylus tayninhensis **sp. nov.**	Vietnam: Tay Ninh Prov., Ba Den Mt	IEBR R.6331	XXXXXX	XXXXXX
Cyrtodactylus tayninhensis **sp. nov.**	Vietnam: Tay Ninh Prov., Ba Den Mt	IEBR R.6335	XXXXXX	XXXXXX
C. arndti	Vietnam: Binh Dinh Prov., Van Canh District	IEBR-R.5077	-	OQ152316
C. arndti	Vietnam: Binh Dinh Prov., Van Canh District	IEBR-R.4930	-	OQ152317
C. badenensis	Vietnam: Tay Ninh Prov., Ba Den Mt	IEBR4976	-	ON145836
C. badenensis	Vietnam: Tay Ninh Prov., Ba Den Mt	TR0001	MT953468	ON145835
C. bidoupimontis	Vietnam: Khanh Hoa Prov., Nha Trang	VNMN03375	MT953470	-
C. bidoupimontis	Vietnam: Lam Dong Prov., Bidoup-Nui Ba NP	ZMMU NAP00080	-	KC016074
C. binhdinhensis	Vietnam: Binh Dinh Prov., Phu Cat District	IEBR R.5207	-	PP441921
C. binhdinhensis	Vietnam: Binh Dinh Prov., Phu Cat District	IEBR R.5212	-	PP441922
C. bugiamapensis	Vietnam: Binh Phuoc Prov., Bu Gia Map NP	IEBR A2011.3B	MT953473	ON145810
C. bugiamapensis	Vietnam: Binh Phuoc Prov., Bu Gia Map NP	KIZ00033	-	KY862173
C. caovansungi	Vietnam: Ninh Thuan Prov., Nui Chua NP	UNS0304	MF169954	-
C. cattienensis	Vietnam: Dong Nai Prov., Cat Tien NP	ZMMU NAP-00117.1	-	HQ967220
C. cattienensis	Vietnam: Dong Nai Prov., Ma Da SFE	UNS0389	MF169956	-
C. cattienensis	Vietnam: Ba Ria - Vung Tau Prov., Binh Chau - Phuoc Buu NR	ZMMU_R14509	-	MG791892
C. cattienensis	Vietnam: Dong Nai Prov., Ma Da SFE	UNS0368	MF169955	-
C. chumuensis	Vietnam: Dak Lak Prov., M'Drak District	IEBR-R.4928	-	OQ152319
C. chumuensis	Vietnam: Dak Lak Prov., M'Drak District	IEBR-R.4929	-	OQ152320
C. chungi	Vietnam: Binh Thuan Prov., Ta Kou NR	IEBR 4581	-	MT576019
C. chungi	Vietnam: Binh Thuan Prov., Ta Kou NR	IEBR 4582	-	MT576020
C. condorensis	Cambodia: Koh Tang Island	ZMMU RAN 1987	-	HM888464
C. condorensis	Vietnam: Ba Ria - Vung Tau Prov., Con Dao Island	UNS 0431	MF169958	MF169910
C. cryptus	Vietnam: Quang Binh Prov., Phong Nha – Ke Bang NP	PNKB 1	-	KF169969
C. cryptus	Laos: Khammouane Prov., Hin Nam No NPA	VNUF A2014.69	MT953476	KX064038
C. cucdongensis	Vietnam: Khanh Hoa Prov., Hon Heo Mountain	UNS0544	MF169959	-
C. cucdongensis	Vietnam: Khanh Hoa Prov., Cuc Dong Cape	VNMN2142	-	MG791884
C. culaochamensis	Vietnam: Quang Nam Prov., Cu Lao Cham Island	LSUHC11413	KT013198	-
C. culaochamensis	Vietnam: Quang Nam Prov., Cu Lao Cham Island	LSUHC11414	KT013199	-
C. dati	Vietnam: Binh Phuoc Prov., Bu Dop	UNS0543	MF169960	-
C. gialaiensis	Vietnam: Gia Lai Prov., Chu Se District	VNUF R.2017.1	MT953479	MG460299
C. gialaiensis	Vietnam: Gia Lai Prov., Chu Se District	VNUF R.2017.4	-	MG460300
C. grismeri	Vietnam: An Giang Prov., Tuc Dup Mt.	ITBCZ 693	-	KF929516
C. grismeri	Vietnam: An Giang Prov., Tuc Dup Mt.	LSUHC8638	JX440538	ON145806

C. huynhi	Vietnam: Dong Nai Prov., Chua Chan Mt.	UNS0413	MF169963	-
C. irregularis	Vietnam: Lam Dong Prov., Lac Duong	ITBCZ10025	-	MG791887
C. irregularis	Laos: Champasal Prov., Pakxong District.	FMNH HERP258697	JX041341	-
C. cf. *irregularis*	Vietnam: Lam Dong Prov., Loc Bac	HLM367	MW713952	-
C. kingsadai	Vietnam: Dak Nong Prov.	VNMN PMT2134	-	ON145834
C. kingsadai	Vietnam: Phu Yen Prov., Tuy Hoa District, Dai Lanh Cape	IEBR A2013.3	MT953483	KF188432
C. orlovi	Vietnam: Ninh Thuan Prov., Thuan Nam District	IEBR 3811	-	MZ440851
C. phnomchiensis	Cambodia: Kampong Thom Prov., Sandan District	CBC 3003	-	MT066405
C. phnomchiensis	Cambodia: Kampong Thom Prov., Sandan District	CBC 3004	-	MT066406
C. phumyensis	Vietnam: Binh Dinh Prov., Phu My District	ZFMK:103153	MW792065	MT210158
C. phumyensis	Vietnam: Binh Dinh Prov., Phu My District	IEBR:4577	-	MT210161
C. phuocbinhensis	Vietnam: Ninh Thuan Prov., Phuoc Binh District	ITBCZ 1518	-	KF169953
C. phuocbinhensis	Vietnam: Khanh Hoa Prov., O Kha Valley	KHReS041	MT953488	ON145822
C. cf. *pseudoquadrivirgatus*	Vietnam	ZMMU R13095.2	-	KP199949
C. pseudoquadrivirgatus	Vietnam: Da Nang Prov., Ba Na	ITBCZ 2532	-	KF169962
C. raglai	Vietnam: Khanh Hoa Prov., Khanh Vinh District	ZMMU R16688	MW675652	MW675653
C. sangi	Vietnam: Khanh Hoa Prov., Cam Ranh	HLM0311	MW713956	-
C. sangi	Vietnam: Ninh Thuan Prov., Nui Chua NP	ITBCZ965	-	KF169952
C. takouensis	Vietnam: Binh Thuan Prov., Ta Kou NR	ITBCZ 2527	-	KF929533
C. takouensis	Vietnam: Binh Thuan Prov., Ta Kou NR	UNS0486	MF169978	-
C. tayhoaensis	Vietnam: Phu Yen Prov., Tay Hoa District	IEBR R.4991	-	OQ603084
C. tayhoaensis	Vietnam: Phu Yen Prov., Tay Hoa District	PYU.DTD 572	-	OQ603085
C. taynguyenensis	Vietnam: Kon Tum Prov., Mang Canh	HLM0337	MW713953	-
C. taynguyenensis	Vietnam: Gia Lai Prov., Kon Ka Kinh	IEBR A2011.11	-	KY862145
C. yangbayensis	Vietnam: Khanh Hoa Prov., Hon Ba NR	UNS0407	MF169980	-
C. yangbayensis	Vietnam: Khanh Hoa Prov., Hon Ba NR	LSUHC11407	KT013202	-
C. ziegleri	Vietnam: Dak Lak Prov., Chu Yang Sin NP	UNS 5007	-	KF169945
C. ziegleri	Vietnam: Dak Lak Prov., Chu Yang Sin NP	ZMMU R-13116-3	-	HQ967210
C. ziegleri	Vietnam: Dak Lak Prov., Yok Don	HLM0345	MW713968	-
Cyrtodactylus sp1	Vietnam: Gia Lai Prov., Kon Ka Kinh	HLM0316	MW713951	-
Cyrtodactylus sp2	Vietnam: Gia Lai Prov., Kon Ka Kinh	HLM0365	MW713950	-
Cyrtodactylus sp3	Vietnam: Kon Tum Prov., Chu Mom Ray NP.	HLM0366	MW713954	-
Cyrtodactylus sp4	Vietnam: Kon Tum Prov., Kon Plong	HLM0354	MW713955	-
Cyrtodactylus sp5	Vietnam: Quang Nam Pro., Song Thanh	NAP08781	MW713949	-
Cyrtodactylus sp6	Vietnam: Thua Thien Hue, Phong Dien District	KIZ010100	-	KY862131
Cyrtodactylus sp6	Vietnam: Thua Thien Hue, Phong Dien District	KIZ010691	-	KY862132

Notes. Prov. = Province, NP = National Park, NR = Nature Reserve, Mt. = mountain

Table 2. Uncorrected (p) distance matrix showing percentage pairwise genetic divergence between *Cyrtodactylus tayninhensis* sp. nov. and closely related species of *Cyrtodactylus* based on a fragment of COI

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Cyrtodactylus tayninhensis* **sp. nov.** IEBR R.6333	-																	
2	*C. arndti* IEBRR4930	15.17	-																
3	*C. badenensis* Tr0001	14.76	18.40	-															
4	*C. bidoupimontis* KC016074	14.85	15.19	17.76	-														
5	*C. binhdinhensis* PP441921	14.61	15.35	15.68	14.86	-													
6	*C. bugiamapensis* IEBR A2011.3B	14.31	14.88	17.50	13.32	14.31	-												
7	*C. chumuensis* IEBRR4928	15.18	15.95	18.56	13.80	15.80	13.19	-											
8	*C. gialaiensis* VNUFR2017.1	14.76	15.33	17.50	15.92	16.29	14.76	17.33	-										
9	*C. irregularis* ITBCZ10025	13.92	15.55	16.82	8.91	15.28	15.13	14.48	15.59	-									
10	*C. kingsadai* IEBRA2013 3	14.61	12.57	16.59	14.99	14.92	14.61	14.57	17.50	15.59	-								
11	*C. phnomchiensis* CBC3003	14.61	15.34	17.50	14.55	15.37	7.15	13.50	14.76	14.98	15.37	-							
12	*C. phumyensis* ZFMK103153	15.22	13.96	18.27	14.54	15.37	14.76	15.34	16.90	13.75	14.92	15.53	-						
13	*C. phuocbinhensis* ITBCZ1518	15.47	13.68	17.80	14.90	15.08	14.69	13.49	14.70	13.99	13.57	15.11	13.21	-					
14	*C. raglai* ZMMU R16688	13.89	13.26	18.95	13.90	14.05	13.41	16.59	17.69	15.56	12.31	14.52	15.30	14.81	-				
15	*C. takouensis* ITBCZ2527	13.13	14.37	17.08	13.27	12.17	11.98	14.59	13.49	13.42	12.84	12.74	11.78	13.82	12.28	-			
16	*C. taynguyenensis* IEBR A2011.11	15.01	14.24	17.03	16.30	16.12	16.81	16.18	17.13	16.28	15.83	17.38	16.34	14.36	15.60	15.82	-		
17	*C. tayhoaensis* IEBR R.4991	14.00	13.34	15.83	14.54	14.46	13.85	13.95	16.13	14.07	4.26	14.76	14.61	12.92	12.64	11.81	14.50	-	
18	*C. ziegleri* ZMMU R13116 3	14.55	15.14	18.17	14.50	15.82	7.36	13.54	15.49	15.51	14.41	5.31	14.56	14.78	14.00	12.59	14.99	13.79	-

Table 3. Uncorrected (p) distance matrix showing percentage pairwise genetic divergence between *Cyrtodactylus tayninhensis* sp. nov. and closely related species of *Cyrtodactylus* based on a fragment of ND2

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Cyrtodactylus tayninhensis* **sp. nov.** IEBR R.6331	-																	
2	*C. badenensis* Tr0001	22.00	-																
3	*C. bidoupimontis* VNMN03375	18.19	21.26	-															

4	*C. bugiamapensis* IEBR A2011.3B	16.44	21.31	16.07	-														
5	*C. cattienensis* UNS0389	19.53	20.71	18.34	19.68	-													
6	*C. cucdongensis* UNS0544	18.59	21.18	17.01	18.27	19.02	-												
7	*C. dati* UNS0543	20.23	23.52	19.24	18.51	19.84	19.09	-											
8	*C. gialaiensis* VNUFR2017.1	18.73	22.57	17.22	17.44	20.96	19.99	20.37	-										
9	*C. huynhi* UNS0413	17.25	22.18	16.11	17.87	16.81	17.18	5.29	19.02	-									
10	*C. kingsadai* IEBRA2013.3	19.41	19.07	18.02	18.66	18.18	19.07	19.14	19.11	19.39	-								
11	*C. phumyensis* ZFMK103153	18.10	21.44	16.91	18.27	19.74	12.04	19.87	20.93	17.59	19.21	-							
12	*C. phuocbinhensis* KHRes041	19.14	20.21	17.20	17.47	13.82	19.34	19.58	18.72	17.32	18.46	18.97	-						
13	*C. raglai* ZMMU R16688	17.82	18.88	16.73	17.54	17.44	18.48	19.03	18.54	18.16	12.08	17.82	16.54	-					
14	*C. sangi* HLM0311	19.01	21.35	17.46	18.37	16.20	19.86	20.31	20.74	18.94	19.48	18.56	14.28	17.46	-				
15	*C. takouensis* UNS0486	16.09	19.47	13.93	15.54	16.60	14.09	15.43	17.83	13.77	15.66	16.10	14.91	15.25	17.13	-			
16	*C. taynguyenensis* HLM0337	19.79	20.30	17.69	17.40	18.49	19.15	20.47	20.61	18.46	16.04	19.88	17.93	14.39	19.16	17.04	-		
17	*C. yangbayensis* LSUHC11407	17.00	20.90	15.08	16.80	19.08	10.51	19.34	18.32	18.19	17.47	10.88	18.18	16.91	18.74	14.36	17.60	-	
18	*C. ziegleri* HLM0345	16.83	21.89	16.09	9.66	19.96	17.91	18.74	17.20	17.31	19.14	18.39	18.01	18.02	19.05	15.15	19.08	16.64	-

Table 4. Summary statistics from the perMANOVA analysis from the loadings of the MFA comparing *Cyrtodactylus tayninhensis* sp. nov. to *C. badenensis*, *C. dati* and *C. nigriocularis*.

Species pairs	**F.Model**	**R2**	**p.value**	**p.adjusted**
C. tayninhensis sp. nov. – *C. badenensis*	155.20	0.93	0.00054	0.0013
C. tayninhensis sp. nov. – *C. dati*	18.65	0.61	0.00054	0.0013
C. tayninhensis sp. nov. – *C. nigriocularis*	194.09	0.94	0.00042	0.0013

Table 5. Measurements (in mm) and morphological characters of the type series of *Cyrtodactylus tayninhensis* **sp. nov.** (* = regenerated or broken tail); bilateral meristic characters are given as (left/right).

Characters	**IEBR.R.6331** (Holotype)	**IEBR.R.6332** (Paratype)	**IEBR.R.6333** (Paratype)	**IEBR.R.6334** (Paratype)	**IEBR.R.6335** (Paratype)	Min-Max
Sex	M	M	M	F	F	
SVL	76.4	73.4	75	79	80	73.4-80
TaL	79.6	76	53.1*	81	79	76-81
HL	22.9	21.7	21.6	22.2	24	21.6-24
HW	15.1	15	14.3	15	16.3	14.3-16.3
HH	8.5	8.5	7.9	8.5	9	7.9-9
OD	5.1	5	5.4	5.1	5.1	5-5.4
SE	9.1	8.8	9.2	10	9.5	8.8-10
EE	6.4	6	5.6	6.7	6.6	5.6-6.7
NE	6.5	6.1	6.7	6.1	7	6.1-7
ED	2.3	2.5	2.2	1.8	1.6	1.6-2.5
ForeaL	12.1	12	11.6	13.2	12.8	11.6-13.2
CrusL	14.6	13.6	14.4	14.2	14.3	13.6-14.6
AG	30.6	31.7	30	33.4	31	30-33.4
BW	14.1	14.1	12.9	14.5	15	12.9-15
IND	2.7	2.5	2.2	2.3	2.4	2.2-2.7
IOD	5.5	5.6	5.8	5.5	6	5.5-6
SL	12/11	10/10	11//10	11//10	10//11	10-12
IL	9/9	7/8	8/8	8/8	8/8	7-9
N	4//4	4//4	4//4	4//4	4//4	5
IN	1	1	1	1	1	1
PM	2	2	2	2	2	2
GST	9	10	10	10	10	9-10
V	39	36	36	39	38	36-39
SLB	171	169	169	171	170	169-171
FP	0	0	0	0	0	0

PP	5	4	6	0	0	4-6 in males
PAT	3/3	3/3	3/3	3/3	3/2	2-3
TubR	13	14	14	14	14	13-14
EFS	0	0	0	0	0	0
NSFIV	15	14	16	15	16	14-16
NSTIV	17	16	16	16	18	16-18

Table 6. Morphological comparisons between *Cyrtodactylus tayninhensis* **sp. nov.** and its 30 congeners from the *Cyrtodactylus irregularis* complex based on examination of specimens and data obtained from the literature (Smith 1921; Heidrich *et al.* 2007; Orlov *et al.* 2007; Nazarov *et al.* 2008; Ngo & Bauer 2008; Rösler *et al.* 2008; Geissler *et al.* 2009; Ngo & Chan 2010; Nazarov *et al.* 2012; Ngo 2013; Nguyen *et al.* 2013; Ziegler *et al.* 2013; Schneider *et al. 20*14; Luu *et al.* 2017; Pauwels *et al.* 2018; Ngo *et al.* 2020; Neang *et al.* 2020; Ostrowski *et al.* 2020, 2021; Nguyen *et al.* 2021; Do *et al.* 2021, 2023; Ngo *et al.* 2023, 2024). (measurements in mm, * = regenerated or broken tail, Max = maximum, other abbreviations defined in the text).

No.	*Taxa*	SVL	TaL	V	DTR	EFS	FP	PP(M)	PP (F)	LD4	LT4	Color patterm of dorsum	Enlarged subcaudals
1	*C. tayninhensis.* **sp. nov.**	73.4–80	76.0–81	36–39	13–14	absent	absent	4–6	0	14–16	16–18	banded	absent
2	*C. arndti*	73.4–80.9	50.1*–91.51	26–38	17–20	5–11	0–2 pitted	6	6 pitted	15–20	17–22	banded	present
3	*C. badenensis*	59.3–74.1	58.6–82.4	25–28	?	absent	absent	0	0	?	18–22	banded	present
4	*C. bidoupimontis*	74.0–86.3	75.0–86	38–43	18–24	8–10	absent	4–6	0	15–20	18–23	banded	absent
5	*C. binhdinhensis*	58.5–80.4	max. 84.7	39–42	19–21	5–6	10	6–7	5–6	17–19	18–21	banded	present
6	*C. bugiamapensis*	58.6–76.8	65.3–83.0	36–46	20–24	6–10	absent	7–11	0–7	15–17	17–20	blotched	absent
7	*C. buchardi*	60.0–65.0	46.0–54.0	30	25	absent	absent	9	0	14	12	blotched	absent
8	*C. caovansungi*	90.4–94	120	38–44	16–18	8	6	9	0	22	23–25	banded	present
9	*C. cattienensis*	43.5–69	51–64.7	28–42	16–22	3–8	absent	6–8	0	12–16	14–19	banded	absent
10	*C. chumuensis*	67.5	51.4*	43–45	20	4–5	0–2	6–7	?	16–19	17–21	banded	absent
11	*C. chungi*	66.6–68.5	62.8*–82.2	30–31	17–18	4–6	absent	7	6	15–18	17–20	banded	absent
12	*C. cryptus*	62.5–90.8	63.5–88.4	47–50	19–20	absent	absent	9–11	0	18–19	20–23	banded	absent

13	*C. cucdongensis*	55.8–65.9	max. 81.3	41–44	16–19	5–9	absent	5–6	4–6	13–18	15–20	banded	absent
14	*C. culaochamensis*	69.8–79.8	89.7–91.2	45–50	20–22	absent	absent	7–8	absent	18–19	20–23	banded	absent
15	*C. dati*	max 70.1	max 57.3	42–48	20–22	4–7	6–7	5–6	?	?	18–19	blotched	absent
16	*C. gialaiensis*	50.1–62.8	?	38–45	16–21	present	absent	9–10	0–8	14–15	15–17	Banded	absent
17	*C. huynhi*	67.2–79.8	61.5–78.6	43–46	16–18	3–5	3–8	7–9	0–8 pitted	14–17	17–21	banded	absent
18	*C. irregularis*	72–86	66.0–74	38–45	???	7–8	absent	5–7	0–6	15–16	18–19	blotched	absent
19	*C. kingsadai*	83–94	max 117	39–46	17-23	9–12	3–7	7–9	4–8	19–21	21–25	banded	present
20	*C. orlovi*	61–77.7	max 71.2	36–39	16–20	3–8	absent	5–6	0	15–17	16–19	banded	absent
21	*C. phnomchiensis*	76.1–80.7	56.9–79.1	45–54	18–20	0–8	absent	4–5	1–7 pitted	18–20	20–23	banded	Absent
22	*C. phumyensis*	63.6–66.8	?	33–41	18–19	5–7	absent	5–7	6 pitted	18–19	18–21	banded	absent
23	*C. phuocbinhensis*	46–60.4	76.1	43–47	???	5	absent	7	0	16–21	17–19	striped/blotched	absent
24	*C. pseudoquadrivirgatus*	48.6–83.3	55.7–82.3	41–57	16–24	absent	absent	5–9	5–10	15–21	16–25	Varied	absent
25	*C. raglai*	87.5–111.7	113.4–119	36–39	???	9–10	0	5	0	?	21–22	banded	present
26	*C. sangi*	49.9–56.3	47.9*	37	19–21	4	Absent	7	4 (Pitted)	?	?	banded	absent
27	*C. takouensis*	74.7–81.1	77.7–91	39–40	9–10	3–5	0–2	3–4	0	16–17	18–20	banded	present
28	*C. tayhoaensis*	82.9–94.2	max 104.3	37–41	20–22	10–11	3-7 males	4–5	0	20–22	22–24	banded	present
29	*C. taynguyenensis*	60.0–85.0	66.0–94.0	42–49	???	absent	absent	6	0	13–18	17–21	blotched	absent
30	*C. yangbayensis*	78.5–92.3	91.3–109.1	39–46	20–23	5–16	0–2	6–8	0	16–19	15–17	banded	Present
31	*C. ziegleri*	84.6–93.0	95.0–107.0	33–39	20–24	8–10	0–6	5–8	0–8	16–19	18–21	banded	Absent

Chapter 6. Speciation by isolation: A new reptile species (Squamata, Gekkonidae) from Hon Tre Island in Khanh Hoa Province, Vietnam

ZooKeys 1253: 195–218 (2025)
DOI: 10.3897/zookeys.1253.149459

Research Article

A new species of *Cyrtodactylus* (Squamata, Gekkonidae) from Hon Tre Island in Khanh Hoa Province, Vietnam

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Abstract

A new species of the genus *Cyrtodactylus* is described from Khanh Hoa Province, South-central Vietnam based on genetic divergence and morphological differences. *Cyrtodactylus arnei* **sp. nov.** is distinguished from the remaining Indochinese bent–toed geckos of the *Cyrtodactylus irregularis* group by having the unique combination of the following characteristics: size medium (SVL 70.9–78.0 mm); dorsal tubercles in 15–17 irregular rows; 34 or 35 ventral scale rows; 12–15 enlarged femoral scales on each side, in continuous series without gap between precloacal and femoral scales; precloacal pores absent in females, 5–7 in males, in a continuous row; femoral pores absent; postcloacal spurs 0–3 on each side; 19–21 lamellae under toe IV; dorsal pattern between limb insertions consisting of four narrow light bands with dark edges and a transversal row of dark spots in the middle; subcaudal scales enlarged, forming broad transverse plates. In phylogenetic analyses, the new species was nested within the *Cyrtodactylus irregularis* group without any clear sister taxon. Genetically, *Cyrtodactylus arnei* **sp. nov.** is divergent from other species within the *Cyrtodactylus irregularis* group by at least 10.97% (COI) and 14.39% (ND2) based on two fragments of the mitochondrial gene.

Key words: *Cyrtodactylus arnei* sp. nov., *Cyrtodactylus irregularis* group, morphology, phylogenetic relationships, taxonomy

OPEN ACCESS

Academic editor: Johannes Penner
Received: 12 February 2025
Accepted: 11 August 2025
Published: 24 September 2025

ZooBank: https://zoobank.org/043DB44D-19FA-4111-B062-C434DF840AFE

Citation: Do QH, Ngo HT, Nguyen TQ, Le MD, Ziegler T, Do DT, Pham CT (2025) A new species of *Cyrtodactylus* (Squamata, Gekkonidae) from Hon Tre Island in Khanh Hoa Province, Vietnam. ZooKeys 1253: 195–218. https://doi.org/10.3897/zookeys.1253.149459

Introduction

South-central Vietnam is considered as a transition zone between the Central Highlands and the coastal areas below 1,000 m in elevation. Its natural habitat is characterized by evergreen forest on low-lying mountains and thorny vegetation coverage, with drought–tolerant trees such as minnows living in windy areas with little rain in coastal areas (Sterling et al. 2006). The region is recognized as a center for new reptile discoveries in Vietnam, as numerous new species have been re-

cently described from the surrounding area, viz. *Acanthosaura murphyi* Nguyen, Do, Hoang, Nguyen, McCormack, Nguyen, Orlov, Nguyen & Nguyen, 2018; *Sphenomorphus yersini* Nguyen, Nguyen, Nguyen, Orlov & Murphy, 2018; *Cyrtodactylus phumyensis* Ostrowski, Do, Le, Ngo, Pham, Nguyen, Nguyen & Ziegler, 2020; *C. chungi* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler, 2021; *C. raglai* Nguyen, Duong, Grismer & Poyarkov, 2021; *Gekko phuyenensis* Nguyen, Nguyen, Orlov, Murphy, & Nguyen, 2021, *Cyrtodactylus orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen, 2021, *C. tayhoaensis* Do, Do, Le, Ngo, Ziegler, Nguyen & Pham, 2023, *C. arndti* Ngo, Horman, Le, Pham, Phung, Do, Ostrowski, Nguyen & Zieger, 2023; *C. binhdinhensis* Ngo, Do, Do, Pham, Bui, Ho, Nguyen, Ziegler & Le, 2024; *Lycodon anakradaya* Nguyen, Duong, Wood & Grismer, 2022; *L. truongi* Nguyen, Duong, Wood & Grismer, 2022; *Trimeresurus cyanolabris* Idiiatullina, Nguyen, Bragin, Pawangkhanant, Le, Vogel, David & Poyarkov, 2024; and *Colubroelaps adleri* Poyarkov, Bragin & Bragin, 2024 (Nguyen et al. 2018a, b, 2021a, b, 2022a; Ostrowski et al. 2020, 2021; Do et al. 2021, 2023; Ngo et al. 2023, 2024; Idiiatullina et al. 2024; Poyarkov et al. 2024).

In terms of species richness of bent–toed geckos, South-central Vietnam harbors 16 species of *Cyrtodactylus*: *C. arndti* Ngo, Horman, Le, Pham, Phung, Do, Ostrowski, Nguyen & Zieger; *C. binhdinhensis* Ngo, Do, Do, Pham, Bui, Ho, Nguyen, Ziegler & Le; *C. caovansungi* Orlov, Nguyen, Nazarov, Ananjeva & Nguyen; *C. chungi* Ostrowski, Do, Le, Ngo, Pham, Nguyen, Nguyen & Ziegler; *C. cucdongensis* Schneider, Phung, Le, Nguyen & Ziegler; *C. culaochamensis* Ngo, Grismer, Pham & Wood; *C. kingsadai* Ziegler, Phung, Le & Nguyen; *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen; *C. phumyensis* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler; *C. phuocbinhensis* Nguyen, Le, Tran, Orlov, Lathrop, MacCulloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang; *C. pseudoquadrivirgatus* Rösler, Vu, Nguyen, Ngo & Ziegler; *C. raglai* Nguyen, Duong, Grismer & Poyarkov; *C. sangi* Pauwels, Nazarov, Bobrov & Poyarkov; *C. takouensis* Ngo & Bauer; *C. tayhoaensis* Do, Do, Le, Ngo, Ziegler, Nguyen & Pham; *C. yangbayensis* Ngo & Chan. All species in this area belong to the *Cyrtodactylus irregularis* group, which occurs in South-central Vietnam and extends into eastern Cambodia and southeast Laos, and includes 30 species to date (Orlov et al. 2007; Ngo and Bauer 2008; Rösler et al. 2008; Ngo and Chan 2010; Nguyen et al. 2013; Ziegler et al. 2013; Schneider et al. 2014; Pauwel et al. 2018; Ngo et al. 2020; Ostrowski et al. 2020, 2021; Do et al. 2021, 2023; Grismer et al. 2021; Nguyen et al. 2021a; Ngo et al. 2023, 2024; Uetz et al. 2025).

Recent field work on Hon Tre Island of Khanh Hoa Province, South-central Vietnam resulted in the collection of nine specimens of a gekkonid species, which can be assigned to the genus *Cyrtodactylus* based on morphological features and phylogenetic analyses. However, this *Cyrtodactylus* population showed genetic divergence and morphological differences from other known species. Thus, we herein describe a new species of *Cyrtodactylus* from Hon Tre Island of Khanh Hoa Province in South-central Vietnam.

Materials and methods

Sampling

A field survey was conducted on Hon Tre Island, Khanh Hoa Province in September 2020 (Fig. 1). Specimens were anaesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons

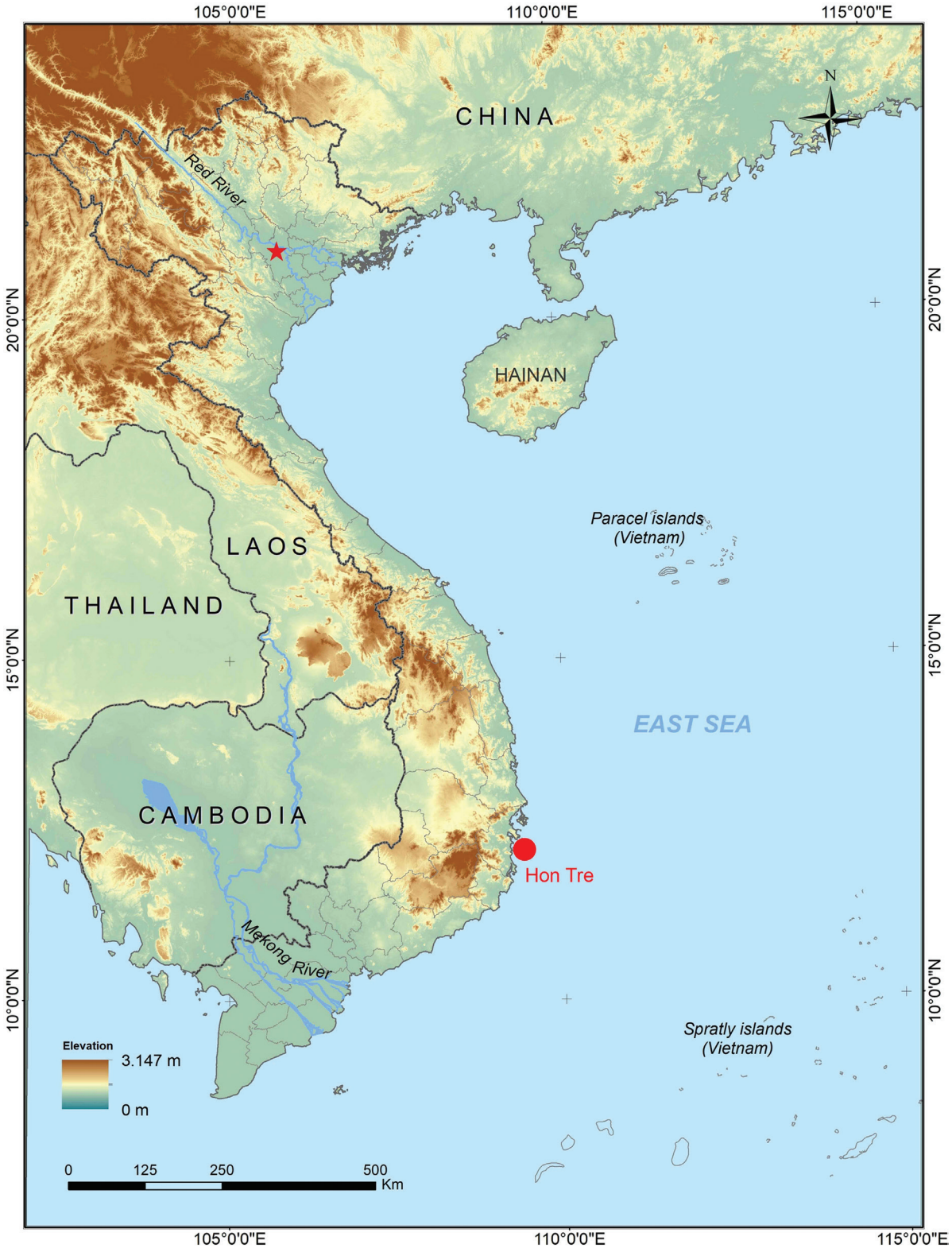

Figure 1. Type locality of *Cyrtodactylus arnei* sp. nov. in Hon Tre Island, Khanh Hoa Province (red dot), Vietnam (Source: NASA Shuttle Radar Topography Mission (SRTM) 2013). The capital Hanoi is indicated with a red star.

2002), fixed in 85% ethanol for eight hours, then later transferred to 70% ethanol for permanent storage. Specimens were deposited in the collections of the Institute of Biology (**IB**) (formerly known as the Institute of Ecology and Biological Resources **IEBR**), Hanoi, Vietnam.

Molecular data and phylogenetic analyses

All recognized species of the *Cyrtodactylus irregularis* group were included in the study, except *C. buchardi* and *C. pseudoquadrivirgatus* sensu stricto (Suppl. material 1). Two taxa, *C. spelaeus* Nazarov, Poyarkov, Orlov, Nguyen, Milto, Martynov, Konstantinov & Chulisov, 2014 and *C. wayakonei* Nguyen, Kingsada, Rösler, Auer & Ziegler, 2010 were used as outgroups based on their phylogenetic relationships to the *C. irregularis* species group as reported by Luu et al. (2017) and Grismer et al. (2021).

DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer's instructions. Extracted DNA was amplified by HotStarTaq PCR mastermix (Qiagen, Germany) with 21 µl volume (10 µl of mastermix, 5 µl of water, 2 µl of each primer at 10 pmol·ml−1 and 2 µl of DNA). PCR conditions were 95 °C for 15 minutes to active the taq; with 35 cycles at 95 °C for 30s, 45 °C for 45s, 72 °C for 60s; and a final extension at 72 °C for 6 minutes. Two fragments of the mitochondrial gene, cytochrome c oxidase subunit I (COI) and NADH dehydrogenase subunit 2 (ND2) were amplified using the primer pair VF1 d (5'–TTCTCAACCAACCACAARGAYATYGG–3') and VR1 d (5'–TAGACTTCTGGGTGGCCRAARAAYCA–3') (Ivanova et al. 2006) and MetF1 (5'–AAGCTTTCGGGCCCATACC–3') and COIR1 (5'–AGRGTGCCAATGTCTTTGTGRTT–3') (Arevalo et al. 1994; Macey et al. 1997). PCR products were visualized using electrophoresis through a 2% agarose gel stained with ethidium bromide. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purification kit (ThermoFisher Scientific, Lithuania). Purified PCR products were sent to FirstBase (Malaysia) for sequencing in both directions.

Each gene dataset was initially aligned separately by ClustalX v. 2.1 (Thompson et al. 1997) with default settings for complete alignment. Combined data (COI + ND2) were analyzed using Bayesian inference (**BI**) as implemented in MrBayes v. 3.2.7 (Ronquist et al. 2012), Maximum likelihood (**ML**) as implemented in IQ–TREE v. 2.4.0 (Minh et al. 2020), and Maximum Parsimony (**MP**) implemented in PAUP*4.0b10 (Swofford 2001). Additionally, each gene dataset was also analyzed using BI and ML for the references. For MP analysis, a heuristic analysis was conducted with 100 random taxon addition replicates using tree–bisection and reconnection (**TBR**) branch–swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support was calculated using 1,000 pseudo–replicates (BP) and 100 random taxon addition replicates. All characters were equally weighted and unordered. For the ML analysis, we employed a single model and 10,000 ultrafast bootstrap replications (**UFB**). The optimal model for nucleotide evolution was determined using jmodeltest v. 2.1.10 (Darriba et al. 2012).

For the Bayesian analyses, we used the optimal model determined by jmodeltest with parameters estimated by MrBayes v. 3.2.7. Two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10^7 generations with a random starting tree and sampled every 1,000 generations. Loglikelihood scores of sample points were plotted against generation time to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn–in function. The posterior probability values (PP) for all clades in the final majority rule consensus tree were provided. Nodal support was evaluated using BP as estimated in PAUP, UFB in IQ–TREE v. 2.4.0, and PP in MrBayes v. 3.2.7. UFB > 95, and PP ≥ 95% and BP ≥ 70% were regarded as strong support for a clade (Hillis and Bull 1993; Ronquist et al.

2012; Hoang et al. 2018). The optimal model for nucleotide evolution was set to GTR+I+G for Bayesian analysis and TIM1+I+G for ML analysis. The cut–off point for the burn–in function was set to delete 25% of the total number of trees generated. Uncorrected pairwise divergences were calculated in PAUP*4.0b10.

Morphological characteristics

Measurements were taken with a digital caliper to the nearest 0.1 mm. Abbreviations are as follows:

SVL snout–vent length (from tip of snout to vent);
TaL tail length (from vent to tip of tail, with * meaning regenerated tail);
HL head length (from tip of snout to retroarticular process of jaw);
HW head width (maximum width of head);
HH head height (from occiput to underside of jaws);
OD orbital diameter (greatest horizontal diameter of orbit);
SE snout to eye distance (from tip of snout to anterior–most point of eye);
EE eye to ear distance (from anterior edge of ear opening to posterior margin of eye);
NE nares to eye distance (from anterior–most point of eye to posterior–most point of nostril);
ED ear length (longest dimension of ear);
ForeaL forearm length (taken on the dorsal surface from the posterior margin of the elbow while flexed 90° to the inflection of the flexed wrist);
CrusL crus length (taken on the ventral surface from the posterior surface of the knee while flexed 90° to the base of heel);
TrunkL trunk length (from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion);
BW body width (the widest distance of body);
IND internarial distance (distance between nares);
IOD interorbital distance (shortest distance between left and right supraciliary scale rows).

Scale counts were taken as follows:

SupL Supralabials (counted from the first labial scale to corner of mouth);
IL infralabials (counted from the first labial scale to posterior corner of mouth);
N nasal scales surrounding nare;
IN postrostrals or internasals;
PM postmentals;
GST granular scales surrounding dorsal tubercles;
V ventral scales in longitudianal rows at midbody counted between ventrolateral folds;
FP femoral pores;
PP precloacal pores;
PAT postcloacal tubercles;
DTR dorsal tubercle rows (counted transversely across the center of the dorsum from one ventrolateral fold to the other);

EFS enlarged femoral scales (number of enlarged femoral scales beneath each thigh);
LD4 number of subdigital lamellae on fourth finger;
LT4 number of subdigital lamellae on fourth toe.

Statistical analyses

All statistical analyses were conducted using R Core Team (2024). The MFA was applied in this study using the R package FactorMineR (Le et al. 2008) and visualized it using the Factoextra package (Kassambara and Mundt 2017). A concatenated dataset comprised of 12 morphometric (SVL, HL, HW, HH, SE, Eye Ear, ED, ForeaL, TrunkL, IND, IOD) and 10 meristic (SupL (left), Infra (left), V, FP (left/right), PP, TubR, EFS (left/right), NST IV) characters was used as an input for FactorMineR. Other morphological characteristics were not incorporated due to limited available data or incomplete sampling (regenerate tail). To remove the effects of allometry, morphometric data were normalized to adjust raw of morphometrics through the allom function in R package GroupStruct (available at https://github.com/chankinonn/GroupStruct). Accordingly, the allometric formula is $X_{adj} = \log_{10}(X) - \beta[\log_{10}(SVL) - \log_{10}(SVL_{mean})]$, where X_{adj} = adjusted value; X = measured value; β = unstandardized regression coefficient for each population and SVL_{mean} = overall average SVL of two populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000; Grismer et al. 2021; Chan and Grismer 2022).

A permutation–based Multivariate Analysis of Variance (perMANOVA) from pairwiseAdonis package in R (available at https://github.com/pmartinezarbizu/pairwiseAdonis/tree/master/pairwiseAdonis) was used to determine if the centroid locations and group clusters of each species were statistically different from each other based on the MFA load scores of dimensions 1–5 (Anderson 2017; Oksanen et al. 2020). A Euclidean (dis) similarity matrix was calculated using 50,000 permutations (Grismer et al. 2024). A pairwise *post hoc* test was also applied to estimate the differences between studied species pairs. A $p < 0.05$ is considered significant difference between the taxa.

Results

Phylogenetic analyses

The matrix of molecular data contained 2,067 aligned characters (COI: 657 and ND2: 1,410), of which 956 were constant, 197 variable and parsimony-uninformative, and 914 parsimony informative. The MP analysis produced a single most parsimonious tree (tree length = 5,435; consistency index = 0.35, retention index = 0.52). Tree topologies from three analyses, ML, MP, and BI based on combined data (COI+ND2) or separate data (COI or ND2) were congruent and similar to those reported in Ngo et al. (2022) and the undescribed species from Hon Tre Island, Khanh Hoa Province was nested within the *Cyrtodactylus irregularis* group without any clear sister taxon (Fig. 2, Suppl. materials 2, 3). Genetically, the new species is divergent from other species within the *Cyrtodactylus irregularis* group by at least ~10.97% (*C. takouensis*) based on a fragment of the mitochondrial COI gene and by at least ~14.39% (*C. bidoupimontis*) based on a fragment of the NADH dehydrogenase subunit 2 (Suppl. material 4: tables S1, S2).

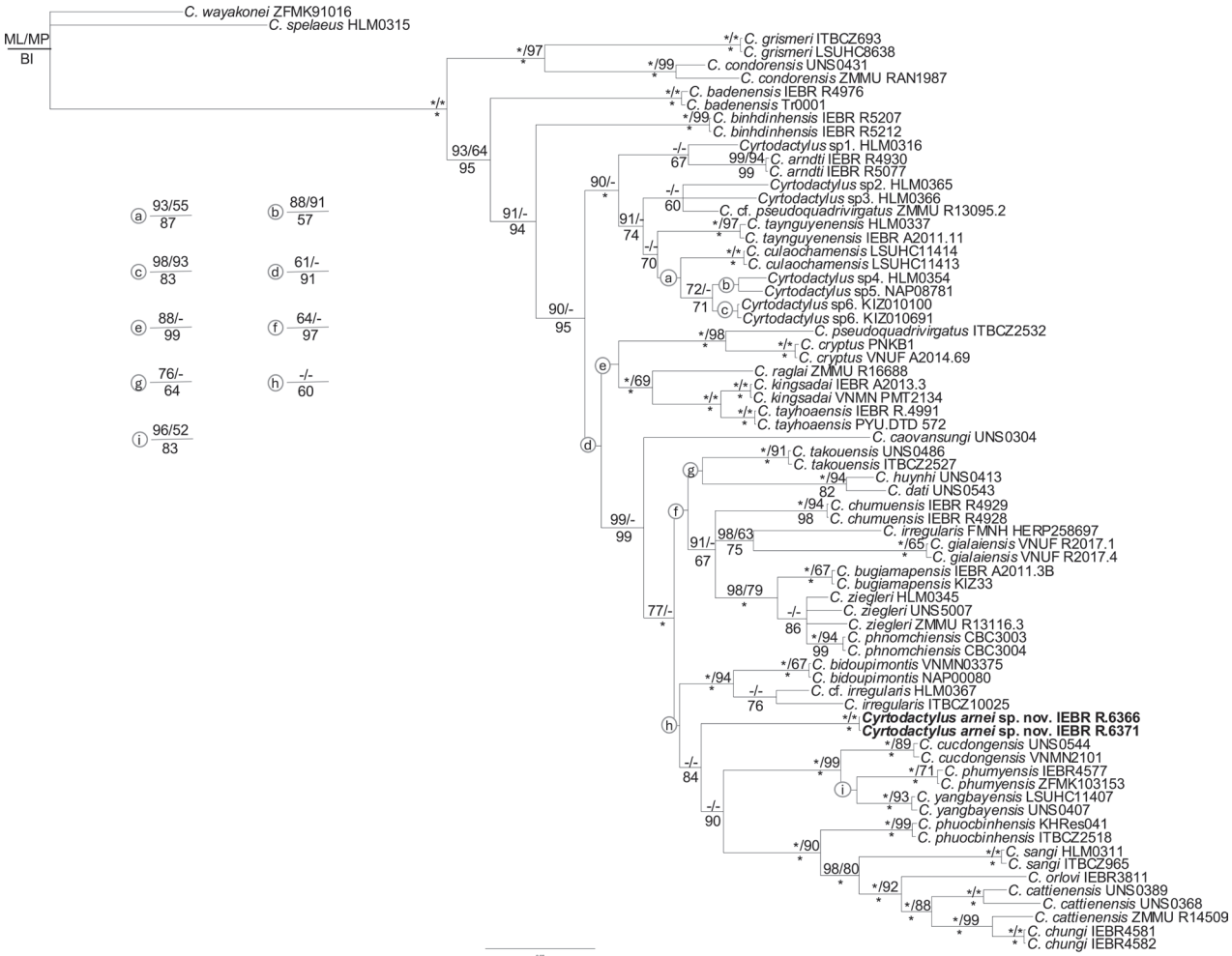

Figure 2. Bayesian phylogram based on the combined matrix of COI and ND2 (+tRNA) genes. Number above and below branches are ML/MP bootstrap and ultrafast bootstrap values and Bayesian posterior probabilities (≥ 50%), respectively.

Morphological analysis. To date, four known species of *Cyrtodactylus* have been recorded in Khanh Hoa Province, consisting of *C. cucdongensis*, *C. raglai* and *C. yangbayensis* and *C. bidoupimontis* (Ngo and Chan 2010; Schneider et al. 2014; Nguyen et al. 2017, 2021a). Therefore, four representative species in Khanh Hoa Province and *C. takouensis* were included in the MFA analysis.

The MFA analysis revealed that although the new population and *C. bidoupimontis*, *C. yangbayensis* are all overlapped along dimensions 1 and 4 that accounted for 21.2% and 14.4% of the variation in the data set (Fig. 3A, B), respectively. They were distinguished from each other along dimensions 2 and 3 which were attributed to additional 19.3% and 17.1% of the variation, respectively (Fig. 3A, B). Furthermore, the perMANOVA analysis indicated that the new population from Hon Tre Island differs significantly in morphospace from *C. bidoupimontis*, *C. cucdongensis*, *C. raglai*, *C. takouensis* and *C. yangbayensis* (Table 1). Morphometric characters contributed most of the variation along dimension 1 and meristic data contributed to the majority of the variation along dimensions 2, 3 and 4. Morphometric character ForeaL followed by morphometric characters HL, SVL and ED provided major variation along dimension 1 (Fig. 3C), while meristic characters TubR, EFS, SL and NST IV made up most of the variation along dimension 2 (Fig. 3D). Meristic characters V, NST IV

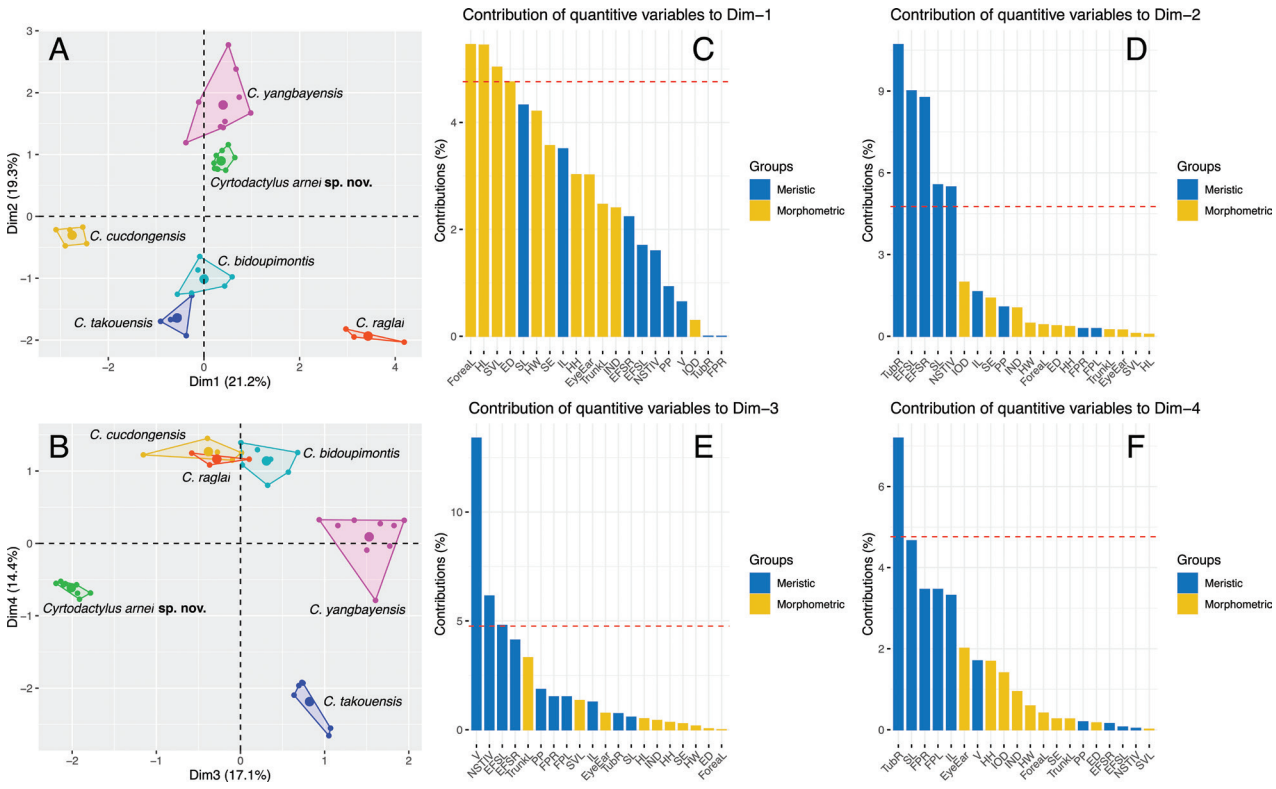

Figure 3. A. MFA of *Cyrtodactylus arnei* sp. nov. and *C. bidoupimontis*, *C. cucdongensis*, *C. takouensis*, and *C. yangbayensis* for Dim 1 and Dim 2 axes; **B.** MFA of *C. arnei* sp. nov. and C. *bidoupimontis*, *C. cucdongensis*, *C. takouensis*, and *C. yangbayensis* for Dim 3 and Dim 4 axes; **C–F.** Percent contribution of the quantitative variables to the dimensions 1–4 of the MFA. Dotted red line is the mean percentage if all values were equal.

Table 1. Summary statistics from the perMANOVA analysis from the loadings of the MFA comparing *Cyrtodactylus arnei* sp. nov. to *C. bidoupimontis*, *C. cucdongensis*, *C. raglai*, *C. takouensis*, and *C. yangbayensis*.

Species pairs	F.Model	R2	p.value	p.adjusted
C. arnei sp. nov. – *C. bidoupimontis*	323.30	0.96	0.10×10^{-3}	0.14×10^{-2}
C. arnei sp. nov. – *C. cucdongensis*	408.97	0.97	0.58×10^{-3}	0.52×10^{-2}
C. arnei sp. nov. – *C. raglai*	360.89	0.97	0.41×10^{-2}	0.021
C. arnei sp. nov. – *C. takouensis*	457.16	0.97	0.22×10^{-3}	0.26×10^{-2}
C. arnei sp. nov. – *C. yangbayensis*.	166.32	0.91	0.60×10^{-4}	0.90×10^{-3}

and EFS contributed to the majority of the variation along dimension 3 (Fig. 3E), and meristic characters TubR, SL, FP and IL accounted for the majority of the variation along dimension 4 (Fig. 3F).

Taxonomic account

***Cyrtodactylus arnei* sp. nov.**

https://zoobank.org/2FF533C8-93B7-44B9-AB0A-387EA9A943F1

Figs 4, 5

Type material. *Holotype*. IEBR R.6365 (Field number KH.2020.7), adult male, collected on 26 September 2020 by C.T. Pham on Hon Tre Island (12°12'58.77"N, 109°15'52.44"E; at an elevation of 74 m asl.), Khanh Hoa

Province, Vietnam. ***Paratypes*.** Five adult males: IEBR R.6366 (Field number KH.2020.5), IEBR R.6367 (Field number KH.2020.8), IEBR R.6368 (Field number KH.2020.11), IEBR R.6369 (Field number KH.2020.12), IEBR R.6370 (Field number KH.2020.13); three adult females: IEBR R.6371 (Field number KH.2020.6), IEBR R.6372 (Field number KH.2020.9), IEBR R.6373 (Field number KH.2020.10), the same data as the holotype.

Diagnosis. The new species can be distinguished from other members of the *Cyrtodactylus irregularis* group by a combination of the following characteristics: size medium (SVL 70.9–78.0 mm); dorsal tubercles in 15–17 irregular rows; 34 or 35 ventral scale rows; 12–15 enlarged femoral scales on each side, in continuous series without gap between precloacal and femoral scales; precloacal pores absent in females, 5–7 in males, in a continuous row; femoral pores absent; postcloacal spurs 0–3 on each side; 19–21 lamellae under toe IV; dorsal pattern between limb insertions consisting four narrow light bands with dark edges and a transversal row of dark spots in the middle; subcaudal scales enlarged, forming broad transverse plates.

Description of holotype. Adult male, medium size, snout–vent length (SVL) 74.8 mm. Head wider than body, elongate (HL 21.9 mm, HL/SVL 0.29), wide (HW 13.8 mm, HW/HL 0.63), relatively depressed (HH 7.9 mm, HH/HL 0.36, HH/HW 0.57), distinct from neck; prefrontal and postnasal regions concave; snout elongate (SE/HL 0.45), round anteriorly, longer than orbit diameter (OD/SE 0.5); scales on snout small, round to oval, granular to weakly conical, mostly homogeneous, larger than those on crown, interorbital and occipital regions; orbit of moderate size (OD/HL 0.23), pupils vertical, supraciliaries short, forming conical spines, larger anteriorly; ear opening vertically oval, small in size (ED/HL 0.09), eye to ear distance longer than orbit diameter (Eye Ear/OD 1.23); rostral much wider than deep with a medial suture, bordered by first supralabial on each side, nostrils, two supranasals and one internasal; nostrils oval, each surrounded by supranasal, rostral, first supralabial and three postnasals; two enlarged supranasals separated from one another anteriorly by one internasal; mental triangular, wider than deep; a single pair of greatly enlarged postmentals in broad contact behind mental, bordered by mental anteriorly, first infralabial laterally, and six enlarged chin scale posteriorly; supralabials 12/11; infralabials 10/10; scales of labial area decreasing in size towards jaw.

Body moderately slender, relatively short (TrunkL/SVL 0.37) with the presence of non–denticulate, ventrolateral skin folds; dorsal scales granular; dorsal tubercles round, conical, present on occipital region and back, each surrounded by eight or nine granular scales, in 16 irregular longitudinal rows at midbody; ventral scales larger than dorsal scales, smooth, oval, subimbricate, largest posteriorly, largest on posterior abdomen and in precloacal region; midbody scale rows across belly between ventrolateral folds 36; gular region with homogeneous, smooth, juxtaposed granular scales; 15 or 16 poreless and pitless enlarged femoral scales each thigh, in continuous series with enlarged precloacal scales; precloacal groove absent; precloacal scales arranged in a diamond shape, precloacal pores seven, in a continuous row, pore–bearing scales enlarged; postcloacal spur each bearing three much enlarged conical scales.

Fore and hind–limbs moderately slender and long (ForeaL/SVL 0.17, CrusL/SVL 0.2); tubercles on dorsum of fore and hind–limbs weakly developed; fingers and toes without distinct webbing; subdigital lamellae: finger IV 17 (with 6 basally broadened lamellae), toe IV 20 (with 7 basally broadened lamellae).

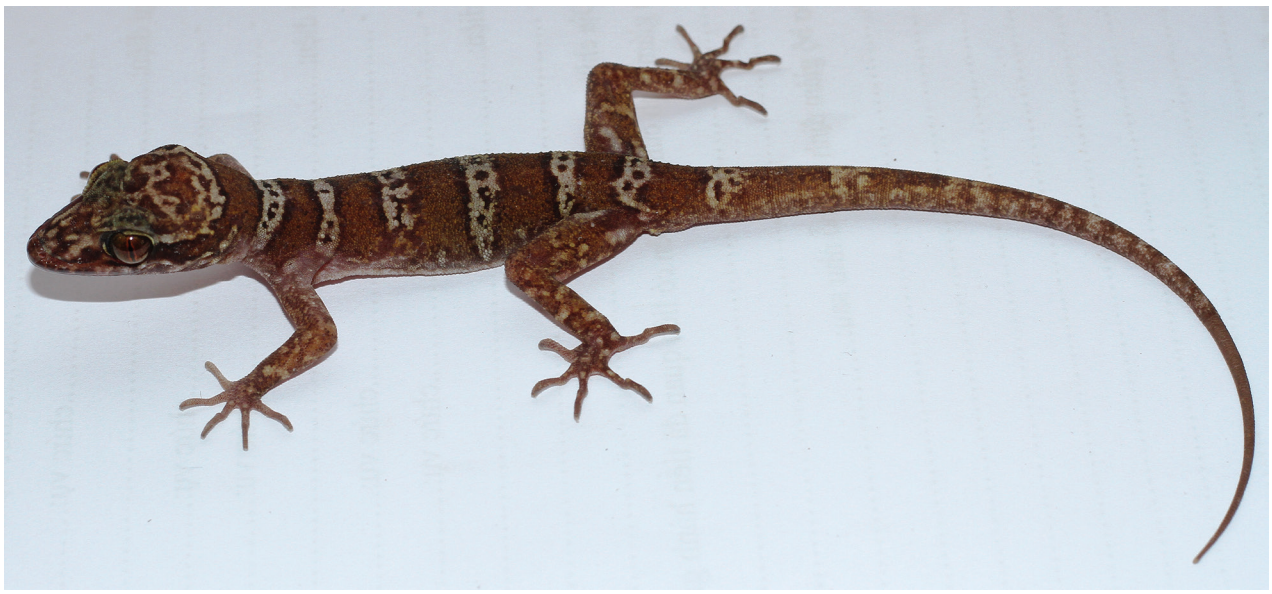

Figure 4. The male holotype of *Cyrtodactylus arnei* sp. nov. (IEBR R.6365, SVL 74.8 mm, TL 107.2 mm) in life. Photograph CTP.

Figure 5. The female paratype of *Cyrtodactylus arnei* sp. nov. (IEBR R.6371, SVL 71.3 mm, TL 103.5 mm) in life. Photos: CTP.

Tail very long (TaL 107.2 mm, TaL/SVL 1.43); subcaudals distinctly transversely enlarged, flat, smooth.

Coloration in life: ground color chocolate brown; dorsal surface of head pale brown with dark reticulated markings, distinct from the lower side by a cream line with dark edge extending from posterior of eye crossing the upper of ear to neck, another line extending from the posterior of labial crossing the ear but interrupted in the neck; eyelids yellowish cream; dorsal pattern consisting of six narrow light bands with dark edges (anterior edges darker than posterior ones) and a transversal row of dark spots in the middle: one on the tail, four between limb insertions and one anterior to the front limbs; original tail chocolate brown, scattered with small darker and cream spots, the same color with front and hind-limbs; ventral side of body cream.

Coloration in preservative: In 70% alcohol, color of this species is slightly faded. The brown turns to grey. Main morphological characters are still clearly visible.

Sexual dimorphism and variation. The females differ from the males in the absence of precloacal pores and hemipenial swellings at the tail base. The number of narrow light band on the tail varies from one to two in each individual. For other morphological characteristics see Table 2.

Distribution. *Cyrtodactylus arnei* sp. nov. is currently known only from the type locality in Hon Tre Island, Khanh Hoa Province (Fig. 1).

Etymology. The new species is named after Dr. Arne Schulze, Executive Director of the Zoological Society for Conservation of Species and Populations (ZGAP) to honor his great commitment and support for herpetological research and conservation in Vietnam, in particular within the scope of the Zoo Species of the Year – The Gecko Conservation Campaign 2024.

Ecological notes. The type series was found from 19:00 to 22:00 hrs on rock boulders and around a small cave along a rocky stream, ~1–2 m above the ground. This is very similar to another granite boulder species in the *C. irregularis* group, *C. raglai* (Nguyen et al. 2021a) to which it is superficially very similar. The surrounding habitat is secondary forest composed of medium and

Table 2. Measurements (in mm) and morphological characters of the type series of *Cyrtodactylus arnei* sp. nov. (* = regenerated or broken tail); bilateral meristic characters are given as (left/right).

Characters	IEBR R.6365	IEBR R.6366	IEBR R.6367	IEBR R.6368	IEBR R.6369	IEBR R.6370	IEBR R.6371	IEBR R.6372	IEBR R.6373	Min–max
	Holotype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	Paratype	
Sex	M	M	M	M	M	M	F	F	F	
SVL	74.8	70.9	73.0	78.0	76.8	75.7	71.3	73.8	71.0	70.9–78.0
TaL	107.2	93.3	104.4	79.8*	100.6	106.8	103.5	106.9	?	93.3–107.2
HL	21.9	20.8	21. 1	22.7	21.7	21.4	21.1	21.0	21.2	20.8–22.7
HW	13.8	13.4	13.8	14.0	13.9	14.0	12.9	12.8	13.1	13.4–14.0
HH	7.9	8.0	8.2	8.0	8.2	8.2	8.0	7.9	7.3	7.3–8.2
OD	4.9	4.9	4.5	5.1	4.7	5.0	5.2	4.7	4.8	4.5–5.2
SE	9.9	9.3	9.3	10.1	9.6	9.2	9.1	9.1	9.6	9.1–10.1
EE	6.1	5.6	5.7	5.7	6.3	5.7	5.4	5.7	5.2	5.2–6.3
NE	7.8	7.0	7.0	7.7	7.2	6.5	6.7	7.2	7.3	6.5–7.8
ED	2.0	1.8	1.8	2.4	2.1	2.1	1.7	1.8	1.7	1.7–2.4
ForeaL	12.4	11.0	12.4	11.9	12.7	12.1	11.7	11.9	11.6	11.0–12.7
CrusL	15.1	14.2	14.5	14.8	15.2	15.0	14.2	14.4	14.8	14.2–15.2
AG	27.8	26.5	26.7	31.3	30.9	29.9	28.9	25.8	26.6	26.5–31.3
BW	13.5	11.3	12.2	12.4	12.0	12.9	12.6	11.8	12.0	11.3–13.5
IND	2.3	2.2	2.3	2.4	2.7	2.2	2.1	2.1	2.1	2.1–2.7
IOD	7.1	5.6	7.2	7.0	6.9	6.4	6.2	5.8	6.2	5.6–7.2
SL	12–11	10–11	11–11	12–11	12–12	11–11	12–11	11–11	11–12	11–12
IL	10–10	10–10	9–9	10–9	10–9	10–10	9–10	10–10	10–9	9–10
N	4–4	4–4	4–4	4–4	3–3	4–4	4–4	4–4	4–4	3–4
IN	1	1	1	1	1	1	1	1	1	1
PM	2	2	2	2	2	2	2	2	2	2
GST	9–9–9	8–9–9	8–9–9	8–9–9	9–9–8	8–9–9	9–9–9	9–9–8	8–9–8	8–9
V	36	35	34	34	34	35	34	35	35	34–35
FP	0	0	0	0	0	0	0	0	0	0
PP	7	6	7	7	5	7	0	0	0	5–7 in males 0 in females
PAT	3–3	2–1	3–2	3–2	3–2	2–3	2–2	1–2	0	0–3
TubR	16	17	16	15	15	16	17	16	16	15–17
EFS	15–16	14	12–13	13–13	14–14	13–15	12–13	12–14	13–13	12–15
NSFIV	17	18	18	17	17	19	17	18	18	17–19
NSTIV	20	21	21	20	19	19	20	21	20	19–21

small hardwoods mixed with shrubs (Fig. 6). Other reptile species found at the site were *Calotes versicolor* (Daudin), *Dixonius vietnamensis* Das, *Gekko* sp., and *Eutropis multifasciatus* (Kuhl).

Comparisons. We compared the new species with its 30 congeners from the *Cyrtodactylus irregularis* group based on examination of specimens and data obtained from the literature (Smith 1921; David et al. 2004; Heidrich et al. 2007; Orlov et al. 2007; Nazarov et al. 2008, 2012; Ngo and Bauer 2008; Rösler et al. 2008; Geissler et al. 2009; Ngo and Chan 2010; Ngo 2013; Nguyen et al. 2013, 2021a; Ziegler et al. 2013; Schneider et al. 2014; Luu et al. 2017; Pauwels et al. 2018; Neang et al. 2020; Ngo et al. 2020; Ostrowski et al. 2020, 2021; Do et al. 2021, 2023; Ngo et al. 2023, 2024).(See Table 3).

Figure 6. A. Macrohabitat; **B.** Microhabitat of *Cyrtodactylus arnei* sp. nov. in Hon Tre Island, Khanh Hoa Province, Vietnam. Photo: CTP.

Table 3. Comparisons of the new species with its 30 congeners from the *Cyrtodactylus irregularis* group (measurements in mm, * = regenerated or broken tail, Max = maximum, other abbreviations defined in the text).

No.	*Taxa*	SVL	TaL	V	EFS	FP	PP(M)	PP (F)	LD4	LT4	Color pattern of dorsum	Enlarged subcaudals
1	*Cyrtodactylus arnei* sp. nov.	70.9–78.0	93.3–107.2	34–35	12–15	absent	5–7	absent	17–19	19–21	banded	present
2	*C. arndti*	73.4–80.9	max. 91.5	26–38	5–11	0–2	6	6	15–20	17–22	banded	present
3	*C. badenensis*	59.3–74.1	58.6–82.4	25–28	absent	absent	absent	absent	?	18–22	banded	present
4	*C. binhdinhensis*	58.5–80.4	max. 84.7	39–42	5–6	10 in males	6–7	5–6	17–18	18–21	banded	present
5	*C. bidoupimontis*	74.0–86.3	75.0–86	38–43	8–10	absent	4–6	absent	15–20	18–23	banded	absent
6	*C. buchardi*	60.0–65.0	46.0–54.0	30	absent	absent	9	?	14	12	blotched	absent
7	*C. bugiamapensis*	58.6–76.8	65.3–83.0	36–46	6–10	absent	7–11	0–7	15–17	17–20	blotched	absent
8	*C. caovansungi*	90.4–94	120	38–44	8	6	9	absent	22	23–25	banded	present
9	*C. cattienensis*	43.5–69	51–64.7	28–42	3–8	absent	6–8	absent	12–16	14–19	banded	absent
10	*C. chungi*	66.6–68.5	62.8*–82.2	30–31	4–6	absent	7	6	15–18	17–20	banded	absent
11	*C. chumuensis*	67.5	51.4*	43–45	4–5	0–2	6–7	?	16–19	17–21	banded	absent
12	*C. cryptus*	62.5–90.8	63.5–88.4	47–50	absent	absent	9–11	absent	18–19	20–23	banded	absent
13	*C. cucdongensis*	55.8–65.9	max. 81.3	41–44	5–9	absent	5–6	4–6	13–18	15–20	banded	absent
14	*C. culaochamensis*	69.8–79.8	89.7–91.2	45–50	absent	absent	7–8	absent	18–19	20–23	banded	absent
15	*C. dati*	max 70.1	max 57.3	42–48	4–7	6–7	5–6	?	?	18–19	blotched	absent
16	*C. gialaiensis*	50.1–62.8	?	38–45	present	absent	9–10	0–8	14–15	15–17	Banded	absent
17	*C. huynhi*	67.2–79.8	61.5–78.6	43–46	3–5	3–8	7–9	0–8 pitted	14–17	17–21	banded	absent
18	*C. irregularis*	72–86	66.0–74	38–45	7–8	absent	5–7	0–6	15–16	18–19	blotched	absent
19	*C. kingsadai*	83–94	max 117	39–46	9–12	3–7	7–9	4–8	19–21	21–25	banded	present
20	*C. orlovi*	61–77.7	max 71.2	36–39	3–8	absent	5–6	absent	15–17	16–19	banded	absent
21	*C. phnomchiensis*	76.1–80.7	56.9–79.1	45–54	0–8	absent	4–5	1–7 pitted	18–20	20–23	banded	Absent
22	*C. phumyensis*	63.6–66.8	?	33–41	5–7	absent	5–7	6 pitted	18–19	18–21	banded	absent
23	*C. phuocbinhensis*	46–60.4	76.1	43–47	5	absent	7	absent	16–21	17–19	striped/ blotched	absent
24	*C. pseudoquadrivirgatus*	48.6–83.3	55.7–82.3	41–57	absent	absent	5–9	5–10	15–21	16–25	Varied	absent
25	*C. raglai*	87.5–111.7	113.4–119	36–39	9–10	0	5	0	?	21–22	banded	present
26	*C. sangi*	49.9–56.3	47.9*	37	4	Absent	7	4 (Pitted)	?	?	banded	absent
27	*C. takouensis*	74.7–81.1	77.7–91	39–40	3–5	0–2	3–4	absent	16–17	18–20	banded	present
28	*C. tayhoaensis*	82.9–94.2	max 104.3	37–41	10–11	3–7 males	4–5	absent	20–22	22–24	banded	present
29	*C. taynguyenensis*	60.0–85.0	66.0–94.0	42–49	absent	absent	6	absent	13–18	17–21	blotched	absent
30	*C. yangbayensis*	78.5–92.3	91.3–109.1	39–46	5–16	0–2	6–8	absent	16–19	15–17	banded	Present
31	*C. ziegleri*	84.6–93.0	95.0–107.0	33–39	8–10	0–6	5–8	0–8	16–19	18–21	banded	Absent

Among the 30 species of the *Cyrtodactylus irregularis* group, *Cyrtodactylus arnei* sp. nov. differs:

- from *C. arndti* Ngo, Horman, Le, Pham, Phung, Do, Ostrowski, Nguyen & Zieger by having more enlarged femoral scales (12–15 vs 5–11 in *C. arndti*), the absence of precloacal pores in females (vs 6 in *C. arndti*), and the difference of dorsal color pattern (four narrow light bands with dark edges and a transversal row of dark spots in the middle vs 6 or 7 irregularly shaped bands in *C. arndti*);
- from *C. badenensis* Nguyen, Orlov & Darevsky by having more ventral scale rows (34 or 35 vs 25–28 in *C. badenensis*), the presence of enlarged femoral scales (12–15 vs absent in *C. badenensis*), and the presence of precloacal pores in males (5–7 vs absent in *C. badenensis*);
- from *C. binhdinhensis* Ngo, Do, Do, Pham, Bui, Ho, Nguyen, Ziegler & Le by having fewer ventral scale rows (34 or 35 vs 39–42 in *C. binhdinhensis*),

more enlarged femoral scales (12–15 vs 5 or 6 in *C. binhdinhensis*), the absence of femoral pores in males (vs 10 in *C. binhdinhensis*), and the absence of precloacal pores in females (vs 5 or 6 in *C. binhdinhensis*);

- from *C. bidoupimontis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler by having a longer tail (TL 93.3–107.2 mm, mean ratio TL/SVL 1.40 vs 75–86 mm, ratio TL/SVL 1.05 in *C. bidoupimontis*), fewer ventral scale rows (34 or 35 vs 38–43 in *C. bidoupimontis*), fewer dorsal tubercle rows (15–17 vs 18–24 in *C. bidoupimontis*), more enlarged femoral scales (12–15 vs 8–10 in *C. bidoupimontis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. bidoupimontis*);
- from *C. buchardi* David, Teynié & Ohler by having a larger size (SVL 70.9–78.0 mm vs 60.0–65.0 mm in *C. buchardi*), more ventral scale rows (34 or 35 vs 30 in *C. buchardi*), the presence of enlarged femoral scales (12–15 vs absent in *C. buchardi*), precloacal pores in males (5–7 vs 9 in *C. buchardi*), more lamellae under finger IV (17–19 vs 14 in *C. buchardi*), more lamellae under toe IV (19–21 vs 12 in *C. buchardi*), and the the difference of dorsal color pattern (banded vs blotched in *C. buchardi*);
- from *C. bugiamapensis* Nazarov, Poyarkov, Orlov, Phung, Nguyen, Hoang & Ziegler by having a longer tail (TL 93.3–107.2 mm, mean ratio TL/SVL 1.40 vs 65.3–83.0 mm, mean ratio TL/SVL 1.08 in *C. bugiamapensis*), fewer ventral scale rows (34 or 35 vs 36–46 in *C. bugiamapensis*), fewer dorsal tubercle rows (15–17 vs 20–24 in *C. bugiamapensis*), more enlarged femoral scales (12–15 vs 6–10 in *C. bugiamapensis*), the difference of dorsal color pattern (banded vs blotched in *C. bugiamapensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. bugiamapensis*);
- from *C. caovansungi* Orlov, Nguyen, Roman, Natalia & Nguyen by having a smaller size (70.9–78.0 mm vs 90.4–94 mm in *C. caovansungi*), fewer ventral scale rows (34 or 35 vs 38–44 in *C. caovansungi*), more enlarged femoral scales (12–15 vs 8 in *C. caovansungi*), the absence of femoral pores in males (vs 6 in *C. caovansungi*), fewer precloacal pores in males (5–7 vs 9 in *C. caovansungi*), and fewer lamellae under finger IV (17–19 vs 22 in *C. caovansungi*), under toe IV (19–21 vs 23–25 in *C. caovansungi*);
- from *C. cattienensis* Geissler, Nazarov, Orlov, Böhme, Phung, Nguyen & Ziegler by having a larger size (SVL 70.9–78.0 mm vs 43.5–69.0 mm in *C. cattienensis*), a longer tail (TL 93.3–107.2 mm, mean ratio TL/SVL 1.40 vs 51–64.7 mm, mean ratio TL/SVL 1.07 in *C. cattienensis*), more enlarged femoral scales (12–15 vs 3–8 in *C. cattienensis*), more lamellae under finger IV (17–19 vs 12–16 in *C. cattienensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. cattienensis*);
- from *C. chungi* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler by having having a larger size (SVL 70.9–78.0 mm vs 66.6–68.5 mm in *C. chungi*), more ventral scale rows (34 or 35 vs 30 or 31 in *C. chungi*), more enlarged femoral scales (12–15 vs 4–6 in *C. chungi*), the absence of precloacal pores in females (vs 6 in *C. chungi*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. chungi*);
- from *C. chumuensis* Ngo, Horman, Le, Pham, Phung, Do, Ostrowski, Nguyen & Zieger by having a larger size (SVL 70.9–78.0 mm vs 67.5 mm in *C. chumuensis*), fewer ventral scale rows (34 or 35 vs 43–45 in *C. chumuensis*), more

enlarged femoral scales (12–15 vs 4 or 5 in *C. chumuensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. chumuensis*);

- from *C. cryptus* Heidrich, Rösler, Vu, Böhme & Ziegler by having a longer tail (TL 93.3–107.2 mm, mean ratio TL/SVL 1.40 vs 63.5–88.4 mm, mean ratio TL/SVL 1.02 in *C. cryptus*), fewer ventral scale rows (34 or 35 vs 47–50 in *C. cryptus*), fewer dorsal tubercle rows (15–17 vs 19 or 20 in *C. cryptus*), the presence of enlarged femoral scales (12–15 vs absent in *C. cryptus*), fewer precloacal pores in males (5–7 vs 9–11 in *C. cryptus*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. cryptus*);
- from *C. cucdongensis* Schneider, Phung, Le, Nguyen & Ziegler by having a larger size (SVL 70.9–78.0 mm vs 55.8–65.9 mm in *C. cucdongensis*), fewer ventral scale rows (34 or 35 vs 41–44 in *C. cucdongensis*), more enlarged femoral scales (12–15 vs 5–9 in *C. cucdongensis*), the absence of precloacal pores in females (vs 4–6 in *C. cucdongensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. cucdongensis*);
- from *C. culaochamensis* Ngo, Grismer, Pham & Wood by having a longer tail (TL 93.3–107.2 mm, mean ratio TL/SVL 1.40 vs 89.7–91.2 mm, mean ratio TL/SVL 1.24 in *C. culaochamensis*), fewer ventral scale rows (34 or 35 vs 45–50 in *C. culaochamensis*), fewer dorsal tubercle rows (15–17 vs 20–22 in *C. culaochamensis*), the presence of enlarged femoral scales (12–15 vs absent in *C. culaochamensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. culaochamensis*);
- from *C. dati* Ngo by having a larger size (SVL 70.9–78.0 mm vs max 70.1 in *C. dati*), a longer tail (TL 93.3–107.2 mm, mean ratio TL/SVL 1.40 vs Max 57.3 mm, mean ratio TL/SVL: 1.06), fewer ventral scale rows (34 or 35 vs 42–48 in *C. dati*), fewer dorsal tubercle rows (15–17 vs 20–22 in *C. dati*), more enlarged femoral scales (12–15 vs 4–7 in *C. dati*), the absence of femoral pores in males (vs 6–7 in *C. dati*), the difference in color pattern of dorsum (banded vs small blotched in *C. dati*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. dati*);
- from *C. gialaiensis* Luu, Tran, Nguyen, Le & Ziegler by having a larger size (SVL 70.9–78.0 mm vs 50.1–62.8 mm in *C. gialaiensis*), fewer ventral scale rows (34 or 35 vs 38–45 in *C. gialaiensis*), fewer precloacal pores in males (5–7 vs 9 or 10 in *C. gialaiensis*), the absence of precloacal pores in adult females (vs 8 pitted scales in *C. gialaiensis*), more lamellae under finger IV (17–19 vs 14 or 15 in *C. gialaiensis*) and under toe IV (19–21 vs 15–17 in *C. gialaiensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. gialaiensis*);
- from *C. huynhi* Ngo & Bauer by having fewer ventral scale rows (34 or 35 vs 43–46 in *C. huynhi*), more enlarged femoral scales (12–15 vs 3–5 in *C. huynhi*), the absence of femoral pores in males (vs 3–8 in *C. huynhi*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. huynhi*);
- from *C. irregularis* (Smith) by having fewer ventral scale rows (34 or 35 vs 38–45 in *C. irregularis*), more enlarged femoral scales (12–15 vs 7 or 8 in *C. irregularis*), the absence of precloacal pores in females (0 vs 0–6 in *C. irregularis*), the difference in color pattern of dorsum (banded vs blotched in *C. irregularis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. irregularis*);

- from *C. kingsadai* Ziegler, Phung, Le & Nguyen by having a smaller size (SVL 70.9–78.0 mm vs 83–94 mm in *C. kingsadai*), fewer ventral scale rows (34 or 35 vs 39–46 in *C. kingsadai*), the absence of femoral pores in males (vs 3–7 in *C. kingsadai*), and the absence of precloacal pores in females (vs 4–8 in *C. kingsadai*);
- from *C. orlovi* Do, Phung, Ngo, Le, Ziegler, Pham & Nguyen by having fewer ventral scale rows (34 or 35 vs 36–39 in *C. orlovi*), more enlarged femoral scales (12–15 vs 3–8 in *C. orlovi*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. orlovi*);
- from *C. phnomchiensis* Neang, Henson & Stuart by having a longer tail (TL 93.3–107.2 mm, mean ratio TL/SVL 1.40 vs 56.9–79.1 mm, mean ratio TL/SVL 0.88 in *C. phnomchiensis*), fewer ventral scale rows (34 or 35 vs 45–54 in *C. phnomchiensis*), more enlarged femoral scales (12–15 vs 0–8 in *C. phnomchiensis*), the absence of precloacal pores in females (vs 1–7 pitted scales in *C. phnomchiensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. phnomchiensis*);
- from *C. phumyensis* Ostrowski, Le, Ngo, Pham, Phung, Nguyen & Ziegler by having a larger size (SVL 70.9–78.0 mm vs 63.6–66.8 mm in *C. phumyensis*), fewer dorsal tubercle rows (15–17 vs 18–19 in *C. phumyensis*), more enlarged femoral scales (12–15 vs 5–7 in *C. phumyensis*), the absence of precloacal pores in females (vs 6 pitted scales in *C. phumyensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. phumyensis*);
- from *C. phuocbinhensis* Nguyen, Le, Tran, Orlov, Lathrop, Macculloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang by having a larger size (SVL 70.9–78.0 mm vs 46.0–60.4 mm in *C. phuocbinhensis*), fewer ventral scale rows (34 or 35 vs 43–47 in *C. phuocbinhensis*), more enlarged femoral scales (12–15 vs 5 in *C. phuocbinhensis*), the difference of dorsal color pattern (banded vs blotched/striped in *C. phuocbinhensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. phuocbinhensis*);
- from *C. pseudoquadrivirgatus* Rösler, Vu, Nguyen, Ngo & Ziegler by having fewer ventral scale rows (34 or 35 vs 41–57 in *C. pseudoquadrivirgatus*), the presence of enlarged femoral scales (12–15 vs absent in *C. pseudoquadrivirgatus*), the absence of precloacal pores in females (vs 5–10 in *C. pseudoquadrivirgatus*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. pseudoquadrivirgatus*);
- from *C. raglai* Nguyen, Duong, Grismer & Poyarkov by having a smaller size (70.9–78.0 mm vs 87.5–111.7 mm in *C. raglai*), fewer ventral scale rows (34 or 35 vs 36–39 in *C. raglai*), and more enlarged femoral scales (12–15 vs 9–10 in *C. raglai*);
- from *C. sangi* Pauwels, Nazarov, Bobrov & Poyarkov by having a larger size (SVL 70.9–78.0 mm vs 49.9–56.3 mm in *C. sangi*), fewer ventral scale rows (34 or 35 vs 37 in *C. sangi*), more enlarged femoral scales (12–15 vs 4 in *C. sangi*), the absence of precloacal pores in females (vs 4 pitted scales in *C. sangi*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. sangi*);
- from *C. takouensis* Ngo & Bauer by having fewer ventral scale rows (34 or 35 vs 39–40 in *C. takouensis*), more dorsal tubercle rows (15–17 vs 9–10 in *C. takouensis*), more enlarged femoral scales (12–15 vs 3–5 in *C. takouensis*), and more precloacal pores in males (5–7 vs 3–4 in *C. takouensis*);

- from *C. tayhoaensis* by having a smaller size (SVL 70.9–78.0 mm vs 82.9–94.2 mm in *C. tayhoaensis*), fewer ventral scale rows (34 or 35 vs 37–41 in *C. tayhoaensis*), fewer dorsal tubercle rows (15–17 vs 20–22 in *C. tayhoaensis*), the absence of femoral pores in males (vs 3–7 in *C. tayhoaensis*), and fewer lamellae under finger IV (17–19 vs 20–22 in *C. tayhoaensis*), and under toe IV (19–21 vs 22–24 in *C. tayhoaensis*);
- from *C. taynguyenensis* Nguyen, Le, Tran, Orlov, Lathrop, Macculloch, Le, Jin, Nguyen, Nguyen, Hoang, Che, Murphy & Zhang by having fewer ventral scale rows (34 or 35 vs 42–49 in *C. taynguyenensis*), the presence of enlarged femoral scales (12–15 vs absent in *C. taynguyenensis*), the difference in color pattern of dorsum (banded vs blotched in *C. taynguyenensis*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. taynguyenensis*);
- from *C. yangbayensis* Ngo & Chan by having fewer ventral scale rows (34 or 35 vs 39–46 in *C. yangbayensis*), fewer dorsal tubercle rows (15–17 vs 20–23 in *C. yangbayensis*), the absence of femoral pores in males (vs 0–2 in *C. yangbayensis*), and more lamellae under under toe IV (19–21 vs 15–17 in *C. yangbayensis*);
- from *C. ziegleri* Nazarov, Orlov, Nguyen & Ho by having a smaller size (SVL 70.9–78.0 mm vs 84.6–93.0 mm in *C. ziegleri*), more enlarged femoral scales (12–15 vs 8–10 in *C. ziegleri*), the absence of femoral pores in males (vs 0–6 in *C. ziegleri*), the absence precloacal pores in females (vs 0–8 in *C. ziegleri*), and the presence of transversely enlarged subcaudal plates (vs absent in *C. ziegleri*).

Discussion

Cyrtodactylus arnei is the fifth species of *Cyrtodactylus* recorded in Khanh Hoa Province. However, *Cyrtodactylus arnei* differs significantly from the four previously known species, *C. bidoupimontis*, *C. cucdongensis*, *C. raglai*, and *C. yangbayensis* by a number of morphological characteristics. Phylogenetically, it is also not closely related to those species (Fig. 2). It bears an uncorrected pairwise sequence divergence of approximately 11.81%, 12.57%, 12.30% and 11.71–11.96% from *C. bidoupimontis*, *C. cucdongensis*, *C. raglai*, and *C. yangbayensis*, respectively (Suppl. material 1). Furthermore, *Cyrtodactylus arnei* is only known from Hon Tre Island in Khanh Hoa Province, where no representative of the genus *Cyrtodactylus* has been reported so far. The new species is the 31st species of the *C. irregularis* group and the 55th species of *Cyrtodactylus* known from Vietnam (Uetz et al. 2025).

Khanh Hoa Province is located in South–central of Vietnam. It has a long coastline and nearly 200 islands. Hon Tre Island is a small island in Nha Trang Bay, Khanh Hoa Province and it is different from another island also named "Hon Tre" located in the Gulf of Thailand in Vietnam. It is located ca 2.1 km from the coast and 5.67 km long from north to south and 12 km wide from west to east (Nguyen et al. 2022b). The island has recently become a popular tourist destination, and rapid tourism development will likely negatively impact the surrounding ecosystems. Therefore, there is an urgent need for further studies to provide a better understanding of the new species' population status, distribution range, and anthropogenic threats.

Acknowledgements

We are grateful to the directorate of Forest Protection Departments of Khanh Hoa Province for their support of our fieldwork and for issuing relevant permits. We thank S.T. Nguyen and T.D. Nguyen (IB, Hanoi) for their assistance in the field. We thank T.T. Nguyen, M.H. Le, A.T.N. Ho for their support in the laboratory. For the fruitful cooperation within joint research projects, we cordially thank A.H. Le (IB, Hanoi), as well as T. Pagel and C. Landsberg (Cologne Zoo). We would like to thank the editors and reviewers for their valuable comments on the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Funding

Doctoral research of H.T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD).

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Species of *Cyrtodactylus* used in the phylogenetic analysis including localities and GenBank accession numbers of the mitochondrial COI and ND2 fragment genes

Authors: Quyen Hanh Do, Hanh Thi Ngo, Truong Quang Nguyen, Minh Duc Le, Thomas Ziegler, Dang Trong Do, Cuong The Pham

Data type: docx

Link: https://doi.org/10.3897/zookeys.1253.149459.suppl1

Supplementary material 2

Bayesian phylogram based on the matrix of COI. Number above and below branches are ultrafast bootstrap values and Bayesian posterior probabilities (≥ 50%), respectively

Authors: Quyen Hanh Do, Hanh Thi Ngo, Truong Quang Nguyen, Minh Duc Le, Thomas Ziegler, Dang Trong Do, Cuong The Pham

Data type: pdf

Link: https://doi.org/10.3897/zookeys.1253.149459.suppl2

Supplementary material 3

Bayesian phylogram based on the matrix of ND2. Number above and below branches are ultrafast bootstrap values and Bayesian posterior probabilities (≥ 50%), respectively

Authors: Quyen Hanh Do, Hanh Thi Ngo, Truong Quang Nguyen, Minh Duc Le, Thomas Ziegler, Dang Trong Do, Cuong The Pham

Data type: pdf

Link: https://doi.org/10.3897/zookeys.1253.149459.suppl3

Supplementary material 4

Pair–wise genetic distance between samples used in this study based on COI and ND2

Authors: Quyen Hanh Do, Hanh Thi Ngo, Truong Quang Nguyen, Minh Duc Le, Thomas Ziegler, Dang Trong Do, Cuong The Pham

Data type: xlsx

Link: https://doi.org/10.3897/zookeys.1253.149459.suppl4

Chapter 7. How many more species are out there? Current taxonomy substantially underestimates the diversity of bent-toed geckos (Gekkonidae, Cyrtodactylus) in Laos and Vietnam

ZooKeys 1097: 135–152 (2022)
doi: 10.3897/zookeys.1097.78127
https://zookeys.pensoft.net

RESEARCH ARTICLE

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How many more species are out there? Current taxonomy substantially underestimates the diversity of bent-toed geckos (Gekkonidae, *Cyrtodactylus*) in Laos and Vietnam

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Academic editor: Johannes Penner | Received 17 November 2021 | Accepted 8 March 2022 | Published 26 April 2022

http://zoobank.org/5666F9CE-D518-4B90-A1D3-EF053D4B0266

Citation: Ngo HT, Do QH, Pham CT, Luu VQ, Grismer L, Ziegler T, Nguyen VTH, Nguyen TQ, Le MD (2022) How many more species are out there? Current taxonomy substantially underestimates the diversity of bent-toed geckos (Gekkonidae, *Cyrtodactylus*) in Laos and Vietnam. ZooKeys 1097: 135–152. https://doi.org/10.3897/zookeys.1097.78127

Abstract

Cyrtodactylus is the most diverse genus of the family Gekkonidae and the world's third largest vertebrate genus. The number of species has increased more than fourfold over the last two decades. Indochina, especially Vietnam and Laos, has witnessed a surge in new species discoveries over the last three decades. The species number reported from Laos and Vietnam has remarkably increased from five in 1997 to 71 species in 2021. However, within the genus, several taxonomic issues have not yet been fully resolved. Based on recently collected samples from Laos and Vietnam, we conducted a comprehensive molecular review of *Cyrtodactylus*

occurring in Laos and Vietnam. Our molecular analysis with support from morphological comparisons showed that *C. thuongae* is a junior synonym of *C. dati* and *C. rufford* is a junior synonym of *C. lomyenensis*. In total, 68 described species distributed in Laos and Vietnam are undisputed with strong support from both molecular and morphological evidence. On the other hand, the molecular analyses revealed that there are at least seven undescribed species in Vietnam and Laos, one in the *C. angularis* group, one in the *C. chauquangensis*, and five in the *C. irregularis* group. This number will likely increase significantly, as previous work suggested that the *C. angularis* and *C. irregularis* groups harbor three and six unnamed lineages, respectively. Based on survey gaps identified in our study, it is clear that additional new species will be discovered in poorly studied regions of central Vietnam and northern and southern Laos. As many species in the genus are facing high extinction risks, several undescribed populations might already be severely threatened by human activities in both countries. Therefore, urgent taxonomic research is needed before conservation assessments of newly discovered taxa can be undertaken to protect them from anthropogenic threats.

Keywords
COI, conservation, Gekkonidae, integrative taxonomy, Southeast Asia, synonymy

Introduction

The bent-toed geckos of the genus *Cyrtodactylus* comprise the most diverse genus of the Gekkonidae with at least 330 nominal species (Uetz et al. 2021). They have a broad distribution extending from tropical South Asia, Indochina, the Philippines, and the Indo-Australian Archipelago to the Solomon Islands (Grismer et al. 2021a, b). Species in the genus can adapt to different habitat types, including limestone karst, granitic montane forests, and lowland evergreen forest. Interestingly, several species have been observed in sympatry, for example, in Phong Nha – Ke Bang National Park in Vietnam (*C. cryptus* Heidrich, Rösler, Vu, Böhme & Ziegler, 2007; *C. phongnhakebangensis* Ziegler, Rösler, Herrmann & Vu, 2003; *C. roesleri* Ziegler, Nazarov, Orlov, Nguyen, Vu, Dang, Dinh and Smith, 2010) and Ba Den Mountain in Vietnam (*C. badenensis* Nguyen, Orlov & Darevsky, 2006; *C. nigriocularis* Nguyen, Orlov & Darevsky; *C. thuongae* Phung, van Schingen, Ziegler & Nguyen, 2006) (Ziegler et al. 2003, 2010, 2013; Nguyen et al. 2006; Heidrich et al. 2007; Phung et al. 2014). Additionally, many new species of *Cyrtodactylus* have been described over the last ten years. *Cyrtodactylus* is therefore recognized as an ideal group for taxonomic, biogeographic, and ecological research as well as a model group for lizard evolution (Grismer et al. 2021a, b; 2022).

Indochina, including Cambodia, Laos, and Vietnam, has long been recognized as a region of global importance in terms of biodiversity richness (Myers et al. 2000). Laos and Vietnam have also been a hotspot of new *Cyrtodactylus* discoveries. From 1997 to present, 66 new species of *Cyrtodactylus* have been described, making it a total of 71 recognized species (Uetz et al. 2021). Remarkably, many cryptic species have recently been described based on either comparison with newly acquired collections or implementation of an integrative approach, i.e., combining evidence from morphological and molecular data. For example, *C. phongnhakebangensis* was split into two species, namely *C. phongnhakebangensis* and *C. roesleri* in 2010 (Ziegler et al. 2010), which were found to occupy different ecological niches (Loos et al. 2012), and *C. irregularis* Smith, 1921 was broken up into

multiple species (Nazarov et al. 2008; Geissler et al. 2009; Grismer et al. 2021a, b). On the other hand, several species have been synonymized. *C. paradoxus* Darevsky & Szczerbak, 1997 was shown to be a junior synonym of *C. condorensis* Smith, 1921 and *C. thochuensis* Ngo & Grismer, 2012 was recommended as a junior synonym of *C. leegrismeri* Chan & Norhayati, 2010 (Orlov et al. 2007; Grismer et al. 2015). In addition, *C. thuongae* was synonymized with *C. dati* Ngo, 2013 based on molecular evidence (Ngo et al. 2017).

In Laos, most new species described in recent years belong to the *Cyrtodactylus angularis* group, which contains at least 16 species recorded in the country. This karst-adapted clade occurs in central Laos and north-central Vietnam (Grismer et al. 2021a). Another five recently discovered species are members of the *C. chauquangensis* group, which occupies northern Laos and northwestern and north-central Vietnam. Two remaining groups include *C. brevipalmatus* and *C. irregularis*. While it is still unclear what species of the former group are present in Laos, the latter likely consists of three species in Laos, *C. buchardi* David, Teynié, Ohler, 2004; *C. cryptus*, and *C. pseudoquadrivirgatus* Rösler, Nguyen, Vu, Ngo & Ziegler, 2008 (David et al. 2004; Rösler et al. 2008; Pauwels et al. 2018; Schneider et al. 2020; Grismer et al. 2021a)

As new species of the genus have been consistently described at a rapid rate, there is a need to review the taxonomic progress and identify areas where future research should focus. Although there have been some attempts to assess the diversity of the group in Vietnam and Laos using molecular data (Nguyen et al. 2015, 2017; Brennan et al. 2017; Ngo et al. 2017), the studies either did not include a thorough taxonomic sampling of species in both countries (Brennan et al. 2017; Grismer et al. 2021a, b) or only focused on Vietnamese or Lao taxa (Nguyen et al. 2017; Ngo et al. 2017; Schneider et al. 2020). To better understand the outstanding taxonomic issues and accurately evaluate the taxonomic diversity of the group, we analyzed 68 of 71 described species and several undescribed populations from across the range of this group in Laos and Vietnam. To determine the distribution ranges of the taxa, we incorporated as many samples as possible from different localities and samples from newly discovered and undescribed populations from previous studies. In some cases where the molecular evidence was equivocal, morphological comparisons were employed to address the pending taxonomic issues.

Materials and methods

Sampling

Field work was conducted between 2009 and 2018 in Laos and Vietnam. Specimens were euthanized with ethyl acetate, fixed in approximately 85% ethanol, then transferred to 70% ethanol for permanent storage. Specimens were subsequently deposited in the collections of the Institute of Ecology and Biological Resources (**IEBR**), Vietnam Academy of Science and Technology, Hanoi, Vietnam; the Vietnam National Museum of Nature (**VNMN**), Hanoi, Vietnam; the Vietnam National University of Forestry (**VNUF**), Hanoi, Vietnam; the National University of Laos (**NUOL**), Laos; and the Zoological Research Museum Alexander Koenig (**ZFMK**), Bonn, Germany.

Morphological analysis

Main morphological characters were rechecked: Measurements were taken with a digital caliper to the nearest 0.1 mm. Abbreviations are as follows: snout-vent length (**SVL**, from tip of snout to anterior margin of cloaca); tail length (**TaL**, from posterior margin of cloaca to tip of tail).

Scale counts were taken using stereo microscopes (Leica S6E, Keyence VHX-500F): ventral scales in longitudinal rows at midbody (**V**) counted transversely across the center of the abdomen from one ventrolateral fold to the other; dorsal tubercle rows (**DTR**) counted transversely across the center of the dorsum from one ventrolateral fold to the other; supralabials (**SL**) and infralabials (**IL**) counted from the first labial scale to the corner of mouth; enlarged femoral scales (**EFS**); femoral pores (**FP**); precloacal pores (**PP**) or the total number of femoral pores and precloacal pores (i.e. the contiguous rows of femoral and precloacal scales bearing pores combined as a single meristic character referred to as the femoroprecloacal pores); number of subdigital lamellae on the fourth finger (**LD4**) and number of subdigital lamellae on the fourth toe (**LT4**) counted from the base of the first phalanx to the claw.

Molecular data and phylogenetic analysis.

Most described taxa of the genus *Cyrtodactylus* in Laos and Vietnam, except for *C. buchardi*, *C. raglai*, and *C. septimontium* were included in the study. In addition, samples of the species from different localities were sequenced to determine their distribution range. In total, 84 new samples from 26 provinces were incorporated (Suppl. material 1: Table S1). The tissue samples of muscle, liver or tail tissue was preserved separately in 70% ethanol. The mitochondrial DNA cytochrome c oxidase subunit I (COI) gene was selected as the markers have been widely used in previous studies and for some geographic populations, only COI data were available (Nguyen et al. 2015, 2017; Luu et al. 2016a; Brennan et al. 2017; Ngo et al. 2017). In several cases, where comparative data for COI were not available, the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) was generated for specimens under consideration. In addition, we obtained 90 sequences of the mitochondrial COI for the ingroup taxa and one outgroup species, *Hemidactylus frenatus*, from GenBank (Wood et al. 2012).

Total genomic DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen, Germany), following protocols by the manufacturer's instructions. PCR was performed using HotStar Taq Mastermix (Qiagen, Germany) to amplify approximately 657 bp fragment of the mitochondrial gene COI and approximately 1200 bp fragment of the mitochondrial gene ND2. We used two primer pairs for PCR with VF1d (5'–TTCTCAACCAACCACAARGAYATYGG-3'), VR1d (5'– TAGACTTCTGGGTGGCCRAARAAYCA–3') (Ivanova et al. 2006) for generating the COI and MetF1 (5'– AAGCTTTCGGGCCCATACC–3'), COIR1 (5'– AGRGTGCCAATGTCTTTGTGRTT–3') (Macey et al. 1997 for the ND2. The PCR volume consisted of 21 µl (2 µl each primer, 5 µl water, 10 µl of Taq mastermix and 1 – 2 µl of DNA depending on the quality of DNA in the final extraction solution). The reactions were performed at 95 °C for 15 min, followed by 35 cycles of 30 s

at 95 °C, 45 s at 48 °C, and 60 s at 72 °C with a final elongation step of 6 min at 72 °C. A negative and positive control was used for every DNA extraction and PCR reactions. PCR products were visualized using electrophoresis through a 1% agarose gel, marker 1 kb, 1X TBE and stained with ethidium bromide and photographed under UV light. Successful amplifications were purified using GeneJet PCR Purification Kit (ThermoFisher Scientific, Lithuania). Cleaned PCR products was sent to 1st Base (Malaysia) for sequencing.

Newly generated sequences were checked by eye using Sequencher v5.4 (Gene Codes Corp, Ann Arbor, MI, USA), aligned by ClustalX v2.1 (Thompson et al. 1997) with default setting. The data were then analyzed using Bayesian inference (BI) as implemented in MrBayes v3.1.2 (Ronquist et al. 2012) and maximum likelihood (ML) analysis using IQ-TREE v.1.6.7.1 (Nguyen et al. 2015) with a single molecular model and 10,000 ultrafast bootstrap (UFBP) replications. For BI, the analysis was conducted with a random starting tree and run for 1×10^7 generations. Four Markov chains, one cold and three heated (utilizing default heating values), were sampled every 1000 generations. Log likelihood scores of sample points were plotted against generation time to detect stationarity of the Markov chains. The burn-in value was set to 59 in the BI analysis, as –lnL scores reached stationarity after 59,000 generations in both runs. The optimal model, GTR+I+G, for nucleotide evolution was set to BI and ML analysis as selected by jModeltest v2.1.4 (Darriba et al. 2012). Nodal support was evaluated using UFBP as estimated in IQ-TREE v1.6.7.1 and posterior probability (PP) in MrBayes v3.2. UFBP and PP ≥ 95% are regarded as strong support for a clade (Ronquist et al. 2012; Nguyen et al. 2015). Uncorrected pairwise distance (*p*) was calculated in PAUP*v4.0b10 (Swofford 2001).

Results

Phylogenetic relationships

We successfully sequenced a fragment of the COI gene for 90 samples and ND2 gene for four samples. The final concatenated matrix consisted of 216 terminals, including 90 from this study, 126 from previous studies, including one outgroup, *Hemidactylus frenatus*, following Wood et al. (2012). Both BI and ML analyses based on a total of 657 aligned characters with no gaps and using a single model of molecular evolution produced very similar topologies (Fig. 1). All species groups were generally recovered with strong support values from both analyses, except for the *C. irregularis* group, which was corroborated only by the BI analysis (Fig. 1). The results show that *Cyrtodactylus* species in Laos and Vietnam fall into six species groups, namely *C. angularis*, *C. brevipalmatus*, *C. chauquangensis*, *C. condorensis*, *C. intermedius*, and *C. irregularis*. While Vietnam harbors members of all groups, Lao species mostly belong to the two karst-dwelling *C. angularis* and *C. chauquangensis* groups (Grismer et al. 2021a, b). Two other groups contain one taxon each from Laos, including *C.* cf. *cryptus* of the *C. irregularis* group (samples of *C. buchardi* and *C. pseudoquadrivirgatus* from Laos not included in the analysis) and *C.* cf. *ngati* of the *C. brevipalmatus* group. Two remaining mostly insular groups, the *C. condorensis* and *C. intermedius* groups, do not have any species from Laos (Fig 1, Suppl. material 1: Table S1).

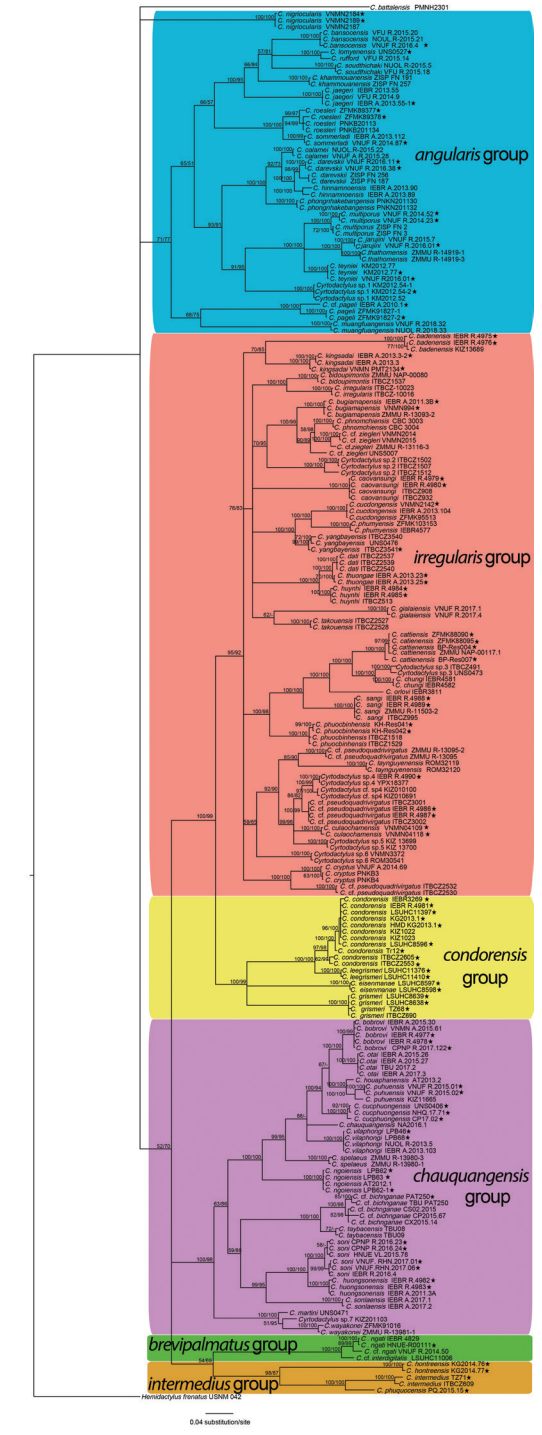

Figure 1. Bayesian cladogram based on 657 bp of the partial COI gene. Numbers above branches are Bayesian posterior probabilities and ultrafast bootstrap values of ML analysis, respectively. ★ = new sequences used in the phylogenetic analyses.

The main difference between this and previous studies is that the *Cyrtodactylus angularis* group was rendered paraphyletic. Nguyen et al. (2015) and Ngo et al. (2017) used the same COI region and confirmed that the monophyly of the group is significantly supported in the BI analysis, while its monophyletic relationship based on 1474 base pairs of the ND2 gene received perfect statistical values from both BI and ML phylogenetic estimates (Grismer et al. 2021a). All other species groups were recovered with strong support values from both analyses, except for the *C. irregularis* group, which was corroborated only by the BI analysis but received perfect statistical support in both analysis in Grismer et al. (2021a).

In the *Cyrtodactylus angularis* group, almost all species are well defined and supported by both analyses. According to our tree, the group contains 19 known species and one undescribed taxon (*Cyrtodactylus* sp. 1) in both countries. There are only three species that have notable genetic sub-structuring, i.e., *C. darevskii*, *C. multiporus*, and *C. pageli*, and samples from genetically distinct populations are labeled as cf. (Fig. 1). Another population from Khammouane Province, Laos with three representative samples, KM2012.52, KM2012.54–1, and KM2012.54–2, is clearly differentiated from other species and likely forms a new species. The former two samples were incorrectly assigned to *C. lomyenensis* in previous studies (Nazarov et al. 2014, Schneider et al. 2014). It is marked as *Cyrtodactylus* sp. 1 on the tree (Fig. 1). The highest pairwise genetic divergence between species of the *Cyrtodactylus angularis* group is 24.04% based on the fragment of COI gene (Suppl. material 2: Table S2). The lowest pairwise genetic divergence between species that are not morphologically well differentiated (SVL, LT4, LD4, Infralabials, EFS, color pattern of dorsum, and enlarged subcaudals specifications, see Tables 2, 4) is 2.44% (*C. rufford* and *C. lomyenensis*). The lowest divergence between species that are morphologically distinct is 4.57% (*C. jarujini* and *C. thathomensis*).

Members of the *Cyrtodactylus brevipalmatus* group, recently discovered in Vietnam (Le et al. 2021), are present in both countries. The genetic divergence between the Vietnamese and Lao populations of *C. ngati* is 2.13% and they are ~ 3.81–4.41% separated from *C.* cf. *interdigitalis* from Thailand. This group contains the lowest number of taxa (Fig. 1, Suppl. material 1: Table S1, Suppl. material 6: Table S6). The *C. chauquangensis* group is also distributed in both countries with 16 described species and one undescribed form, *Cyrtodactylus* sp. 7, from Vietnam. Samples of purported *C. bichnganae* are all labeled as cf. because genetic sequences of the true *C. bichnganae* have not been made available (see Pham et al. 2019). The second and third smallest groups, the *C. intermedius* and *C. condorensis* groups, have three and four species, respectively. All of the species occur in Vietnam and mostly inhabit offshore islands in the southern part of the country.

The largest group, the *Cyrtodactylus irregularis* group, consists of more than 30 species with at least five undescribed forms, *Cyrtodactylus* sp. 2 – *Cyrtodactylus* sp. 6. The highest and lowest pairwise genetic divergence that exists between species of the *C. irregularis* group are 21.41% and 0.74% (Suppl. material 3: Table S3). In addition, *C.* cf. *pseudoquadrivirgatus* is recovered in three distinct places of the tree, one close to *C. taynguyenensis*, one embedded within a clade comprising *Cyrtodactylus* sp. 4, *Cyrtodactylus* sp. 5, *C. culaochamensis*, and the other was recovered as a sister taxon to *C. cryptus*. Finally, *C. dati* and *C. thuongae* are not highly divergent in terms of pairwise genetic distance and only separated by 0.74% (Table 3, Suppl. material 3: Table S3).

The *Cyrtodactylus condorensis* group is composed of four well defined species with pairwise divergences of 5.48 – 18.05% (Suppl. material 4: Table S4). The *Cyrtodactylus chauquangensis* group is composed of 17 species which have remained stable in many analyses of phylogenetic relationships. The genetic divergences between the members of the group are ~ 3.81–19.54% (Suppl. material 5: Table S5).

Taxonomic issues

Our results based on a fragment of the mitochondrial gene COI show that the lineage containing *Cyrtodactylus thuongae* with the holotype and paratype from Tay Ninh Province and *C. dati* from the Lam Dong, Binh Phuoc populations is divided into two sub-lineages. However, the PP value is insignificant (Fig. 1). These populations are separated by no greater than 1% in sequence divergence using COI data (Table 3). In addition, genetic distances between the holotype of *C. dati* (UNS 0543) and the holotype and paratype of *C. thuongae* (IEBR A.2013.23, IEBR A.2013.25) are 2.21% and 1.85%, respectively, based on 951 bp of a fragment of the mitochondrial ND2 gene. At the time of the latter species' description, molecular data for both species were not available for comparison. Instead, *C. thuongae* was described on the basis of its highly variable morphology (Table 1). Morphological examination of specimens of *C. thuongae* and published data of *C. dati* show that morphologically they do not have a high level of distinction (size, number of ventral scales, infralabials, EFS, FP, LT4, enlarged subcaudals, color pattern of dorsum. The only differences between the two species are: DTR: 17 or 18 in *C. thuongae* vs. 20–22 in *C. dati*, SL: 7–9 in *C. thuongae* vs. 10–12 in *C. dati* and 0 or 1 pitted scales in males in *C. thuongae* vs. five or six in *C. dati*.

Much the same is true for *C. lomyenensis* from Khammouan (paratype – UNS0527) and *C. rufford* also from Khammouan (holotype – VFU R.2015.14). Genetic divergence between the two species is less than 2.44% based on a fragment of mitochondrial gene COI. According to our morphological examinations between specimens of

Table 1. Morphological characters of *Cyrtodactylus dati*, *C. huynhi*, *C. thuongae.*

	C. dati	***C. thuongae***	***C. huynhi* without UNS 0327**	***C. huynhi* UNS 0327 paratype, M**
Article	Ngo 2013	Phung et al. 2014	Ngo et al. 2008	Ngo and Bauer 2008
Locality	Binh Phuoc	Tay Ninh	Dong Nai	Dong Nai
SVL	max 70.1	57.3 – 77.6	67.2 – 79.8	54.8
TaL	max 57.3	max 78.1	61.5 – 78.6	29.1
V	42 – 48	29 – 44	43 – 46	44
DTR	20 – 22	17 – 18	16 – 18	18
SL	10 – 12	7 – 9	?	?
IL	8 – 10	7 – 10	?	?
EFS	4 – 7	2 – 5	3 – 5	/
FP	3 – 4 each	0 – 3 (pitted scales)	3 – 8	4+4
PP in males	5 – 6	0 – 1 (pitted scales)	7 – 9	9
PP in females	?	0	0 – 8 (pitted scales)	?
LD4	?	14 – 17	14 – 17	15
LT4	18 – 19	14 – 20	17 – 21	17
Color pattern of dorsum	blotched	blotched	banded	banded
Enlarged subcaudals	absent	absent	absent	absent

Table 2. Morphological characters of *Cyrtodactylus rufford* and *C. lomyenensis.*

	C. lomyenensis	***C. rufford***
Article	Ngo and Pauwels 2010	Luu et al. 2016b
Locality	Khammouan Province	Khammouan Province
SVL	max 71.2	max 72.5
TaL	max 86.1 (Reg)	max 96.8
V	35 – 36	27 – 29
DTR	20 – 24	14 – 16
SL	13 – 14	11 – 12
IL	11	9 – 11
EFS	17 – 18	17 – 18
Total of FP and PP in males	39 – 40	42 – 43
LD4	16 – 19	19 – 20
LT4	19 – 23	18 – 19
Color pattern of dorsum	four narrow yellowish-cream transversal bands	three or four light transverse bands
Enlarged subcaudals	medially enlarged	medially enlarged

Table 3. Uncorrected ("p") distance matrix showing percentage genetic divergence (COI) between *Cyrtodactylus dati*, *C. thuongae*, and closely related species. Numbers in bold are the lowest percentages.

Species	1	2	3	4	5	6	7	8	9	10	11	12
1. *C. bidoupimontis* HQ967215	–											
2. *C. bugiamapensis* IEBR A.2011.3B	13.24	–										
3. *C. caovansungi* NT.2016.2	15.07	14.0	–									
4. *C. cucdongensis* VNMN PMT 2142	13.70	14.16	15.68	–								
5. *C. cryptus* KX064038	14.31	15.53	15.07	14.92	–							
6. *C. dati* KF929508	14.71	15.05	17.07	17.04	16.78	–						
7. *C. huynhi* KF169948	14.18	15.24	16.71	16.68	16.77	**4.18**	–					
8. *C. irregularis* KP199951	8.86	15.13	16.96	14.05	14.97	14.71	14.89	–				
9. *C. takouensis* KF929533	13.26	11.98	13.45	12.32	15.31	13.64	12.36	13.42	–			
10. *C. thuongae* IEBR A.2013.23	14.61	14.61	16.44	16.44	15.53	**0.74**	**3.83**	14.52	13.46	–		
11. *C. yangbayensis* ITBCZ 3540	12.79	12.33	15.22	7.92	15.22	15.22	14.31	12.83	11.60	14.46	–	
12. *C. ziegleri* HQ967210	14.41	7.36	14.41	15.34	15.17	15.42	15.07	15.51	12.59	15.02	13.15	–

Table 4. Uncorrected ("p") distance matrix showing percentage genetic divergence (COI) between *Cyrtodactylus lomyenensis*, *C. rufford* and closely related species. Number in bold is the lowest percentage.

Species	1	2	3	4	5	6	7	8
1. *C. bansocensis* KU175573	–							
2. *C. jaegeri* KT004364	15.09	–						
3. *C. khammouanensis* HM888469	12.04	14.92	–					
4. *C. lomyenensis* UNS0527	11.58	15.22	11.72	–				
5. *C. sommerladi* KJ817437	15.55	15.83	16.44	15.98	–			
6. *C. soudthichaki* KX077904	12.65	14.16	13.55	14.00	15.55	–		
7. *C. roesleri* KF929531	15.97	15.27	16.39	16.13	6.22	15.11	–	
8. *C. rufford* KU175572	11.43	14.61	11.87	**2.44**	17.20	14.46	16.28	–

C. rufford and published data of *C. lomyenensis*, *C. rufford* differs from *C. lomyenensis* by having fewer ventral scale rows (27–29 vs. 35 or 36), fewer supralabials (10–12 vs. 13 or 14), and more femoral and precloacal pores in males (42 or 43 vs. 39 or 40) (Table 2). The differences are quite small, except for the ventral scale row. In addition, *C. rufford* is similar to *C. lomyenensis* in size and coloration: head dorsum yellowish with irregular brown blotches, dorsal pattern with transverse bands, rings on original tail with dark brown transversal bands wider than light brown spaces, and median

row of enlarged subcaudal scales. In addition, genetic distance between the paratype of *C. lomyenensis* (UNS0527) and holotype of *C. rufford* (VFU R.2015.14) is 0.21% based on 413 bp of a fragment of the mitochondrial ND2 gene.

Discussion

In general, the phylogenetic relationships supported by this study are similar to those corroborated by previous studies using the same genetic marker (Nguyen et al. 2013; Luu et al. 2016a; Brennan et al. 2017; Ngo et al. 2017). However, some outstanding issues remain unresolved. Specifically, this study shows that *C. badenensis* is a member of the *C. irregularis* group, although Grismer et al. (2021a) based on ND2 suggested that the species belongs to *C. condorensis* or an independent lineage. The *C. irregularis* group contains several taxonomically unconfirmed populations. For example, *C.* cf. *ziegleri* revealed to be at least two taxa, while the phylogenetic placement of the true *C. ziegleri*, similarly to the previously reported cases in *C. bichnganae* in the *C. chauquangensis* group, has not been clarified in previous studies. Our study also confirms that although COI is a good marker for DNA barcoding it is limited by its length and lacks characters to resolve deeper nodes.

Cyrtodactylus has a complex taxonomic history and at least two species have been synonymized before this study. Grismer et al. (2015) examined the taxonomy of *C. condorensis* and *C. intermedius* complex using 100 samples from 30 localities and their analyses based on the mitochondrial ND2 suggested that *C. paradoxus* is a junior synonym of *C. condorensis* and *C. thochuensis* is a junior synonym of *C. leegrismeri*. Based on the similarities of morphological and molecular data, we consider *C. thuongae* Phung, van Schingen, Ziegler & Nguyen, 2014 a junior synonym of *C. dati* Ngo, 2013 and place *C. rufford* Luu, Calame, Nguyen, Le, Bonkowski & Ziegler, 2016b in the synonymy of *C. lomyenensis* Ngo & Pauwels, 2010. With two more species synonymized in this study, *Cyrtodactylus* from Vietnam and Laos currently consists of 69 valid species, including 47 from Vietnam and 22 from Laos (of these, *C. cryptus* and *C. roesleri* are known from both countries). Independent of morphological evidence, our molecular phylogenetic results confirm that other lineages represent undisputed species.

The number of *Cyrtodactylus* species within six identified species groups will likely change as new discoveries continue to be made at a rapid rate. At least seven unnamed lineages are confirmed by our study, one in the *C. angularis* group, one in the *C. chauquangensis* group, and five others in the *C. irregularis* group. In addition, several species complexes, such as *C. pseudoquadrivirgatus* and *C. ziegleri*, warrant further taxonomic clarification and future studies will probably reveal that some of the lineages within the complexes turn out to be new taxa. Of these, *C. pseudoquadrivirgatus* is most problematic because it was described using the type series from a wide distribution while at the moment, many members of the genus *Cyrtodactylus* are known for their notable site-restricted endemism. It is recommended that the species definition be redefined to the holotype of *C. pseudoquadrivirgatus* from A Luoi in Thua Thien Hue Province (including voucher/field numbers ITBCZ3001, ITBCZ3002, AL.2017.125, AL.2017.126), or to topotypic specimens, viz. the series in case they can be clearly proven to represent that taxon.

Grismer et al. (2021a) also suggest that several other unnamed lineages are present in Vietnam and Laos. The *Cyrtodactylus angularis* group comprises three undescribed taxa from Laos, while the *C. irregularis* group includes six. The results of this and our study clearly demonstrate that the *C. irregularis* group is the most speciose within the genus, but there exist many more cryptic species that have not been formally described thus far, in particular from southern Vietnam, a hotspot of this group. It is also noted that several areas in Vietnam and Laos are still poorly studied. More surveys in the areas, in particular the karst region in the northern Annamites, the southern Annamites, and the Central Highlands in Vietnam and northern karst region in northern Laos and the lowland area in southern Laos, will certainly yield more new taxa for science (Fig. 2).

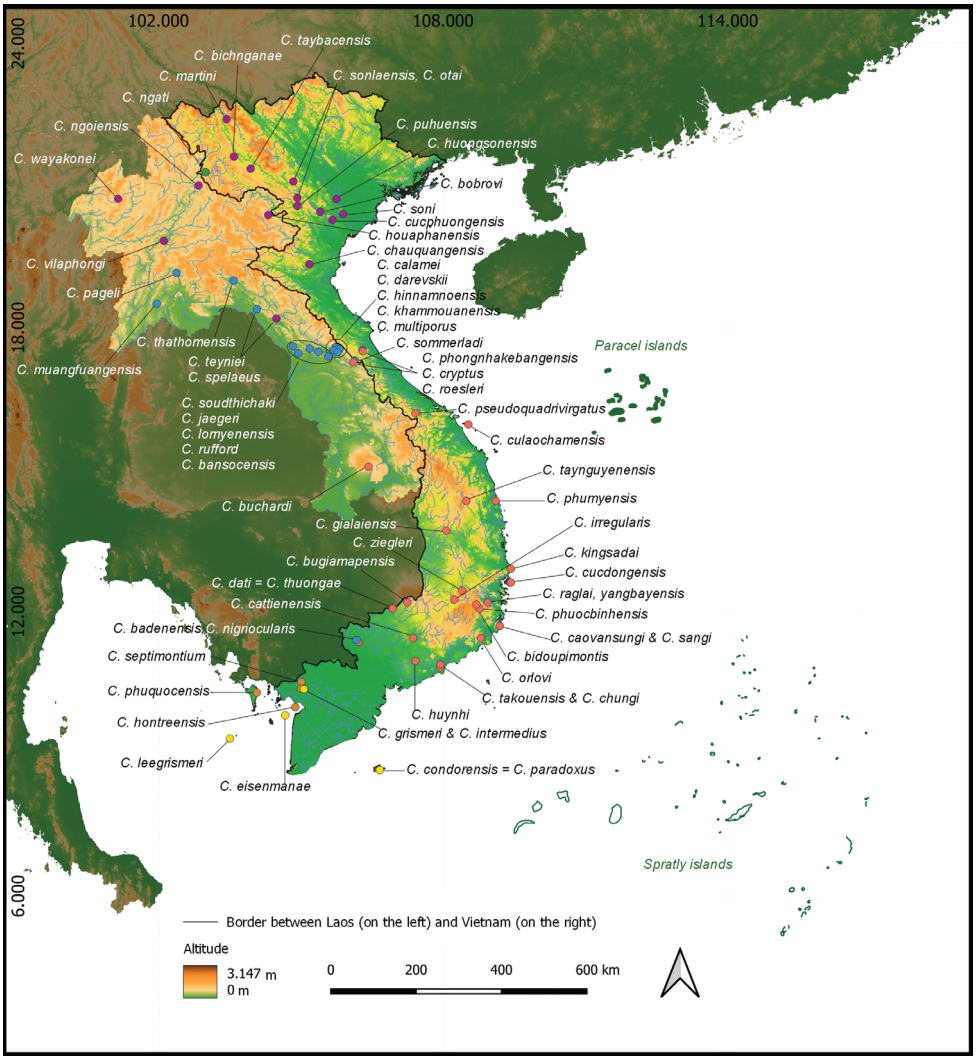

Figure 2. Type localities of all *Cyrtodactylus* taxa occurring in Laos and Vietnam (the altitude data based on GADM database of Global Administrative Areas, 2021).

According to the IUCN Red List, several species of this genus in Laos and Vietnam are facing exceedingly high extinction risks, including four species listed as Critically Endangered, three Endangered, and eight Vulnerable (IUCN Red List 2021). The recently described *Cyrtodactylus gialaiensis* is only known from a single locality with a distribution range of less than 10 km^2 and a population of fewer than 50 individuals (Luu et al. 2017, 2020). However, a majority of *Cyrtodactylus* still need to be carefully evaluated and it is likely that additional assessment will result in a higher number of species to be listed in the IUCN Red List in the future. Taxonomic uncertainty is also hindering conservation efforts, as some undescribed populations might already be critically threatened by human activities in both countries. Urgent research is therefore needed to resolve pending taxonomic issues before conservation assessments for the taxa can be undertaken.

Information on biogeographic ranges of six *Cyrtodactylus* groups occurring in Laos and Vietnam was detailed in Grismer et al. (2021a). According to geographic distribution of our newly collected samples, several species have broader ranges than previously documented. For example, while the occurrences of *C. cattienensis* were reported for the first time from Dong Nai and Ba Ria-Vung Tau provinces, we herein record it from Dong Phu District, Binh Phuoc Province. Distribution of *C. cucdongensis* is extended to Dak Nong Province, *C. huongsonensis* to Lac Thuy District, Hoa Binh Province, *C. kingsadai* to Khanh Hoa and Dak Nong provinces, and *C. phuocbinhensis* to Khanh Hoa Province. With more sampling of the members of the genus in Laos and Vietnam, our knowledge of its taxonomy, distribution, and conservation in the two countries will be improved in the future.

Acknowledgements

We thank A.V. Pham, T.V. Nguyen, D.T. Le, T.M. Phung for their assistance in the field. T.V. Nguyen, H.T. Duong, G.H.T. Cao, T.T. Nguyen, N.D. Pham, T.P.T. Bui, A.N.T. Ho assisted laboratory work. This research was funded by the Vietnam National Foundation of Science and Technology Development (NAFOSTED) under grant number 106.05-2020.24. Research of H. T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD). Comments from Aaron Bauer, Mark D. Scherz, the editor, and two anonymous reviewers greatly improved the paper.

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Supplementary material 1

Table S1

Authors: Hanh Thi Ngo, Quyen Hanh Do, Cuong The Pham, Vinh Quang Luu, L. Lee Grismer, Thomas Ziegler, Van Thi Hong Nguyen, Truong Quang Nguyen, Minh Duc Le

Data type: Docx file.

Explanation note: Samples used in this study.

Link: https://doi.org/10.3897/zookeys.1097.78127.suppl1

Supplementary material 2

Table S2

Authors: Hanh Thi Ngo, Quyen Hanh Do, Cuong The Pham, Vinh Quang Luu, L. Lee Grismer, Thomas Ziegler, Van Thi Hong Nguyen, Truong Quang Nguyen, Minh Duc Le

Data type: Docx file.

Explanation note: Uncorrected ("p") distance matrix showing percentage genetic divergence (COI) (highlighted in bold are the lowest and highest percentage) between species in the *Cyrtodactylus angularis* group.

Link: https://doi.org/10.3897/zookeys.1097.78127.suppl2

Supplementary material 3

Table S3

Authors: Hanh Thi Ngo, Quyen Hanh Do, Cuong The Pham, Vinh Quang Luu, L. Lee Grismer, Thomas Ziegler, Van Thi Hong Nguyen, Truong Quang Nguyen, Minh Duc Le

Data type: Docx file.

Explanation note: Uncorrected ("p") distance matrix showing percentage genetic divergence (COI) (highlighted in bold are the lowest and highest percentage) between species in the *Cyrtodactylus irregularis* group.

Link: https://doi.org/10.3897/zookeys.1097.78127.suppl3

Supplementary material 4

Table S4

Authors: Hanh Thi Ngo, Quyen Hanh Do, Cuong The Pham, Vinh Quang Luu, L. Lee Grismer, Thomas Ziegler, Van Thi Hong Nguyen, Truong Quang Nguyen, Minh Duc Le

Data type: Docx file.

Explanation note: Uncorrected ("p") distance matrix showing percentage genetic divergence (COI) (highlighted in bold are the lowest and highest percentage) between species in the *Cyrtodactylus condorensis* group.

Link: https://doi.org/10.3897/zookeys.1097.78127.suppl4

Supplementary material 5

Table S5

Authors: Hanh Thi Ngo, Quyen Hanh Do, Cuong The Pham, Vinh Quang Luu, L. Lee Grismer, Thomas Ziegler, Van Thi Hong Nguyen, Truong Quang Nguyen, Minh Duc Le

Data type: Docx file.

Explanation note: Uncorrected ("p") distance matrix showing percentage genetic divergence (COI) (highlighted in bold are the lowest and highest percentage) between species in the *Cyrtodactylus chauquangensis* group.

Link: https://doi.org/10.3897/zookeys.1097.78127.suppl5

Supplementary material 6

Table S6

Authors: Hanh Thi Ngo, Quyen Hanh Do, Cuong The Pham, Vinh Quang Luu, L. Lee Grismer, Thomas Ziegler, Van Thi Hong Nguyen, Truong Quang Nguyen, Minh Duc Le

Data type: Docx file.

Explanation note: Uncorrected ("p") distance matrix showing percentage genetic divergence (COI) (highlighted in bold are the lowest and highest percentage) between species in the *C. brevipalmatus and C. intermedius* group.

Link: https://doi.org/10.3897/zookeys.1097.78127.suppl6

3.2. Investigating phylogenetic relationships, evolutionary process and biogeographic history of *Cyrtodactylus*

Chapter 8. Molecular phylogeny and biogeography of the Cyrtodactylus angularis group: Diversification in a biodiversity hotspot

(Submitted to Ecology and Evolution)

Molecular phylogeny and biogeography of the *Cyrtodactylus angularis* group: Diversification in a global biodiversity hotspot

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ABSTRACT

Background The bent-toed gecko genus *Cyrtodactylus* is the most speciose of the family Gekkonidae and the third-largest vertebrate genus on the planet with more than 380 nominal species. Consisting of least 21 species, the *C. angularis* species group represents a diverse clade in Indochina, a main center of the genus' radiation. Recent works, which attempt to resolve the phylogenetic relationships between the congeners, have primarily used a couple of mitochondrial loci and, as a result, could not provide a robust phylogenetic hypothesis for the group. In addition, important aspects of its evolutionary and biogeographic history have not been addressed for all members of this group in previous research.

Results To better understand the phylogeny, origin, evolution, and biogeography of the species group, we analyzed data from three mitochondrial loci and four nuclear loci for 21 species of this group. The largest dataset to date helps produce strongly congruent phylogenetic relationships among the species, recover six evolutionarily independent clades, and resolves some poorly supported nodes in the group with high confidence.

Conclusion Our study shows that the group first emerged in the Oligocene, while a majority of other lineages started to diverge in the Miocene, when the East Asia summer monsoon developed and might have brought a large-scale vegetation change as a result of increased precipitation in the region. Biogeographic analysis suggests that the Mekong Lowlands is likely the ancestral area of the species group, whereas most of the remaining members originate from the Annamite Mountain Range. Our study once again highlights the importance of the Annamite landscape, a global biodiversity hotspot, in maintaining evolutionary processes for many endemic and evolutionarily distinct vertebrates.

Keywords: Ancestral area, Annamite Mountain Range, Bent-toed Gecko, Indochina, Miocene.

BACKGROUND

Cyrtodactylus is by far the most speciose and ecologically diverse genus of the family Gekkonidae [1–4]. It currently contains 381 nominal species [4] and is distributed from Tibet, tropical South Asia to Indochina, the Philippines, through the Indo-Australian Archipelago, to the Solomon Islands in the East [2, 5, 6]. New species discoveries have surged in recent years, as only 70 species were known to science at the turn of this century [4]. The use of molecular data has helped to uncover many cryptic species across its range. Although the current number of the taxa makes *Cyrtodactylus* the third largest vertebrate genus on Earth, its diversity is still largely underestimated with many undescribed forms awaiting to be named [1, 2, 7, 8].

Members of the genus inhabit a broad array of habitat types ranging from intertidal, arboreal, cave, karst, granite, and swamp, to coffee plantations [1, 3, 9, 10]. Bent-toed geckos stunning abilities have resulted in many shifts from one habitat preference to another. Specifically, Grismer et al. [10] documented at least 39 transitions from the general habitat preference—the ancestral trait of the genus—to eight other specific types, 11 events in reverse, and 16 others between different specific habitats. With that remarkable adaptability, *Cyrtodactylus* has been shown to have evolved into one of the most successful vertebrate groups on the planet with several major radiations in Indochina, Sundaland, Wallacea, and the Philippines since the Eocene from its ancestral area in the proto-Himalaya. Among the regions, Indochina and the Sundaland form two centers of group diversity and radiations [3, 5].

The *Cyrtodactylus angularis* group is one of the largest Indochinese lineages with at least 21 nominal species, excluding *C. zhaoi* because of its taxonomic invalidity, and several undescribed forms as of Grismer et al. [2]. Even though the type species was discovered in the early 20th century [11], like other species groups within *Cyrtodactylus*, most of its diversity had not been recognized

until the early 21st century [4]. A large portion of the genus occur in a special ecotype – karst [1, 10]. The color pattern of these species is diverse, which include a dorsal pattern formed by blotches (e.g., *C. teyniei*), irregularly shaped separate spots (e.g., *C. multiporus*) and bands (e.g., *C. bansocensis* and *C. darevskii*) [12–14]. Most species of the group are localized and only known from their type localities in Thailand (i.e., *C. angularis, C. chanhomeae*), Laos (i.e., *C. bansocensis, C. calamei, C. darevskii, C. hinnamnoensis, C. jaegeri„ C. khammouanensis, C. lomyenensis, C. multiporus, C. muangfuangensis, C. pageli, C. sommerladi, C. soudthichaki, C. thathomensis, C. teyniei*) or Vietnam (i.e., *C. hangvaensis*, *C. nigriocularis*, and *C. phongnhakebangensis*), while *C. jarujini* are reported in Laos and Thailand and *C. roesleri* are reported in both Laos and Vietnam [15–19].

Interestingly, aside from two species known only from Thailand, all other species, except for the Black-eyed Bent-toed Gecko (*C. nigriocularis*), occupy the northern Annamite Mountain Range, a region that spreads between the Laos and Vietnam borders and is well known for its high level of endemicity and evolutionary distinctiveness [20–24]. On the Vietnamese side, the northern Annamites, despite its relatively small size, harbors 115 species of reptiles or approximately 24% of the country's reptile diversity, of which 12 species are endemic [24].

In addition to reptiles, other endemic and flagship species in the landscape include such Critically Endangered vertebrates as Saola (*Pseudoryx vuquangensis*), the Large-antlered Muntjac (*Muntiacus vuquangensis*), the Southern White-cheeked Gibbon (*Nomascus siki*), and the Edwards's Pheasant (*Lophura edwardsi*) [25–28]. The small mammal fauna here is represented by micro-endemic species, e.g., the Laotian Rock Rat (*Laonastes aenigmamus*) and Paulina's Limestone Rat (*Saxatilomys paulinae*) that are restricted to karst habitat [22, 29]. In particular, the presence of the Laotian Rock Rat, considered a living fossil as all of its relatives found all over

Asia went extinct around 11 million years ago, clearly shows that the Annamites have been an ancient refuge for many different taxa throughout a long history of Indochina [23, 30, 31]. However, little research has been undertaken to better understand the tempo and mode of evolutionary processes that have driven radiations in karst habitats in the Annamites (but see Nicolas et al. [22], Le et al. [23], Le et al. [31]). Therefore, further studies on the evolution and biogeography of the *Cyrtodactylus angularis* group may provide more information about karst habitats in the Annamite region.

Although several studies have been carried out to recover the phylogeny and estimate divergence times and the historical diversification of *Cyrtodactylus*, previous work has not focused on the phylogeny, evolution, and biogeography of members of *C. angularis* group or included all representative species of the group [1, 3, 5, 32]. Additionally, previous phylogenetic analyses have primarily been restricted to the use of one or two mitochondrial genes of members of *C. angularis* group [2, 3, 32, 33]. To improve our understanding of the phylogenetic relationships and radiation of this group, especially in the Annamite hotspot, we reconstructed a phylogeny for 21 species of this group using three mitochondrial, cytochrome c oxidase subunit I (*COI*), cytochrome *b* (*Cytb*), and NADH dehydrogenase subunit 2 (*ND2*) (including tRNA), and four nuclear genes, *Cmos, PDC, Rag1, Rpl35*, which represents the largest molecular dataset employed in any studies of *Cyrtodactylus* to date in terms of the number of loci and the length of the matrix. We also calibrated time divergence of the group congeners using the Bayesian relaxed clock method and optimized biogeographic patterns to infer the historical diversification of this group.

92 **METHODS**

93 **Taxon sampling**

94 In total, 36 samples of 21 described taxa of the *C. angularis* group in Thailand, Laos and Vietnam
95 and one sample of an undescribed taxa were included in the present study. Four outgroup species,
96 namely *C. ayeyarwadyensis*, *C. brevidactylus*, *C. condorensis*, and *C. grismeri* were selected [1,
97 2]. In total, 199 new sequences of 7 genes from 36 samples were incorporated in this study (Table
98 1). In addition, 68 published sequences were also included in our analyses. Detailed information
99 of the newly generated sequences and GenBank accession numbers of the existing ones used in
100 the study are given in Table 1.

101 **DNA extraction, PCR and DNA sequencing**

102 Genomic DNA was extracted using Dneasy Blood and Tissue (Qiagen, Germany) or GeneJet
103 Genomic DNA Purification kit (Thermo Fisher Scientific, Lithuania) following the manufacturer's
104 instructions. Seven molecular markers, comprising three mitochondrial, *COI*, *Cytb*, and *ND2*
105 (including tRNA) and four nuclear loci*, Cmos, PDC, Rag1*, and *Rpl35* were employed in this study.
106 The primers used to amplify the fragments are listed in Table 2. PCR amplification was performed
107 in a total volume of 21 µl that contained 2 µl template DNA, 2 µl of each primer and 10 µl
108 DreamTaq Mastermix (Thermo Fisher Scientific, Lithuania) or HotStarTaq Mastermix (Qiagen,
109 Germany) and 5 µl of nuclease free water. The reaction was carried out with an initial denaturation
110 at 95ºC for 15 min with HotStarTaq Mastermix or 5 min with DreamTaq Mastermix, followed by
111 35 cycles of amplification (denaturation at 95ºC for 30 s, annealing at 48ºC – 58ºC for 45 s, and
112 extension at 72ºC for 1 min), with final extension at 72ºC for 10 min. Negative and positive
113 controls were also used in all amplifications and extractions to check for possible contamination.

The PCR products were visualized by agarose gel electrophoresis and stored at -4ºC after visualization. The PCR products were purified using GeneJet PCR Purification kit (Thermo Fisher Scientific, Lithuania), in accordance with the manufacturer's instructions. The sequences of the forward and reverse strands were determined for all taxa using the sequencing service from 1st Base (Malaysia).

Sequence alignment and phylogenetic analyses

Three datasets that contained mitochondrial fragments (mt dataset) (*COI* + *Cytb* + *ND2* + tRNA*),* nuclear genes (nu dataset) (*Cmos* + *Rag1* + *PDC* + *Rpl35*) and combined loci *(COI* + *Cytb* + *ND2* + tRNA + *Cmos* + *Rag1* + *PDC* + *Rpl35* sequences) were analyzed separately. The combined dataset was divided into 21 partitions based on codon positions (first, second, and third). Each gene was initially aligned separately using ClustalX v2.1 [47] with default settings for complete alignment. Data were analyzed using maximum likelihood (ML) implemented in IQtree v2.3.6 [48], Bayesian inference analysis (BI) as implemented in MrBayes v3.2.7 [49], and maximum parsimony (MP) as implemented in PAUP v4.0b10 [50] to assess phylogenetic relationships of the *C. angularis* group. Genetic divergences between members of the group were calculated using uncorrected p-distance in PAUP.

For BI and ML analyses, the optimal substitution models were determined using the Akaike Information Criterion (AICc) as implemented in jModeltest v2.1.10 [51] and PARTITIONFINDER v1.1.1 [52] with parameters estimated by MrBayes. All the best models for the three datasets are shown in Table 3. Two simultaneous analyses with four Markov chains (one cold and three heated) were run for 10^7 generations with a random starting tree and sample every 1,000 generations. Log-likelihood scores of sample points were plotted against generated time to determine stationarity of Markov chains. The cut-off point for the burnin function was set to 25%

of the total number of trees generated in the Bayesian analyses. For ML analysis, 10,000 ultrafast bootstrap replications (UFBP) were run. For MP analysis, heuristic analysis was conducted for the combined dataset with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch-swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap (BP) support was calculated using 1,000 pseudo-replicates and 100 random taxon addition replicates. Nodal support was evaluated using UFBP, as estimated in IQtree v2.3.6, posterior probability (PP), as calculated in MrBayes v3.2.7, and BP, as assessed in PAUP v4.0b10. UFBP > 95% and PP ≥ 0.95 and BP ≥ 70% are regarded as strong support for a clade [49, 53, 54].

Time divergence analysis

In order to calibrate time divergences at the nodes using the relaxed molecular method, one sample for each described species and one sample for an undescribed species were selected. Consequently, the combined dataset, consisting of 26 taxa with 22 ingroups (including 21 described species and one undescribed species) and four outgroups, was employed in BEAST v2.7.6 [55] with the substitution models unlinked but with the molecular clock and trees linked for each gene partition. One calibration point, approximately 27 million years ago (MYA), was set to the node representing the split between *C. angularis* + *C. chanhomeae* and all other species within the *C. angularis* group, except for *C. nigriocularis* [5]. Another calibration point, approximately 33 million years ago (MYA), was used for the node representing the split between *C. nigriocularis* and all other species within the *C. angularis* group [3]. The substitution models applied to the data matrix were determined using jModeltest v2.1.10 (Table 4) [51]. The dating analysis was run for 300,000,000 generations with sampling every 5,000 generations. After the dataset with the above settings was analyzed in BEAST, the resulting likelihood profile was then examined by the program Tracer

v1.7.1 to confirm the ESS > 200 for all parameters. The final tree with calibration estimates was computed using the program TreeAnnotator v2.7.6 [55] as recommended by the program manual.

Inferring historical biogeography

Bayesian binary MCMC (BBM) analysis [56, 57] as implemented in Reconstruct Ancestral State in Phylogenies (RASP v4.3) [58] and BioGeoBEARS as implemented in R [59], were used to reconstruct historical biogeography for the *C. angularis* group. The tree generated from the BEAST program was used as the input data for the analysis. The geographic distributions for the *C. angularis* group were assigned to three areas: (A) northern Annamite, (B) Mekong Lowland, and (C) West Indochina [21]. For BioGeoBEARS, all biogeographic models, including dispersal-vicariance (DIVA), dispersal-extinction-clado-genesis (DEC), and Bayesian analysis of biogeography when the number of areas is large (BayAreaLike), with and without the jump dispersal parameter (j), were run in this study. The total number of areas each species allowed to occupy was set to three.

RESULTS

Phylogenetic analysis

The combined dataset *(COI* + *Cytb* + *ND2* + tRNA + *Cmos* + *Rag1* + *PDC* + *Rpl35*) consisted of 5,275 aligned characters (*COI:* 657 + *Cytb:* 1,031 + *ND2* and tRNA: 1,247 + *Cmos*: 354 + *PDC*: 395 + *Rag1*: 1,038 + *Rpl35*: 553), of which 3,534 were constant, 314 variable and parsimony-uninformative, and 1,427 parsimony informative. In the MP analysis, after 4,360,791 rearrangements were attempted, and the single most parsimonious tree with 5,417 steps was recovered (Consistency index = 0.48; Retention index = 0.52). The tree obtained from ML, MP,

BI and Bayesian partitioned analyses with branch length estimated by the Bayesian partitioned analysis based on the combined dataset are shown in Fig. 1. The aligned mt dataset (*COI* + *Cytb* + *ND2* + tRNA) comprises 2,935 positions, of which 1,476 were constant, 187 variable and parsimony-uninformative, and 1,272 parsimony informative. In the MP analysis, after 4,076,905 rearrangements were undertaken and the single most parsimonious tree with 5,070 steps was restored (Consistency index = 0.45; Retention index = 0.55). The tree obtained from ML, MP and BI analyses with branch length estimated by the Bayesian analysis based on the mt dataset are shown in Fig. 2. The nu matrix (*Cmos* + *Rag1* + *PDC* + *Rpl35*) includes 2,340 characters, of which 2,058 were constant, 127 variable and parsimony-uninformative and 155 parsimony informative. In the MP analysis, the single most parsimonious tree with 342 steps was recovered (Consistency index = 0.87; Retention index = 0.13). The tree inferred from the ML, MP and BI analyses with branch length estimated by the Bayesian analysis based on nu dataset are shown in Fig. 3.

Overall, phylogenies generated of the combined dataset (mt-nu) were very similar to those produced by the mt dataset and significantly different from those of nu dataset, especially in terms of the number of unresolved nodes in the trees from nu dataset. However, there were some areas of agreement between the three datasets. For example, almost all trees from the combined dataset, mt, and nu datasets strongly corroborated the monophyly of the *C. angularis* group, except for the trees from ML and MP analyses of the mt dataset (UFBP = 81 and BP = 65). In addition, the monophyly of Group I, Group II, Group III as well as the clades encompassing Group II – Group VI, Group III – Group VI, and Group IV – Group VI was recovered by analyses of all three datasets, even though MP did not support the monophyly of Group III and the clade consisting of Group III – Group VI and nodal values of the branches were generally low in the trees from both

analyses of the nu dataset. Nonetheless, the monophyly of Group IV – Group VI were not corroborated by the trees of nu dataset in contrast with the phylogenetic signals derived from trees of mt and combined matrices (Fig. 1, Fig. 2, and Fig. 3).

In cases where analyses of both mt and nu datasets recovered the same deeper nodes, the nodal statistical values were improved in trees based on the combined matrix. For example, nodes representing the *C. angularis* group, Group II, Group III, and Group III – Group VI in trees from the combined dataset all received higher values regardless of the analyses compared to those resulting from the mt dataset (Fig. 1 and Fig. 2). On the other hand, significant support values for Group IV – Group VI all came in trees from the mt data as the analyses of nu dataset resulted in largely unresolved or weakly corroborated phylogenetic relationships. Moreover, four species *C. darevskii*, *C. jaegeri*, *C. roesleri*, *C. sommerladi* were paraphyletic, while *C. hinnamnoensis* was polyphyletic in trees supported by the nu dataset (Fig. 3).

In general, all nodes in the trees from combined dataset were well corroborated by at least three analyses, except for three poorly supported nodes, i.e., the placements of *C. bansocensis*, *C. khammouanensis*, and *C. multiporus*. *C. nigriocularis* was recovered as the sister species to all other members of *C. angularis* group. The remaining *C. angularis* species clustered in five major subclades with significant statistical values, except for Group III where the MP analysis did not recover the same relationship: Group II (UFBP = 97, BP = 77, PP = 1 and 1), Group III (UFBP = 96, PP = 1 and 1), Group IV (UFBP = 100, BP = 92, PP = 1 and 1), Group V (UFBP = 99, BP = 95, PP = 1 and 1), and Group VI (UFBP = 100, BP = 98, PP = 1 and 1). When combined with geographic data, five distinct phylogenetic groups are primarily distributed in distinct areas. Specifically, the earliest diverging group, Group I is restricted to Ba Den Mountain, southern Vietnam and Group II with two species from eastern Thailand (*C. angularis, C. chanhomeae*),

Group III – Group VI occur almost exclusively in northern Annamites, in which Group III consists of two species from northern Laos (*C. muafuangensis, C. pageli*), Group IV occupying central Vietnam and eastern Laos (*C. bansocensis, C. hangvaensis, C. jaegeri, C. lomyenensis, C. roesleri, C. sommerladi, C. soudthichaki*), group V spreading from eastern Thailand (*C. jarujini*) to central and northern Laos (*C. jarujini*, *C. multiporus, C. teyniei*, *C. thathomensis* and one undescribed species), and Group VI ranging from central Laos to northcentral Vietnam (*C. calamei*, *C. darevskii*, *C. hinnamnoensis*, *C. phongnhakebangensis*) (Fig. 1 and Fig. 4A). Within four groups, the lowest and highest pairwise genetic divergence ranges from 3.65 (between *C. darevskii* and *C. hinnamnoensis*) to 22.98 (between *C. hinnamnoensis* and *C. nigriocularis)* based on fragments of *COI* gene; from 3.10 (between *C. darevskii* and *C. hinnamnoensis*) to 22.56 (between *C. chanhomeae* and *C. teyniei*) based on fragments of *cytb* and from 2.31 (between *C. darevskii* and *C. hinnamnoensis*) to 22.99 (between *C. angularis* and *C. lomyenensis*) based on fragments of *ND2* gene (Supplementary 1).

Divergence dating analysis

The combined dataset for seven genes consisted of 26 taxa (Fig. 5). The results of divergence time estimation showed that *C. nigriocularis* and all other members of *C. angularis* group split (Node 3) in the early Oligocene (29.46 to 35.48 MYA) (Node 3; Fig. 5, Table 5). Group II, Group III and Group IV – VI begun to emerge in the Oligocene (Node 4, 5; Fig. 5, Table 5). Two species of Group II were also split in the late Oligocene (Node 6; Fig. 5, Table 5). The remaining lineages of the *C. angularis* group diverged in the Miocene (23.03 million to 5.3 million years ago) (Fig. 5, Table 5). The most recent speciation event, i.e., the split between *C. hinnamnoensis* and *C. darevskii*, took place in the Pleistocene around 2.06 MYA (Node 25; Fig. 5, Table 5). Besides *C. hinnamnoensis* and *C. darevskii, C. calamei*, *C. jaegeri*, *C. jarujini*, *C. roesleri*, *C. soudthichaki*,

C. sommerladi, *C. thathomensis* are youngest members of the group, which started to speciate from the early Pliocene, ca. 4.8 – 2.75 MYA (Node 21–24; Fig. 5, Table 5) [61].

Inferring historical biogeography

The inferred historical biogeographic scenarios from analyses using RASP are shown in Fig. 4B. In the reconstruction of the group ancestral geographic range, several areas of endemism contribute differentially with the Mekong Lowland (purple) receiving the highest probability of 73.67%, followed by West Indochina of 12.55% and northern Annamite of 10.91% (Node 43 in Fig. 4B). The most probable ancestral areas for the remaining five groups (Node 42 in Fig. 4B) were northern Annamite (45.66%) and West Indochina (42.43%). The ancestral area with the highest probability for Group II (Node 23 in Fig. 4B) was West Indochina (99.35%). Northern Annamite was recovered as the most probable ancestral area for Groups III, IV, V and VI (≥ 99.85%) (Node 30, 34, 37 and 40 in Fig. 4B).

The BioGeoBEARS analysis, recovered DIVALIKE model as the best-fit to the data and most likely to infer the correct ancestral range at each node with the highest LnL and the lowest AIC and AICc (Supplementary 3). However, time-calibrated biogeographic reconstruction of the DIVALIKE model did not provide informative results for *C. angularis* group (Supplementary data 4).

DISCUSSION

Phylogenetic relationships

By using both mitochondrial and nuclear markers, the largest dataset to date for any groups of *Cyrtodactylus*, we were able to recover a generally well-supported the phylogenetic tree of all members of the *C. angularis* group. Although in two major lineages, the monophyly of Group III

and Group III + Group IV + Group V + Group VI in the trees based on combined dataset, are not corroborated by the MP analysis and three other nodes, i.e., the placements of *C. bansocensis*, *C. khammouanesis*, and *C. multiporus*, receive low statistical values, all other analyses consistently recover six distinct phylogenetic groups. It is also important to note that while mitochondrial markers produce well-resolved topologies, combining mt and nu data results in enhanced statistical nodal values, especially for the deep nodes due to their slower rate of evolution but not the more recently divergences, i.e., nodes representing the monophyly of the *C. angularis* species group, monophyly of Group III, and monophyly of Group III + Group IV + Group V + Group VI.

Similar to those reported by Grismer et al. [1, 3] using *ND2* and Brennan et al. [37] and Duong et al. [32] using a combination of *COI* and *ND2*, the phylogenetic hypotheses supported by analyses based on the combined dataset in our study show that the *C. angularis* group is monophyletic although the group was rendered paraphyletic in the review study by Ngo et al. [7] using *COI* gene, likely due to the low number of base pairs compare to other genes. Our study therefore confirms that even though *COI* is a good marker for DNA barcoding of *Cyrtodactylus*, it is limited by its length and lacks characters to resolve deeper nodes. Overall, the addition of nuclear data enhances the support level at the deeper nodes compared to support values reported in previous studies.

Several discrepancies between the results derived from our study and Grismer et al. [1] and Duong et al. [32] remain. Specifically, according to the combined trees in this study, *C. pageli* is placed as a sister taxon to *C. muafuangensis* and the two species together form a distinct lineage, Group III. Nevertheless, Grismer et al. [1] and Duong et al. [32] based on *ND2* and *COI* or combined *ND2, Rag1, MXRA5* and *PDC* suggested that *C. pageli* clusters with species in Group II (*C. angularis* and *C. chanhomeae*). In addition, despite similar taxa compositions in Group IV – Group VI, the positions of *C. bansocensis*, *C. teyniei* and *C. jaegeri* + *C. soudthichaki* differ between the

three studies. Although we did not include the newly described *C. hangvaensis* in our analysis, this species likely belongs to Group IV based on its close affinity to *C. roesleri* and *C. sommerladi* [32]. Given these analyses are sensitive to sample size and gene coverage, we prefer our mito-nuclear phylogeny to those of Grismer et al. [1] and Duong et al. [32].

Grismer et al. [1], Duong et al. [32], and our analyses illustrate that the current taxonomy underestimates the diversity of the group. At least, six populations, two from Thailand and four from Laos, are shown to be potential new species in three studies. Additionally, the large area across central Indochina would suggest other species likely occur between the range of groups I and II and the remaining groups (Fig. 4A). Furthermore, this study reveals several species with genetically distinct lineages, including *C. darevskii*, *C. hinnamnoensis*, *C. pageli*, *C. sommerladi*. Interestingly, our analyses of nu dataset suggest that at least five species, namely *C. darevskii*, *C. hinnamnoensis*, *C. jaegeri*, *C. roesleri*, *C. sommerladi*, are either paraphyletic or polyphyletic (Fig. 3). While the nature of the relationships in *C. jaegeri* could result from incomplete lineage sorting, the other species may experience historical gene flow between closely distributed taxa. Future studies using genome-wide single nucleotide polymorphisms and microsatellites will be able to shed more lights on the issues.

Time calibration and biogeography

Our time-calibrated molecular results resemble those reported by Grismer et al. [3] and reveal that *C. nigriocularis* and other three clades, Groups II, III, and IV+V+VI emerged in the Oligocene. As the Mekong Lowland is regarded as the most likely ancestral area of the *C. angulari*s species group, some of the earliest speciation events probably took place in the region. The group might then have colonized West Indochina and the Annamites around the late Oligocene, as the two distribution ranges were recovered as ancestral areas of Groups II and Groups II – VI, respectively.

All of the most recent common ancestors of Groups II – VI, which make up a majority of the nodes (65% or 13/20 nodes) within the species group, arose in the Miocene (Fig. 4B and Fig. 5).

It is suggested that the burst of radiation within the *C. angularis* group corresponds remarkably well to the development of the East Asian summer monsoon around the Oligocene and Miocene boundary [62, 63]. During this period, increased precipitation from the monsoon likely accelerated the dissolution of the limestone substrate and deeply influenced the development of the karst region [62, 63]. Open habitats created in the newly formed evergreen broad-leaf forests might have contributed to the expansion and divergence of the *C. angularis* group, especially in the northern Annamites. This wave of colonization from the nearby region, the Mekong Lowland, also coincides with the abrupt change from the ancestral habitat preference of granite cave in *C. nigriocularis* to karst specialist in all remaining species in the group, except for *C. lomyenensis*, which reverses to the original state of granite habitat. The *C. angularis* species group also forms one of two largest karst-associated radiations in the genus *Cyrtodactylus* with the other comprising *C. chauquangensis*, *C. oldhami*, *C. sadansinensis*, and *C. yathepyanensis* species groups, which occupies karst landscapes in China, Laos, Myanmar, Thailand, and Vietnam [1].

When *C. angularis* group began to disperse out of the Mekong Lowland to the northern Annamites and eastern Thailand (West Indochina) in the late Oligocene, other groups, including animals, fungi, and plants, also migrated into the karst landscape in subtropical East Asia [63]. During the Miocene, the geckos adapted well to the new habitat and experienced a surge in speciation rate (Figure 5). Miocene must have been also a special epoch for other Annamites endemic taxa because some unique and endemic species evolved in the mountain range during this time, including the cavernicolous genus of scorpions, *Aemngvantom* in the early Miocene *ca*. 18 MYA [64], Saola in the mid Miocene around 16.1 – 13.3 MYA [65], and the Laotian rock rat in the late

Miocene approximately 7 MYA [22, 31]. As the favorable climate conditions of higher rainfall vanished along with the change in vegetation around four MYA [63], the rate of diversification within the *C. angularis* species group also slowed down. Nonetheless, the central Annamites has still experienced a higher rainfall compared to the northern and southern regions and is dominated by evergreen forests, which help sustain ecological evolutionary processes for many endemic and evolutionarily distinct species [20, 23].

CONCLUSIONS

Using multiple molecular markers, including mitochondrial and nuclear loci, this study has been able to illustrate the evolutionary and biogeographic history of one of the largest radiations within the speciose gecko genus *Cyrtodactylus* in Indochina. In particular, the *Cyrtodactylus angularis* species group's rapid rate of speciation during the Miocene might have been substantially facilitated by the physical factors, i.e., paleoclimate patterns and the limestone substrate, and the subsequent large-scale development of evergreen forest habitat in the Miocene. Although previous studies have reported the same results that several unique taxa, including mammals and insects, in the Annamites emerged in the same geological era, none has shown the increased rate of speciation during this period. We expect that further research in other ancient groups of both animals and plants in the region will reveal more interesting phenomena in this remarkable and understudied mountain ranges. Our study once again contributes to a growing body of evidence, which demonstrates the importance of the landscape in maintaining the regional unique fauna and flora.

SUPPORTING INFORMATION

Supplementary 1. Uncorrected p-distance of species in the *C. angularis* group (highlighted in bold are the lowest and highest percentage)

Supplementary 2. Detailed output from the S-DIVA analysis

Supplementary 3. Model testing for the BioGeoBEARS analysis with and without found-event speciation (+J)

Supplementary 4. Time-calibrated biogeographic reconstruction of the DIVALIKE model from the BioGeoBEARS analysis

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not Applicable

Availability of data and materials

The data generated during the current study have been deposited in GenBank under accession numbers PV454673-PV454681, PV463222-PV463384, and PV463222-PV463384 accessible at https://www.ncbi.nlm.nih.gov.

Competing interests

The authors declare no competing interests.

Funding

This research was funded by the Vietnam National Foundation of Science and Technology Development (NAFOSTED) under grant number 106.05-2020.24. Doctoral research of H.T. Ngo in Germany is supported by the German Academic Exchange Service (DAAD).

Authors' contributions

HTN, MDL conceived the study, performed all the analyses, and drafted the manuscript. HTN, DNP, ATNH, VTHN carried out the laboratory work. VQL, TQN, CTP, TZ undertook the fieldwork and provided logistical support. All authors have read, reviewed, and agreed to the published version of the manuscript.

Acknowledgements

We thank Ha N.V., Thanabuaosy K., Homsaysombat T. for their assistance in the field and Nguyen T.V., Duong H.T., Cao G.T.H., Nguyen T.T., Le M.H. for their assistance in the laboratory. Nguyen T.T. provided tissue samples of *C. nigriocularis* and Nguyen A.T. helped with map design.

Authors' information (optional)

Not Applicable

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Figure 1. Phylogenetic consensus tree inferred from mixed-model ML, MP and Bayesian analyses with branch length estimated by the Bayesian mixed-model analysis based on three mitochondrial loci, *COI, Cytb, ND2*, and four nuclear markers, *Cmos, PDC, Rag1,* and *Rpl35*. Numbers above and below branches are ML ultrafast bootstrap/MP bootstrap values and Bayesian single-model posterior probabilities/Bayesian mixed-model posterior probabilities, respectively.

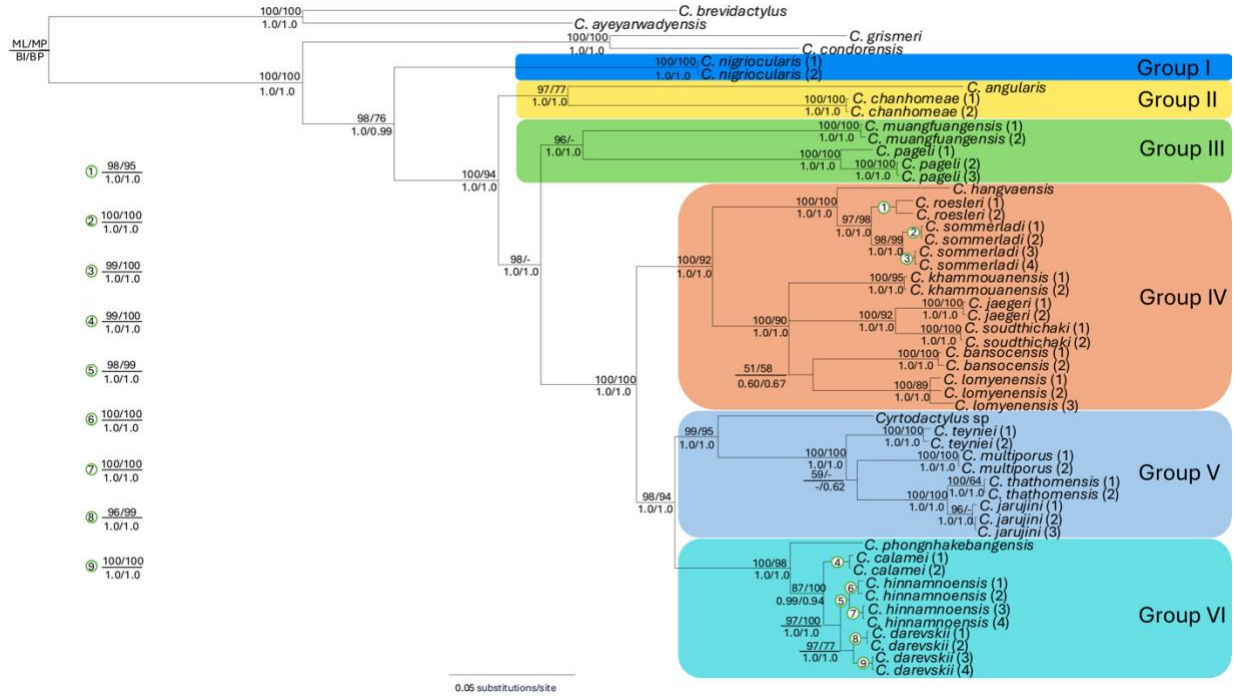

Figure 2. Phylogenetic consensus tree inferred from ML and Bayesian single-model analyses with branch length estimated by the Bayesian analysis based on three mitochondrial genes, *COI, Cytb,* and *ND2*. Numbers above and below branches are ML ultrafast bootstrap/MP bootstrap values and Bayesian posterior probabilities, respectively.

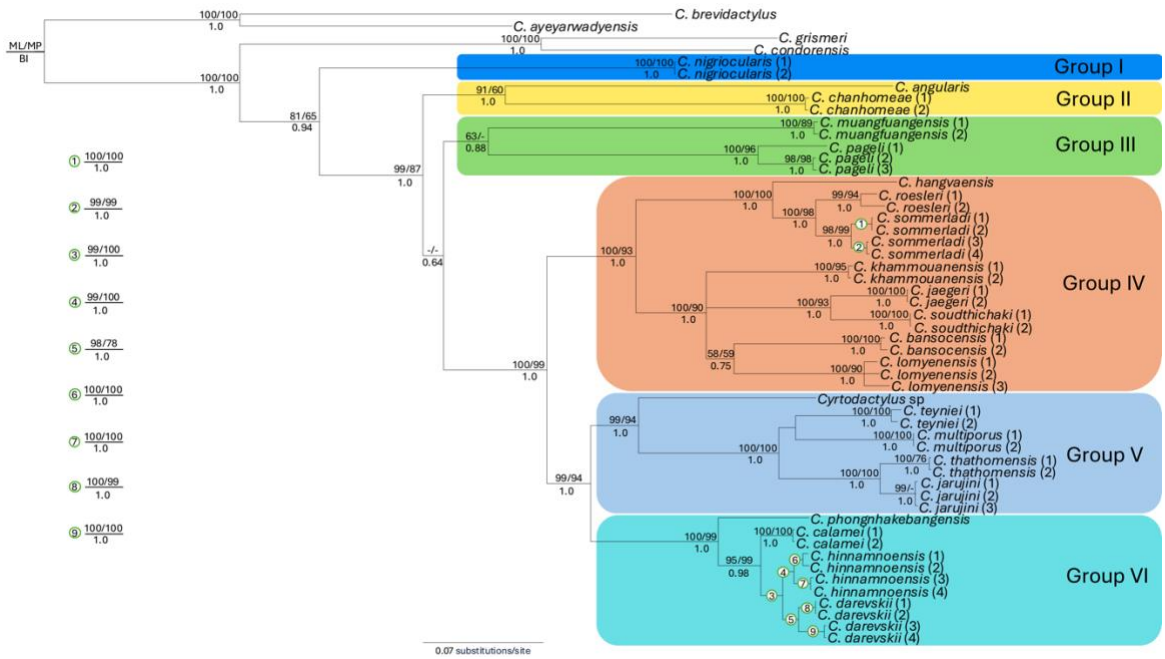

Figure 3. Phylogenetic consensus tree inferred from ML and Bayesian single-model analyses with branch length estimated by the Bayesian analysis based on four nuclear loci, *Cmos, PDC, Rag1,* and *Rpl35.* Numbers above and below branches are ML ultrafast bootstrap/MP bootstrap values and Bayesian posterior probabilities, respectively.

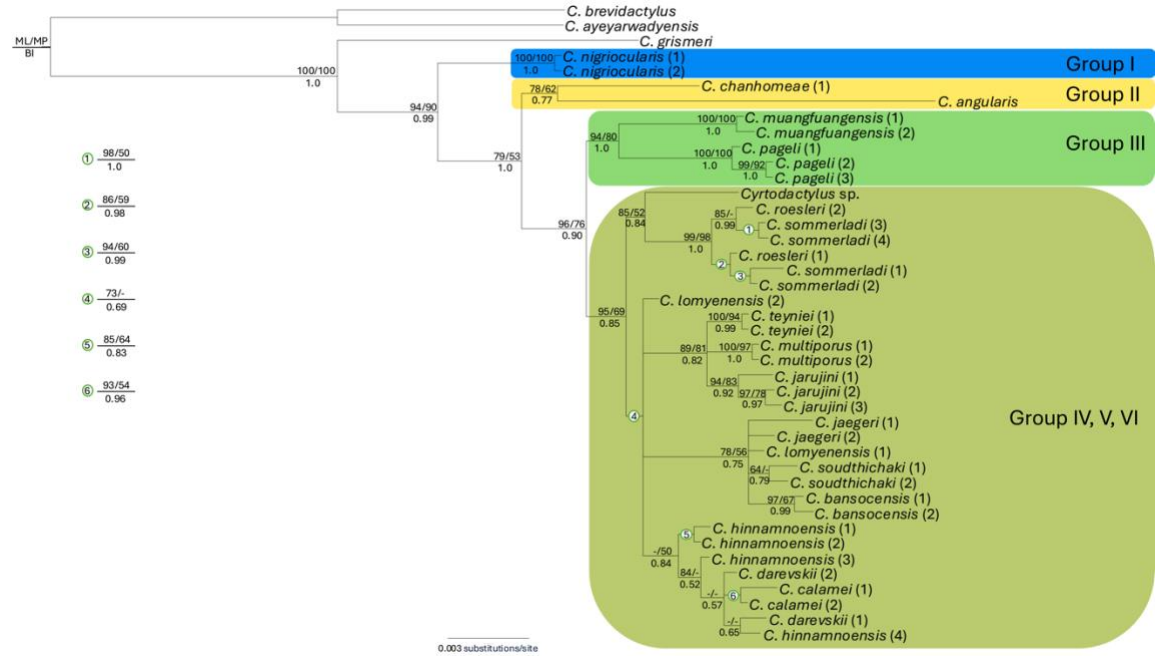

Figure 4. (A) Distribution of the *C. angularis* species group (the altitude data based on GADM database of Global Administrative Areas, 2021). MEK: Mekong Lowland, NAN: Northern Annamites, WINDO: West Indochina. (B) Divergence time estimation and ancestral area reconstruction of the *C. angularis* species group using the combined dataset. Each node is labeled with a number, which can be used to check statistical details in Supplementary 2.

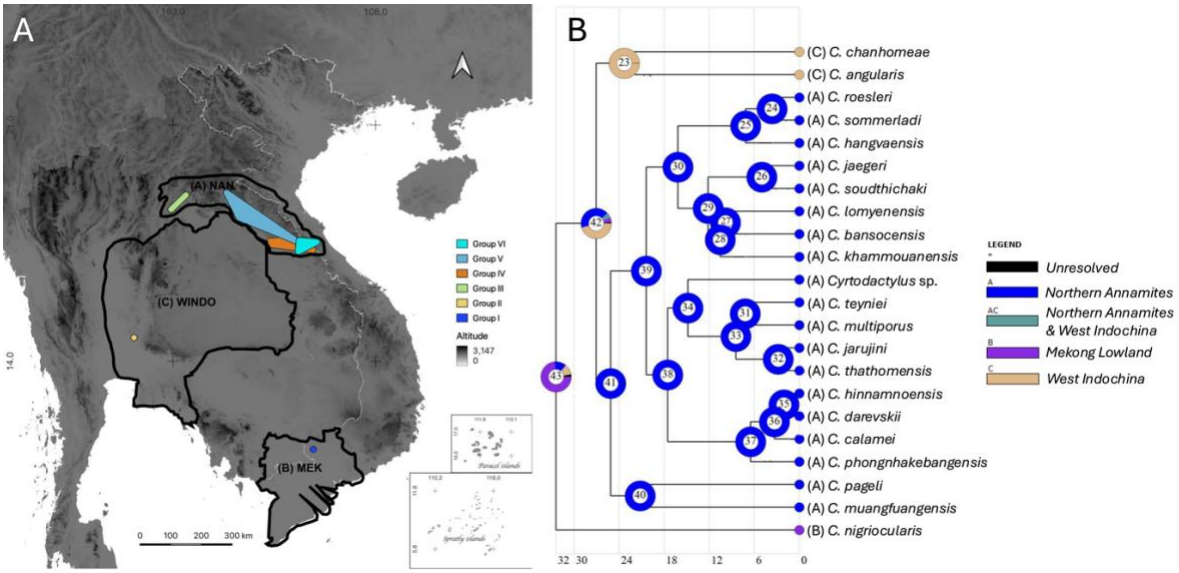

Figure 5. Time calibration using the BEAST. The blue bars show 95% highest posterior densities (HPD). (C) represents the calibration point (Node 3 and 4).

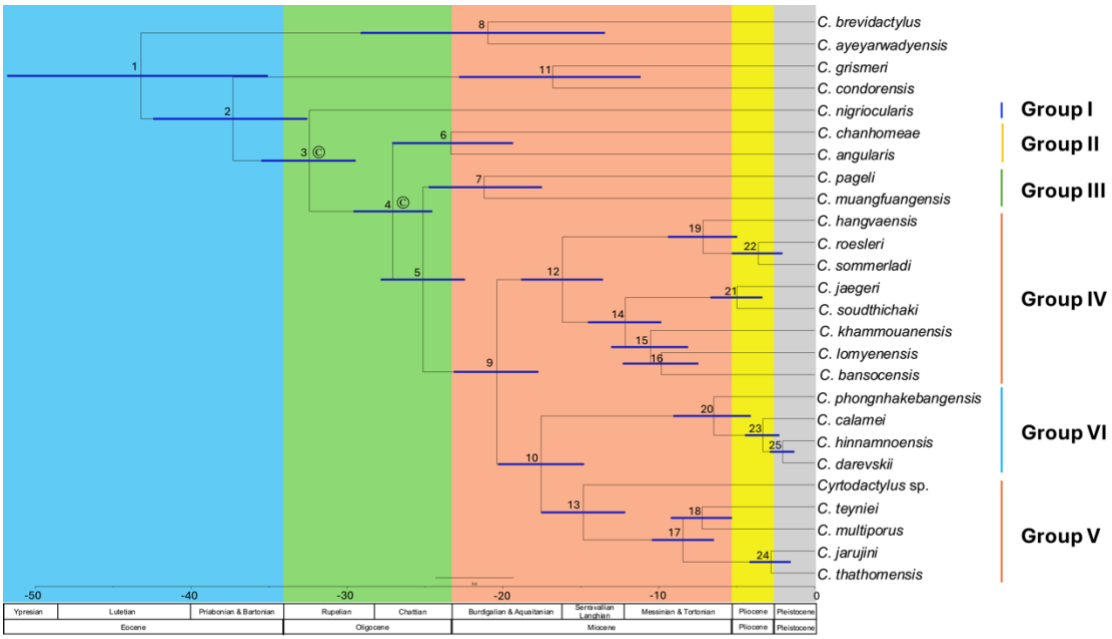

Table 1. Information of sequences used in this study

Species	Voucher number	Genbank accessions							References
		COI	Cytb	ND2	Rpl35	Rag1	PDC	Cmos	
C. ayeyarwadyensis	CAS216446	-	EU268380	EU268348	GU458039	EU268287	EU268317	JQ945550	[34–36]
C. brevidactylus	CAS214104	MF169905	-	-	GU458037	JX440687	JX440636	-	[5, 35, 37]
C. condorensis	UNS0431	MF169910	-	MF169958	-	-	-	-	[37]
C. grismeri	LSUHC8638	-	-	JX440538	-	JX440698	JX440647	-	[5]
C. angularis[*]	FMNH265815	-	-	JX041340	GU458041	JQ945301	JX440632	JQ945549	[5, 35, 36]
C. bansocensis (1)	VFU R.2015.20	KU175573	PV463231	MT953469	-	PV463332	PV463286	PV463272	This study
C. bansocensis (2)[*]	VNUF R.2016.4	ON145865	PV463232	PV475491	PV463382	PV463331	PV463283	PV463273	This study
C. calamei (1)	NUOL R.2015.22	KX064043	-	PV475485	PV463378	PV463350	PV463294	PV463266	This study
C. calamei (2)[*]	VNUF R.2015.28	KX064044	-	PV475486	PV463379	PV463338	PV463293	PV463265	This study
C. chanhomeae (2)	AP018117	AP018117	AP018117	AP018117	-	-	-	-	[38]
C. chanhomeae (1)[*]	CUMZ2003.62	MF169908	-	JX440529	-	JX440688	JX440637	-	[5, 37]
C. darevskii (1)*	VNUF R.2016.306	PV454675	PV463243	PV475483	PV463375	PV463336	PV463309	PV463254	This study
C. darevskii (2)	VNUF R.2016.342	PV454676	Pv463244	PV475484	PV463377	PV463321	PV463310	PV463264	This study
C. darevskii (3)	ZISP FN187	HQ967223	-	-	-	-	-	-	NCBI
C. darevskii (4)	ZISP FN256	HQ967221	-	-	-	-	-	-	NCBI
C. hangvaensis[*]	DBN000132	PP865549	-	PP853075	-	-	-	-	[32]
C. hinnamnoensis (1)[*]	IEBR A.2013.89	KX064045	PV463239	PV475479	PV463373	PV463323	PV463292	PV463269	This study
C. hinnamnoensis (2)	VNUF R.2017.11	PV454679	PV463240	PV475480	PV463376	PV463322	PV463311	PV463259	This study
C. hinnamnoensis (3)	VNUF A.2015.3	KX064046	PV463241	PV475481	-	PV463342	PV463302	PV463263	This study
C. hinnamnoensis (4)	VNUF R.2014.489	PV454678	PV463242	PV475482	PV463374	PV463337	PV463296	PV463253	This study
C. jaegeri (1)[*]	NUOL R.2013.1	KT004365	PV463228	PV475489	PV463380	PV463333	PV463285	PV463268	This study
C. jaegeri (2)	VFU TK.914	KT004366	PV463229	-	PV463381	PV463334	PV463284	PV463262	This study
C. jarujini (1)	FMNH255472	-	-	JX440541	-	JQ945303	JX440651	JQ945552	[5, 36]
C. jarujini (2)	VNUF R.2015.7	KX077907	-	PV475476	PV463370	PV463345	PV463300	PV463252	This study
C. jarujini (3)[*]	VNUF R.2016.01	ON145876	PV463236	PV475477	PV463369	PV463346	PV463291	PV463261	This study
C. khammouanensis (1)[*]	ZISP FN191	HM888467	-	-	-	-	-	-	NCBI
C. khammouanensis (2)	ZISP FN192	HM888468	-	-	-	-	-	-	NCBI
C. lomyenensis (1)[*]	VFU R.2015.14	KU175572	PV463233	PV475490	-	-	PV463287	PV463255	This study
C. lomyenensis (2)	UNS0527	ON145866	-	PV475471	-	-	PV463289	PV463257	This study
C. lomyenensis (3)	UNS0534	-	-	MF169966	-	-	-	-	[37]
C. multiporus (1)[*]	ZMMU R-13985-2	PV454680	PV463237	PV475474	PV463371	PV463347	PV463297	PV463270	This study
C. multiporus (2)	ZMMU R-13985-3	PV454681	PV463238	PV475475	PV463372	PV463348	PV463301	PV463271	This study
C. muangfuangensis (1)[*]	VNUF R.2018.32	MN395826	PV463222	PV475492	PV463365	PV463351	PV463312	PV463281	This study
C. muangfuangensis (2)	NUOL R.2018.33	MN395827	PV463223	-	PV463366	PV463352	PV463313	PV463282	This study
C. nigriocularis (1)	VNMN 2184	ON145793	-	MT953485	PV463353	PV463319	PV463317	PV463247	This study
C. nigriocularis (2)[*]	VNMN 2189	ON145794	PV463246	PV475466	PV463354	PV463320	PV463318	PV463248	This study
C. pageli (1)[*]	IEBR A.2010.1	KJ817431	-	-	PV463362	PV463339	PV463314	PV463249	This study
C. pageli (2)	NQT 2010.37	KX077902	-	-	PV463363	PV463341	PV463315	PV463250	This study
C. pageli (3)	ZFMK91827	KX077903	-	MT953487	PV463364	PV463340	PV463316	PV463251	This study
C. phongnhakebangensis[*]	PNKN2011.19	KF929528	-	-	-	-	-	-	[39]
C. roesleri (1)	ZFMK89377	ON145862	-	-	PV463359	PV463324	PV463308	PV463274	This study
C. roesleri (2)[*]	ZFMK89378	ON145863	-	-	PV463358	PV463325	PV463307	PV463275	This study
C. sommerladi (1)	HNN68	KJ817437	PV463224	PV475467	PV463356	PV463329	PV463303	PV463276	This study
C. sommerladi (2)[*]	IEBR A.2015.57	KX064040	PV463225	PV475468	PV463355	PV463328	PV463306	PV463277	This study

C. sommerladi (3)	VNUF R.2017.28	PV454673	PV463226	PV475469	PV463357	PV463326	PV463305	PV463278	This study
C. sommerladi (4)	VNUF R.2017.206	PV454674	PV463227	PV475470	PV463360	PV463327	PV463304	PV463279	This study
C. soudthichaki (1)*	VFU R.2015.18	KX077905	PV463230	PV475487	PV463383	PV463335	PV463299	PV463260	This study
C. soudthichaki (2)	IEBR A.2015.34	KX077906	-	PV475488	PV463384	PV463330	PV463288	PV463258	This study
Cyrtodactylus sp*	IEBR KM2012.54	KJ817436	PV463245	PV475478	PV463361	PV463349	PV463290	PV463280	This study
C. thathomensis (1)	ZMMU R14919.2	MG791875	-	-	-	-	-	-	[40]
C. thathomensis (2)*	ZMMU R14919.3	MG791874	-	-	-	-	-	-	[40]
C. teyniei (1)*	IEBR KM2012.77	KJ817430	PV463234	PV475472	PV463368	PV463343	PV463295	PV463267	This study
C. teyniei (2)	VNUF R.2016.302	ON145872	PV463235	PV475473	PV463367	PV463344	PV463298	PV463256	This study

* samples used in BEAST analysis

Table 2. PCR primers used in this study

Gene	Primer	Primer sequences (5' – 3')	Reference
COI	VF1d	TTCTCAACCAACCACAARGAYATYGG	[13]
	VR1d	TAGACTTCTGGGTGGCCRAARAAYCA	
Cytb	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	[41]
	H16064	CTTTGGTTTACAAGAACAATGCTTTA	
ND2 + tRNA	MetF1	AAGCTTTCGGGCCCATACC	[42]
	COIR1	AGRGTGCCAATGTCTTTGTGRTT	[43]
Rpl35	N66	GCTAAACAAGCACAGAGTTGATCC	[35]
	N67	TCAGGCTCAGAAAGRACTATTATGG	
Rag1	R13	TCTGAATGGAAATTCAAGCTGTT	[44]
	CyrRag1	CTCCTTGTGRCTAGAAAGAT	This study
PDC	PHOF1	AGATGAGCATGCAGGAGTATGA	[45]
	PHOR1	TCCACATCCACAGCAAAAAACTCCT	
Cmos	G73	GCGGTAAAGCAGGTGAAGAAA	[46]
	G74	TGAGCATCCAAAGTCTCCAATC	

Table 3. Best-fit models for all the datasets in MrBayes

Gene			**Model**		
Mitochondrial dataset					
COI + *cytb* + *ND2* + tRNA					GTR+I+G
Nuclear dataset					
Rpl35 + *Rag1* + *PDC* + *Cmos*					GTR+G
Combined dataset					
COI + *cytb* + *ND2* + tRNA + *Rpl35* + *Rag1* + *PDC* + *Cmo*s					GTR+I+G
Partitioned datasets					
COI					
Codon position 1	GTR+I+G	Codon position 2	K81uf+I	Codon position 3	GTR+G
cyt*b*					
Codon position 1	GTR+G	Codon position 2	TVM+I+G	Codon position 3	TIM+I+G
ND2					
Codon position 1	TVM+I+G	Codon position 2	GTR+I+G	Codon position 3	GTR+I+G
Rpl35					
Codon position 1	K81uf+G	Codon position 2	K80+I	Codon position 3	K81uf+G
Rag1					
Codon position 1	TrN	Codon position 2	HKY+G	Codon position 3	TVM+G
PDC					
Codon position 1	TVM+G	Codon position 2	TrN	Codon position 3	F81+I
Cmos					
Codon position 1	TrN	Codon position 2	F81+I	Codon position 3	TVM+G

Table 4. Best-fit models for each gene in BEAST

Gene	Model	Gene	Model
COI	GTR+I+G (gamma category count = 4, shape = 1.107, proportion invariant = 0.554, rate AC = 0.4791, rate AG = 9.4493, rate AT = 0.4121, rate CG = 0.1979, rate CT = 3.0282, rate GT = 1.0)	*ND2*	GTR+I+G (gamma category count = 4, shape = 0.948, proportion invariant = 0.34, rate AC = 0.3961, rate AG = 3.9842, rate AT = 0.4257, rate CG = 0.2887, rate CT = 2.7116, rate GT = 1.0)
Cytb	TIM1+I+G (gamma category count = 4, shape = 1.589, proportion invariant = 0.481)	*Rag1*	TPM1uf+G (gamma category count = 4, shape = 0.501)
Rpl35	HKY+G (kappa = 2.0, gamma category count = 4, shape = 0.906)	*PDC*	K80+G ((gamma category count = 4, shape = 0.207)
		Cmos	TrN

Table 5. Time calibration for nodes in the phylogeny. Node numbers defined in Fig. 5

Node	**Age estimate (million years)**	**95% HPD (million years)**	**Node**	**Age estimate (million years)**	**95% HPD (million years)**
1	43.22	35.09-51.79	13	14.84	12.16-17.54
2	37.32	32.55-42.43	14	12.15	9.85-14.54
3	32.42	29.46-35.48	15	10.53	8.13-13.05
4	27.07	24.54-29.58	16	9.85	7.48-12.31
5	25.13	22.44-27.84	17	8.46	6.48-10.44
6	23.34	19.36-27.09	18	7.22	5.32-9.23
7	21.21	17.51-24.76	19	7.17	4.98-9.41
8	20.96	13.47-29.12	20	6.48	4.11-9.07
9	20.40	17.73-23.15	21	5.00	3.37-6.68
10	17.55	14.80-20.31	22	3.62	2.07-5.31
11	16.81	11.18-22.80	23	3.33	2.28-4.46
12	16.18	13.59-18.82	24	2.81	1.55-4.18
			25	2.06	1.31-2.88

Chapter 9. Molecular phylogeny and biogeography of the Cyrtodactylus chauquangensis group

(To be submitted to Biology)

Molecular phylogeny and biogeography of the *Cyrtodactylus chauquangensis* group

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Abstract

The *Cyrtodactylus chauquangensis* species group is a large limestone karst radiation with at least 28 nominal species and has a broad distribution range with seven species found in northwestern Thailand, five in south-central China, five in northern Laos and 11 species in northern Vietnam. Interestingly, 26 species in this group are known only from their type locality. To trace the biogeographic pattern of this group, we reconstruct its phylogenetic relationships and diversification history using three mitochondrial genes and four nuclear genes. Our results showed that *C. chauquangensis* is a monophyletic group and might have originated from the Northwest Uplands of the Indochina region, including northern Laos and part of northwestern Vietnam, during the Miocene and subsequently dispersed into northwestern Thailand and southern China and finally reached the remaining part of northern Vietnam. Several lineages within this group diverged during the Miocene era when the East Asia monsoon was developed and increased precipitation in the region.

KEYWORDS: bent-toed gecko, East Asia monsoon, evolution, karst, Miocene

1. Introduction

Karst regions (hereafter referred to simply as karsts) may be defined as landscapes and caves that developed on soluble rocks such as carbonates (limestone, dolomite and marble), evaporite rock (anhydrite, gypsum, and halite), and some partially soluble non-carbonates such as quartzite and siliceous sandstones (Ford and Williams 2007; Lewin and Woodward 2009; Goldscheider et al. 2020). They are found in widely scattered sections across all continents of the world (Goldscheider et al. 2020; Huang et al. 2022). Karsts cover an area of approximately 2.2 x 10^7 square kilometers (km^2), and they account for around 15% of the global land area (Falkowski et al. 2000). Karsts offer a variety of valuable natural resources such as unique landscapes and caves with high cultural, historical and tourism value (Goldscheider 2019); raw material (carbonate rock) used to manufacture commercially valuable products, mainly cement production (Hobbs and Gunn 1998; Clements et al. 2006); thermal and mineral water for geothermal energy production (Goldscherder et al. 2010) and freshwater for agricultural and other activities of human life (Bakalowicz 2005). Karsts are also recognized as important ecosystems and contain high levels of biodiversity and unique and endemic species both at the land surface and underground (Clement et al. 2006; Grismer et al. 2021a). For example, around 80% of the total land snail fauna have been found on karsts in Malaysia, or 1/3 of the total flora occurs on karsts in Brazil (Clement et al. 2006; Nielsen et al. 2017; Bystriakova et al. 2019). Therefore, they

have served as ‘natural laboratories’ for ecological, evolution, and cultural studies (Clements et al. 2006).

In Southeast Asia (including southern China), karsts cover an area of around 900,000 km^2, making it one of the premier karst regions in the world, with a limestone area (Day & Urich 2000; Clements et al. 2006; Quah et al. 2021; Zhu 2007). It is widely distributed in nine of the 12 countries, including Thailand, China, Indonesia, Vietnam, Laos, Philippines, Myanmar, Malaysia, and Cambodia (Chen et al. 2017; Goldscheider et al. 2020). Of these, China, Laos, and Vietnam have long been recognized as regions of global importance with regard to biodiversity (Myers et al. 2000) and the karst ecosystems of China, Laos, and Vietnam are known to support high levels of micro-endemism in plants, e.g., *Calocedrus rupestris* and genus *Vietorchis* are endemic to limestone mountains of northern Vietnam (Averyanov et al. 2008; Samigulin et al. 2024); primates, e.g., Francois’ langur (*Trachypithecus francoisi*) occurs only in south China and northern Vietnam, Delacour’s langur (*Trachypithecus delacouri*) is endemic in Vietnam, Hatinh langur (*Trachypithecus hatinhensis*) is restricted in northern Vietnam, southern China and eastern Laos, Cat Ba langur (*Trachypithecus poliocephalus*) is only found in northern Vietnam and southern China (Nadler et al. 2003); small mammals, e.g., the Laotian Rock Rat (*Laonastes aenigmamus*) and Paulina’s Limestone Rat (*Saxatilomys paulinae*) are only recorded in central Laos and central Vietnam (Sterling et al. 2006; Nguyen et al. 2014). However, the karsts of China, Laos, and Vietnam, with steep and rocky slopes, may be vulnerable to many kinds of disasters, such as drought, flood, and acid rain. In addition, karsts are vulnerable to various anthropogenic disturbances, such as logging, commercial exploitation, and shifting cultivation. Many karst outcrops are being quarried for limestone (Fig. 1), which not only makes it more challenging to deal with disaster prevention measures but also poses a threat to the karst’s unique ‘ark of biodiversity’ (Clements et al. 2006).

The gekkonid genus *Cyrtodactylus* Gray is by far the most speciose genus of the family Gekkonidae and the third largest vertebrate group (Grismer et al. 2021a, b; 2022; Ngo et al. 2022). It currently contains 372 nominal species (as of 11 September 2024; Uet et al. 2024), ranging from South Asia to the Solomon Islands (Grismer et al. 2021a, b). As a result of being broadly distributed, species in this genus bear various habitat types, ranging from terrestrial to arboreal and from intertidal to swamp, volcanic and limestone karst (Grismer et al. 2020, 2021, 2022; Ngo et al. 2022). According to Grismer et al. (2020), at least nine major habitat preferences were identified (including general, arboreal, trunk, karst, granite, cave, terrestrial, swamp, and intertidal), and further habitat preferences might be recorded due to the provision of detailed microhabitat information in further studies. Among nine

habitat preferences, karst habitats have been recognized as the second most common habitat preference and are the foci of speciation (Grismer et al. 2020, 2021b, 2022). In addition, the remarkable increase in the discovery of new species in the karst habitat makes it underscores their unrealized biodiversity (Grismet et al. 2020, 2021b; Wood et al. 2017).

The *Cyrtodactylus chauquangensis* group is one of the two largest groups, including species endemic to limestone karst (Grismer et al. 2020, 2021a, b, 2022; Ngo et al. 2022). It is composed of 28 nominal species in northwestern Thailand (*C. auribalteatus, C. dumnuii, C. doisuthep, C. erythrops, C. kunyai, C. phamiensis, C. phukhanensis*), south-central China (*C. caixitaoi, C. gulinqingensis, C. hekouensis, C. menglianensis, C. zhenkangensis*), northern Laos (*C. houaphanensis, C. ngoiensis, C. spelaeus, C. vilaphongi, C. wayakonei*), and northwestern Vietnam (*C. bichnganae, C. bobrovi, C. cucphuongensis, C. chauquangensis, C. huongsonensis, C. martini, C. puhuensis, C. otai, C. soni, C. sonlaensis, C. taybacensis*) (Grismer et al. 2021a, b, 2024). Until recently, no *Cyrtodactylus chauquangensis* group species were known from west of the Red River in Vietnam, and the first species of this group was discovered north of the Red River, *C. luci* (Tran et al. 2024; Grismer et al. 2024). Among them, 26 species in the *Cyrtodactylus chauquangensis* group are known only from type localities (Nguyen et al. 2015, 2017; Pham et al. 2019; Grismer et al. 2024). It indicates that limestone karsts are extremely important not only for *Cyrtodactylus* diversity but also for protecting *Cyrtodactylus* species, especially *Cyrtodactylus chauquangensis* group.

Although several studies have attempted to build phylogenetic trees and estimate divergence times and historical diversification of the genus *Cyrtodactylus* (Wood et al. 2012; Brennan et al. 2017; Grismer et al. 2020, 2021b, 2022; Ngo et al. 2022), studies either did not reconstruct phylogenetic trees for the *Cyrtodactylus chauquangensis* group or did not include all its members to date. Additionally, previous studies have primarily been restricted to the use of one or two mitochondrial genes (Grismer et al. 2024). Therefore, the goal of this study is to elucidate the phylogenetic relationships for 28 species of the *Cyrtodactylus chauquangensis* group and to infer its historical biogeography based on three mitochondrial genes (cytochrome c oxidase subunit I – COI, NADH dehydrogenase subunit II – ND2, cytochrome b – cytb) and four nuclear genes (Cmos, PDC, RAG1, Rpl35). Knowing details about how and when the group originated and dispersed can provide insight into understanding the plausible biogeographic affinity between Indochina, northwestern Thailand and south-central China.

2. Materials and methods

2.1. Taxon sampling and Data collection

28 samples were used for each of the 15 species of *Cyrtodactylus chauquangensis* group (Table 1). Based on the results from previous phylogenetic analyses of the genus *Cyrtodactylus* (Wood et al. 2012; Grismer et al. 2021a,b, 2022), three outgroup species, namely *C. elok, C. interdigitalis,* and *C. loriae* were selected. Detailed information of the newly generated sequences in this study and GenBank accession numbers of the existing ones are given in Table 1.

2.2. DNA extraction, Amplification and Sequencing

The bent-toed gecko lizard genomic DNA was extracted from leg muscle or tail tissue of 28 specimens using DNeasy Blood and Tissue (Qiagen, Germany) or the GeneJET Genomic DNA Purification kit (Thermo Fisher Scientific, Lithuania), following the manufacturer's instructions. Seven molecular markers, comprising three mitochondrial loci, cytochrome c oxidase subunit I (COI), cytochrome b (cytb), and NADH dehydrogenase subunit 2 (ND2) (including tRNA) and four nuclear loci, Cmos, phosducin (PDC), recombination activating protein 1 (Rag1), ribosomal protein L35 (Rpl35) were used in this study. PCR amplification was performed in a total volume of 21 µl that contained 2 µl template DNA, 2 µl of each primer and 10 µl DreamTaq Mastermix (Thermo Fisher Scientific, Lithuania) or HotStarTaq Mastermix (Qiagen, Germany). The primers used to amplify the fragment's loci are listed in Table 1. The reaction was carried out with an initial denaturation at 95ºC for 15 min with HotStar Taq Mastermix or 5 min with Dream Taq Mastermix, followed by 35 cycles of amplification (denaturation at 95ºC for 30 s, annealing at 48ºC – 58ºC for 45 s, and extension at 72ºC for 1 min), with final extension at 72ºC for 10 min. Negative and positive controls were also used in all amplifications and extractions to check for possible contamination. The PCR products were visualized by agarose gel electrophoresis and stored at -4ºC after visualization. The PCR products were purified using GeneJET PCR Purification kit (Thermo Fisher Scientific, Lithuania), in accordance with the manufacturer's instructions. The sequences of the forward and reverse strands were determined for all taxa using the sequencing service from 1st Base (Malaysia). The resulting sequences were edited using Sequencher v5.4.6 (Gene Codes Corporation). Each gene was initially aligned separately using ClustalX v1.8.3 (Thompson et al. 1997) with default settings for complete alignment. All sequences generated for this study were deposited in GenBank under accession numbers shown in Table 1.

2.3. Phylogenetic analyses

The mitochondrial and nuclear datasets (COI + Cytb + ND2 + tRNA and Cmos + Rag1 + PDC + Rpl35) were analyzed both separately and simultaneously using Maximum parsimony (MP) as implemented in PAUP v4.0b10 (Swofford 2001), Bayesian inference (BI) as implemented in MrBayes v3.2.7 (Ronquist et al. 2012) and Maximum likelihood (ML) as implemented in IQtree v1.6.12 (Nguyen et al. 2015). For MP analyses, heuristic analyses were conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch-swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) was calculated using 1,000 pseudo-replicates and 100 random taxon addition replicates. All characters were equally weighted and unordered. For BI analyses, the Markov chain Monte Carlo (MCMC) algorithms were run for 10^7 generations with one cold and three heated chains, starting from random trees and samples one out of every 1,000 generations. The burn-in and convergence diagnostics were graphically accessed using Tracer v1.7.1 to confirm ESS > 200 for all parameters (Rambaut et al. 2018). The cut-off point for the burn-in function was set to 25% of the total number of trees generated. The remaining trees were assumed to be representative of the posterior probability (PP) distribution. For ML analyses, 10,000 ultrafast bootstrap replications (UFBP) were run. Models of nucleotide substitution were selected based on the Akaike Information Criterion (AIC) as determined by jModeltest v2.1.10 (Darriba et al. 2012). All the best models for the three datasets are shown in Table XX. We regard BP ≥ 70% and UFB and PP ≥ 95% as strong support (Hillis and Bull 1993; Ronquist et al. 2012; Bui et al. 2013). We considered nodes with UFB and PP values of 90–94 as well-supported.

The mitochondrial and nuclear dataset was also partitioned into 21 parts by codon positions (first, second, and third) to be used in the multiple-model BI analysis with the above settings for the Markov chains and generations. The best partition scheme and evolutionary model for each partition were selected using PARTITIONFINDER v2.1.1 (Table 3) (Lanfear et al. 2012).

2.4. Divergence time estimation

The partitioned by gene of the mitochondrial and nuclear sequences, consisting of seven loci and 33 taxa, were used to estimate the divergence time of *Cyrtodactylus chauquangensis* group using a Bayesian MCMC approach to co-estimate topology, substitution rates and node ages as implemented in BEAST v2.7.6 (Drummond et al. 2012; Bouckaert et al. 2019). BEAUti was used to set criteria for the analysis, in which the substitution models were unlinked (Table 4), but the molecular clock and trees were linked for each gene partition. A Yule tree prior model was implemented in the analysis, with rate variation across branches assumed to be uncorrelated and lognormally distributed

(Drummond et al. 2006). The dating analysis was run for 200,000,000 generations, with sampling every 1,000 generations. After the dataset with the above settings was analyzed in BEAST, the resulting likelihood profile was then examined by the program Tracer v1.7.2 to confirm the ESS > 200 for all parameters. The final tree with calibration estimates was computed using the program TreeAnnotator v2.7.6 as recommended by the program manual.

For the fossil calibrations, we followed the strategy adopted by Grismer et al., 2022. One calibration point, approximately 22 million years ago, was set to the node representing the first split between members of the *C. chauquangensis* group (Grismer et al. 2022). The substitution models applied to the data matrix were determined using jModeltest v2.1.10 (Table 4) (Darriba et al. 2012).

2.5. Inferring historical biogeography

Considering the areas of endemism and tectonic history of region and subregions suggested by Bain and Hurley (2011), we use six areas for the *Cyrtodactylus chauquangensis* taxa: (A) Northwestern Thailand, (B) South-central China, (C) Northern Annamites, (D) Northeast Lowlands, (E) Northeast Uplands, (F) Northwest Uplands. Each sample was assigned to its respective area according to its contemporary distribution range. Biogeographic inferences were obtained by applying both Bayesian binary MCMC analysis (BBM) and BioGeoBears implemented in RASP v4.3 with default settings (Yu et al. 2010). For BBM analysis, a subset of 1,000 randomly selected trees from the posterior distribution output of BEAST and a final tree from TreeAnnotator v2.7.4 were used and the maximum number of individual unit areas was set to six. The probability of dispersal between areas was maintained as equal. For the BioGeoBears, all biogeographic models, dispersal-vicariance (DIVA); dispersal-extinction-clado-genesis (DEC); and Bayesian analysis of biogeography when the number of areas is large (BayArea), with and without the jump dispersal parameter (j) was tested using "Compare six models using BioGeoBears" function. Afterward, the best-fit model for the data was applied to reconstruct the time-calibrated biogeographic of *C. chauquangensis* group. The maximum number of areas for ancestral distribution was set to six, and the remaining parameters used default settings.

3. Results

3.1. Sequence alignments and data partitioning

The mitochondrial and nuclear (hereafter referred to simply as mt-nu) datasets include 222 sequences, 5,651 bp (COI, 47 sequences, 701 bp; Cytb, 11 sequences, 1,142 bp; ND2, 52 sequences, 1,414 bp;

Cmos, 28 sequences, 387 bp; PDC, 29 sequences, 421 bp; Rag1, 30 sequences, 957 bp; Rpl35, 25 sequences, 267 bp) whereas the mitochondrial (hereafter referred to simply as mt) dataset includes 110 sequences, 3,257 bp (COI, 47 sequences, 701 bp; Cytb, 11 sequences, 1,142 bp; ND2, 52 sequences, 1,414 bp) and the nuclear (hereafter referred to simply as nu) dataset includes 111 sequences, 2,394 bp (Cmos, 27 sequences, 387 bp; PDC, 29 sequences, 421 bp; Rag1, 30 sequences, 957 bp; Rpl35, 25 sequences, 267 bp) (Table 1).

3.2. Phylogenetic analysis

The mt-nu datasets (COI + Cytb + ND2 + tRNA and Cmos + Rag1 + PDC + Rpl35) consisted of 5,651 aligned characters, of which 4,088 were constant, 1,315 were parsimony-informative and 248 variable characters were parsimony-uninformative. In the MP analysis, the single most parsimonious tree with 4,829 steps was recovered (Consistency index = 0.47, retention index = 0.71). The tree obtained from ML, MP and BI and Bayesian partitioned analyses based on the mt-nu datasets are shown in Fig. 2. The aligned mt dataset (COI + Cytb + ND2+ tRNA) comprises 3,257 positions, of which 1,856 were constant, 1,220 were parsimony-informative and 181 variable characters were parsimony-uninformative. The single most parsimonious tree with 4,624 steps was recovered (Consistency index = 0.46, retention index = 0.71). The nu matrix (Cmos + Rag1 + PDC + Rpl35) includes 2,394 characters, of which 2,232 were constant, 95 were parsimony-informative and 67 variable characters were parsimony-uninformative. The single most parsimonious tree with 204 steps was recovered (Consistency index = 0.82, retention index = 0.86). The tree inferred from the ML, MP and BI analyses are shown in Fig. 3 and Fig. 4.

Overall, the ML, MP and BI analyses of both separately and simultaneously mitochondrial and nuclear datasets retrieved very similar topologies, recovering 48 of the same 54 nodes (88.89%) in phylogenies generated by the mt dataset, 15 of the same 24 nodes (62.50%) by the nu dataset and 48 of the same 55 nodes (87.27%) by mt-nu datasets, even though PP, BP, UFB were weakly within some nodes (Fig. 2 – Fig. 4). Phylogenies generated by the mt-nu datasets were very similar to those produced by the mt dataset and significantly different from those of the nu dataset. All analyses resulted from mt-nu datasets and the mt dataset strongly supported the monophyly of the *Cyrtodactylus chauquangensis* group by grouping all members together with high support values (PP = 100, BP = 100, and UFB = 100 in both two datasets). In comparison, the monophyly of the *Cyrtodactylus chauquangensis* group was only strongly supported in MP analysis by nu dataset (UFB = 84, BP = 99 and PP = 90). In addition, the monophylies of Group I, Group II, Group IV, Group V,

Group VI and Group VII were recovered by all analyses of mt-nu and mt datasets (except the MP analysis of Group II, Group V and Group VI by both mt-nu and mt datasets and the ML and BI analyses of Group II by mt dataset). In contrast, the phylogenies inferred by the nu dataset were only supported the monophyly of Group I in all analyses and Group VII in BI analysis (Fig. XX – Fig. XX). Moreover, the nu phylogenies did not resolve interspecific relationships within *Cyrtodactylus chauquangensis* group, particularly the relationships of species within and between the species subgroups in *Cyrtodactylus chauquangensis* group. For example, the relationship between *C. bobrovi, C. cucphuongensis, C. houaphanensis, C. otai,* and *C. puhuensis* in Group VII was unresolved and weakly supported values for the relationship of *C. huongsonensis, C. luci, C. soni,* and *C. sonlaensis* (Group VI).

In comparing the results of the mt dataset with mt-nu datasets, congruent topology was recognized in all nodes (Fig. 2 and Fig. 3). However, the nodal statistic values were improved in trees based on the mt-nu datasets. For example, a node representing Group II from the mt-nu datasets received well-supported values of the ML and BI analyses compared to those resulting from the mt dataset.

The BI, BP, MP, and ML analyses of the mt-nu datasets show similar tree topologies, albeit with variation within some clades (Fig. 2). For example, the MP analysis was not supported or lower supported in several basal nodes, whereas all the latter analyses were strongly or well supported, such as a node that includes Group III-VII (UFB = 94, BP unsupported, PP = 100 and 100) or a node composed of *C. auribalteatus* and *C. kunyai* (UFB = 98, BP = 61, PP = 100 and 100) or a node consist of *C. gulinqingensis, C. hekouensis, C. huongsonensis, C. luci, C. soni* and *C. sonlaensis* (UFB = 98, BP = 64, PP = 100 and 100). In general, the *C. chauquangensis* species clustered in seven major subclades with significant statistical values, except for Group II, V and VI where the MP analysis recovered weakly relationship: Group I (UFB = 100, BP = 100, PP = 100 and 100), Group II (UFB = 96, BP = 54, PP = 98 and 92), Group III (UFB = 100, BP = 100, PP = 100 and 100), Group IV (UFB = 100, BP = 98, PP = 100 and 100), Group V (UFB = 98, BP = 61, PP = 100 and 100), Group VI (UFB = 98, BP = 64, PP = 100 and 100), Group VII (UFB = 100, BP = 100, PP = 100 and 100). When combined with geographic data and subregions based on Bain and Hurley (2011), seven distinct phylogenetic groups are distributed in several areas of endemism. Specifically, while species in Group I, Group II, Group III and Group V are restricted to only one subregion (Northwest Uplands or Northwestern Thailand), members of Group IV, VI and VII occur in several different subregions. In particular, Group I consists of two species (*C. bichnganae* and *C. taybacensis*) belonging to Northwest Uplands (NWU); Group II, Group III and Group V are composed of four (*C. doisuthep, C. erythrops,*

C. phamiensis and *Cyrtodactylus* sp), one (*C. dumnuii)* and two species (*C. auribalteatus* and *C. kunyai*), respectively, that occur in Northwestern Thailand (NWTL); Group IV includes six species (*C. caixitaoi, C. martini, C. menglianensis, C. phukhaensis, C. zhenkangensis,* and *C. wayakonei*) spreading from South-central China to NWU and NWTL; Group VI contains six species (*C. gulinqingensis, C. hekouensis, C. huongsonensis, C. luci, C. soni* and *C. sonlaensis*) ranging from South-central China, Northeast Uplands (NEU) to NWU and Northeast Lowlands (NEL); Group VII consist of nine species occupying NWU, NEL and Northern Annamites (NAN) (Fig. 5).

3.3. Divergence dating analysis

The combined dataset for seven genes consisted of 33 taxa (Fig. 6). The results of divergence time estimation showed that all the significant divisions of *C. chauquangensis* occurred during the Miocene Period, except nine divisions in the Pliocene and Pleistocene (Fig. 6, Table 5). The diversification of *C. chauquangensis* began *ca.* 22.29 Mya (19.69-24.94 Mya; node 4; Fig. 6, Table 5) in the Miocene and it was directly split into two ancestral lineages. The first lineage consisted of Group I and Group II, and other lineage included the remaining group. In particular, Group I and Group II began to emerge *ca.* 21.11 Mya (18.46-23.88 Mya; node 5; Fig. 6; Table 5) while Group IV and Group III, V, VI, VII formed *ca.* 20.29 Mya (17.52-23.03 Mya; node 6; Fig. 6; Table 5). Specifically, Group VII and Group III, V, VI diverged *ca.* 19.49 Mya (16.88-22.25 Mya; node 7; Fig. 6; Table 5), whereas the diversification between Group V and Group III-IV tool place ca. 18.75 Mya (15.98-21.56; node 9; Fig. 6, Table 5). In addition, the most recent speciation event was the split between *C. houaphanensis* and *C. puhuensis*, which occurred in the Pleistocene *ca.* 2.2 Mya (1.56-2.86; node 32; Fig. 6; Table 5). Besides *C. houaphanensis* and *C. puhuensis, C. bobrovi* and *C. otai* are the youngest members of the group, which started to speciate from the early Pleistocene, *ca.* 2.43 Mya (1.75-3.16; node 31; Fig. 6; Table 5).

3.4. Inferring historical biogeography

For the BioGeoBEARS analysis, model comparisons revealed that the Bayaralike + J model represents the best-fit biogeographic model to the data and is most likely to infer the correct ancestral range at each node with the highest LnL and the AICc_wt and the lowest AICc (Table 6). In general, both ancestral state reconstructions from BBM and Bayaralike +J analyses indicated a similar pattern of *C. chauquangensis* biogeographic history, with slight variations. Here, the results described are based on the Bayarealike + J analysis (Fig. 7; Supplementary 1). The result of the BBM analysis is shown in Supplementary 2 and 3.

In the reconstruction of the group's ancestral geographic range, several areas of endemism contribute differentially with the Northwest Uplands (NWU) receiving the highest probability of 73.96%, followed by North-western Thailand of 22.19%, south-central China of 1.47%, Northeast Lowlands of 1.36%, Northeast Uplands (NEU) of 1.10% and other areas < 1% (Node 59 in Fig. 7). The most probable ancestral areas for Group I and Group II were the Northwest Uplands (72.19%) and North-western Thailand (27.81%) (Node 58 in Fig. 7). Group III, Group IV, and Group V originated in North-western Thailand (93.16%) (Node 38 in Fig. 7). The ancestral area with the highest probability for Group VI was Northwest Uplands (67.46%) (Node 53 in Fig. 7). Northwest Uplands was also recovered as the most probable ancestral area for Group VII (97.37%) (Node 46 in Fig. 7).

4. Discussion

4.1. Phylogenetic analysis

In this study, we provide our original mt-nu datasets for the *Cyrtodactylus chauquangensis* group, consisting of seven loci: COI, Cytb, ND2, Cmos, PDC, Rag1 and Rpl35. We used our dataset for phylogenetic reconstruction with the separate and simultaneous mitochondrial and nuclear datasets. Importantly, our research is the first study to include all described *Cyrtodactylus chauquangensis* species up to date. Furthermore, comparing the results of our mt analyses with our mt-nu results indicates that combining the mt-nu loci is more effective for the nodal statistic values than only using the mitochondrial loci.

In comparison to the previously published trees, the topology of the tree generated herein is similar to that of Grismer et al. (2024) based on both codon-partitioned and unpartitioned ND2 gene (plus tRNA), Tran et al. (2024) based on one substitution model for ND2 and tRNA, the review study of Ngo et al. (2022) based on COI and Grismer et al. (2021b) based on three substitution model for ND2, tRNA, and Rag1+PDC+Maxr5 genes. All the phylogenetic hypotheses supported by analyses in this study and those studies show that the *C. chauquangensis* group is monophyletic. However, the topology of the tree inferred by Grismer et al. (2024) and Tran et al. (2024) recovered three nodes of seven groups with lower support values than herein. In particular, nodes representing Group II, Group V and Group VI herein received higher values regardless of the ML and/or BI analyses compared to Grismer et al. (2024) (Group II: UFB = 96, PP = 98 *vs.* UFB = 89 and PP = 60 in Grismer et al. 2024, Group V: PP = 100 *vs.* PP = 80 in Grismer et al. 2024, Group VI: PP = 100 *vs.* PP = 90 in Grismer et al. 2024). In comparison, nodes regarding Group IV, Group V and Group VI herein received significant statistics supported in the ML and MP analyses compared to Tran et al. (2024) (Group IV:

UFB = 100, BP = 98 *vs.* UFB = 96, BP = 85 in Tran et al. 2024, Group V: UFB = 98, BP = 61 *vs.* UFB = 92, BP = 56 in Tran et al. 2024, Group VI: UFB = 98, BP = 64 *vs.* UFB = 88, BP = 57 in Tran et al. 2024). The detailed comparison of Grismer et al. (2021b), Ngo et al. (2022) and other previously published trees is difficult because they used only one gene or had incomplete species coverage of the group (Brennan et al. 2017; Grismer et al. 2021b; Ngo et al. 2022). Comparing the topologies of the trees in both our study, Grismer et al. (2024) and Tran et al. (2024) show that the *C. chauquangensis* group is monophyletic although the group was rendered paraphyletic in the study of Chomdej et al. (2022) based on one substitution model for ND2 and tRNA. The increase in statistical support values and the differences in the recovery of the monophyletic may be due to the choice of outgroups.

Grismer et al. (2021b, 2024) and our analyses illustrate that the group's diversity is currently underestimated. At least one population from Thailand is shown to be a potential new species in three studies. Further herpetological surveys in karstic areas of south-central China, northwestern Thailand and northern Indochina will continue to discover numerous new populations of the *C. chauquangensis* species group.

4.2. Time calibration and biogeography

Our time-calibrated molecular results based on three mitochondrial genes and four nuclear genes resemble those reported by Grismer et al. (2022) based on one mitochondrial gene and reveal that diversification of almost major lineages of *C. chauquangensis* occurred during the Miocene Period. In addition, both studies agreed that *C. bobrovi, C. houaphanensis, C. otai* and *C. puhuensis* are the youngest members of the group (Grissmer et al. 2022). However, our results of divergence time estimation showed that the *C. chauquangensis* group began to split between Group I+II and the remaining groups, whereas the *C. chauquangensis* group started to diverge between Group I and the remaining groups in the study by Grismer et al. (2022). Afterward, the split between Group VI and III+IV+V+VII appeared, while Grismer et al. (2022) recovered the emergence between Group III+IV+V and VI+VII. In addition, according to our divergence dating results, Group I appeared at 4.69 Mya, later than a previous study, which proposed 8.91 Mya and Group II emerged at 19.26 Mya, earlier than Grismer et al. (2022), which suggested 11.48 Mya. The difference might be the result of increasing the number of loci. In this study, we used three mitochondrial genes (COI, Cytb, ND2) and four nuclear genes (Cmos, PDC, Rag1 and Rpl35), while a study by Grismer et al. (2022) included only one mitochondrial gene (ND2).

The results of this study indicate that the ancestor of the *C. chauquangensis* group most probably lived in the Northwest Uplands (area NWU) during the Oligocene – Miocene boundary (~23 Mya); some of their descendants speciated within this area, whereas others dispersed to North-western Thailand (area NWTL), South-central China (area SCC), Northern Annamites (area NAN), Northeast Lowlands (area NEL) and Northeast Uplands (area NEU) before their descendants gave rise to the extant taxa. There were at least two independent dispersals from the central basin to the North-western Thailand zone, including one descendant speciated within this area and another one dispersed northward to South-central China (area SCC) and returned to Northwest Uplands (area NWU) (Fig. 7).

Dispersal of *C. chauquangensis* may have been enhanced by the development of the East Asian monsoon and the accompanying copious precipitation (especially the winter precipitation) around the Oligocene and Miocene boundary. During this period, a transition from broadleaf vegetation to evergreen broadleaf vegetation and plant diversity increased, which probably provided suitable habits and hosts for members of the *C. chauquangensis* group. In addition, precipitation from the East Asia monsoon likely accelerated the dissolution of the limestone substrate and deeply influenced the development of the karst region, which possibly contributed to the expansion and divergence of this group (Li et al. 2021, 2022; Chen et al. 2023).

5. Conclusions

In this study, we used three mitochondrial genes and four nuclear genes to construct the most comprehensive phylogenies of *C. chauquangensis* group to date. These results have updated our understanding of intrageneric relationships within this group. Our phylogenies were based on the mitochondrial dataset and combined dataset of mitochondrial nuclear support monophyly of the *C. chauquangensis* group. Our biogeographical analyses indicate interesting distribution patterns for this group. The *C. chauquangensis* group is most likely to have originated in the area corresponding to the present-day Northwest Uplands, followed by dispersal northwards to Southcentral China and dispersal eastwards to southeastwards to Northwestern Thailand and dispersal westwards to Northeast Lowlands and Northeast Uplands.

Supporting information

Supplementary 1. Detailed output of the BioGeoBears analysis

Supplementary 2. Divergence time estimation and ancestral area reconstruction of the *C. chauquangensis* species group using BBM analysis.

Supplementary 3. Detailed output of the BBM analysis

Acknowledgments

We thank T.V. Nguyen, H.T. Duong, G.H.T. Cao, T.T. Nguyen, H.M.T. Le for their support in the laboratory. This research was funded by … Doctoral research of H.T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD).

Conflicts of Interest

The authors declare no conflicts of interest.

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Figure 1. Karst quarried for limestone in Son La Province, Vietnam.

Figure 2. Phylogenetic consensus tree inferred from ML, MP and Bayesian analyses with branch length estimated by the Bayesian multiple-model analysis. The phylogeny was recovered using 5,651 aligned characters of a concatenated data set based on three mitochondrial loci (COI, cytb, ND2) and four nuclear loci (Cmos, PDC, Rag1 and Rpl35). Numbers above and below branches are ML ultrafast bootstrap/MP bootstrap values, respectively and Bayesian single-model posterior probabilities/Bayesian multiple-model posterior probabilities, respectively. Asterisk indicates 100% value.

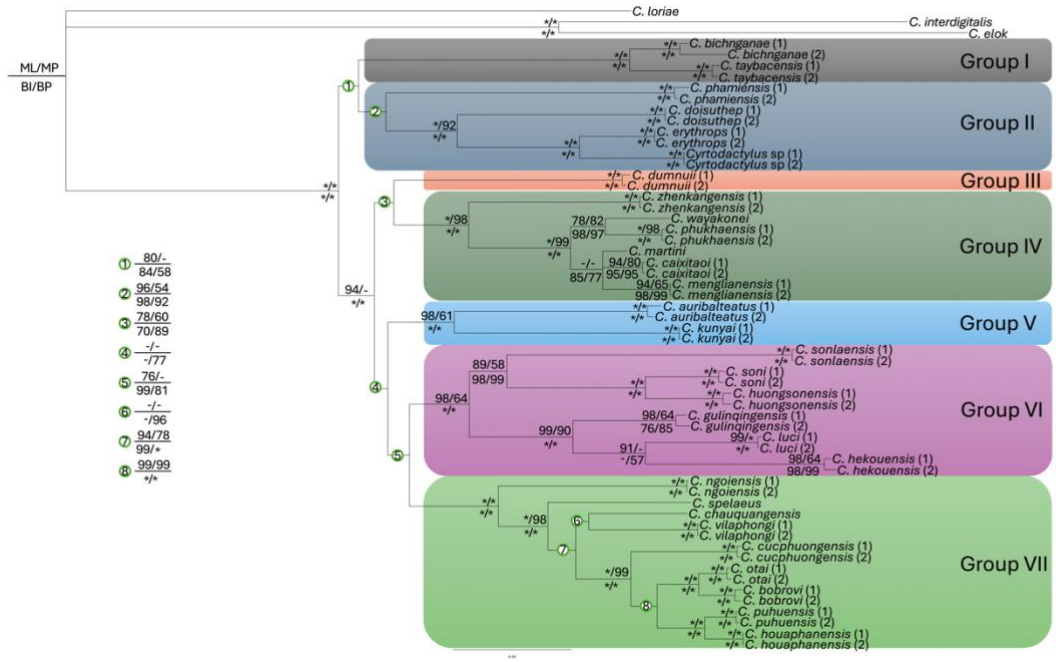

Figure 3. Phylogenetic consensus tree inferred from ML, MP and Bayesian single-model analyses with branch length estimated by the Bayesian analysis. The phylogeny is based on 3,257 aligned characters from three mitochondrial genes, COI, Cytb, and ND2. Numbers above and below branches are ML ultrafast bootstrap values and Bayesian posterior probabilities, respectively. Asterisk indicates 100% value.

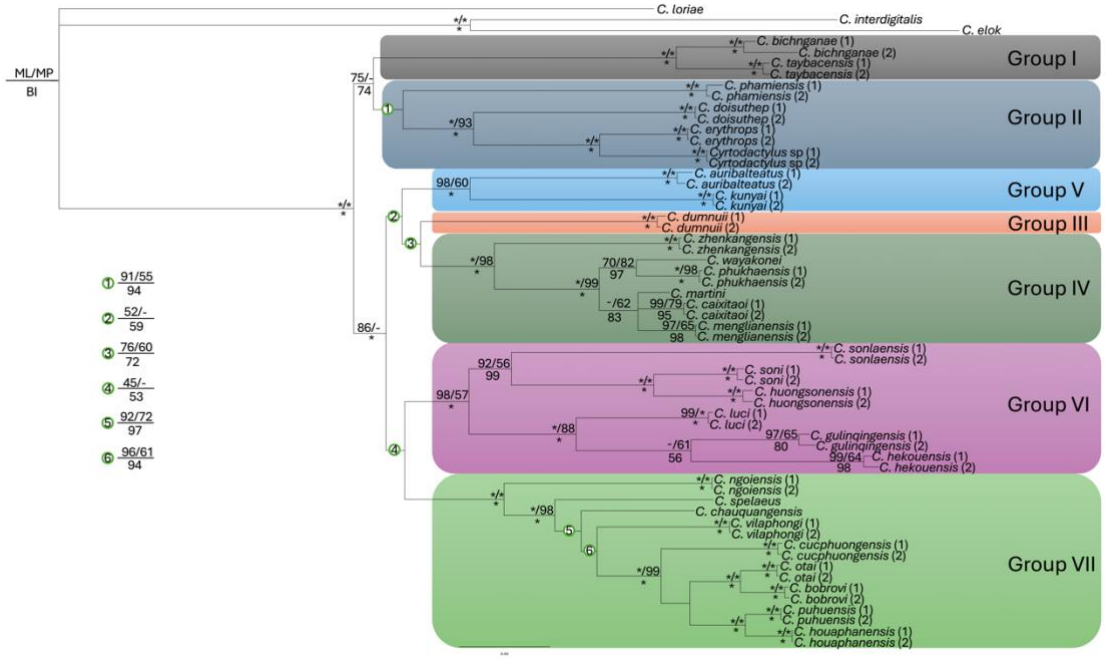

Figure 4. Phylogenetic consensus tree inferred from ML, MP and Bayesian single-model analyses with branch length estimated by the Bayesian analysis. The phylogeny is based on 2,394 aligned characters from four nuclear loci, Cmos, PDC, Rag1, and Rpl35. Numbers above and below branches are ML ultrafast bootstrap values and Bayesian posterior probabilities, respectively. Asterisk indicates 100% value.

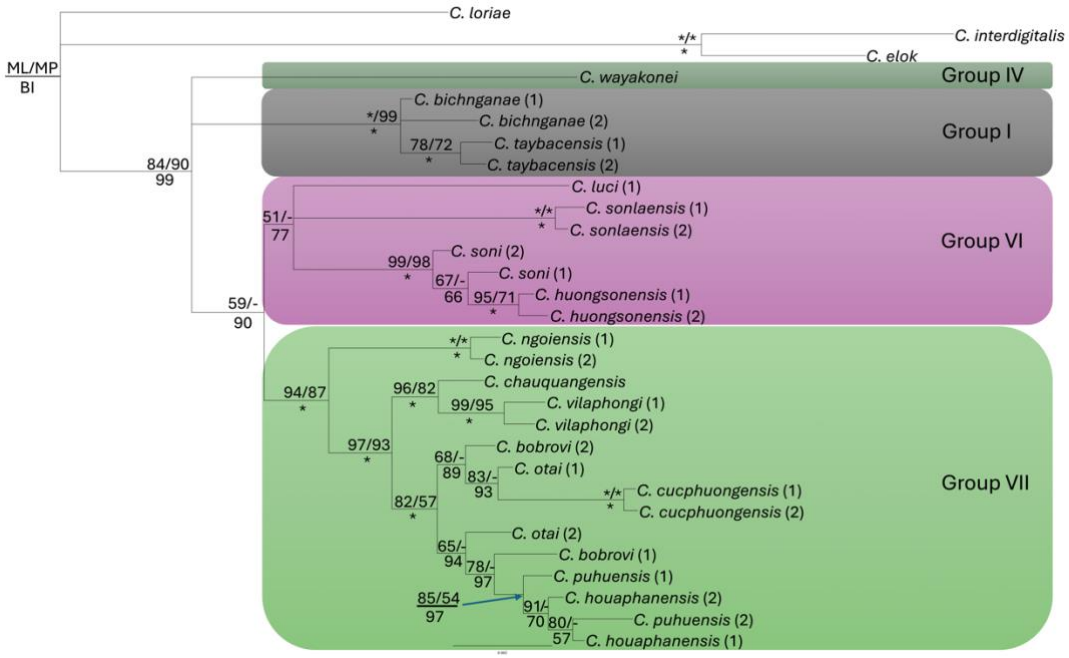

Figure 5. Distribution of the *C. chauquangensis* species group (the altitude data based on GADM database of Global Administrative Areas, 2021)

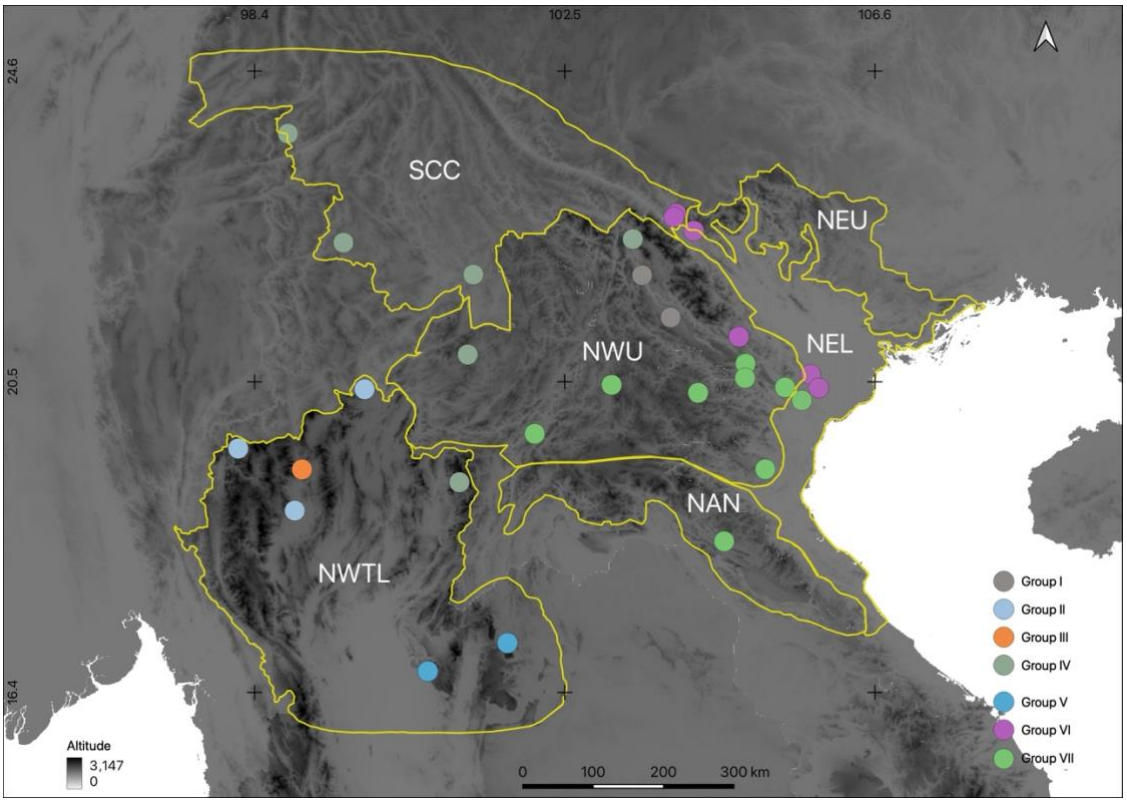

Figure 6. Time calibration using the BEAST. The blue bars show 95% higher posterior densities (HPD)

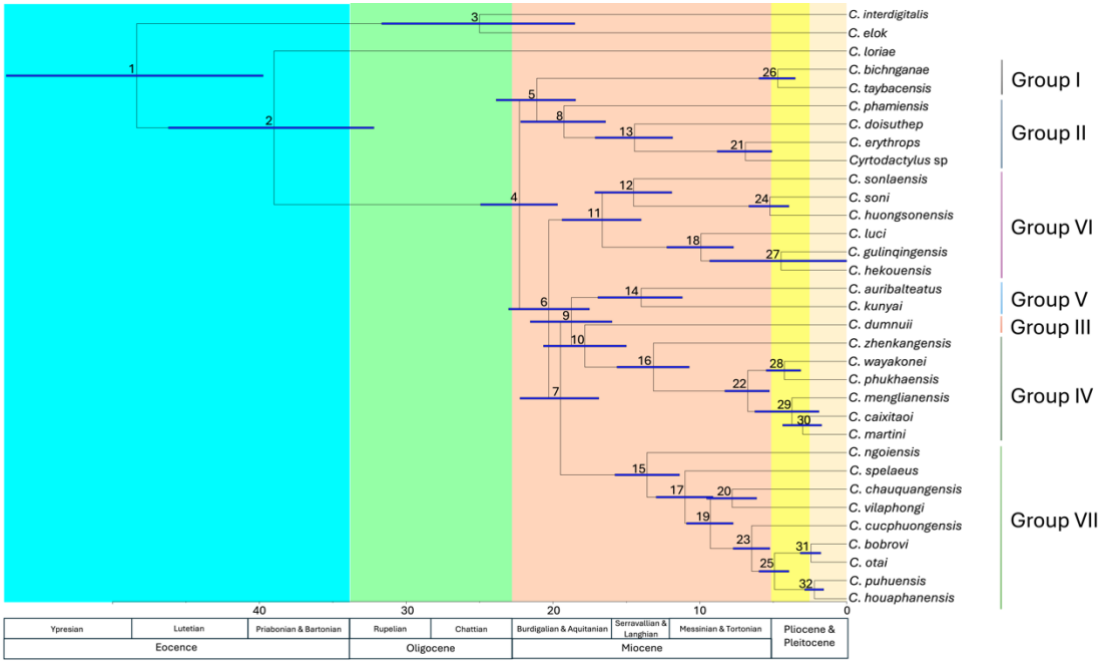

Figure 7. Divergence time estimation and ancestral area reconstruction of the *C. chauquangensis* species group using the combined dataset. Each node is labeled with a number, which can be used to check statistical details in Supplementary 1.

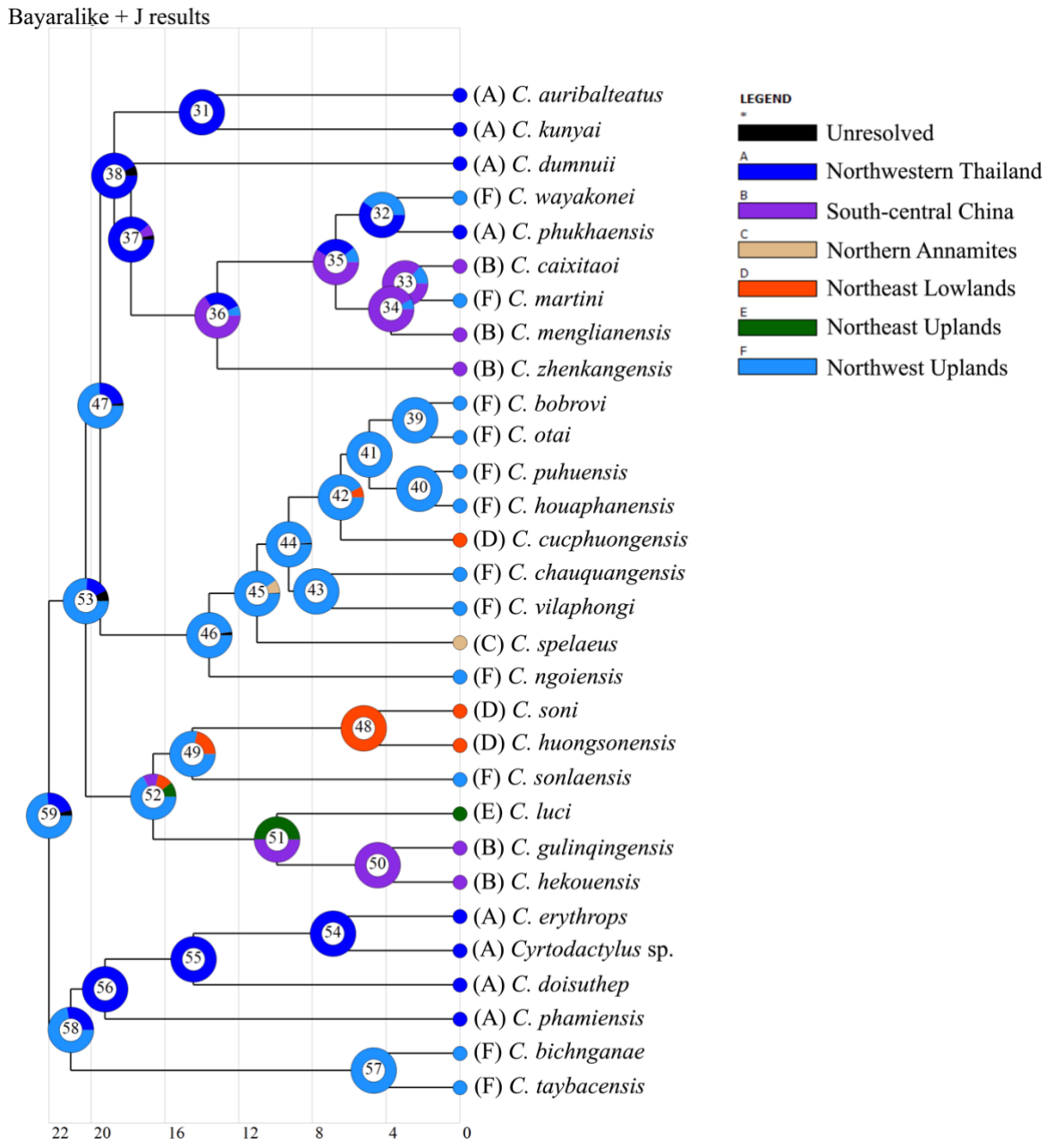

Table 1. Information of sequences used in this study

Species	Catalog number	Genbank accessions						
		COI	Cytb	ND2	Rpl35	Rag1	PDC	Cmos
C. elok	LSUHC6471	-	-	JQ889180	-	JX440694	JX440643	-
C. interdigitalis	FMNH255454	MF169919	-	JQ889181	-	JX440700	JX440648	-
C. loriae	FK7709	MF169925	EU268382					
C. auribalteatus (1)	AP018116	AP018116	AP018116	AP018116	-	-	-	-
C. auribalteatus (2)	AUP01745	MZ439906	-	MZ439914	-	-	-	-
C. bichnganae (1)	Ct120	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. bichnganae (2)	Ct130	XXXXXX	XXXXXX	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX
C. bobrovi (1)	Ct118	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. bobrovi (2)	Ct170	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. caixitaoi (1)	KIZ201904002	OR515257	-	-	-	-	-	-
C. caixitaoi (2)	KIZ201904003	OR515258	-	-	-	-	-	-
C. chauquangensis	Ct141	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. cucphuongensis (1)	Ct380	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. cucphuongensis (2)	Ct382	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. doisuthep (1)	AUP00774	-	-	MT550626	-	-	-	-
C. doisuthep (2)	AUP00777	MZ439890	-	MT497801	-	-	-	-
C. dumnuii (1)	AUP00769	MZ439884	-	MT497802	-	-	-	-
C. dumnuii (2)	AUP00770	MZ439885	-	MT497803	-	-	-	-
C. erythrops (1)	AUP00771	MZ439886	-	MT497806	-	-	-	-
C. erythrops (2)	AUP00772	-	-	MW713958	-	-	-	-
C. gulinqingensis (1)	KIZ061813	-	-	MZ782150	-	-	-	-
C. gulinqingensis (2)	KIZ061814	-	-	MZ782151	-	-	-	-
C. hekouensis (1)	SFU002880	MW067127	-	-	-	-	-	-
C. hekouensis (2)	SFU002881	MW067128	-	-	-	-	-	-
C. houaphanensis (1)	CtL1	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. houaphanensis (2)	CtL2	XXXXXX	XXXXXX	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX
C. huongsonensis (1)	Ct157	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. huongsonensis (2)	Ct7	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. kunyai (1)	AUP01747	MZ439908	-	MZ439916	-	-	-	-
C. kunyai (2)	AUP01748	MZ439909	-	MZ439917	-	-	-	-
C. luci (1)	Ct548	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. luci (2)	Ct550	XXXXXX	-	XXXXXX	-	-	-	XXXXXX
C. martini	UNS0471	MF169929	-	-	-	-	-	-
C. menglianensis (1)	KIZ20210714	-	-	OM296043	-	-	-	-
C. menglianensis (2)	KIZ20210716	-	-	OM296044	-	-	-	-
C. ngoiensis (1)	CtL4	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. ngoiensis (2)	CtL5	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX

C. otai (1)	Ct104	XXXXXX	-	-	XXXXXX	XXXXXX	-	XXXXXX
C. otai (2)	Ct185	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. phamiensis (1)	ZMKUR01085	-	-	PP430582	-	-	-	-
C. phamiensis (2)	ZMKUR01086	-	-	PP430585	-	-	-	-
C. phukhaensis (1)	KIZ042649	-	-	MZ439912	-	-	-	-
C. phukhaensis (2)	KIZ042652	MN871846	-	MZ439913	-	-	-	-
C. puhuensis (1)	Ct134	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. puhuensis (2)	Ct135	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. sonlaensis (1)	Ct183	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. sonlaensis (2)	Ct184	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. soni (1)	Ct133	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. soni (2)	Ct181	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Cyrtodactylus sp. (1)	AUP01576	MZ439895	-	MT468908	-	-	-	-
Cyrtodactylus sp. (2)	HLM0357	-	-	MW713961	-	-	-	-
C. spelaeus	HLM0315	-	-	MW713962	-	-	-	-
C. taybacensis (1)	Ct308	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. taybacensis (2)	Ct309	XXXXXX	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. vilaphongi (1)	CtL7	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. vilaphongi (2)	CtL8	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. wayakonei	ZFMK91016	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
C. zhenkangensis (1)	KIZL2020046	MW593136	-	-	-	-	-	-
C. zhenkangensis (2)	KIZL2020047	MW593137	-	MW792062	-	-	-	-

Table 2. PCR primers used in this study

Gene	Primer	Primer sequences (5' – 3')	Reference
COI	VF1d	TTCTCAACCAACCACAARGAYATYGG	Nazarov et al. 2012
	VR1d	TAGACTTCTGGGTGGCCRAARAAYCA	
Cytb	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink et al. 2000
	H16064	CTTTGGTTTACAAGAACAATGCTTTA	
ND2 + tRNA	MetF1	AAGCTTTCGGGCCCATACC	Macey et al. 1997
	COIR1	AGRGTGCCAATGTCTTTGTGRTT	Arevalo et al. 1994
Rpl35	N66	GCTAAACAAGCACAGAGTTGATCC	Siler et al. 2010
	N67	TCAGGCTCAGAAAGRACTATTATGG	

Rag1	R13	TCTGAATGGAAATTCAAGCTGTT	Growth and Barrowclough 1999
	CyrRag1	CTCCTTGTGRCTAGAAAGAT	This study
PDC	PHOF1	AGATGAGCATGCAGGAGTATGA	Bauer et al. 2007
	PHOR1	TCCACATCCACAGCAAAAAACTCCT	
Cmos	G73	GCGGTAAAGCAGGTGAAGAAA	Saint et al. 1998
	G74	TGAGCATCCAAAGTCTCCAATC	

Table 3. Best-fit models for all the datasets in MrBayes

Gene					**Model**
Mitochondrial dataset					
COI + *cytb* + *ND2* + tRNA					GTR+I+G
Nuclear dataset					
Rpl35 + *Rag1* + *PDC* + *Cmos*					GTR+I
Combined dataset					
COI + *cytb* + *ND2* + tRNA + *Rpl35* + *Rag1* + *PDC* + *Cmo*s					GTR+I+G
Partitioned datasets					
COI		cyt*b*		ND2	
Codon position 1	Trn+G+X	Codon position 1	GTR+I+X	Codon position 1	TIM+I+G+X
Codon position 2	TIM+I+G+X	Codon position 2	Trn+I+G+X	Codon position 2	GTR+I+G+X
Codon position 3	K81uf+I+X	Codon position 3	TIM+G+X	Codon position 3	GTR+I+G+X
Cmos		PDC		Rag1	
Codon position 1	HKY+I+X	Codon position 1	Trn+I+X	Codon position 1	TIM+I+X
Codon position 2	K80+I+G	Codon position 2	Trn+I+X	Codon position 2	HKY+I+X
Codon position 3	Trn+I+X	Codon position 3	K81uf+I+X	Codon position 3	Trn+G+X

	Rpl35	
	Codon position 1	TVMef+I
	Codon position 2	K81uf+G+X
	Codon position 3	K81uf+G+X

Table 4. Best-fit models for each gene in BEAST

Gene	Model	Gene	Model
COI	GTR+I+G (gamma category count = 4; shape = 1.546; proportion invariant = 0.606; rate AC = 1.6791; rate AG = 23.3486; rate AT = 1.6791; rate CG and GT = 1.0; rate CT = 12.5128)	*Rpl35*	HKY+I (proportion invariant = 0.594; kappa = 2.2423)
Cytb	GTR+I (proportion invariant = 0.576; rate AG = 10.9226; rate AC, AT, CG, GT = 1.0; rate CT = 4.6927)	*Rag1*	HKY+G (gamma category count = 4; shape = 0.227, kappa = 5.8268)
ND2	GTR+I+G (gamma category count = 4; shape = 0.734; proportion invariant = 0.306; rate AC = 0.4387; rate AG = 5.305; rate AT = 0.3188; rate CG = 0.2804; rate CT = 2.0102; rate GT = 1.0)	*PDC*	GTR+G (gamma category count = 4; shape = 0.1; rate AC, AT, CG and GT = 1.0; rate AG = 2.0643; rate CT = 10.7642)
		Cmos	HKY+I (proportion invariant = 0.885; kappa = 2)

Table 5. Time calibration for nodes in the phylogeny. Node numbers defined in Fig. 6

Node	Age estimate (million years)	95% HPD (million years)	Node	Age estimate (million years)	95% HPD (million years)	Node	Age estimate (million years)	95% HPD (million years)
1	48.36	39.75-57.25	12	14.51	11.90-17.15	23	6.46	5.23-7.73
2	39	32.20-46.20	13	14.45	11.85-17.13	24	5.23	3.93-6.66
3	25.01	18.51-31.68	14	14.00	11.19-16.94	25	4.91	3.92-5.97
4	22.29	19.69-24.94	15	13.60	11.38-15.79	26	4.69	3.94-5.97
5	21.11	18.46-23.88	16	13.16	10.72-15.64	27	4.47	0.00-9.33
6	20.29	17.52-23.03	17	11.01	9.10-12.97	28	4.24	3.12-5.48
7	19.49	16.88-22.25	18	9.92	7.70-12.25	29	3.73	1.88-6.25
8	19.26	16.42-22.21	19	9.28	7.72-10.90	30	2.99	1.70-4.36
9	18.75	15.98-21.56	20	7.80	6.12-9.54	31	2.43	1.75-3.16
10	17.84	15.00-20.65	21	6.90	5.08-8.82	32	2.20	1.56-2.86
11	16.64	14.00-19.39	22	6.73	5.26-8.29			

Table 6. Model testing for the BioGeoBears analysis with and without found-event speciation (+J)

a. Relative probability of each of six models, using AICc

Models	LnL	number of parameters	d	e	j	AICc	AICc_wt
DEC	-41.64	2	0.02	0.81	0	87.72	0.081
DEC+J	-39.66	3	1.00E-12	0.71	0.072	86.24	0.17
DIVALIKE	-40.52	2	0.022	2.69	0	85.48	0.25
DIVALIKE+J	-39.85	3	0.006	3.24	0.046	86.63	0.14
BAYAREALIKE	-42.01	2	0.013	0.43	0	88.47	0.056
BAYAREALIKE+J	-39.08	3	1.00E-07	0.19	0.057	85.08	0.3

b. AIC results and relative probability of each pair of models

alt	null	LnLalt	LnLnull	DFalt	DFnull	DF	Dstatistic	pval	test	tail	AIC1	AIC2	AICwt1	AICwt2
DEC+J	DEC	-39.66	-41.64	3	2	1	3.96	0.047	chi-squared	one-tailed	85.31	87.27	0.73	0.27
DIVALIKE+J	DIVALIKE	-39.85	-40.52	3	2	1	1.33	0.25	chi-squared	one-tailed	85.7	85.03	0.42	0.58
BAYAREALIKE+J	BAYAREALIKE	-39.08	-42.01	3	2	1	5.87	0.015	chi-squared	one-tailed	84.16	88.03	0.87	0.13

Chapter 10. Assessing the suitability of mitochondrial genetic markers for molecular systematics of Cyrtodactylus: A case study of the phylogenetic performance based on twelve mitogenomes

(To be submitted to ZooKeys)

What is the suitability of mitochondrial genetic markers for molecular systematics and the phylogenetic relationship of *Cyrtodactylus*? The case study is a comparison of the phylogenetic performance based on twelve mitogenomes

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ABSTRACT

Mitochondrial genetic markers have become widely used in molecular systematics, species identification, evolution, and biogeography of the genus *Cyrtodactylus* due to their typically non-recombining, maternally inherited, single-copy, recognizable and variable. However, determining the suitability of a genetic marker for a specific study can be complicated by its properties and the goals of the research. To provide a reference for future studies, we assessed the suitability of genetic markers for molecular systematics and species identification within *Cyrtodactylus* using twelve complete mitochondrial genomes from ten different *Cyrtodactylus* species. Overall, our results indicate that phylogenies inferred from longer mitochondrial protein-coding genes (PCGs) (such as ND5, ND4, ND2, ND1, Cytb, and COXI) performed better similar to that of the 13 PCGs compared to those inferred from shorter ones (including ATP6, ATP8, COXII, COXIII, ND3, ND6, ND4L). Among these, phylogenies inferred from ND5 most closely resembled the topologies derived from the 13 PCGs.

KEYWORDS: genetic markers, molecular systematics, species identification, mitochondrial, *Cyrtodactylus*

INTRODUCTION

The genus *Cyrtodactylus* was first described by Gray in 1827 based on the digital and precloacal scale characteristics of a single species, *Cyrtodactylus pulchellus*, (Gray, 1827). Since then, the number of species within the *Cyrtodactylus* genus has increased more than 380 times over the past 200 years, largely due to the great advantage of molecular data (Uet et al. 2024; Grismer et al. 2021, 2022; Ngo et al. 2022). Unlike traditional methods that rely on morphological traits, molecular data offer numerous comparative characters that are less biased (Pierce 2010). As a result, it addresses the limitations of conventional species identification and offers a great opportunity to resolve several cryptic and complex *Cyrtodactylus* taxa (Nazarov et al. 2012; Murdoch et al. 2019). For example, the *Cyrtodactylus irregularis* group was originally considered to comprise only one taxon with

an irregular dorsal pattern, fermoral scales, if present, are isolated from preanal scales (Nguyen et al. 2017; Grismer et al. 2021). Due to the similar morphological characteristics, distinguishing members of this group is challenging for researchers. As supported by molecular data, it is recognized as the most speciose group within the genus *Cyrtodactylus* to date, with at least 30 known species (Ngo et al. 2024). Moreover, molecular data also provide a phylogenetic framework for understanding relationships within *Cyrtodactylus* or *Cyrtodactylus* and other geckos (Grismer et al. 2015, 2021; Brennan et al. 2017; Ngo et al. 2022). Nowadays, the molecular data published so far cover more than 80% of species and provide a first idea of their evolution and biogeography of *Cyrtodactylus* (Wood et al. 2012; Grismer et al. 2021, 2022, 2023; Ngo et al. 2022; NCBI 2024).

Following the literature on mitochondrial markers for *Cyrtodactylus* studies, a combination of the 12S and 16S ribosome RNA genes was the first genetic marker to assess phylogenetic relationships within eublepharid genera and gekkonid taxa and identify *Cyrtodactylus* species (Ota et al. 1999; Ziegler et al. 2010; Schneider et al. 2011). Then, a genetic marker selection has transformed into a fragment of cytochrome c oxidase subunit I (COI or COXI) for numerous studies of *Cyrtodactylus* following the recommendation by the Consortium for the Barcode of Life and available sequences at the BOLD systems website (http://www.boldsystems.org) (Hebert et al. 2003, Nazarov et al. 2012). After that, other genetic markers have also been successfully used in several studies of *Cyrtodactylus* for systematics and identification purposes, making at least four different fragments of mitochondrial genes (including COXI, the NADH dehydrogenase subunit II - ND2, cytochrome b - Cytb, NADH dehydrogenase subunit 4) used as genetic markers among *Cyrtodactylus* studies (Welton et al. 2010; Grismer et al. 2014). Within them, COI or ND2 is the most commonly used (Nguyen et al. 2013; Nazarov et al. 2014; Grismer et al. 2014; NCBI 2024). However, COXI has been found inefficient for *Cyrtodactylus* to resolve deeper nodes within the genus *Cyrtodactylus* due to the limitations of the length and lack of characters (Ngo et al. 2022, 2024). While ND2 has been found to have a faster evolutionary rate than other mitochondrial protein-coding and rRNA genes in several species (Near et al. 2003), it might not be the best target for interspecies identification. Therefore, the best marker gene for the systematics and identification studies of *Cyrtodactylus* needs to be investigated.

In addition, the development of DNA sequencing technology has enabled the sequencing of large amounts of gene fragments and even complete mitochondrial genomes. Consequently, sequences of the single gene of thousands of *Cyrtodactylus* have been produced and are freely available in Genbank (https://www.ncbi.nlm.nih.gov) (NCBI 2024). They provide a huge chance to increase the phylogenetic studies from multiple genes, which may improve our understanding of the phylogenetic relationships and evolutionary histories (Brennan et al. 2017; Liu et al. 2017; Sivayyapram et al. 2023). However, handling and choosing the right genetic marker for multigene analyses can be daunting due to the different rates of sequence evolution of different genes. Hence, exploring other priority markers for combining different genes in phylogenetic analyses of *Cyrtodactylus* is important.

In contrast, for complete mitochondrial genomes, only six species have been sequenced from the third most speciose genus, *Cyrtodactylus,* which contains more than 372 species (Areesirisuk et al. 2018; Uetz et al. 2024). It occupied only 1.6% of the bent-toed gecko mitogenomes that have been accessed so far. Moreover, no mitogenome-based phylogenetic study of *Cyrtodactylus* has explored priority different markers. Therefore, we sequenced twelve mitogenomes of ten species, including two new species, to (1) assess the suitability of mitochondrial genetic markers for molecular systematics and identification purposes by comparing the topology from each mitochondrial protein-coding gene to that from all 13 protein-coding genes and (2) provide references for future genome research.

MATERIALS AND METHODS

Taxonomic sampling

In total, twelve new samples of *Cyrtodactylus* were incorporated into the analysis. Of these, eleven were collected from six provinces in Vietnam, including Binh Dinh, Binh Phuoc, Binh Thuan, Dak Nong, Khanh Hoa, and Tay Ninh Provinces between 2010 and 2022 and one was a paratype of *C. cucdongensis* (Schneider et al. 2014). The remaining samples were from Genbank for *Cyrtodactylus* species based on Areesirisuk et al. (2018) and Kumazawa Y. (unpublished) and two for the closely related taxon, *Hemidactylus bowringii* (NC025938) and *Hemidactylus frenatus* (NC012902) to be used as an outgroup species (Table XX).

DNA extraction and complete mitogenome sequencing

A piece of muscle samples was collected and stored in a freezer with 70% ethanol (Merck, Germany). Total genomic DNA was extracted using the Dneasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's instructions for animal tissue. The integrity and quality of each genomic extraction were checked by electrophoresis and spectrophotometry, respectively. The samples were sent to BGI, China, for library construction and sequencing.

Assembly and annotation

All bioinformatic analysis was carried out using SOAPnuke software by BGI and the Galaxy server (https://usegalaxy.org.au and https://usegalaxy.eu). After removing adapters and N reads using SOAPnuke software, raw data was assessed to generate a broad overview of some summary statistics using FastQC v0.12.1 (Andrews 2023). The low-quality sequences were filtered using Trimmomatic v0.39 (Bolger et al. 2014). Clean data was assembled using SPADES v3.15.5 with default setting (Bankevich et al. 2012; Nurk et al. 2013; Prjibelski et al. 2014; Antipov et al. 2015; Vasilinetc et al. 2015). Mitogenome sequences were annotated and identified the two rRNA genes (12S and 16S rRNA) and 13 protein-coding genes (PCGs) using MITOS v2.1.7 (Arab et al. 2017; Donath et al. 2019) and CHLOROBOX-GeSeq-Annotation of Organellar Genomes (https://chlorobox.mpimp-golm.mpg.de/geseq.html) (Tillich et al. 2017). The CG View/Proksee map online server V1.1.6 (Grant et al. 2023) was used to draw the maps of the complete mitogenome of *Cyrtodactylus* (https://cgview.ca/, accessed on 31st December 2024). The CG and AT skew were calculated with the following formula: AT skew = (A – T) : (A + T), GC skew = (G – C) : (G + C) (Arab et al. 2017; Donath et al. 2019).

Sequence alignments and phylogenetic analysis

In order to determine whether topologies derived from individual mt genes were capable of reproducing the complete set of 13 PCGs topology, phylogenetic analyses were applied to 14 datasets that contained 13 single mitochondrial PCGs and the complete set of 13 PCGs. Individual mt gene was initially aligned separately using ClustalX v2.1 (Thompson et al.

1997) with default settings for complete alignment. Each dataset was analyzed using maximum likelihood (ML) as implemented in IQtree v1.6.12 (Nguyen et al. 2015) and Bayesian phylogenetic inference analysis (BI) as implemented in MrBayes v3.2.7a (Ronquist et al. 2012) to reconstruct topologies. The optimal substitution models were determined using the Akaike Information Criterion (AICc) as implemented in jmodeltest v2.1.10 (Darriba et al. 2012) and PARTITIONFINDER v2.1.1 (Lanfear et al. 2012). All the best models for each dataset are shown in Table XX. For BI analyses, Markov chain Monte Carlo (MCMC) was run for 10,000,000 generations with four chains, and trees were sampled every 1,000 generations. The convergence of parameters was assessed using TRACER v1.7.2 and the first 25% of the samples from the cold chain were discarded (burninfrac = 0.25). All the datasets were used one single nucleotide model. The complete set of 13 PCGs was also partitioned into 13 parts by genes and 39 parts by codon positions (first, second and third). For the ML analysis, a single model was applied for a single gene, and partitioned models into 39 parts by codon position were performed for 13 PCGs set. Ten thousand ultrafast bootstrap replications (UFBF) were used. Nodal support was evaluated using UFB, as estimated in IQtree and posterior probability (PP) as estimated in MrBayes. UFB and PP $\geq$ 95% are regarded as strong support for a clade and values of < 95%, as weak support (Ronquist et al. 2012; Nguyen et al. 2015). We considered nodes with UFBF and PP values of 90-94 as well-supported.

RESULT

Mitogenome sequencing

Assembly for twelve complete mitogenome lengths between 16,587 bp (*C. bugiamapensis*) and 16,990 bp (*C. chungi*) (Table XX), and their corresponding GenBank accession number are XXXXX – XXXXX. With the typical circular structure, the mitogenome arrangements of *Cyrtodactylus* include 13 PCGs (including ATP6, ATP8, COXI-III, Cytb, ND1-6, ND4L), two rRNA genes (12S rRNA and 16S rRNA) and 22 tRNAs, along with a non-coding region (D-loop) between tRNA-Pro and tRNA-Phe (Figure 1). Of these, ND6 and 8 tRNAs (including tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu

and tRNA-Pro) were encoded in the light-strand (L-strand) and the remaining 28 genes were encoded in the heavy-strand (H-strand). Positive AT skews and negative GC skews were found in all twelve mitogenomes of *Cyrtodactylus* (Table XX). The highest percentage of A + T was found in *C. nigriocularis* (56.84%), and the lowest was in *C. bugiamapensis* (49.71%) (Table XX).

Protein coding genes

The A+T content of PCGs ranged from 49.56% (*Cyrtodactylus* sp.) to 57.58% (*C. nigriocularis*) in the twelve mitogenomes analyzed (Table XX). The total length of the 13 PCGs was between 11,337 bp (*C. yangbayensis*) and 11,362 bp (*C. badenensis*). The sizes of the different protein-coding genes were between 159 bp and 1,809 bp, with NAD5 being the longest (1,809 bp) and ATP8 the shortest (159 bp – 162 bp).

For start codons, ATG was used in most protein-coding genes (including COXI - III, ATP6, ATP8, ND4L, ND4, ND5 and Cytb), whereas GTG was used in ND1 and ATA was used in ND2, ND3 and ND6. TAN (TAA/TAG) was often found as the stop codon (genes) in the twelve sequences. In addition, AGG/AGA was used as the stop codon in some protein-coding genes (COXI and ND4) (Table XX).

Transfer and ribosomal RNAs

The two ribosomal RNAs (12S rRNA and 16s rRNA) were separated by tRNA-Val and the full sizes of 12S rRNA were 936 bp – 950 bp. Similarly, the total lengths of 16S rRNA for twelve mitogenomes were 1,481 bp – 1,537 bp. The AT content of 12S rRNA and 16S rRNA ranges from 48.39% and 53.48% of *Cyrtodactylus.* The AT-skew of 12S rRNA and 16S rRNA are positive and greater than the GC-skew, suggesting that there are more As and Cs than Ts and Gs (Table XX).

The tRNAs ranged from 54 to 76 bp and there was a positive AT-skew, except in the light strand tRNA. The skew analysis of tRNA indicated the use of As and Cs in the heavy-strand, while there was an obvious bias toward the use of Ts and Gs in the light-strand.

Control region

198 The size of the CR for 12 mitogenomes ranged from 1,278 to 1,684 bp, and the AT content
199 ranged from 51.48% to 60.02% (Table XX).

200 **Assessment of suitable genetic markers for molecular identification**

201 The complete set has 11,527 aligned characters (ATP6: 687 + ATP8: 169 + COXI: 1,564 +
202 COXII: 688 + COXIII: 785 + Cytb: 1,148 + ND1: 970 + ND2: 1,050 + ND3: 420 + ND4:
203 1,388 + ND5: 1,815 + ND6: 545 + ND4L: 298). Our results based on 13 PCGs demonstrate
204 that mitogenomes were effective for resolving phylogenetic relationships of *Cyrtodactylus*
205 with strong statistic support values. Although single mtDNA genes generally performed
206 poorly in phylogenetic analyses, some mt genes were significantly better at reproducing a
207 topology consistent with that of the 13 PCGs matrix. In particular, phylogenies inferred from
208 longer mt PCGs (including ND5, ND4, ND2, ND1, Cytb, COXI) individually tended to
209 perform better similar to that of the 13 PCGs than shorter ones (including ATP6, ATP8,
210 COXII, COXIII, ND3, ND6, ND4L). Of these, phylogenies inferred from ND5 most
211 frequently recovered a topology related to that of the 13 PCGs (Fig. 2). Both topologies show
212 that 16 *Cyrtodactylus* species in this study were clustered into three major groups: group I,
213 composed of five species from Thailand (*C. auribalteatus, C. louisiadensis, C. peguensis, C.*
214 *thirakhupti,* and *C. tigroides*); Group II contained seven described species and two
215 undescribed species from Vietnam (*C. arndti, C. badenensis, C. bugiamapensis, C. chungi,*
216 *C. cucdongensis, C. phumyensis, C. takouensis, C. yangbayensis,* and *Cyrtodactylus* sp) ; and
217 Group III, included two species from eastern Thailand and southern Vietnam (*C. chanhomeae*
218 and *C. nigriocularis*). However, phylogenetic reconstructions based on the ND5 gene showed
219 no statistical support for group I (65% Bayesian probability and 58% ultrafast bootstrap
220 support), while it formed significant support based on the 13 PCGs (100% Bayesian
221 probability and 93% ultrafast bootstrap support). In addition, the UFB and PP values in ND5
222 topologies were lower in some nodes compared with the PCGs topologies, e.g., nodes
223 representing Group II from the analyses of 13 PCGs received higher values regardless of the
224 analyses compared to those resulting from the ND5. On the other hand, *C. auribalteatus* was
225 recovered as the sister taxon to *C. tigroides* with strong support values in ND5 topologies
226 (PP = 100, UFB = 96). In contrast, it was placed as a member in a clade consisting of *C.*

227 *peguensis, C. thirakhupti,* and *C. tigroides* without any clear sister species in the PCGs
228 topologies. Much the same is true for *C. cucdongensis* and *C. phumyensis, C. cudongensis*
229 was recovered as the sister taxon to *C. phumyensis* with a strong support value in BI analysis
230 in the PCGs topologies, while it was located without any clear sister species in a clade
231 consisting of *C. phumyensis* and *C. yangbayensis* in ND5 topologies (Fig. 2).

232 In comparing the topologies of 13 PCGs with the two most commonly used mt fragments in
233 previous taxonomy and identification studies, phylogenies inferred from the COXI gene were
234 performed better similarly to the 13 PCGs than those from the ND2 gene in several external
235 nodes. However, the nodal statistical values (UFB and PP) in several nodes of the topologies
236 from the ND2 genes were significantly higher than those from the COXI gene. In particular,
237 the analyses from the COXI gene were performed well in recovering the monophyly of the
238 three groups (even though the nodal values of Group III were generally low). In contrast, the
239 analyses from the ND2 gene did not corroborate the monophyly for the three groups.
240 Moreover, phylogenies generated by the ND2 were recovered *C. louisiadensis* as a basal
241 node, and it was placed as the sister species to all remaining species in this study, while this
242 species was nested in Group I in both analyses of COXI and 13 PCGs. Although *C. peguensis*
243 was located as the sister species to *C. thiralhupti* with strong support values in both analyses
244 of COXI and 13 PCGs, it was recovered as the sister species to both *C. thirakhupti* and *C.*
245 *tigroides* in the trees based on ND2. On the other hand, the nodal statistical values in several
246 nodes of the topologies from the ND2 genes were significantly higher than those from the
247 COXI gene. For example, nodes representing Group III from ND2 received higher values
248 regardless of the analyses compared to those resulting from the COXI (UFB = 100 and PP =
249 97 in ND2 topologies *vs.* UFB = 60 and PP = 46 in COXI topologies). In addition, significant
250 support values for a node representing *C. bugiamapensis, C. cucdongensis, C. phumyensis,*
251 *C. takouensis, C. yangbayensis,* and *Cyrtodactylus* sp. in the BI analysis (PP = 100, UFB =
252 89) came from the ND2 analyses, and while this lineage was not well supported in both
253 analyses of COI. Moreover, the nodal statistical values of lineages consisting of *C.*
254 *auribalteatus, C. thirakhupti, C. tigroides,* and *C. peguensis* also received higher values in
255 the trees based on ND2 compared with the analyses from COXI. Furthermore, phylogenies
256 inferred from the ND2 gene were performed greater similarly to the 13 PCGs than those from

the COXI gene in some internal nodes compared, e.g., all analysis results from ND2 and 13 PCGs strongly supported *C. arndti* as the sister species to the species *C. bugiamapensis, C. chungi, C. cucdongensis, C. phumyensis, C. takouensis C. yangbayensis* and *Cyrtodactylus* sp., while it was placed as the sister species to the *C. bugiamapensis, C. cucdongensis, C. phumyensis, C. takouensis C. yangbayensis* and *Cyrtodactylus* sp. in the analyses of COXI (Fig. 2). COXI was the second longest mt PCGs and ND2 was the fifth longest mt PCGs of *Cyrtodactylus* species, suggesting that COXI might not be a poorer marker than ND2.

ND4, the third longest mt PCG, surprisingly performed similarly in several external and terminal nodes compared with the PCGs topologies, i.e., both topologies show that 16 *Cyrtodactylus* species in this study were clustered into three major groups. Both analyses based on the ND4 gene supported the monophyly of Groups I, II, and III with well-supported values (exclude UFB for Group II). However, the topologies based on the ND4 gene were still limited to resolving several internal nodes compared to the PCGs topologies, i.e., the position of *C. arndti, C. chungi, C. takouensis, C. tigroides*. Much the same is true for ND1 and Cytb, all the analyses showed the different positions of *C. arndti, C. louisiadensis, C. takouensis, tigroides* when compared to the PCGs topologies (Fig. 2). It suggests that a single mitochondrial gene may not be adequate for inferring phylogenetic relationships among *Cyrtodactylus* species.

DISCUSSION

Mitogenome sequencing

Similar to those of other *Cyrtodactylus* and other gecko lizards in previous studies, our results show that twelve mitogenomes of the ten *Cyrtodactylus* species were determined to have a genome size of 16-17 kb and consist of 13 PCGs, two rRNA genes, and 22 tRNAs, along with a non-coding region (D-loop). Positive AT skews and negative GC skews were found in all twelve mitogenomes of *Cyrtodactylus,* suggesting a higher content of As and Cs compared with Ts and Gs (Areesirisuk et al. 2018; Zhou et al. 2006; Kumazawa et al. 2014; Kim et al. 2015). In a comparison of six available mitogenomes of *Cyrtodactylus* species in NCBI, both studies revealed that ATG, ATA and GTG were common start codons of protein-coding genes

285 and TAA, TAG, AGA, and AGG (except for COXIII) appeared to commonly stop codons.
286 Among 13 PCGs, ND5 was the longest gene, followed by COXI, ND4, Cytb, ND2, ND1,
287 COXIII, COXII, ATP6, ND6, ND3, ND4L, ATP8 (Table. XX) (this study, Areesirisuk et al.
288 2018).

289 **Suitable genetic markers for molecular identification**

290 Our results showed that at least four encoding proteins of the NADH dehydrogenase subunits
291 (NADH) performed topologies similar to that of the 13 PCGs set. It means that among genes,
292 those encoding proteins of the NADH dehydrogenase subunits (NADH) were more effective
293 at reproducing a topology consistent with that of the 13 PCGs set than those encoding the
294 cytochrome carriers (COX, Cytb), similar to a previous study by Havird & Santos (2014)
295 based on mitogenomic data from 1,865 metazoan taxa. The reason might be that NADH
296 genes are numerically dominant in the metazoan mitogenomes (7 of 13 protein-coding genes
297 compared with 3 COX genes of 13 protein-coding genes), therefore, their phylogenetic signal
298 exerts the greatest influence on the overall 13 PCGs topologies. Moreover, COX or Cytb are
299 more conserved than NADH genes, and therefore, NADH genes might provide an additional
300 resolution that would represent the phylogenetic signal found in the 13 PCGs topologies.
301 Although single mitochondrial gene phylogenies generally performed limited to resolving
302 external nodes and/or several internal nodes presented here, genes with long lengths,
303 including ND5, COXI, ND4, Cytb, ND2, and ND1, were generally a good predictor of
304 reproducing a topology consistent with that of the 13 PCGs set (Fig. 2). Among these,
305 phylogenies inferred from the ND5 performed significantly better than those from the
306 concatenation of the six longest mt genes compared to the 13 PCGs phylogenies. It is
307 suggested that the inconsistency and efficiency in recovering the correct nodes depend on the
308 length of the individual markers. This result is similar to those Havird & Santos (2014)
309 reported using the mitogenomic data from 1,865 metazoan taxa. The ND5 gene encodes one
310 subunit of NADH dehydrogenase complex that is part of the oxidative phosphorylation
311 machinery and is one of the fastest-evolving mitochondrial genes for statistical analyses (Su
312 et al. 1996). Until now, no studies have picked up ND5 as a selection genetic marker to assess
313 phylogenetic relationships within *Cyrtodactylus* taxa and identify *Cyrtodactylus* species.

However, the ND5 gene was previously used as a powerful marker in elucidating the level of genetic and phylogenetic divergence of several animal groups (Kim et al. 2000; Doosey et al. 2010). Therefore, ND5 may be the best marker for the genus *Cyrtodactylus* when only one gene can be chosen.

The COXI and ND2 markers were the most widely utilized genetic markers in previous research on the genus *Cyrtodactylu*s, as well as several species within the Gekkonidae family (Grismer et al. 2014, 2021, 2022; Ngo et al. 2022). However, all the studies of the genus *Cyrtodactylus* have only used a fragment of COXI due to full COXI being too long for typical PCR-based methods and, as many samples are degraded. Based on our results, the topologies based on COXI performed no worse at recreating the 13 PCGs topologies than ND2, in contrast to previous studies (Ngo et al. 2022; Grismer et al. 2022). The reason might be the length of COXI used in the earlier studies. In our study, full COXI with ranges from 1,518 – 1,600 bp were analyzed, whereas in previous studies, only fragments of COXI (approximately 650 bp) were explored. Therefore, researchers should be cautious when basing hypotheses regarding the evolution of *Cyrtodactylus* lineages solely on phylogenies derived from a fragment/or only one mitochondrial gene. Specifically, further studies should be considered to reconstruct the phylogenetic trees based on two or more mitochondrial genes instead of only one gene or one fragment.

Cytb shows little intraspecies variation compared to NADH genes but has sufficient interspecies variation (Linacre 2012). Consequently, the gene is one of the most commonly used genetic loci across reptiles (mostly in turtles), and it is applied in both taxonomy, forensic sciences, phylogenetic relationship, and biogeographical history studies (Le et al. 2006, 2008, 2013; Linacre 2012). However, the gene was not widely used in Gekkonidae studies compared to ND2 and COXI, and rarely previous studies selected cytb as the best mitochondrial marker for phylogenetic relationships (Lajmi et al. 2016). Other studies used cytb as one of two mitochondrial markers to resolve some complex groups (Yan et al. 2010, Vasconcelos et al. 2014). For the genus *Cyrtodactylus*, only four studies were recorded using cytb together with ND2 and/or ND4 to describe new species or to reconstruct the phylogeny of the gekkonid species (Bauer et al. 2008, Agarwal 2016, Agarwal et al. 2016, 2018). Based on our study, while the topologies derived from the cytb gene were limited in resolving some

344 internal nodes, this gene would serve as a valuable marker for further studies of the genus
345 *Cyrtodactylus* using multiple genetic approaches markers.
346 Similar to the ND5 gene, ND4 and ND1 encodes NADH hydrogenase complex subunits that
347 are part of a large enzyme complex known as complex I. They are also the third and sixth
348 longest mt PCGs in reptile mitogenomes (Zhou et al. 2006; Yan et al. 2014; Areesirisuk et al.
349 2018). Within the mitogenome of *Cyrtodactylus,* the lengths of ND4 and ND1 ranged from
350 1,353 – 1,381 bp and 961 – 969 bp, respectively (this study, Areesirisuk et al. 2018). To date,
351 no studies have been reported using the ND4 and ND1 gene sequences to identify the species
352 of *Cyrtodactylus* or studies on phylogenetic relationships among the genus *Cyrtodactylus.*
353 However, these markers are widely used in several reptile description species or
354 phylogeographic studies, for example, the genus *Phrynosoma* (Phrynosomatidae) (Blair &
355 Bryson 2017), the *Psammodromus algirus* species (Lacertidae*)* (Díaz et al. 2017), the genus
356 *Afroedura* (Gekkonidae) (Makkhubo et al. 2015, Busschau et al. 2019), the genus
357 *Hemidactylus* (Gekkonidae) (Vasconcelos et al. 2014), the genus *Lygosoma* (Scincidae)
358 (Heitz et al. 2016, Karin et al. 2018), etc. In this study, phylogenies inferred from ND4 and
359 ND1 were surprisingly performed similarly in several external and terminal nodes compared
360 with the 13 PCGs topologies. Therefore, ND4 and/or ND1 might be good markers when
361 several genes can be selected**.**

362 **Phylogenetic relationship and genetic divergence**

363 Similar to the previous study by Ostrowski et al. (2020) based on a fragment of COXI, our
364 phylogenetic analysis shows that *C. phumyensis* was recovered as a sister taxon to *C.*
365 *cucdongensis* with high statistical values from BI analyses (Fig. 2). Importantly, two samples
366 (Ct386, Ct388) of unnamed lineages were included in this study and recovered with strong
367 statistical support in all analyses as members of *C. irregularis* group. They were collected
368 from Dak Nong Province (Table XX, Fig. 2). In terms of genetic divergences, Ct386, Ct388
369 were separated from other species in this study by at least 15.07% based on 13 PCGs,
370 16.67%, 19.14%, 12.23%, 11.34%, 10.84%, 13.86%, 12.94%, 14.89%, 16.09%, 16.54%,
371 13.81%, 17.75%, 17.05% based on ATP6, ATP8, COXI, COXII, COXIII, cytb, ND1, ND2,
372 ND3, ND4, ND4L, ND5, ND6, respectively (Supplementary 1). It was also at least 6.54%

and 11.85% genetically divergent from available sequences of *Cyrtodactylus* species based on a fragment of COXI and ND2, respectively, using BLAST (Basic Local Assignment Search Tool) on GenBank.

Limitations

This study was limited by the availability of the mitogenome sequences in the NCBI database, which restricted the number of taxa that we could compare and analyze together across the genetic markers. Inadequate sampling might affect clade arrangement and marker selection. Therefore, future studies should be undertaken to provide more mitogenome sequences of the species sampled.

CONCLUSIONS

In this study, we reported twelve complete mitogenomes from ten bent-toed species. The mitogenome structures of these ten bent-toed species were similar to the typical mitogenomes of reptiles, featuring a genome size of 16-17 kb, which includes 13 protein-coding genes (PCGs), two rRNA genes, and 22 tRNAs, along with a non-coding region. For phylogenetic purposes, our analyses of the mitogenomic data demonstrated that ND5, COXI, ND4, Cytb, ND2, and ND1 performed best as molecular markers for systematic investigations, while ATP6, ATP8, COXII, COXIII, ND3, ND6, and ND4L showed the worst results. Importantly, our study indicated that ND5 could be the optimal marker for the genus *Cyrtodactylus* when only a single gene is selected. Based on our results, we recommend utilizing multiple mtDNA markers for systematic investigations of *Cyrtodactylus*.

ACKNOWLEDGMENTS

We thank Q.H. Do, H.Q. Nguyen, A.V. Pham, T.Q. Phan for their assistance in the field and T.T. Nguyen, M.H. Le for their support in the laboratory. This research is funded by the Vietnamese Ministry of Science and Technology's Program 562. Doctoral research of H.T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD).

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Figure Legends

Fig. 1. Circular visualization maps of the complete mitogenomes of *Cyrtodactylus* spp. The three circles from the outside to the inside show the gene map (PCGs, rRNAs, tRNAs, D-loop), GC skew and the GC content, respectively.

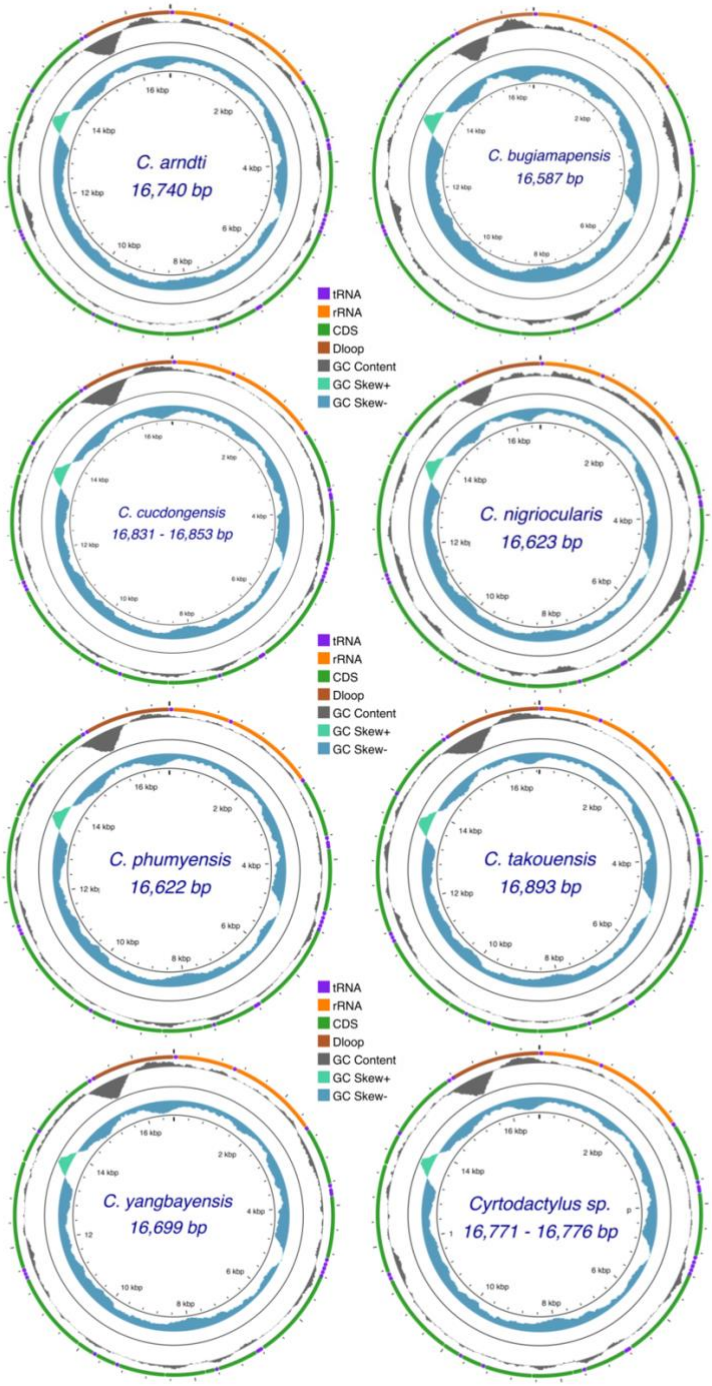
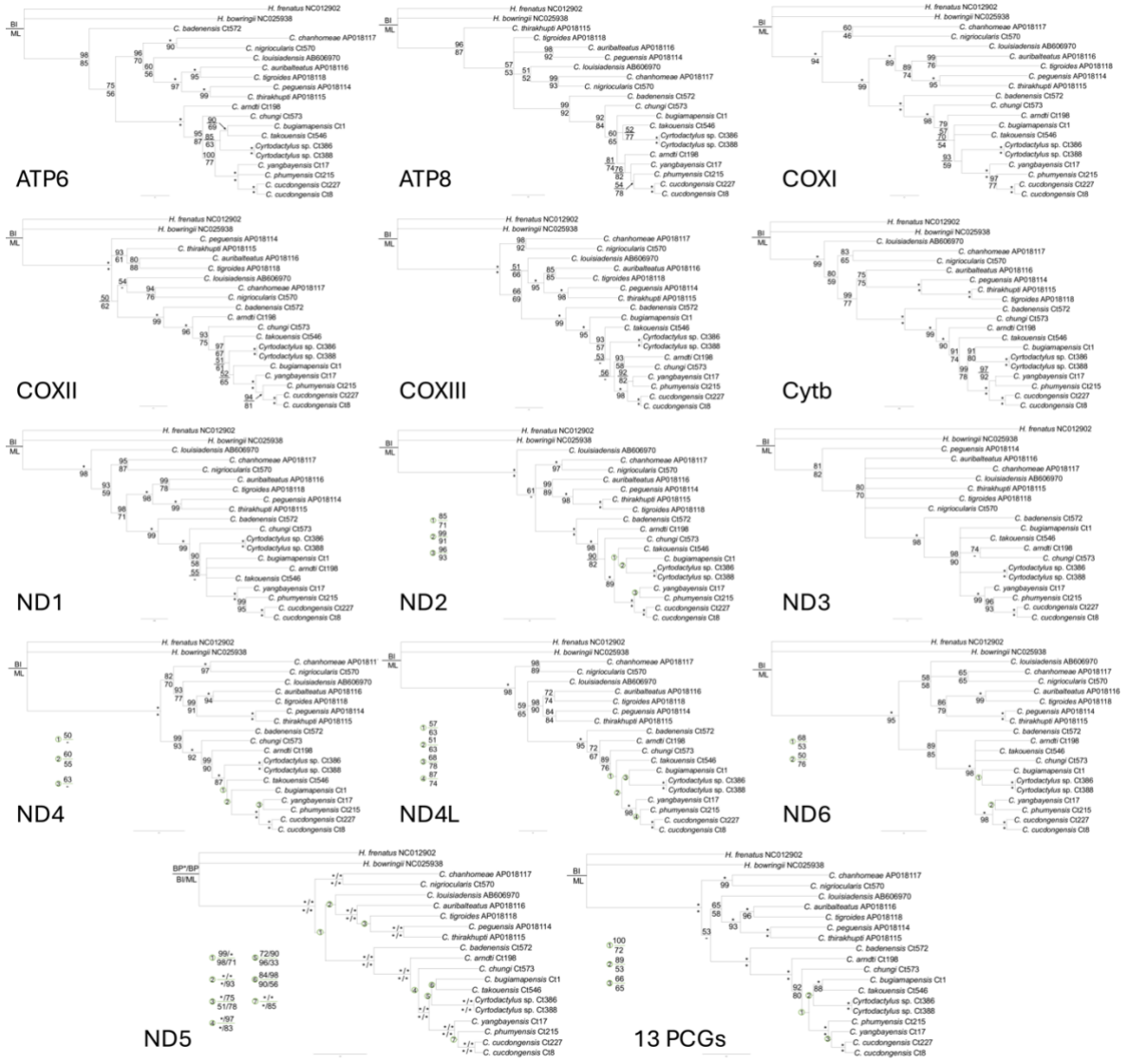


Fig. 2. Phylogenetic consensus tree inferred from ML and Bayesian analyses with branch length estimated by the Bayesian multiple-model analysis. Numbers above and below branches are Bayesian single-model posterior probabilities and ML ultrafast bootstrap values, respectively (except for 13 PCGs topology). For 13 PCGs topology, numbers above are Bayesian single-model posterior probabilities and Bayesian multi-model (13) posterior probabilities, respectively. Number below are Bayesian multi-model (39) posterior probabilities and ML ultrafast bootstrap values, respectively. Asterisk indicates 100% value and dash indicates < 50% value. See Table XX for models of sequence evolution used.



3.3. Ecological features and population status of two endemic and critically endangered species

Chapter 11. Population status and ecological features of the endemic and Critically Endangered Ta Kou bent-toed gecko (Cyrtodactylus takouensis) in Vietnam

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Population Status and Ecological Features of the Endemic and Critically Endangered Ta Kou Bent-Toed Gecko (*Cyrtodactylus takouensis*) in Vietnam

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Academic Editor: Antoni Margalida

Received: 19 May 2025
Revised: 21 August 2025
Accepted: 2 September 2025
Published: 11 September 2025

Citation: Ngo, H.T.; Do, Q.H.; Ngo, H.N.; Nguyen, H.Q.; Pham, A.V.; Pham, C.T.; Nguyen, L.T.; Trinh, H.L.T.; Nguyen, T.Q.; Ziegler, T.; et al. Population Status and Ecological Features of the Endemic and Critically Endangered Ta Kou Bent-Toed Gecko (*Cyrtodactylus takouensis*) in Vietnam. *Conservation* **2025**, *5*, 52. https://doi.org/10.3390/conservation5030052

Abstract

Population estimates and microhabitat characteristics are widely used to support conservation decisions. However, there had been no surveys focusing on the population status of the endemic and Critically Endangered Ta Kou bent-toed gecko to inform conservation actions across its distribution range. In this study, we conducted the first field surveys to assess its population status using the mark–capture–recapture method, determine microhabitat characteristics, and identify anthropogenic threats to the species' survival in Binh Thuan Province, Vietnam. Based on our study results, *Cyrtodactylus takouensis* was only recorded on granitic rocks at various elevations from 265 to 694 m a.s.l. In total, 148 individuals of *C. takouensis* were detected in the dry season, and 95 individuals of *C. takouensis* were encountered in the rainy season. Of these, 73 and 51 adults were documented during the two seasons, respectively. The estimated total population size of *C. takouensis* was 315 individuals in the dry season (95% confidence intervals ranging between 189 and 581 individuals), whereas it comprised 149 individuals, calculated using the Petersen–Lincoln and Schnabel formula, in the rainy season (95% confidence intervals ranging between 108 and 361 individuals). The estimated difference in total population size was probably due to several factors, such as the rapid growth of interlaced vines making parts of the surveyed transects inaccessible during the rainy season, weather variations, and

differences in survey effort and detection probability. Additionally, several microhabitat variables and species behaviors were investigated in both seasons. However, humidity was the only significant environmental variable when compared between the two seasons. Moreover, we found that tourism activities and parasites could pose threats to *C. takouensis* on Ta Kou Mountain. However, no structured or quantitative framework was employed to assess these risks in this study. Further research is needed to quantify factors affecting the species' survival.

Keywords: Binh Thuan Province; granitic rocks; mark-capture-recapture; microhabitat characteristics; direct threats

1. Introduction

The bent-toed gecko lizard genus *Cyrtodactylus* represents one of the most diverse vertebrate genera in the world, with 381 recognized species and still many undescribed forms [1–4]. However, this genus is one of the most neglected vertebrate groups in terms of conservation attention since only a few studies have been conducted to provide information on its population status and the main anthropogenic threats to threatened species [5–7]. To date, although 381 species of this genus have been discovered, as many as 142 species, approximately 37.3%, have not been assessed by the International Union for Conservation of Nature's (IUCN) Red List of Threatened species [4,5]. This is the highest number of unassessed species compared to several other genera in the Gekkonidae family, such as *Hemidactylus* (31.66%), *Lepidodactylus* (14.89%), *Lygodactylus* (29.47%), *Pachydactylus* (1.72%), and *Phelsuma* (1.87%) [4,5]. Consequently, there is an urgent need to evaluate the population status and identify threats to these species in order to support necessary conservation programs.

Additionally, among those assessed, 13.39% (51 species) of species are categorized as Data Deficient, 33.07% (126 species) as Least Concern, 7.87% (30 species) as Near Threatened and Vulnerable, 4.99% (19 species) as Endangered, and 2.89% (11 species) as Critically Endangered [5]. Nevertheless, current assessments have largely been based on general surveys, observations related to their relative commonness or rarity, and the coverage of their habitats by protected areas [5]. Only few of the assessed species have been evaluated for the population status, distribution range, and habitat suitability [5,6]. Furthermore, threats have mostly been recorded by observation during general herpetological surveys, and interviews have barely been undertaken to better understand their extinction risks [5]. Therefore, there is a significant gap in our knowledge of the population status of and main anthropogenic threats to many threatened species of *Cyrtodactylus*.

Located in the Indo-Burma hotspot, Vietnam harbors an unprecedented diversity of *Cyrtodactylus*, with 56 species, and many new ones wait to be discovered [4,8,9]. At the same time, several bent-toed geckos in Vietnam are suffering from anthropogenic threats, such as habitat loss and degradation and pollution due to infrastructure and tourism development, as well as agricultural expansion [5,6,10–13]. These impacts might negatively affect their survival, especially for threatened endemic species, even when their habitats are covered by protected areas [3,5]. According to the IUCN Red List, there are three species listed as Critically Endangered, three as Endangered, and five as Vulnerable, all of which are endemic to the country [5] (Figure 1). However, only one of them (*C. gialaiensis*) has been preliminarily surveyed to estimate its population size [6]. As a result, additional information on population status and main anthropogenic threats should be collected for the remaining species.

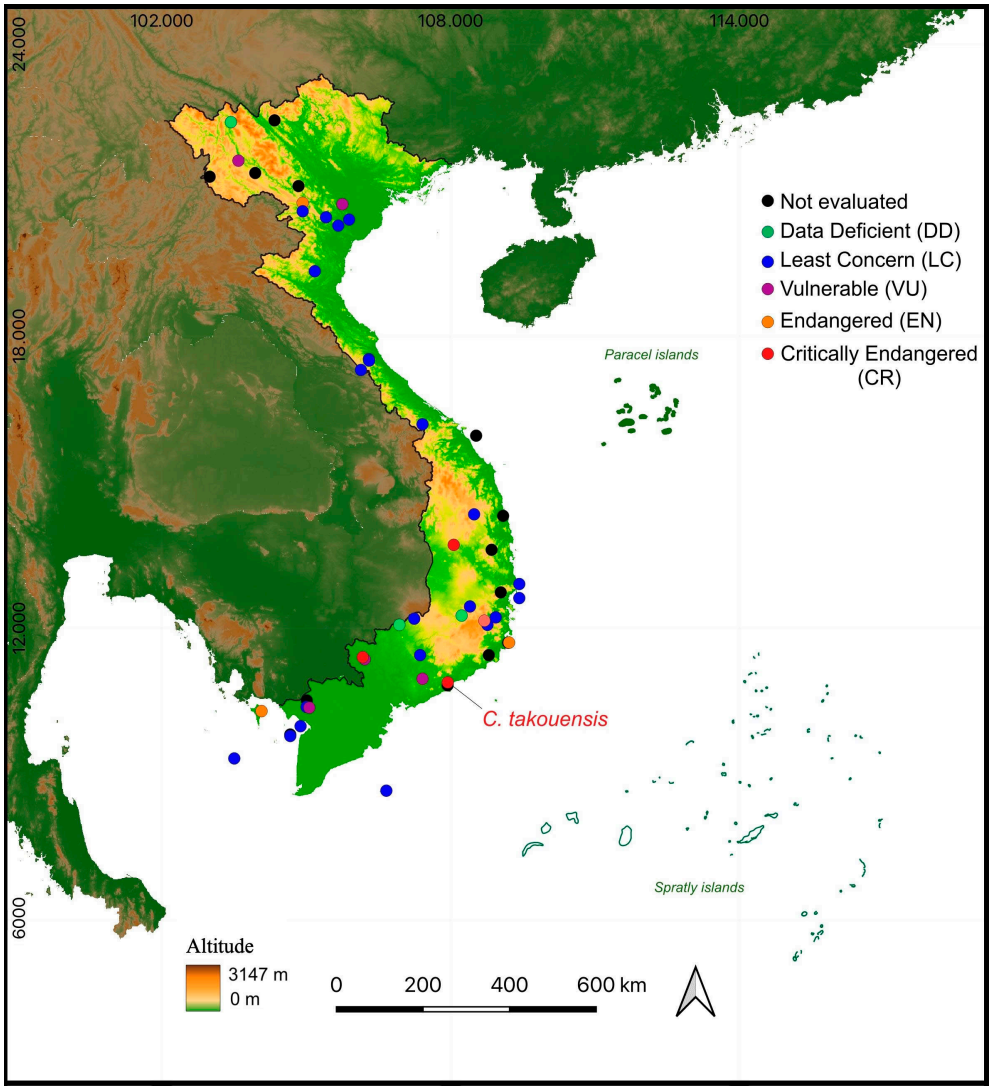

Figure 1. Type localities of all *Cyrtodactylus* species occurring in Vietnam and their threat status on the IUCN Red List. Approximately 61% of the taxa are only known from their type locations. The target species, *C. takouensis*, is marked in red color. The map was plotted using data from https://diva-gis.org/data.html (accessed on 12 November 2011).

The Ta Kou bent-toed gecko (*Cyrtodactylus takouensis* [14]) is locally endemic to Ta Kou Mountain in Binh Thuan Province, Vietnam. This species inhabits the lowland deciduous forests and is often found in association with granite rocks and deep caves [14]. Until now, *C. takouensis* has only been known from its type locality—Hang To Cave, Ta Kou Mountain [14,15]. Consequently, the species has been classified as Critically Endangered (CR) by the IUCN Red List since 2017 due to its extremely restricted range characterized by an extent of occurrence (EOO) of just 16 km^2 [15]. Nonetheless, like other bent-toed geckos in the country, *C. takouensis* has been largely neglected by conservation initiatives. To date, no studies have been conducted to provide information about its population status and microhabitat preferences. Furthermore, all identified threats have been based solely on herpetofauna surveys rather than targeted surveys.

In this study, we carried out field surveys at the type locality of *C. takouensis* and its surrounding areas to answer four key questions: (1) Can *C. takouensis* be found elsewhere within Ta Kou Mountain or in nearby regions? (2) Is the natural population of *C. takouensis* small and fragmented? (3) Does *C. takouensis* prefer specific microhabitats, and does its habitat preference change between different seasons? (4) What potential threats are

adversely affecting the *C. takouensis* population? Based on the study results, we provide recommendations to mitigate risks associated with a declining population size.

2. Materials and Methods

2.1. Field Surveys

Surveys were conducted at all potential localities of *C. takouensis* in the southern central coastal region of Vietnam based on our review of previous herpetofauna surveys from 2010 to 2024 and the literature [14,16–19], as well as interviews with local people and rangers. All the surveys were conducted in both the dry (April 2022) and the rainy seasons (October 2022, January 2024, and November 2024) on Ta Kou Mountain, on Ta Dang Mountain, and in Nui Ong NR, Binh Thuan Province. A total of twenty-one transects were set up, consisting of nine on Ta Kou Mountain, six in Nui Ong Nature Reserve, and five on Ta Dang Mountain. The survey area covered approximately 92.28 km^2, at elevations ranging from 265 m above sea level (a.s.l.) to 694 m a.s.l.

The transects where *C. takouensis* was found spanned a total area of approximately 11.42 km^2 in the dry season and a smaller area of approximately 7.84 km^2 in the rainy season, which was measured by Quantum GIS software (QGIS Version 3.12.0, Development Team. 2020; available online at http://qgis.osgeo.org [accessed on 8 October 2024]). A team of three researchers and one local ranger participated in each survey, which was carried out after sunset between 18:30 and 00:30 to guarantee the highest detection probability. Although two researchers and one local ranger participated, and the time frame was consistent in both seasons to reduce bias, differences in detection probability and observer effects could still influence the seasonal results (please see more detail in the Discussion Section). Geckos were captured by hand and subsequently released at the collection site after being photographed and measured for their SVL (snout–vent length, from tip to snout to vent) with a digital caliper to the nearest 0.1 mm. Coordinates were recorded using a GPSmap 62s (Garmin, Olathe, KS, USA) in a WGS84 datum. Coordinate data can be shared by the authors upon request.

2.2. Population Estimation

To estimate the population size of *C. takouensis*, a "capture–mark–recapture" method, which has been successfully used for many lizard species in Vietnam [6,20–24], was employed. In particular, each individual was marked only once on the head or middle of the body with a correction fluid pen (Tipp-Ex, Eltville, Germany) using a series of dots (for juveniles—SVL < 67 mm [14]) or numbers (for adults—SVL $\geq$ 67 mm [14]) to identify and record the recaptured ones. A series of dots and numbers was assigned continuously each day. For example, on the first day on transect 1, four individuals were captured and labeled as 1 to 4 (adults represented by 1, 2, 3, and 4, and juveniles shown as dots from one to four). The next day, four newly caught adult individuals were labeled from 5 to 8.

Each transect was surveyed at least twice, with an interval of 1–2 days between visits to minimize potential population changes (such as births and deaths) during the survey period. Since there had been no studies on the home range of the species, we considered the home range of the Ta Kou bent-toed gecko to be very small (approximately 0.0001 km^2), based on data of another gekkonid species, *Gekko japonicus* [25]. We therefore assumed that the populations remained closed with no immigration or emigration during the 5–7 days of each survey. In case of two sampling periods, the "Petersen–Lincoln Index" was used following the formula $P = (n_1 \times n_2)/m_2$, where P is the estimated population size; n_1 is the number of marked individuals released in the first sample; n_2 is the size of the second sample; and m_2 is the number of recaptured marked animals. Since $m_2/n_2 > 0.1$, a binomial confidence interval was applied to obtain confidence intervals for the Petersen–

Lincoln estimates [26]. In case of at least three sampling times (one capture and two recaptures), the "Schnabel Index" was applied following the formula below to estimate the population size ($\widehat{N}$) [26,27]:

$$\widehat{N} = \frac{\sum_{i=1}^{t}(C_i M_i)}{\sum_{i=1}^{t} R_i}$$

$$\text{Variance } s^2\left(\frac{1}{\widehat{N}}\right) = \frac{\sum_{i=1}^{t} R_i}{\left(\sum_{i=1}^{t}(C_i M_i)\right)^2}$$

$$\text{Standard error } s_{\bar{x}}\left(\frac{1}{\widehat{N}}\right) = \sqrt{s^2\left(\frac{1}{\widehat{N}}\right)}$$

where M_i is the total number of previously marked animals at time i; C_i is the number of animals caught at time i; and R_i is the number of marked animals caught at time i. Confidence intervals for the Schnabel population estimate were obtained from the Poisson distribution because the total number of recaptures ($\sum R_i$) was less than 50. In particular, a 95% confidence interval on ΣR_i was calculated as follows [28,29]:

$$\text{Lower 95\% confidence limit } = \frac{\sum(C_i M_i)}{\sum R_i}$$

$$\text{Upper 95\% confidence limit } = \frac{\sum(C_i M_i)}{\sum R_i}$$

Regarding population structure, individuals of *C. takouensis* were classified into two different age groups, including juveniles (SVL < 67 mm) and adults (SVL ≥ 67 mm) [14]. The sex of adults was determined based on the presence of large swollen hemipenal bulges in males and un-swollen ones in females [30,31]. Population densities of all individuals and only adults were further calculated per square kilometer (indiv./km^2) with reference to each surveyed transect and day (indiv./km^2/day).

2.3. Microhabitat Characterization and Behaviors

To record microclimatic parameters, the air temperature (°C) and relative air humidity (%) were measured with a digital thermometer (TFA Dostmann/Wertheim Kat. No. 30.5015, Wertheim, Germany) at each location where animals were captured. A digital infrared thermometer (Mestek, Shenzhen, China) was used to measure temperatures (°C) at the substrate surface and at the ventral body surface of the animals. Moreover, two HOBO pendant temperature data loggers (HOBO, Eichstetten am Kaiserstuhl, Germany) and one Elitech RC-51H USB temperature and humidity data logger, 3200 (Elitech, London, UK), were also employed to record the air temperature (°C) twice per day between April and October 2022 at three transects where animals were found.

Other microhabitat characteristics were also documented: the substrate type (classified as dead leaves, branches, dead wood, rock, roots, soil, trunk), rocky surface (classified as bare or covered with moss and lichen, tree roots), position (outside or inside a rocky cave/crevice), canopy (percentage of vegetation coverage above each animal—estimated by direct observation), substrate condition (dry or wet), substrate angle (between the substrate surface axis and the horizontal axis, ranging from 0° to 180°), and elevation of capture locations using the GPSmap 62s (Garmin, Olathe, KS, USA). The animal posture (hanging—identified as the animals being attached to the underside of rocks; standing—identified as the animals being positioned on top of rocks), activity (resting—identified as the animals standing when encountered; feeding—identified as the animals eating when detected; or moving—identified as animals moving when encountered), and encounter time were further recorded.

2.4. Statistical Analysis

Statistical analysis was performed using Rv4.4.3 [32]. A Chi^2 test was applied to test the difference in population structure between the dry and rainy seasons. For microhabitat characteristics, six categorical variables (i.e., substrate type, rocky surface, location, substrate condition, animal posture, and activity) were also seasonally compared using Chi^2 tests. Other microhabitat traits (i.e., canopy, air temperature, substrate temperature, animal temperature, and humidity) were estimated for normal distribution using Shapiro–Wilk tests and tested for differences by Wilcoxon tests. For all these tests, a significant difference was determined using the *p*-value ($p < 0.05$) and effect sizes ($r \geq 0.5$).

2.5. Threat Identification

To detect impacts of human activities on the species in Vietnam, two large local markets near Ta Kou NR were visited, and ten local people, each having lived near Ta Kou NR for at least two generations and knowledgeable of the area, and one ranger were also interviewed to determine the local use of the species (Appendix A). Nocturnal and diurnal surveys were also carried out to obtain evidence of human disturbances and direct threats to the species, such as poaching, deforestation, direct-burning incenses in some caves, and littering on Ta Kou Mountain and along transects in the surrounding areas. Additionally, data related to direct effects on the species were collected during the field surveys.

3. Results

3.1. Population Status

The Ta Kou bent-toed gecko was not found on Ta Dang Mountain (an isolated mountain belonging to Ta Kou NR) or in Nui Ong Nature Reserve, and it was only detected along three transects (transects 1, 2, and 3) on Ta Kou Mountain. A total of 243 individuals of *C. takouensis* were encountered along the transects, including 118 captured individuals (73 adults—62%) and 30 escaped ones during the dry season and 76 captured individuals (51 adults—67%) and 19 escaped ones during the rainy season (Table 1). We also documented 61 recaptures in both seasons (37 and 24 individuals in the dry and rainy seasons, respectively). In terms of the sex ratio among adult captured individuals, the number of females was higher than that of males in both seasons (Figure 2, F:M = 42:31 in the dry season and 29:22 in the rainy season). However, there were no significant differences in sex ratio between the dry and rainy seasons ($Chi^2 = 0.995$, effective size = 0.072, CI lower = 0.00, CI upper = 0.19, df = 2, $p = 0.608 > 0.05$). According to the Petersen–Lincoln and Schnabel indices, the total population size of *C. takouensis* was estimated at about 315 individuals in the dry season (95% confidence intervals ranging between 189 and 581 individuals) and 180 individuals in the rainy season (95% confidence intervals ranging between 108 and 361 individuals) (Table 1).

In general, the average densities of *C. takouensis* were similar between the two seasons, around 12–13 individuals/km^2 (6–7 adults/km^2). When recaptures on surveyed days were incorporated, the average density in the rainy season (7.5 individuals/km^2/day) was higher than that in the dry season (3.8 individuals/km^2/day) (Table 1). There was substantial variation among the surveyed transects in terms of the population density (minimum: 2.97 individuals/km^2/day along T1 during the dry season; maximum: 13.47 individuals/km^2/day along T2 during the rainy season).

Table 1. Total observed numbers and estimated population size of *Cyrtodactylus takouensis* on Ta Kou Mountain. D_A = density estimation of adult individuals per km^2; D_{AD} = density estimation of adult individuals per km^2 per day; D = overall density estimation; D_D = overall density estimation per km^2 per day; N = estimated total individuals. * During the capture–recapture event, this transect was surveyed two times. Therefore, Nicole Peterson's formula was applied for estimation.

Transects	Transect 1 (T1)	Transect 2 (T2)	Transect 3 (T3)	Total
Dry Season—April 2022				
Total Adults	14	21	38	73
Total Obs	19	42	87	148 (NA = 30)
Area (km^2)	2.36	2.97	6.09	11.42
D_A (Adults/km^2)	5.93	7.07	6.24	6.39
D_{AD} (Adults/km^2/day)	1.48	1.77	1.56	1.60
D (ind./km^2)	8.05	14.14	14.29	12.96
D_D (ind./km^2/day)	2.01	3.54	3.57	3.24
N–Total Schnabel	27	109	179	**315**
Variance	1.48×10^{-6}	1.19×10^{-5}	0.0156	0.015638
Standard error	0.00122	0.00345	0.125	0.1297
Lower 95% confidence limits	119	56	14	**189**
Upper 95% confidence limits	294	233	54	**581**
Rainy season—September 2022				
Total Adults	8	7	36	51
Total Obs	11	12	72	95 (NA = 19)
Area (km^2)	1.23	0.52	6.09	7.84
D_A (Adults/km^2)	6.50	13.46	5.91	6.51
D_{AD} (Adults/km^2/day)	2.17	4.49	1.97	2.17
D (ind./km^2)	8.94	23.08	11.82	12.12
D_D (ind./km^2/day)	2.98	7.69	2.96	3.03
N–Total Schnabel	14	24 *	142	**180**
Variance	0.00108	-	2.93×10^{-6}	-
Standard error	0.00171	-	0.0329	-
Lower 95% confidence limits	6	10	92	**108**
Upper 95% confidence limits	35	75	251	**361**
Human impacts	Severely disturbed	Intact	Disturbed	

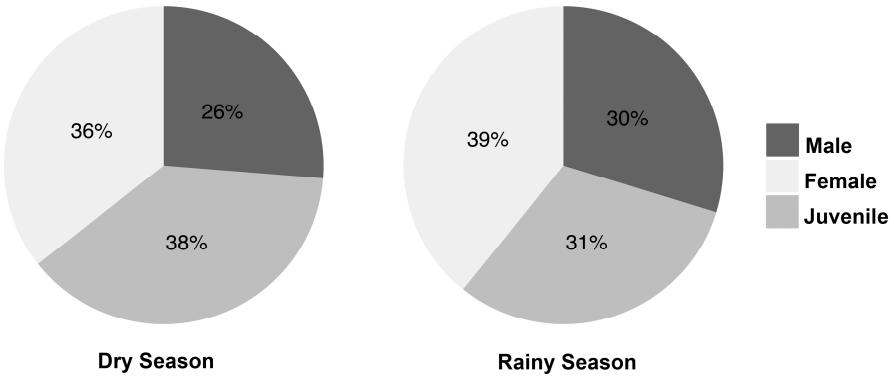

Figure 2. Pie charts representing age and sex ratios of the *Cyrtodactylus takouensis* population.

3.2. Microhabitat Selection and Behaviors

Cyrtodactylus takouensis was only found on granitic rocks, both with and without the coverage of evergreen broad-leaved forest intermixed with ferns, shrubs, and vines on Ta Kou Mountain, at elevations from 265 m to 694 m a.s.l. (Figure 3). The gecko species was not found at dragon fruit plantation sites, nor in some small bamboo areas. A majority of individuals were sighted on granite rocks in both seasons (83% and 92% in the dry and rainy seasons, respectively) (Figure 4A–D). Consequently, there were no significant differences in substrate type between the two seasons (Chi^2 = 11.01, effective size = 0.17,

CI lower = 0.00, CI upper = 0.28, df = 6, $p = 0.088 > 0.05$; Figure 4A,B). Concerning rocky substrates only, the species was mostly found on the bare surface of granite rocks in both seasons (65% and 57% in the dry and rainy seasons, respectively), followed by rocks covered by moss/lichen (32.7% and 41.9% in the dry and rainy seasons, respectively) or small tree roots (2% and 1% in the dry and rainy seasons, respectively). However, there were no significant differences in the surfaces of rocky substrates between the two seasons ($Chi^2 = 2.31$, effective size = 0.10, CI lower = 0.00, CI upper = 0.21, df = 2, $p = 0.315 > 0.05$; Figure 4B). Therefore, substrate types and rocky surfaces may not be important environmental factors when comparing between the two seasons.

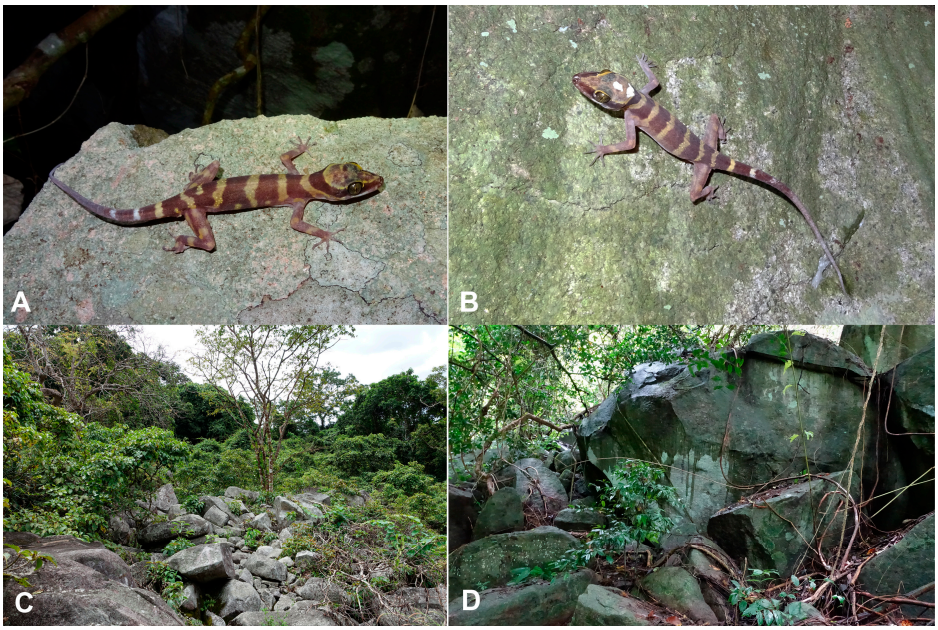

Figure 3. (**A**). *Cyrtodactylus takouensis* on Ta Kou Mountain; (**B**). marked *C. takouensis* on occurrence site; (**C**). macrohabitat of *C. takouensis* along T1; (**D**). microhabitat of *C. takouensis* along T3.

Additionally, 80% of captured individuals of *C. takouensis* were observed on dry substrates in the dry season, while 69.9% of individuals were on wet substrates in the rainy season, with a significant difference between the two seasons ($Chi^2 = 59.02$, effective size = 0.50, CI lower = 0.37, CI upper = 0.63, df = 1, $p < 0.0001$; Figure 4D). In terms of behaviors, the species was mainly encountered hanging (dry: 56.6%, rainy: 74.7%) ($Chi^2 = 7.75$, effective size = 0.18, CI lower = 0.05, CI upper = 0.31, df = 1, $p = 0.005 < 0.05$), outside granite caves (78% and 51.6%, respectively) ($Chi^2 = 16.65$, effective size = 0.28, CI lower = 0.14, CI upper = 0.41, df = 1, $p = 4.48 \times 10^{-5} < 0.05$), but mostly resting (more than 80%) ($Chi^2 = 3.02$, df = 2, $p > 0.05$) in both seasons, with no difference between the two seasons (Figure 4C,D,F).

The canopy coverage was much less dense during the dry season (86.71 ± 2.3%; $n = 123$) compared to the rainy season (90.54 ± 2.57, $n = 93$). However, there were no differences in canopy coverage between the dry and rainy seasons (W = 4757.5, r = 0.18 < 0.5, CI lower = 0.05, CI upper = 0.32, $p = 0.007 < 0.05$; Figure 5; Table 2). The microclimatic niche of *C. takouensis* was characterized by overlapping ranges of air temperatures (23.3–30.9 °C, $n = 238$) and substrate temperatures (19.1–27.3 °C, $n = 237$) across both seasons, with no statistically significant differences between the dry and rainy seasons ($p > 0.05$; Figure 5; Table 2). In contrast, the mean relative humidity at microsites was 62.16 ± 0.87% (40–85%, $n = 145$) during the dry season, significantly lower than that of 81.77 ± 0.58% (69–88%, $n = 93$) during the rainy season (W = 644; $p < 0.001$; Figure 5; Table 2). Therefore, canopy,

air temperature, substrate temperature, and animal temperature might not be significant determining environmental variables when comparing between the two seasons.

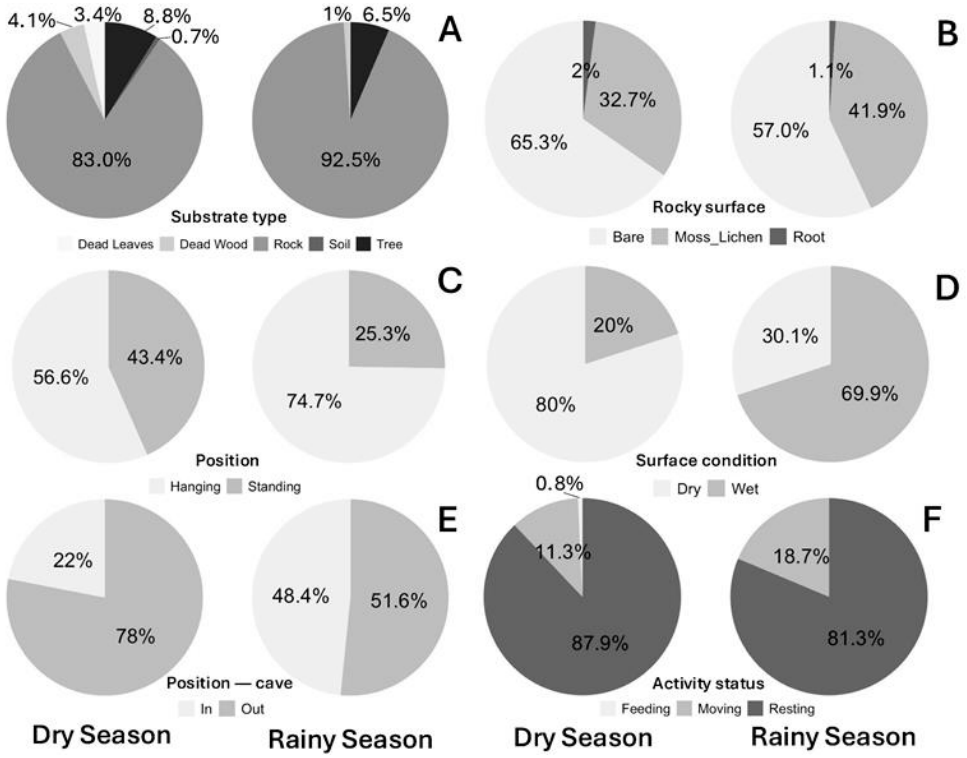

Figure 4. Comparison of microhabitat characteristics and behaviors of *Cyrtodactylus takouensis* between dry and rainy seasons: (**A**) substrate type; (**B**) rocky surface; (**C**) posture of *C. takouensis*; (**D**) surface condition; (**E**) location outside or inside cave; (**F**) activity status.

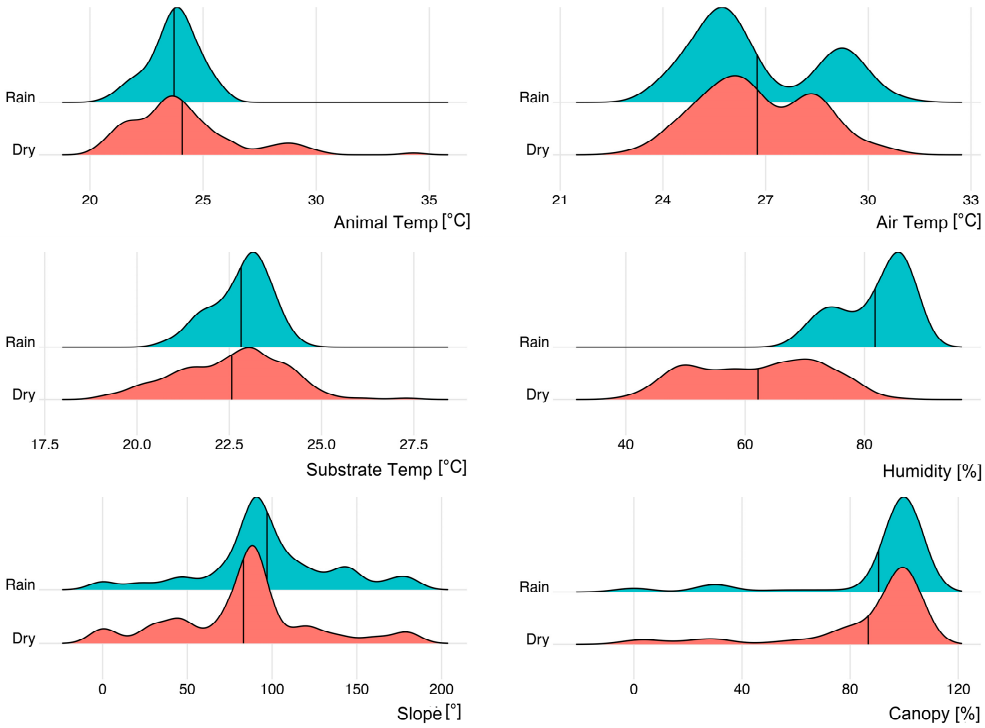

Figure 5. Comparisons of environmental parameters characterizing the microhabitat of *Cyrtodactylus takouensis* in the dry and rainy seasons.

Table 2. Environmental parameters characterizing the microhabitat of *Cyrtodactylus takouensis*.

Parameters	Dry Season	Rainy Season	Wilcoxon Test
Canopy cover [%]	0–100 (86.71 ± 2.3) (n = 123)	0–100 (90.54 ± 2.57) (n = 93)	W = 4757.5, r = 0.18 < 0.5, CI lower = 0.05, CI upper = 0.32, p-value = 0.007 < 0.05 -> no significant seasonal differences
Elevation [m]	267–700 (434.25 ± 14.15) (n = 146)	265–678 (358.14 ± 0.05) (n = 93)	
Animal Temp. [°C]	20.3–34.2 (24.08 ± 0.22) (n = 118)	20.8–25.8 (23.72 ± 0.12) (n = 76)	W = 4450, r = 0.006 < 0.5, CI lower = 0.002, CI upper = 0.16, p-value = 0.93 > 0.05 -> no significant seasonal differences
Substrate Temp. [°C]	19.1–27.3 (22.57 ± 0.12) (n = 144)	20.8–24.4 (22.82 ± 0.08) (n = 93)	W = 6034.5, r = 0.08 < 0.5, CI lower = 0.01, CI upper = 0.20, p-value = 0.20 > 0.05 -> no significant seasonal differences
Air Temp. [°C]	23.3–30.5 (26.76 ± 0.13) (n = 145)	23.8–30.9 (26.75 ± 0.19) (n = 93)	W = 6958, r = 0.03 < 0.5, CI lower = 0.002, CI upper = 0.16, p-value = 0.68 > 0.05 -> no significant seasonal differences
Air Humidity [%]	40–85 (62.16 ± 0.87) (n = 145)	69–88 (81.77 ± 0.58) (n = 93)	W = 644, r = 0.76 > 0.5, CI lower = 0.72, CI upper = 0.80, p-value < 2.2×10^{-16} < 0.05 -> significant seasonal differences
Slope [°]	0–180 (83.12 ± 3.51) (n = 136)	0–180 (97.03 ± 4.04) (n = 91)	W = 4682.5, r = 0.21 < 0.5, CI lower = 0.09, CI upper = 0.34, p-value = 0.002 < 0.05 -> no significant seasonal differences

Three temperature dataloggers at different times (dry season: one month; rainy season: five months) recorded air micro-temperatures ranging from 22.43 °C to 28.95 °C (Figure 6).

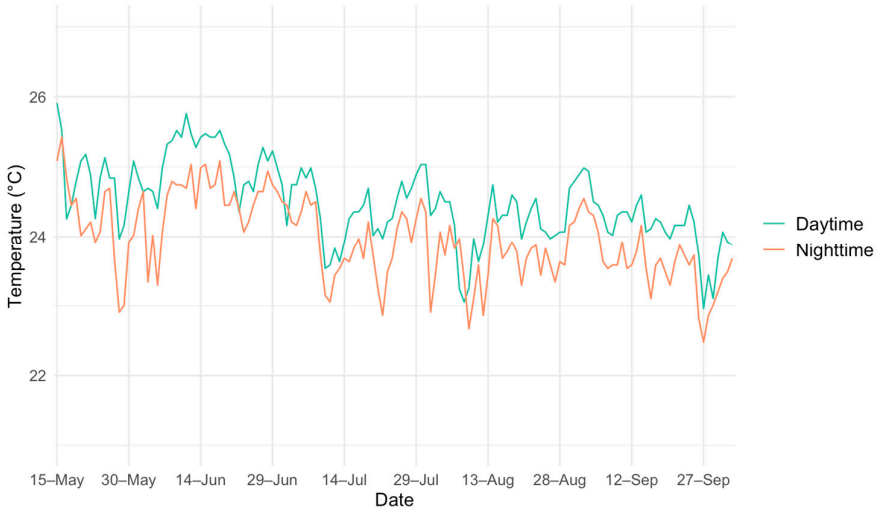

Figure 6. Average daytime and nighttime temperature per day from 15 May 2022 to 30 September 2022 in the microhabitat of *Cyrtodactylus takouensis*.

We further measured the body temperature of *C. takouensis* individuals (dry: 24.08 ± 0.22 °C, rainy: 23.72 ± 0.12 °C, W = 4374, p > 0.05; Table 2). We also noted that the body temperature of *C. takouensis* was correlated with the substrate temperature (r = 0.4, t = 6.06, df = 192, $p = 7.14 \times 10^{-9}$ < 0.05) and air temperature (r = 0.19, t = 2.63, df = 192, p = 0.009 < 0.05). Regarding its active time, *C. takouensis* was found at all night survey times, with the main peak occurring between 19:00 and 20:00 in both seasons (Figure 7).

Figure 7. Numbers of encountered (including captured and escaped) *Cyrtodactylus takouensis* individuals in one-hour intervals during survey times.

3.3. Main Threats

Even though individuals of *C. takouensis* are neither collected for food nor poached for the pet trade, tourism activities and parasites are considered important threats to its wild populations. In particular, infrastructure developments associated with the cable car built a decade ago are still expanding to accommodate the growing tourism around transect 1, since Ta Kou NR has become a popular tourist destination in southern Vietnam in recent years. Additionally, transect 1 was visited every day and flashlit every night by tourists in both seasons, while another surveyed transect (transect 3) was visited at least twice or three times a month. During surveys along transects in both seasons, a large amount of plastic waste left by tourists was observed at several locations where *C. takouensis* was found (Figure 8A,B). Furthermore, many individuals (dry: 22; rainy: 26) were detected with ticks as ectoparasites and an unidentified parasite as endoparasites on the body surface (Figure 8C,D). Although we could not identify the parasites, they were common in adult individuals of *C. takouensis* (23% during the dry season and nearly 43% during the rainy season). Further studies are therefore needed to provide more information on the parasites and how they affect the fitness of *C. takouensis* in the future.

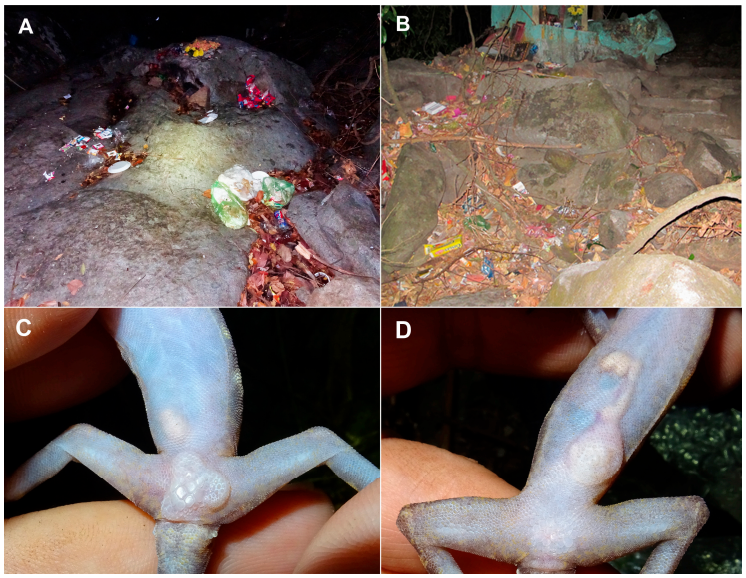

Figure 8. (**A**,**B**) Plastic trash was observed at several locations where *C. takouensis* was found; (**C**,**D**) endoparasites were found in *C. takouensis*. Photos by L.T. Nguyen.

4. Discussion

4.1. Population Status

Using capture–mark–recapture data, we provided the first estimate of the Ta Kou bent-toed gecko population. Our results show that this endemic and Critically Endangered species possesses a very small population size in our study area. The effective population size only reflects the assessment at the three known sites of the species on Ta Kou Mountain. Potential observer bias limits the ability to generalize our findings to other areas where the species may exist. The total population size of the species is assumed to be limited and does not exceed the size of a minimum viable population required for long-term stability, which is estimated to be at least 3000 to 7000 individuals [33,34], because this species almost exclusively occupies granite habitats. Therefore, this range-restricted species is especially imperiled by additional anthropogenic threats.

This study also revealed wide seasonal fluctuation in population estimates, which was probably caused by several factors. Firstly, the rapid growth of interlaced vines made parts of surveyed transects 1 and 2 inaccessible in the rainy season. In the dry season, 2.36 km^2 and 2.97 km^2 were assessed in transects 1 and 2, respectively, while only 1.23 km^2 and 0.52 km^2, respectively, were evaluated during the rainy season (Table 2). Secondly, variations in survey effort and detection probability may account for fluctuations in population estimates. Throughout both seasons, the number of researchers and rangers involved in each survey remained unchanged, with three-quarters of the team members consisting of the same individuals to minimize bias. However, differences in detection probability still occurred among survey members due to personal health or individual circumstances. Moreover, weather conditions such as wind speed and humidity, microhabitat surface wetness, and gecko behavior may also have impacted the detection probability of species in the two seasons. Therefore, population estimates across seasons may not be ecologically meaningful. Future studies should incorporate detectability-corrected frameworks (e.g., N-mixture or occupancy models) to provide more accurate seasonal estimates of the total population. Our results suggest that the mean density of *C. takouensis*, at 12–13 individual/km^2, is significantly lower than that of other Critically Endangered gecko species (Table 3).

Table 3. Comparison of density estimations between *C. takouensis* and other species.

Species	Locality	Density Estimation	Method Notes	References
Cnemaspis thackerayi	Salam District, India	1,000,000–2,000,000 individual/km^2 (10–15 individuals/7.5 m^2)	Herpetofauna surveys	[35]
Gonatodes daudini	Grenadines and Saint Vincent	8700–21,900 individuals/km^2 (87–218 individuals/ha)	These figures resulted from surveys targeting four cryptic reptile species. The process involved sifting through litter and lifting various types of cover, such as rocks, logs, and deadfall, by two researchers along the transects, while a third person monitored the edges to observe any escaping animals	[36,37]
Lygodactylus williamsi	Tanzania	35,300 individuals/km^2 (353 individuals/ha)	Observations by three researchers in the mornings and afternoons	[38]

4.2. Microhabitat Selection and Behaviors

Cyrtodactylus takouensis is considered a microhabitat specialist only occupying bare granitic boulders or those covered with moss, lichen, and small tree roots in evergreen forest intermixed with ferns, shrubs, and vines. As it is an ectothermic gecko, the basic physiological functions of *C. takouensis*, such as locomotion, growth, and reproduction, and its microhabitat selection are assumed to be influenced by environmental conditions [39]. Resembling another insular gecko, *Cnemaspis psychedelica*, in southern Vietnam, *C. takouensis* also exhibits seasonal variation in its microhabitat selection, including the substrate condition (dry or wet), canopy coverage, and humidity [40]. Using the ordination test, we demonstrated that canopy and humidity are the most important parameters explaining the seasonal variation in the microhabitat selection of *C. takouensis*.

4.3. Main Threats

Tourism development has not only substantially fragmented but also resulted in the loss and degradation of animal habitats worldwide [41]. For *C. takouensis*, due to its very small population size, annual tourism growth could threaten the species' long-term survival, particularly through habitat disturbance, if not properly managed. Although the impact of plastic trash was not assessed quantitatively, the presence of plastic waste implies potential habitat degradation, which could negatively impact the quality of the limited microhabitat of this species. Furthermore, the prevalent parasites detected in *C. takouensis* in this study might also adversely affect the population of this species. However, no structured or quantitative framework was employed to assess these risks in this study. Further research is needed to quantify the factors affecting the species' survival.

4.4. Conservation

In the Vietnam Red Data Book, Nguyen et al. [42] reassessed the conservation status of the species and classified it as Endangered (B1ab(iii)), based on an estimated extent of occurrence (EOO) of approximately 120 km^2. However, our results suggest that *C. takouensis* as currently known only occurs on Ta Kou Mountain, where the species occupies a specific microhabitat and is restricted to a small area of the mountain. The population size of *C. takouensis* is also quite small, with an estimation of 315 individuals, with the number of adult individuals ranging from 51 to 73. Using localities where the species is found, we recalculated the EOO utilizing the GeoCAT tool (available at "https://geocat.iucnredlist.org/editor (accessed on 12 May 2025)"), which was introduced by the IUCN Red List, based solely on the GPS data from our surveys [43]. The EOO of *C. takouensis* was estimated to be approximately 2 km^2, which falls within the Critically Endangered category (B2ab(iii)) [44].

Besides its very small range, this species might be particularly vulnerable to infrastructure development, which often results in habitat loss and fragmentation. We therefore recommend that Ta Kou NR closely monitor the construction works along transect 1 and enforce a higher protection level within the granite habitat of *C. takouensis*, such as limiting visitors from entering the granite areas of transect 1 and transect 3. Further studies should be undertaken to better understand the severity of the parasites found on the species. To save this species in the long term, it is essential to raise awareness among the local community and visitors with regard to littering and habitat protection. Under supported projects, we trained the protected-area staff in using the Spatial Monitoring and Report Tool (SMART) for patrolling and designed and printed signboards and leaflets for educational purposes, but additional long-term conservation measures need to be implemented in the nature reserve.

Author Contributions: Conceptualization, M.D.L., T.Q.N. and T.Z.; methodology, H.T.N., Q.H.D., H.Q.N., A.V.P., H.N.N., M.D.L., C.T.P., T.Q.N. and L.T.N.; software, H.T.N. and H.N.N.; validation, H.T.N., Q.H.D., H.Q.N., A.V.P., H.N.N., M.D.L., C.T.P. and T.Q.N.; formal analysis, H.T.N. and H.N.N.; investigation, H.T.N., H.N.N. and M.D.L.; resources, M.D.L. and H.L.T.T.; data curation, H.T.N.; writing—original draft preparation, H.T.N., H.N.N. and M.D.L.; writing—review and editing, all authors; supervision, C.T.P., M.D.L. and T.Z.; project administration, M.D.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Critical Ecosystem Partnership Fund (CEPF-111890), the Asian Species Action Partnership (ASAP), the Zoological Society for the Conservation of Species and Populations (ZGAP), and the Rufford Foundation. Cologne Zoo partially funded field surveys by H.T.N., and H.T.N.'s research in Germany was funded by the German Academic Exchange Service (DAAD).

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board of the Center for Environment and Community Assets Development (date of approval: 7 October 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available on request to the first author (H.T.N.).

Acknowledgments: For supporting fieldwork and issuing relevant permits, we thank the Forest Management Department of Ta Kou Nature Reserve, its staff, and the Department of Agriculture and Rural Development of Binh Thuan Province. We are very thankful to S.N. Nguyen (Institute for Tropical Biology) for advice and M.V. Le (University of Da Nang—University of Science and Education, Da Nang, Vietnam) for assistance in the field. Comments from four reviewers greatly improved the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Interview questions on the threat of the Ta Kou bent-toed gecko lizard

Team member:.....
Date:.....
Time:.....
Interview No.....
Commune name:.....

1. How many years have you lived in this local region?
2. What do you do in your commune?
3. Do you usually go to the forest? ☐ Yes ☐ No

If yes,
What do you do in the forest?

If no,

4. Do you know the Ta-Kou bent-toed gecko lizard? ☐ Yes ☐ No

If yes,
- How will I recognize the species?
- Are there any local names for the Ta-Kou bent-toed gecko lizard?
- What do you do if you see the Ta-kou bent-toed gecko lizard?
- Do you know where I can find the Ta-kou bent-toed gecko lizard?
- Do you know anyone else who are known about the Ta-kou bent-toed gecko lizard?

(If yes, we ask some general information: name, where we can find him/her?)
We show them the photo of the Ta-Kou bent-toed gecko lizards to confirm the species.

If no, show the photo of the Ta-Kou bent-toed gecko lizards. If they know this species, our conversation can continue. If they don't know, the conversation stops.

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Chapter 12. Decades of habitat destruction – Is there any chance left for the Critically Endangered and endemic Cyrtodactylus gialaiensis in Vietnam?

(Nature Conservation – Minor Revision)

Decades of habitat destruction – Is there any chance left for the endemic and Critically Endangered *Cyrtodactylus gialaiensis* in Vietnam?

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Abstract

The discovery of the Gia Lai Bent-toed Gecko (*Cyrtodactylus gialaiensis*) on a coffee farm in Chu Se District, Gia Lai Province, raises concerns about its natural habitats, threats, and conservation status. Restricted to a small range of less than 10 km^2 in Vietnam and distributed outside of any protected area in the country, the species has been classified as Critically Endangered in the IUCN Red List soon after its description. A recent survey identified a small population of the gecko in Chu Se District, but other potential sites have not been investigated. In this study, we expand the survey area to assess population status and distribution of the Critically Endangered species. Our results show that *C. gialaiensis* occurs in at least three districts of Gia Lai Province, including Chu Se, Duc Co, and Chu Pah. The subpopulation size in Chu Se is estimated to be extremely small with fewer than 100 individuals, but still larger than those found in Duc Co and Chu Pah. We identify habitat loss, degradation, and fragmentation and uncertainties related to land use and land cover as the main the drivers of its alarming population decline and potential imminent extinction of its wild population in the near future. Although some conservation actions are implemented as part of this study, there remains an urgent need to strengthen both in situ and ex situ conservation efforts for this highly imperilled species.

Keywords: Conservation efforts, extinction risks, Gia Lai Bent-toed Gecko, land use change, population decline

Introduction

Habitat loss, referring to the decline in areas suitable for wildlife, typically occurs with habitat fragmentation, which divides the continuous habitat into smaller and isolated patches (Pimm and Raven 2000; Wilson et al. 2016). They are among the main threats to global biodiversity and the foremost causes of species extinction (Pimm and Raven 2000; Wilson et al. 2016; Hogue and Breon 2022) by substantially reducing effective population size and distribution range of resident species and subsequently disrupting the interspecies interactions essential for ecosystem functionality (Haddad et al. 2015; Newbold et al. 2015). While there can be evolutionary advantages associated with habitat fragmentation within some animal populations, this process may not occur rapidly enough to benefit many species facing the challenges of habitat degradation (McClure et al. 2008; Allendorf et al. 2013). Consequently, habitat loss is associated with over 85% of species classified as threatened by the IUCN Red List (Hogue and Breon 2022; IUCN Red List of Threatened species). Of these, it has a strong negative effect on reptile populations when compared to other vertebrate groups, such as birds and mammals, due to small distributional ranges, elevated levels of endemism, limited ability of dispersal, and specific ecological requirements (Todd et al. 2010; Böhm et al. 2013; Meiri et al. 2018). In addition, Böhm et al. (2013) reported that many reptile species have been adversely impacted by habitat loss in both areas of higher (tropical regions) and lower reptilian species richness. In particular, Southeast Asia is situated at the center of the threat due to its extremely high rate of deforestation (Böhm et al. 2013).

Located in the Southeast Asia, Vietnam has been recognized as one of the most biodiverse countries globally, featuring a wide range of ecosystems, including tropical forests, streams, lagoons, and coral reefs (Myers et al. 2000; Sterling et al. 2006). However, like many tropical regions, Vietnam has suffered dramatic losses in the primary forest due to agricultural expansion over recent decades (Nguyen 2000; Meyfroid and Lambin 2008, 2009; Bourgoin et al. 2019; MARD 2020; Xiao et al. 2023). Between 1930s and 1992, the nation's forest cover substantially shrank from 42.7% to 24.7% of its total land area, based on land cover changes (Nguyen 2000; Meyfroid and Lambin 2008, 2009; MARD 2020). Apart from massive overexploitation, habitat loss has resulted in a decline of several reptilian populations across the country (van Schingen et al. 2015; Le et al. 2020; Ngo et al. 2022). Since 1992, national reforestation initiatives aimed at

rehabilitating barren land have facilitated the recovery in forest cover, which reached approximately 41.89% coverage in 2019 (Fig. 1) (Meyfroid and Lambin 2009; Bourgoin et al. 2019; MARD 2020). However, reforestation efforts have predominantly concentrated on industrial plantations utilizing fast-growing monocultures of exotic species, such as *Eucalyptus* sp., *Acacia* sp., and *Pinus* sp. or native species like *Coffea* sp., *Piper nigrum*, *Durio zibethinus* (Turnbull et al. 1997; Kha et al. 2003). These monoculture plantations are generally ineffective in restoring original biodiversity (Barlow et al. 2007; Matthews et al. 2002; Newmaster et al. 2011) and can never replace the conservation value of natural ecosystems. Moreover, plantations with single species are easier to log (Kelty 2006) or convert to other crops, leading to uncertainty in land use. Species inhabiting plantation areas are therefore generally more susceptible to human disturbances than those living in natural habitats.

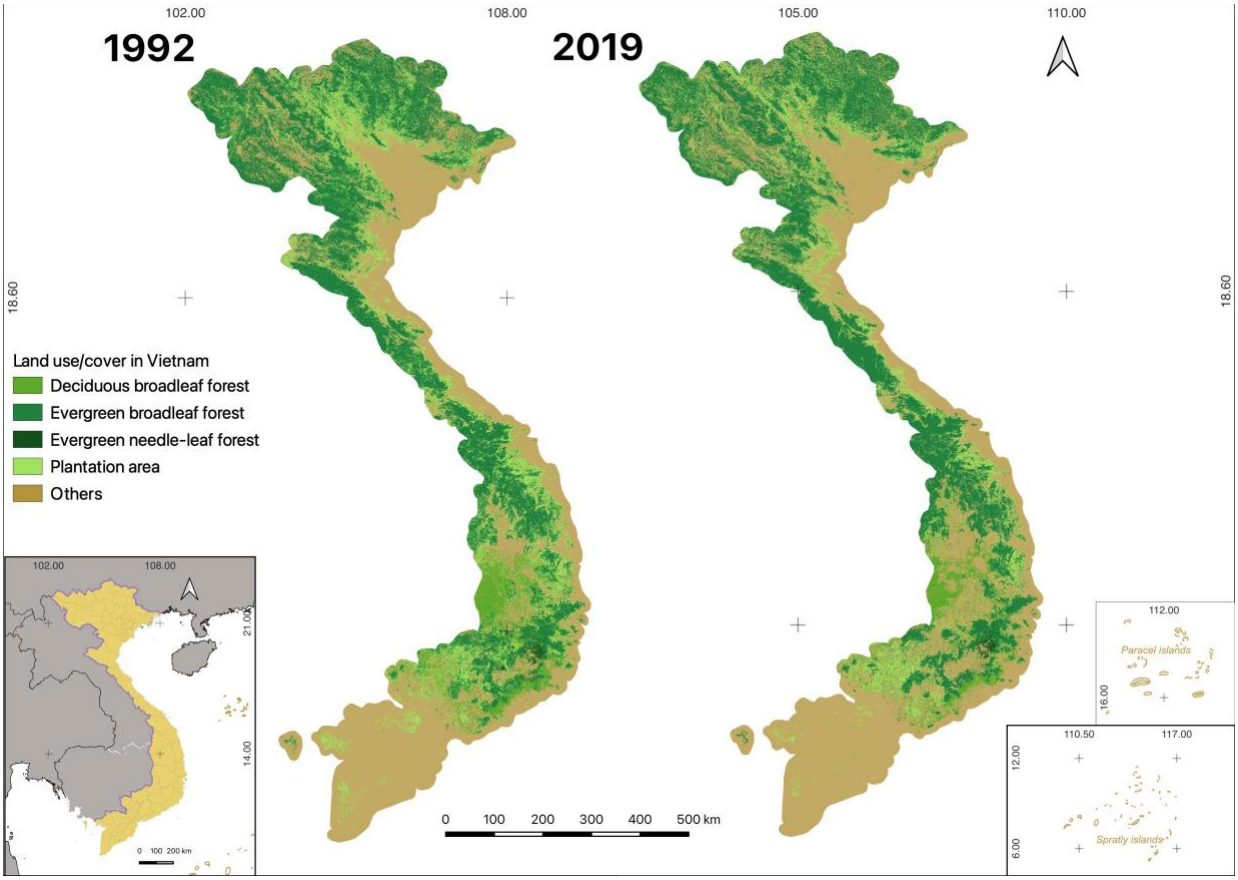

Figure 1. Forest cover change in Vietnam from 1992 to 2019 (map based on ALOS Science Project, Japan Aerospace Exploration Agency).

The Gia Lai Bent-toed Gecko, *Cyrtodactylus gialaiensis*, was first discovered in 2017 (Luu et al. 2017; Luu 2018; Luu et al. 2020). Until now, it has only been found in a coffee plantation site or the soil cliff between the coffee and rubber plantations in Chu Se District, Gia Lai Province, Vietnam (Fig. 2) (Luu 2018; Luu et al. 2020). This endemic species is estimated to occur within a very restricted distribution range of less than 10 km^2, located entirely outside protected areas (Luu 2018; Luu et al. 2020). As a result, *C. gialaiensis* has been classified as Critically Endangered (CR) by the IUCN Red List since 2018 (Luu 2018). To date, the species has been subject to many threats, such as habitat loss because of land use change and the risk of predation by domesticated animals (Luu 2018; Luu et al. 2020). However, recent surveys were conducted only over a small area around the type locality of *C. gialaiensis* in Chu Se District, Gia Lai Province, Vietnam (Luu 2018; Luu et al. 2020). Other potential areas in Chu Se District and suitable habitats surrounding Chu Se have not been investigated. To better understand the population status and distribution range of the Critically Endangered gecko, we conducted extensive surveys of the surrounding areas and identified threats facing this species. Based on the findings, conservation measures are proposed to better protect the species from extinction risks.

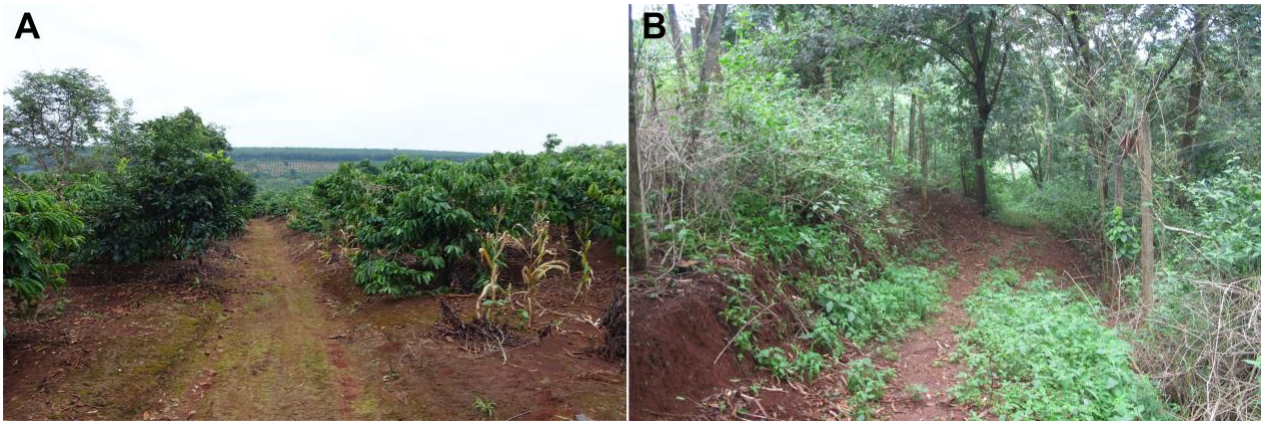

Figure 2. Habitat of *Cyrtodactylus gialaiensis*. A) The coffee plantation area is a locality of *C. gialaiensis*. B) soil cliff near coffee plantation area.

Materials and Methods

Field surveys

Field surveys were carried out in March 2022, October 2022, June 2023, August 2023, July 2024, August 2024, and April 2025 in Chu Se, Duc Co, Chu Pah, Chu Prong Districts, Gia Lai Province. All transects were set up based on data published in previous studies (i.e., Luu et al. 2017, 2018, 2020), and direct observations. Each transect was visited three times after sunset between 19:00 and 00:30 h when the species is expected to be most active to guarantee the highest detection probability. A total of 28 transects in Chu Se (14 transects), Duc Co (five transects), Chu Prong (one transect) and Chu Pah (eight transects), which covered coffee, rubber, durian, padauk, passion fruit, and coconut palm plantations, were visited. During our surveys, we also screened for two types of soils, including adobe (10 transects in total with four transects in Duc Co and six transects in Chu Pah) and basalt soils (18 transects in total with 14 transects in Chu Se, one in Duc Co, one in Chu Prong, and two in Chu Pah). Coordinates of each surveyed transect at the starting and ending points and locations of captured animals were recorded using GPS units (Garmin GPSmap62s, WGS84 datum).

Species identification

Molecular data

Due to the similar morphological characteristics, distinguishing members within the genus *Cyrtodactylus* is challenging for researchers. Therefore, molecular analysis was applied in the study. In particular, one or two tail tissue samples were collected for each subpopulation of *Cyrtodactylus* observed. In total, eighteen DNA samples from Chu Se (four samples), Duc Co (five samples) and Chu Pah (nine samples) districts were collected and preserved separately in 70% ethanol (Merck, Germany) for DNA extraction. Total DNA was then extracted using QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions. Extracted DNA was amplified by DreamTaq Mastermix (Thermo Fisher Scientific, Lithuania) with 21 µl volume (10 µl of mastermix, 5 µl of water, 2 µl of each primer at 10 pmol.µml-1 and 2 µl of total DNA). PCR conditions were 95^{o}C for 5 minutes to activate the taq; with 35 cycles at 95^{o}C for 30s, 45^{o}C for 45s, 72^{o}C for 60s; and a final extension at 72^{o}C for 6 minutes. A fragment of the mitochondrial gene, NADH dehydrogenase subunit 2 (ND2), was amplified using the primer pair MetF1 (5'-AAGCTTTCGGGCCCATACC-3') and COIR1 (5'-AGRGTGCCAATGTCTTTGTGRTT-3') (Arevalo et al. 1994; Macey et al. 1997). PCR products were visualized using electrophoresis through a 2% agarose gel stained with ethidium bromide. Successful amplifications were purified

to eliminate PCR components using GeneJET PCR Purification kit (Thermo Fisher Scientific, Lithuania). Purified PCR products were sent to 1st Base (Malaysia) for sequencing in both directions. Afterward, sequences were validated with Sequencher v4.10 (Gene Codes, Ann Arbor, MI) with default setting and compared with data available on Genbank using BLAST Tool as implemented in the National Center for Biotechnology Information (NCBI 2025). The newly obtained sequences were uploaded on GenBank under accession numbers XXXXX-XXXXX.

Morphological analysis

Measurements followed Ziegler et al. (2022) and Luu et al. (2017) and were taken with a slide-caliper to the nearest 0.1 mm. Measurements were taken on the right side of the specimens unless otherwise indicated. Abbreviations are as follows: SVL: snout-vent length, measured from tip of snout to vent; HL: head length, measured from tip of snout to retroarticular process of jaw; HW: head width, maximum width of head; HH: head height, from occiput to underside of jaws; OrbD: orbital diameter, greatest diameter of orbit; BW: body width, the widest distance of body; TrunkL: Trunk length, distance from axilla to groin measured from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; ForeaL: forearm length, from base of palm to tip of elbow; CrusL: crus length, from base of heel to knee.

Population estimation

The population size was estimated by applying a "capture-mark-recapture" method. Each individual was captured by hand, measured to collect morphological data, marked only once on the head or middle of the body with correction fluid pens (Tipp-Ex, Germany) using a series of dots (for juveniles – SVL < 47 mm) or numbers (for adults – SVL ≥ 47 mm) (Luu et al. 2017) to denote the capture and released on the same spot. A series of dots and numbers was assigned continuously each day. For example, on the first day on transect 1, four individuals were captured and labelled as 1 to 4 (adults represented by 1, 2, 3, and 4, and juveniles shown as dots from one to four). The next day, four newly caught adult individuals were labelled from 5 to 8.

Each transect was surveyed three times, with an interval of 1 to 2 days between visits. Since there had been no studies on the home range of the species, we considered the home range of *C. gialaiensis* to be very small (approximately 0.0001 km^2), based on data of another gekkonid species, *Gekko japonicus* (Park et al. 2019). We therefore assumed that the populations remained

closed with no immigration or emigration during the 5–7 days of each survey. The "Schnabel Index" methods were applied to estimate the population size of *C. gialaiensis* (Schnabel 1938). In particular, the population size (N^) was calculated following the formula:

$$\widehat{N} = \frac{\sum_{i=1}^{t}(C_i M_i)}{\sum_{i=1}^{t} R_i}$$

Variance Standard error of

$$s^2\left(\frac{1}{\widehat{N}}\right) = \frac{\sum_{i=1}^{t} R_i}{\left(\sum_{i=1}^{t}(C_i M_i)\right)^2} \qquad s_{\bar{x}}\left(\frac{1}{\widehat{N}}\right) = \sqrt{s^2\left(\frac{1}{\widehat{N}}\right)}$$

Where M_i is the total number of previously marked animals at time i, C_i is the number of animals caught at time i, and R_i is the number of marked animals caught at time i. A 95% confidence interval was also obtained to reduce the bias (Crow and Gardner 1959; Krebs 2014) as follows:

$$\text{Lower 95\% confidence limit} = \frac{\sum(C_i M_i)}{\sum R_i} \qquad \text{Upper 95\% confidence limit} = \frac{\sum(C_i M_i)}{\sum R_i}$$

Where the 95% confidence interval for ΣR_i was obtained from the Poisson distribution. In addition, population densities of *C. gialaiensis* (adults only) were also calculated per square kilometer (indiv./km^2) for each surveyed transect and per day (indiv./km^2/day).

Microhabitat assessment

Regarding microclimatic parameters, the air temperature (°C) and relative air humidity (%) were measured with a digital thermometer (TFA Dostmann/Wertheim Kat. No. 30.5015, Germany) at each location with 0-1 m high from animals. A mestek digital infrared thermometer pyrometer laser (Mestek, China) was also used to measure temperatures (°C) at the substrate surface and the body surface of animals (on the belly). Mean and the standard error of the mean of morphometric measurements and microhabitat characteristics were calculated using R v4.4.2 (R Core Team 2025).

Threats identification

The effects of human activities on the species were observed during field surveys. This study did not use a formal or quantitative method to evaluate these risks.

Results

Species identification

We successfully sequenced a fragment of the ND2 gene from a total of 18 samples. Among these, nine samples collected from Chu Se (four samples), Duc Co (five samples), and Chu Pah (two samples) were identical and 95.53 - 99.22% similar to the holotype sequence of *C. gialaiensis* with GenBank accession number MT953497 (voucher number VNUF R.2017.1). Other remaining seven sequences originating from seven samples collected in Chu Pah were identified as belonging to different species. However, as only one or two individuals were encountered per night across various transects in Duc Co and Chu Pah, data derived from four transects in Chu Se District were selected to present morphometric measurements, microhabitat characteristics and an estimation of population size. In particular, morphometric measurements for the subpopulations fell within the range of data collected from the holotype and paratypes of *C. gialaiensis* (Table 1).

Table 1. Morphometric measurements taken from adult samples of *C. gialaiensis*.

Measurements	Min – max (Transect 1) (n = 8 ind.)	Min – max (Transect 2) (n = 16 ind.)	Min – max (Transect 3) (n = 11 ind.)	Min – max (Transect 4) (n = 9 ind.)	Min – max (n = 3) (Luu et al. 2017)
SVL	53.3 – 67.3	61.4 – 73.7	47.9 – 77.0	59.2 – 77.7	56.3 – 62.8
HL/SVL	0.27 – 0.30	0.23 – 0.31	0.26 – 0.31	0.26 – 0.30	0.28 – 0.30
HW/SVL	0.19 – 0.21	0.19 – 0.23	0.18 – 0.23	0.19 – 0.21	0.20 – 0.21
HH/SVL	0.10 – 0.13	0.10 – 0.13	0.11 – 0.14	0.11 – 0.14	0.12 – 0.15
OrbD/SVL	0.05 – 0.07	0.05 – 0.07	0.05 – 0.07	0.05 – 0.06	0.07 – 0.08
TrunkL/SVL	0.38 – 0.57	0.43 – 0.56	0.45 – 0.53	0.44 – 0.50	0.44 – 0.49
ForeaL/SVL	0.14 – 0.16	0.13 – 0.17	0.14 – 0.16	0.14 – 0.16	0.15 – 0.19
CrusL/SVL	0.16 – 0.18	0.16 – 0.20	0.15 – 0.19	0.17 – 0.19	0.17 – 0.20

Species distribution and new records

According to our surveys, *C. gialaiensis* were active in both the wet and dry seasons. This species was detected in both basalt and adobe soil types. Our findings also show that *C. gialaiensis* occurred not only in Chu Se District but also in Duc Co and Chu Pah Districts, representing its first records in these locations.

Population status

A total of 56 individuals were captured, and 17 recaptured events took place in four transects (Transect 1 – Transect 4). Based on the calculated Schnabel Index, the total population of *C.*

gialaiensis in Chu Se was estimated to comprise about 96 individuals (95% confidence interval ranged from 40 to 314 individuals), from which only 47 were considered to be adults (Table 2). The highest density of *C. gialaiensis* was found in transect 4 (eight individuals per km^2), while lowest densities were detected in transect 3 (four individuals per km^2). In terms of its population recruitment, a low number of juveniles (16.98%) was observed.

Table 2. Total number of observed, estimated population size and abundances of observed *C. gialaiensis* in Chu Se District; D_A = density estimation of adult individual per km^2, D_{AD} = density estimation of adult individual per km^2 per day, D = overall density estimation, DD = overall density estimation per km^2 per day, N = estimated total individuals.

Transects	Transect 1	Transect 2	Transect 3	Transect 4	Total
Oct – 22					
Total Adults	8	19	11	9	47
Total Obs	12	21	13	10	56
Area (km^2)	2.90	2.90	3.3	1.23	9.10
DA (Adults/km^2)	2.76	6.55	3.33	7.32	5.16
DAD (Adults/km^2/day)	0.92	2.18	1.11	2.44	1.72
D (ind./km^2)	4.14	7.24	3.94	8.13	6.15
DD (ind./km^2/day)	1.38	2.41	0.98	2.71	1.54
N – Total Schnabel	19	21	23	23	96

Microhabitat characteristics

Cyrtodactylus gialaiensis was observed to be most active between 20:00 h and 22:00 h (Fig. 3) in hole cliff (Fig. 4A) or soil cliff, tree trunk, and shrub between the coffee plantation and the road (Fig. 4B and C), or coffee plantation areas (Fig. 4D) with elevations ranging from 557 m a.s.l to 650 m a.s.l. It was found in the areas with lower temperatures and high humidity. Among four survey sites, mean air temperatures were 23.00 ± 0.14°C (n = 71, Table 3) with humidity of 81.42 ± 1.01% (n = 71, Table 3). The mean canopy coverage was 35.00 ± 5.09% (n = 71, Table 3), and sighted locations ranged from 0 – 250 cm above the ground (mean = 41.69 ± 6.24, n = 71). The species was detected on the substrate with low temperature (mean = 21.63 ± 0.16°C, n = 57), and the mean body' temperature was 22.09 ± 0.14°C (Table 3, n = 69).

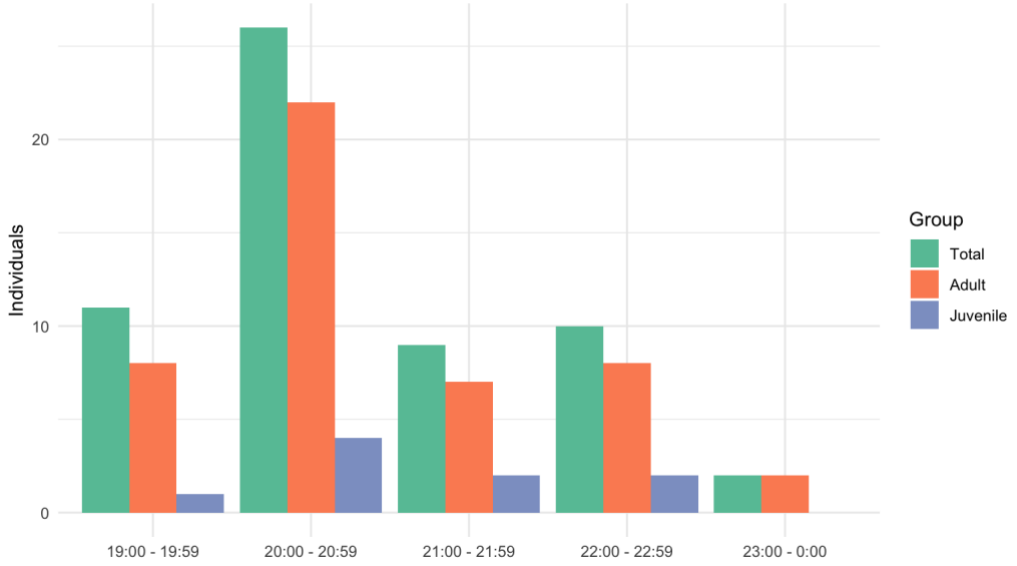

Figure 3. Activity period of *Cyrtodactylus gialaiensis* (n = 68) during the surveyed time.

Figure 4. *Cyrtodactylus gialaiensis* in the wild. A) An adult of *C. gialaiensis* inside a small hole cliff, B) An adult of *C. gialaiensis* on the soil cliff, C) An adult on a tree trunk, D) An adult on a coffee stump.

Table 3. Microhabitat characteristics of *C. gialaiensis.*

Transects	Number of observations	Min – Max	Mean ± SE
Humidity [%]	56	74 – 95	84.79 ± 0.81
Air temperature [°C]	56	20.3 – 25.6	23.13 ± 0.14
Substrate temperature [°C]	56	19.3 – 24.2	21.65 ± 0.16
Animal temperature [°C]	56	19.8 – 24.8	22.22 ± 0.15
Canopy coverage [%]	56	0 – 100	40.27 ± 6.24
Distance to ground	56	0 – 250	52.76 ± 7.22

Main threats

Among all recorded threats, habitat loss because of land use change represented as the most critical risk to all populations of *C. gialaiensis*. During surveys in two different seasons, most individuals of *C. gialaiensis* were found inside the coffee plantations or between the coffee plantations and the local roads. A small subset of the populations continued to inhabit isolated patches of natural vegetation, primarily containing shrubs and small native trees. However, all these subpopulations face the uncertainty in land use change, driven by the profit motives of landowners. For example, during our visit in October 2022, we found a shift in land use from bamboo to coconut palm plantations at the site T2. Land use changes could create unsuitable habitats for species and may also raise chemical pollutants that harm the habitat of this species.

Furthermore, none of the studied populations were situated within any existing protected areas, which renders them vulnerable to threats posed by land conversion activities (Fig. 5A and B). A large amount of garbage, including plastics and chemical products, was also observed around the species' habitat in Chu Se (Fig. 5C-F). In addition, a significantly high percentage of individuals exhibiting regenerated tails was recorded at various surveyed locations. In particular, a record of tail conditions across four transects in Chu Se indicated that 58.33%, 50%, 38.46%, and 60% of the encountered individuals had regenerated tails, respectively.

Figure 5. Threats to the population of *Cyrtodactylus gialaiensis.* A-B) Land use change, C-F) Plastic bag in the habitat of *C. gialaiensis*.

Discussion

Gia Lai is the second-largest province in Vietnam, covering an area of approximately 15,000 square kilometers (People's Committee of Gia Lai Province 2025; Le et al. 2024). It plays an important role within the Central Highlands' socioeconomic development, which has resulted in various challenges related to land resource management. A comparative analysis of forest cover between 1992 and 2019 based on forest cover maps derived from the database managed by ALOS Science Project, Japan Aerospace Exploration Agency, reveals that deforestation, afforestation, agricultural expansion, and urbanization along with other extensive development activities substantially contributed to the deterioration of natural forests (Fig. 6) (ALOS Science Project, Japan Aerospace Exploration Agency). Additionally, strategies aimed at converting traditional

crops to high-value ones and adapting to climate change during the period of 2023 to 2025, with a vision toward 2030, were proposed in 2023. These strategies recommend replacing the current unproductive crops, such as rubber, pepper, coffee, and sugarcane, with more economically viable plants, including passion fruit, potato, pineapple, banana, chili, watermelon, and soybean (Law Library 2025). This transition represents a major short- and medium-term threat to wildlife survival (Mas et al. 2010; Quesada et al. 2009; Sala et al. 2000).

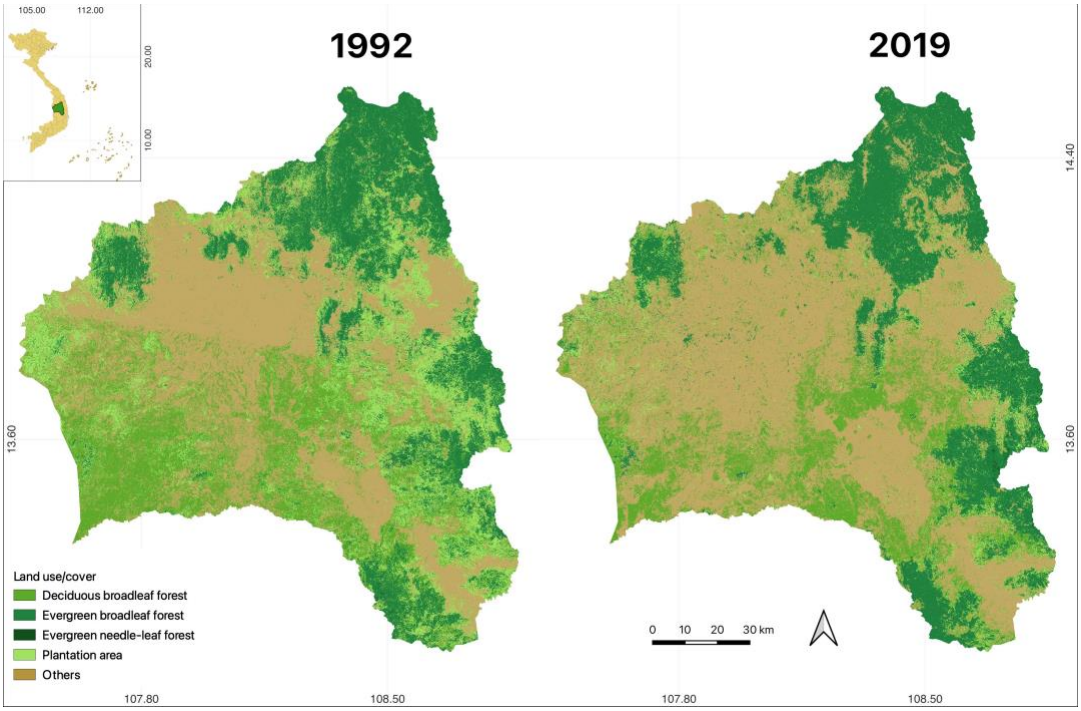

Figure 6. Land use change in Gia Lai Province from 1992 to 2019.

The distribution of *C. gialaiensis* is documented in three districts of Gia Lai Province, including Chu Se, Duc Co, and Chu Pah. The distances measured between the nearest subpopulations are as follows: 22 km between Chu Se and Duc Co, 41 km between Duc Co and Chu Pah, and 56 km between Chu Pah and Chu Se based on Google Earth Pro v7.3.6.10201 (64-bit) (13 January 2025). The data suggest that *C. gialaiensis* may have had a more extensive distribution across the Gia Lai Province in the past. However, this species was not detected in 16 out of 28 surveyed transects. In addition, the rarity of *C. gialaiensis* in other locations implies that much of its suitable habitat has been lost and that it can only persist in remnant patches of natural vegetation or has to inadvertently adapt to monoculture plantations, such as coffee and rubber (Luu 2018; Luu et al. 2020). There is an urgent need for formulation of effective conservation measures to avert its imminent extinction.

Population size is a critical determinant of wildlife survival, with species that have limited distributions being especially vulnerable (Foufopoulos and Ives 1999; Reed et al. 2003; van Schingen et al. 2014). As such, obtaining accurate information on population size should be prioritized in conservation planning (Shaffer et al. 2002). According to Reed et al. (2003), for any vertebrate species, an effective population size of at least 7,000 individuals is required to ensure a long-term survival. In the case of *C. gialaiensis*, our preliminary assessment provides an estimate of approximately 96 individuals in Chu Se and even fewer numbers documented in Duc Co and Chu Pah, much lower than the minimum viable population, making it difficult for the species to withstand environmental and demographic stochasticity.

Furthermore, our surveys suggest that there might have been no migration between the subpopulations due to the long distances separating them and no suitable habitat corridor connecting the metapopulations. In the long run, habitat loss and fragmentation may reduce genetic diversity and increase the risks of genetic drift and inbreeding depression, which potentially led to higher extinction vulnerability the subpopulations (Levins 1969; Hanski and Gilpin 1997). When adding other external threats, such as climate change and disease, it is clear that *C. gialaiensis* requires immediate conservation measures, especially because its distribution range falls outside the country's protected area network.

Luu (2018) assessed the conservation status of the species and classified it as Critically Endangered (B1ab(iii)+2ab(iii)). This classification is based on an estimated extent of occurrence (EOO) and area of occupancy (AOO) of less than 10 km², emphasizing that its distribution is known to exist at only a single location. Additionally, there is a continuing decline observed, estimated, inferred, or projected in any aspect of its area, extent, and/or quality of habitat. In contrast, the Vietnam Red Data Book revised the conservation status of the species and downgraded it to Endangered (B1ab(iii)). This listing relies on an estimated extent of occurrence (EOO) of less than 5,000 km², with its range either being severely fragmented or known to exist at only a single location, as well as a continuing decline in habitat area, extent, or quality (Nguyen et al. 2024). Using all recorded localities, we recalculated the EOO and AOO using the GeoCAT tool (GeoCAT Tool 2025), which was introduced by the IUCN Red List (Red List Technical Working Group). The estimated EOO for *C. gialaiensis* is approximately 2,124 km², while the AOO is valued at 56 km². However, even though *C. gialaiensis* has been recorded in three districts

in Gia Lai Province, all subpopulations are estimated with fewer than 50 mature individuals and highly fragmented. Furthermore, we believe that the population of *C. gialaiensis* is likely to decline significantly in the coming years due to uncertainties in land use and the increasing use of chemical products. Consequently, *C. gialaiensis* fits the criteria for the Critically Endangered category C2a(i) (IUCN Standards and Petitions Committee).

Conclusions

Our surveys revealed the distribution range of *C. gialaiensis* to be extended from Chu Se to Duc Co and Chu Pah districts (Fig. 7). Nonetheless, the species occurs in very low numbers in both adobe and basalt soils, and is severely hampered by habitat fragmentation and degradation as a result of land use changes. To safeguard the habitat at its type locality, a payment for ecosystem service scheme has been implemented in recent years. In receiving the payment, the landowner has agreed not to use any chemicals, convert the coffee plantation to other crops or carry out any activities potentially harmful to the species. According to our study results, *C. gialaiensis* is found not only in the plantation, but also in very narrow tracts of natural vegetation adjacent to the farm. Nevertheless, none of those sites is located inside any protected area. We strongly recommend that more field surveys be conducted to identify areas with better vegetation cover, where a suitable site can be set aside as a protected area or species conservation area to ensure the long-term survival of the species. In addition, a captive breeding program should be initiated to strengthen the small and fragmented wild population in the near future.

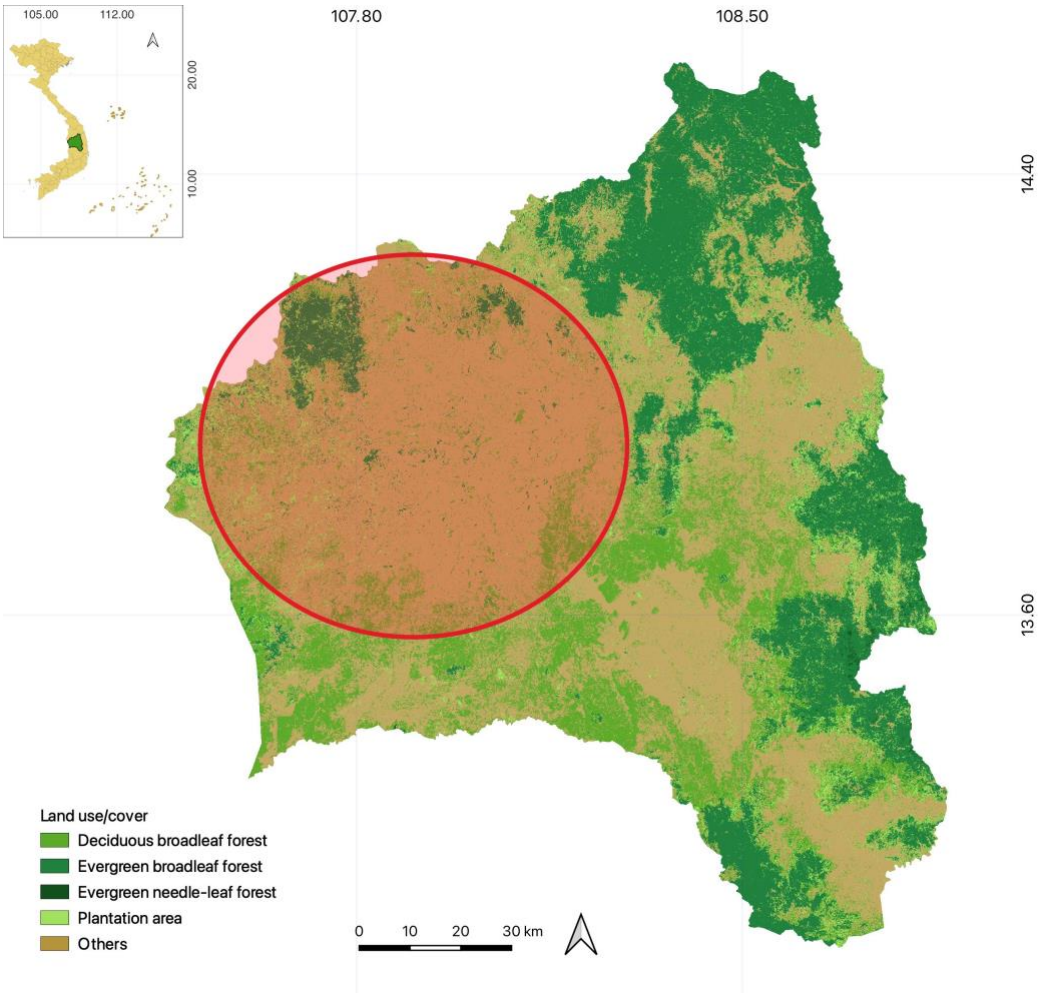

Figure 7. Geographic distribution of *Cyrtodactylus gialaiensis* in Gia Lai Province shown in the red circle.

Acknowledgement

We are grateful to the Gia Lai Department of Agriculture and Rural Development and the Forest Protection Department of Gia Lai for their support of our fieldwork and for issuing relevant permits. Thanks to C.V. Hoang, N.H. Nguyen, H.N. Truong, for assistance in the field. This research was funded by the Critical Ecosystem Partnership Fund, grant number CEPF-111890, the Asian Species Action Partnership (ASAP), the Zoological Society for the Conservation of Species and Populations (ZGAP), and the Confederation of European Forest Owners. Research of Hanh Thi Ngo in Germany was funded by the German Academic Exchange Service (DAAD).

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Chapter 13. Assessment of the threat status of bent-toed geckos (Cyrtodactylus) in Indochina: implementation of the One Plan Approach

Integrative Conservation

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RESEARCH ARTICLE OPEN ACCESS

Assessment of the Threat Status of Bent-Toed Geckos (*Cyrtodactylus*) in Indochina: Implementation of the One Plan Approach

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Received: 30 December 2024 | **Revised:** 9 July 2025 | **Accepted:** 14 July 2025

Editor-in-Chief & Handling Editor: Ahimsa Campos-Arceiz

Keywords: Cambodia | *Cyrtodactylus* | discovery rate | endemic species | Laos | protected area | species diversity | threat analysis | Vietnam

ABSTRACT

Cyrtodactylus is the most diverse genus of the family Gekkonidae, as well as the third-largest vertebrate genus worldwide. In this study, we investigate the diversity, conservation status, and distribution of species within the genus *Cyrtodactylus* in Indochina. There are 84 species known from the Indochina region (Vietnam, Laos, and Cambodia), representing 22.6% of the global diversity of the genus. The majority (90.5%) of the taxa are endemic, with a significant portion being microendemic and therefore restricted to specific regions. Recent rapid rise in species discoveries has been driven by molecular analyses of morphologically cryptic species. However, many newly described species remain unassessed in terms of their population status, creating a gap in conservation priorities, with up to 34.5% of currently recognized bent-toed geckos remaining unevaluated. Our study also reveals that over half of the currently recognized *Cyrtodactylus* species (54.8%) are not covered by any protected area, and 85.7% of threatened species lack any protection measure. Moreover, ex-situ conservation efforts, such as conservation breeding, have not been targeted in conservation plans for bent-toed geckos in Indochina, leaving many species being further threatened in the wild. The findings call for a more integrated approach to conservation in line with IUCN's One Plan Approach, to ensure the long-term survival of these species, advocating for improved taxonomic research and comprehensive conservation assessments for the IUCN Red List. Expanding protected areas and initiating ex-situ programmes are essential to ensure the long-term survival of the bent-toed geckos in the region.

1 | Introduction

Reptiles, though crucial components of ecosystems, are often overlooked in conservation efforts (Farooq et al. 2024). They are especially vulnerable to climate change and other anthropogenic threats. Both reptiles and amphibians are considered to be among the taxonomic groups most severely affected by climate change, one of the most significant threats to global biodiversity, because the taxa rapidly respond to environmental changes (Bickford et al. 2010; Farooq et al. 2024).

Reptiles are experiencing serious declines in populations, resulting in an increasing number of threatened species, and extinction rates are accumulating rapidly as the modern

Summary

This study assesses the diversity, conservation status, and distribution of geckos within the genus *Cyrtodactylus* in Indochina. We compiled an updated checklist comprising 84 species, which are known to occur in Vietnam, Laos, and Cambodia. Most of the species in Indochina occur only in this area, where a high proportion of taxa has very restricted geographic distributions. During the last decade, a rapid rise in species discoveries has taken place. According to the IUCN Red List, many recently identified species (up to 34.5%) remain unassessed in terms of their population status. This lack of information hampers targeted conservation planning. Spatial analyses revealed that about half of the species are not covered by any protected area. This is more pronounced with regard to threatened species, where more than three-fourth of them live in unprotected habitats. Conservation breeding has not been established for any species from Indochina. Our results highlight the importance to develop a more integrated approach to conservation in line with IUCN's One Plan Approach. This appears to be pivotal to protect the species from current anthropogenic threats. There is an urgent need for continued taxonomic research and comprehensive conservation assessments for the IUCN Red List and habitat protection.

- **Practitioner Points**
  - The diversity of the genus *Cyrtodactylus* is still underestimated in Indochina, evident by sharply increasing description rates.
  - Many recently discovered species lack baseline information for conservation and are currently not assessed in the IUCN Red List.
  - Ex-situ conservation measures are currently not in place for any species from Indochina.

biodiversity crisis is getting worse (Clulow and Clulow 2016). The threat assessment of reptiles has identified at least 21.1% of reptile species that are under a high risk of extinction (Cox et al. 2022). Whilst certain reptiles require urgent and targeted measures to avoid extinction, widespread efforts to protect, such as habitat preservation, management of trade and controlling invasive species, are likely to yield benefits to numerous reptile species (Cox et al. 2022). A considerable number is severely endangered due to overharvesting for either domestic or international trade, for traditional medicine and food (Stenger et al. 2023). For reptiles, harvesting and habitat loss are key threats to their populations (Clulow and Clulow 2016). A high conservation priority has been assigned to 36 biodiversity hotspots with high rates of species richness and local endemism, such as Vietnam (Stenger et al. 2023). Southeast Asian's biodiversity is especially vulnerable to habitat loss, not only through climate change (Sodhi et al. 2009). As a result of numerous threats to biodiversity in Indochina, including illegal hunting and habitat destruction, climate change is expected to result in the contraction of species ranges and to increase the probability of local species extinctions (Nguyen et al. 2022). This could result in a predicted loss of 13%–85% of the total biodiversity in Southeast Asia, which has one of the highest proportions of threatened vascular plant, reptile, bird, and mammal species, by 2100 (Sodhi et al. 2009).

Herein, we have focused on the bent-toed gecko genus *Cyrtodactylus* in the Indochina region (Vietnam, Laos, and Cambodia). Currently, 372 species of *Cyrtodactylus* are recognized (Uetz et al. 2024a), making it the third-largest vertebrate genus worldwide, thus serving as a model group for ecological and evolutionary studies (Riedel et al. 2024). *Cyrtodactylus* is also the most diverse genus within the family Gekkonidae (Ngo et al. 2022), comprising currently 1637 recognized species (Uetz et al. 2024b).

Cyrtodactylus is a genus that exhibits a significant number of recently discovered cryptic diversity (Korshunova et al. 2019; Davis et al. 2020; Hending 2024), with many of them having minute distribution ranges according to our current knowledge. Consequently, there is a severe lack of data essential to support conservation efforts due to the recent taxonomic changes and species descriptions (Hending 2024).

Thus, we herein aim to demonstrate the influence that the recent high species discovery rate has on the knowledge and conservation of *Cyrtodactylus* and showcases which of the examined species are threatened and unprotected. To achieve that goal, the species of the genus *Cyrtodactylus* in Indochina were examined regarding their rate of assessment, threat status, grade of endemism, distribution within or outside of protected areas in Indochina, and their in-situ and ex-situ populations. Ultimately, this way critical gaps in current conservation efforts were demonstrated.

2 | Materials and Methods

2.1 | Data Collection

A list of all currently recognized species of *Cyrtodactylus* in Vietnam, Laos and Cambodia was compiled by utilizing data retrieved from the Reptile Database on the 9th of September 2024 (Uetz et al. 2025) and from the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (International Union for Conservation of Nature IUCN 2024) between the 9th of September and the 11th of September 2024. Subspecies and extinct species were excluded.

To obtain a complete list of all recognized species of *Cyrtodactylus* in Indochina, the use of the advanced search option in the Reptile Database is necessary. The search parameters included the genus name *Cyrtodactylus* combined with the Boolean 'OR' operator regarding the target countries Vietnam, Laos, and Cambodia to ensure the result will yield all current *Cyrtodactylus* in these countries without excluding any species regardless of whether their distributions overlap (Uetz et al. 2025). The Information gathered from the IUCN Red List was extracted through the rredlist package on R (Gearty and Chamberlain 2025).

Four species were excluded on account of being synonyms of other species: *Cyrtodactylus thochuensis* (Ngo and Grismer 2012) is regarded as a junior synonym of *C. leegrismeri*, *Cyrtodactylus paradoxus* (Darevsky and Szczerbak 1997) is a synonym of *C. condorensis*, *Cyrtodactylus thuongae* (Phung et al. 2014) is a synonym of *C. dati*, and *Cyrtodactylus rufford* (Luu et al. 2016a) is considered as a junior synonym of *C. lomyenensis* (Grismer et al. 2015; Ngo et al. 2022).

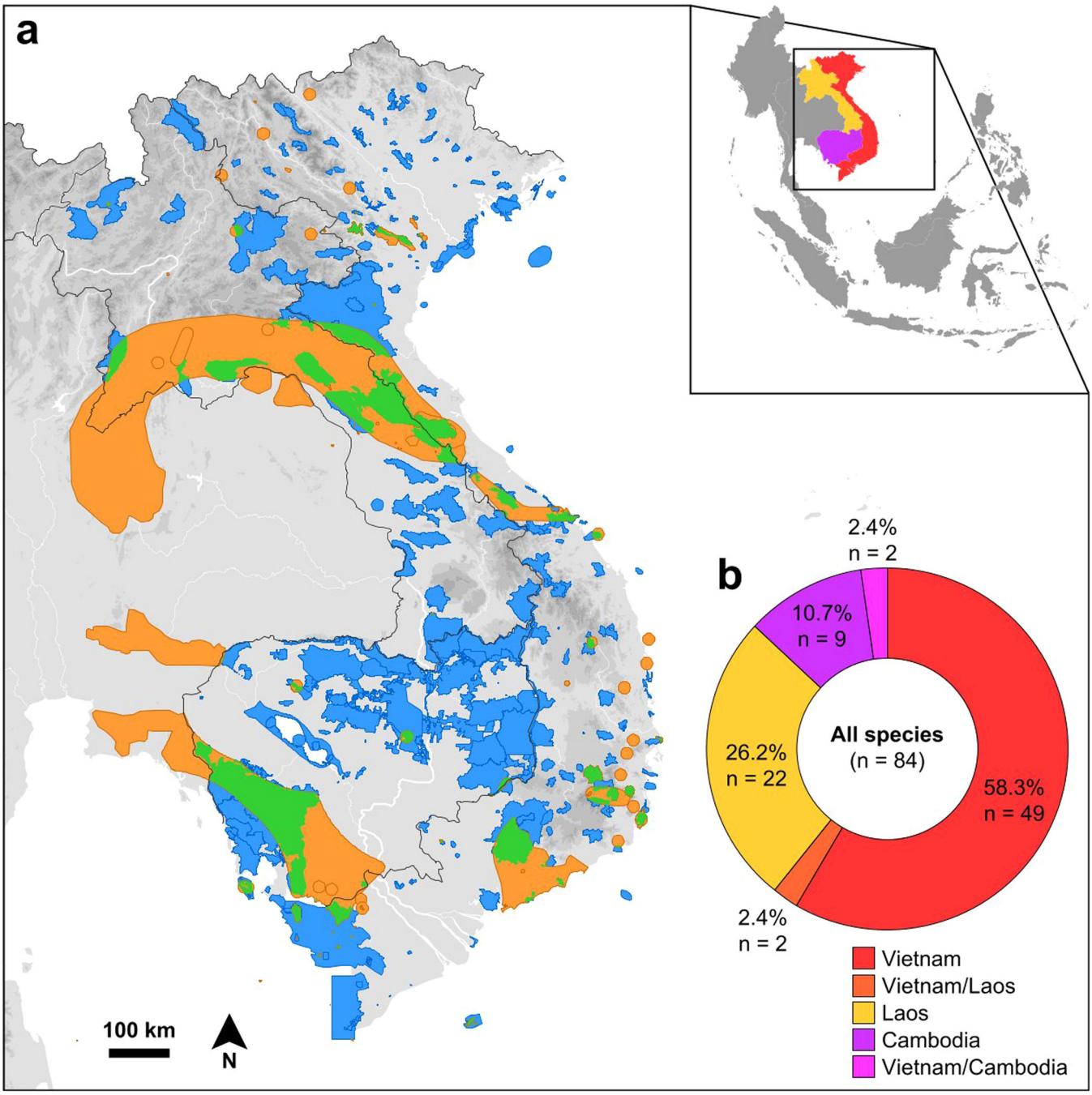

FIGURE 1 | (a) Map of Indochina visualizing the protected areas (blue), the distribution of all examined *Cyrtodactylus* ($n = 84$; orange) and the overlap of these polygons illustrating active protected areas (green). (b) Country distribution of all examined *Cyrtodactylus* ($n = 84$).

The threat assessment was conducted on the basis of various conservation databases and their assessment methods. Between the 13th of September 2024 and the 28th of December 2024 data was compiled from the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Convention on International Trade in Endangered Species of Wild Fauna and Flora CITES 2024), IUCN Red List (International Union for Conservation of Nature IUCN 2024), The Zoological Information Management System (ZIMS) (Species360 2024) and the Vietnam Red Data Book (Vietnam Red List 2024). In case of conflicting data, the most recent assessment was prioritized. None of the examined *Cyrtodactylus* species has been listed in either the CITES appendices or the ZIMS database (Convention on International Trade in Endangered Species of Wild Fauna and Flora CITES 2024; Species360 2024).

The IUCN threat assessments and the corresponding range polygons were retrieved for each of the included species, and based on the distribution, all species were classified into one of four categories of endemism or defined as non-endemic. The spatial data of all *Cyrtodactylus* species currently assessed by the IUCN Red List were downloaded from the IUCN Red List on 28th of September 2024. Range polygons missing for species that are not assessed by the IUCN Red List were therefore separately mapped in QGIS Version 3.34.11 (QGIS Development Team 2024)

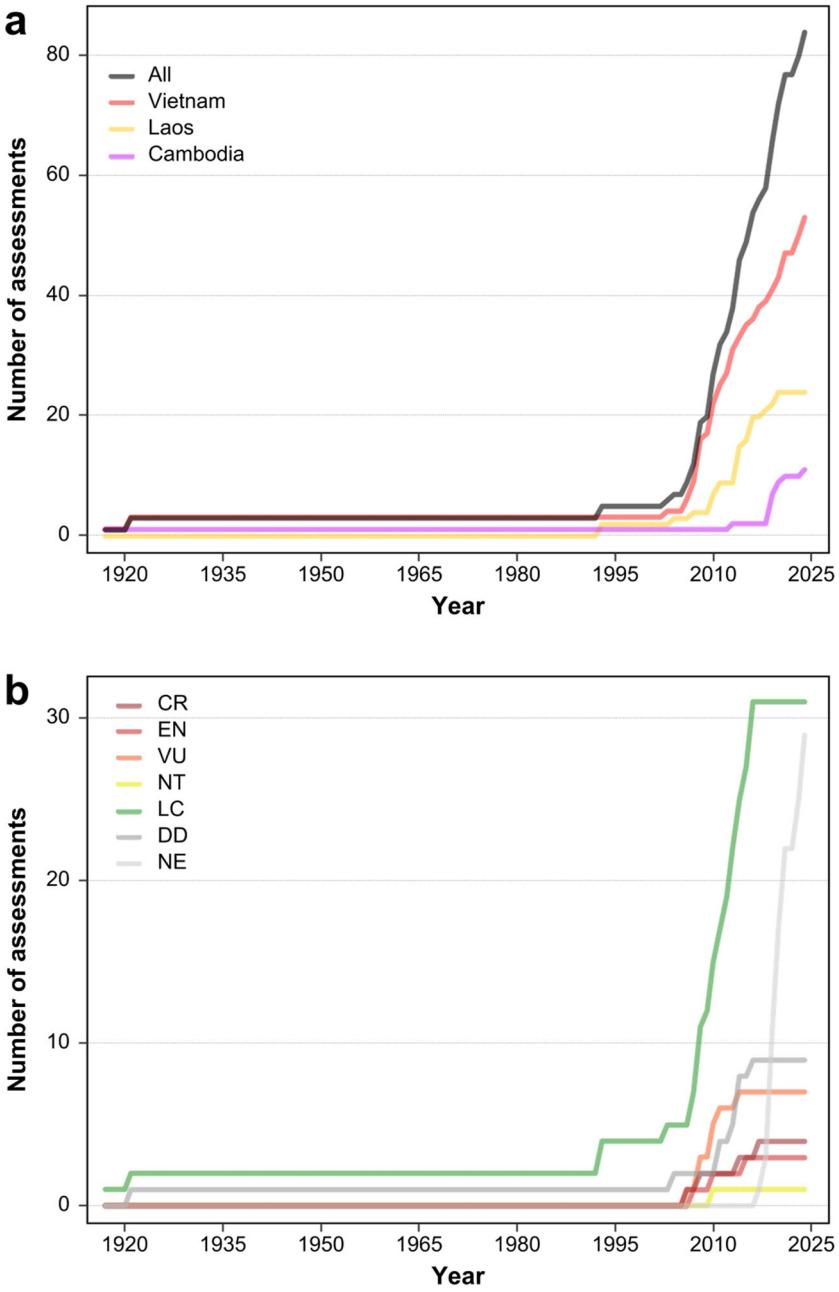

FIGURE 2 | (a) Discovery rate of all examined *Cyrtodactylus* ($n = 84$), divided by country distribution. (b) Discovery rate of all examined *Cyrtodactylus* ($n = 84$), divided by IUCN categories.

based on coordinates extracted from literature, oftentimes their original descriptions (references are provided in Appendix I). The spatial data of all conservation areas in Vietnam, Laos, and Cambodia were acquired from the World Database on Protected Areas (WDPA) on the 28th of October 2024 (UNEP-WCMC and IUCN 2024).

2.2 | Endemism

The knowledge about the biodiversity and the distribution of reptiles is severely lacking, and, therefore, reptiles are poorly represented in the protected areas of the global network (Roll et al. 2017). Reptiles in Southeast Asia are often highly endemic (Pratihar et al. 2014), the severity of the endemism, however, differs. In this study, the *Cyrtodactylus* species are divided into different endemism categories.

Microendemic (ME) species are frequently known only from the type locality or are species restricted to an area smaller than 100 km^2 (Meiri et al. 2017). Subregion-endemic (SRE) includes species that are found in one of 19 subregions (Bain and Hurley 2011). Region-endemic (RE) species that reside in only one of four regions (Stenger et al. 2023). The regions are divided into the Uplands, Lowlands, Coasts, and the Islands, each with own distinct climatic conditions and vegetation characteristics (Stenger et al. 2023). Locations above 450 meters are considered to be the Uplands, and locations below 450 meters are defined as the Lowlands (Bain and Hurley 2011). The category country-endemic (CE) describes species, that are neither microendemic nor

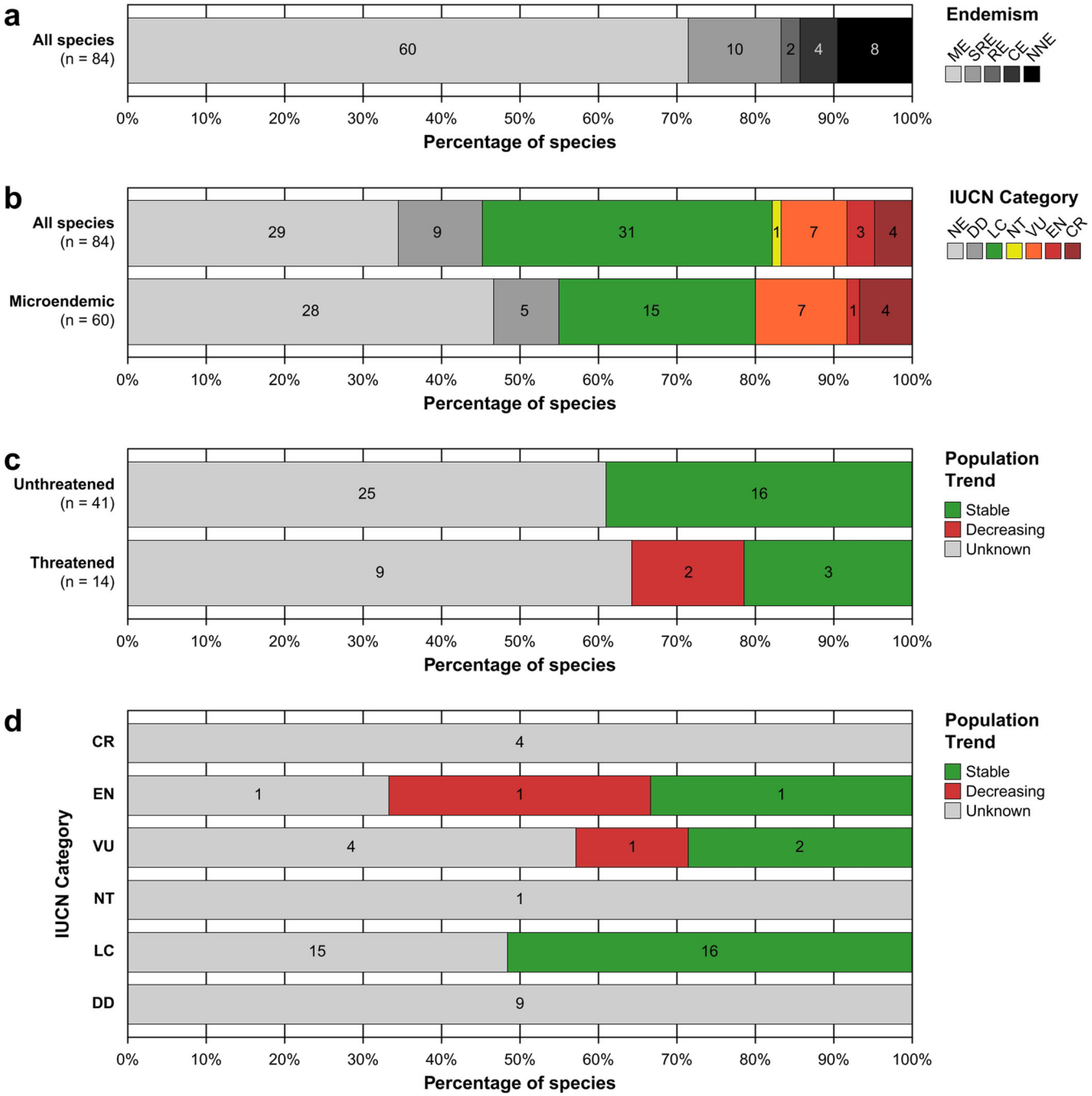

FIGURE 3 | (a) Endemism categories of the examined *Cyrtodactylus* ($n = 84$). (b) IUCN Red List categories of all examined *Cyrtodactylus* ($n = 84$) and of all examined microendemic *Cyrtodactylus* ($n = 60$). (c) IUCN Red List population trends divided by threat category of all IUCN assessed *Cyrtodactylus* in Indochina ($n = 55$). (d) IUCN Red List population trends divided by IUCN categories of all IUCN assessed *Cyrtodactylus* in Indochina ($n = 55$).

region-endemic, and are distributed only within the borders of one country. Widely occurring species are going to be referred as non-endemic (NNE).

3 | Results

3.1 | Species List

The final species list contains 84 species of *Cyrtodactylus* occurring in Vietnam, Laos, and Cambodia (Appendix I). The examined species constitute 22.58% of all 372 recorded *Cyrtodactylus* species (Uetz et al. 2024a), of which 49 (58.33%) species are found solely in Vietnam, 22 (26.19%) species are restricted to Laos, and 9 (10.71%) species are distributed in Cambodia. There are two (2.38%) species of *Cyrtodactylus* resident in Vietnam and Cambodia, and Vietnam and Laos, respectively (Figure 1b).

The description of *Cyrtodactylus* species began in 1917 with *C. intermedius*, followed by *C. condorensis* and *C. irregularis* in 1921. There were no new records of *Cyrtodactylus* until 1993, and then 10 years later in 2003. The discovery rate increased

significantly after 2008, with 2022 being the only year in which no new species of *Cyrtodactylus* were discovered in either Vietnam, Laos, or Cambodia (Figure 2a).

Of the examined countries, Vietnam has the most species of *Cyrtodactylus* and follows a similar growth trend to Figure 2a. The first species was discovered in 1917, and the next two species were found in 1921. Since 2006, there has been a consistent increase of species discoveries, with the exception of 2022. The most *Cyrtodactylus* found in one year were seven new species in 2008.

In Laos, the first two species were described in 1993. The next two species were uncovered in 2004 and 2007. Nearly half ($n = 11$) of the *Cyrtodactylus* species occurring in Laos were described between the years 2014 and 2016. There have been no new species discoveries since 2020.

Cambodia's first *Cyrtodactylus* discovery was also *C. intermedius*. However, the next recognized species was *C. dati* in 2013. The majority of species were discovered between the years 2019 and 2021, with approximately half ($n = 5$) of the new species being described solely in 2019. *C. regicavernicolus* is the most recent species found in Cambodia and was published 2024 (Figure 2a).

3.2 | Endemism

Out of the 84 examined *Cyrtodactylus* species, eight (9.52%) species are non-endemic. The remaining 76 (90.48%) species are endemic to Indochina.

Approximately 4.76% ($n = 4$) of the examined species are CE. The region-endemic species constitute 2.38% ($n = 2$) of all examined species, and 11.9% ($n = 10$) of species are subregion-endemic. The majority of the *Cyrtodactylus* are microendemic (60 species), which represent 71.43% of all examined species (Figure 3a).

A total of 41 species are located in the Lowlands of the examined region. The majority of these species are found in the Upper Mekong Lowlands with 16 (38.10%) resident species, followed by the Mekong Delta ($n = 8$, 19.05%), the Central-South Vietnam Lowlands ($n = 7$, 16.67%), the Interior Cambodian Lowlands ($n = 5$, 11.90%), the Northeast Lowlands ($n = 5$, 11.90%) and the Southern Lao Lowlands ($n = 1$, 2.38%).

Most of the species of the Uplands region occupy either the Northwest Uplands ($n = 12$, 30%) or the Southern Annamites ($n = 12$, 30%). Seven (17.5%) species occur in the Northern Annamites, five (12.5%) species in the Cardamom Uplands, three (7.5%) species in the Central Annamites, and one (2.5%) species in the Northeast Uplands.

The Islands are comprised of six species, with 83.33% ($n = 5$) in the Southern Islands and 16.67% ($n = 1$) in the Northern Islands. The last four species are distributed along the Coast, with three (75%) species at the Central Coast and one (25%) species at the Southern Coast.

3.3 | IUCN Red List

Up to 55 (65.48%) of analyzed *Cyrtodactylus* species have been evaluated for the IUCN Red List. Of the evaluated species, 14 (16.67%) have been classified for one of the threatened categories, including seven (8.33%) VU species, three (3.57%) EN species, and four (4.76%) CR species. The remaining 41 (48.81%) species are in the unthreatened categories, with one (1.19%) NT species and 31 (36.90%) LC species. For nine (10.71%) species, there is insufficient data, and therefore they have been listed as DD. Up to 29 (34.52%) species have not yet been assessed by the IUCN Red List (Figure 3b).

Out of the 60 examined microendemic species, 32 (53.33%) have been assessed by the IUCN Red List with 15 (25%) LC, seven (11.67%) VU, one (1.67%) EN, four (6.67%) CR and five (8.33%) DD. The remaining 28 (46.67%) species have not been evaluated by the IUCN Red List (Figure 3b).

The population trends of the evaluated species within one of the threatened categories are largely unknown ($n = 9$, 64.29%). Populations of three (21.43%) species are stable, and two (14.29%) are declining in number. The remaining 41 species in the unthreatened categories consist of 16 (39.02%) species with stable populations and of 25 (60.98%) species with unknown population trends (Figure 3c).

There is no information regarding the population trends of the CR species. Out of the EN species population trends, one (33.33%) species is stable, one (33.33%) species is declining, and one (33.33%) species is unknown. Roughly 28.57% ($n = 2$) of the VU species show stable population trends, one (14.29%) species is decreasing, and four (57.14%) are unknown. The current state of the NT species in terms of their populations is unknown. Approximately 16 (51.61%) of the LC species have stable population trends, and the other 15 species (48.39%) are unknown. Due to the insufficient data regarding the DD species, their population trends are also unknown (Figure 3d).

Approximately 34.52% ($n = 29$) of the examined species are NE. The NE species constitute 46.67% ($n = 28$) of the examined microendemic taxa. The distribution of these species is limited to their type locality, with *C. kohrongensis* additionally being restricted to Koh Rong Island, which only reaches a surface area of 74 km^2 (Thaung 2013). *C. culaochamensis* is a country-endemic species, which is the sole NE species with a different endemism category.

As many as 28 of NE species were described in the last 5 years, starting in 2018. *C. sonlaensis* is the only exception, with its description published already in 2017 (Nguyen et al. 2017).

There has been no increase in any of the discovery rates of the other IUCN Red List categories since then (Figure 2b).

The rate of DD species starts increasing in 2004, with the exception of *C. irregularis* in 1921, until 2018, where it stays stagnant as the third-highest category. The first species of both the CR and VU species were determined in 2006. The rates stabilized in 2017 and 2014, respectively, with CR ranking as the fifth highest and VU ranking as the fourth-highest rate. In 2007

the initial EN species was found and described. This number grew until 2015, and the rate is the second to last in magnitude. *C. wayakonei*, the sole NT species, was discovered in 2010, resulting in the lowest rate among all classifications.

The highest discovery rate belongs to the LC species ($n = 31$), beginning with *C. intermedius* in 1917. There were not many increases in the numbers, with only a few discoveries in 1921, 1993, and 2003, until 2007. Between 2007 and 2016 the number of new discoveries increased strongly and only barely surpassed the number of NE species (Figure 2b).

3.4 | Vietnam Red Data Book

Of the examined species four *Cyrtodactylus* species were assessed by the Vietnam Red Data Book. The species *Cyrtodactylus badenensis*, *Cyrtodactylus gialaiensis*, and *Cyrtodactylus takouensis* are classified as EN. *Cyrtodactylus nigriocularis* is the only other species and considered to be CR (Nguyen 2024).

3.5 | Protected Areas (PAs)

There exists a total of 284 protected areas in Vietnam, Laos, and Cambodia. As many as 236 (83.1%) of these conservation units protect terrestrial habitats and inland waters, 40 (14.08%) protective areas are solely marine habitats, and eight (2.82%) areas cover both terrestrial and marine habitats.

Vietnam, with 184 (64.79%) sites, has the majority of protected areas. As many as 76.09% ($n = 140$) of the Vietnamese protected areas focus on terrestrial habitats, 36 (19.57%) of them are marine zones, and eight (4.35%) conserve both marine and terrestrial species. These areas cover 7.58% (24,689 km^2) of the total land regions in Vietnam (325,838 km^2) and 0.49% (3687 km^2) of the total marine and coastal habitats (752,465 km^2; UNEP-WCMC 2024a).

Approximately 10.92% ($n = 31$) of all examined conservation zones are found in Laos. They are all terrestrial and the 43,362 km^2 wide coverage constitutes 18.82% of the total land area (230,382 km^2; UNEP-WCMC 2024b).

Up to 69 (24.3%) of the protected areas are in Cambodia. Roughly 5.8% ($n = 4$) of these zones are marine, and the remaining 94.2% ($n = 65$) are terrestrial. The 72,058 km^2 of land covered by protected areas constitutes 39.78% of Cambodia's total land area (181,145 km^2; UNEP-WCMC 2024c). 1.48% (719 km^2) of the marine and coastal area (48,719 km^2) is preserved by protected areas (UNEP-WCMC 2024c).

As many as 211 (74.3%) of the protected areas have no overlap with the range estimate of *Cyrtodactylus* species, 54 (19.01%) areas have one species at a time, 11 (3.87%) areas have two, four (1.41%) areas have three and three (1.06%) areas have four different species. The protected area (0.35%) with the majority of *Cyrtodactylus* species is the Hin Nam No National Park harbouring seven species (Figure 1a).

Out of all the examined *Cyrtodactylus* species, 54.76% ($n = 46$) species do not inhabit any of the protected areas. Only 21 (25%) species are found in one of the protected areas, seven (8.33%) species occupy two areas, four (4.76%) species are distributed in two, and two (2.38%) species are covered by five protected areas.

The remaining four categories, which make up 1.19% of the examined *Cyrtodactylus,* respectively, are all at least country-endemic and therefore present in more places. The country-endemic species *C. condorensis* is distributed throughout seven and *C. pseudoquadrivirgatus* throughout 11 different PAs. *C. interdigitalis* and *C. intermedius* are non-endemic species and are found in 15 and 17 separate protected areas, respectively (Figure 4).

Of the 60 microendemic *Cyrtodactylus*, 66.67% ($n = 40$) of the species are not found in any of the protected areas. Sixteen (26.67%) species are reported in one of the protected areas,

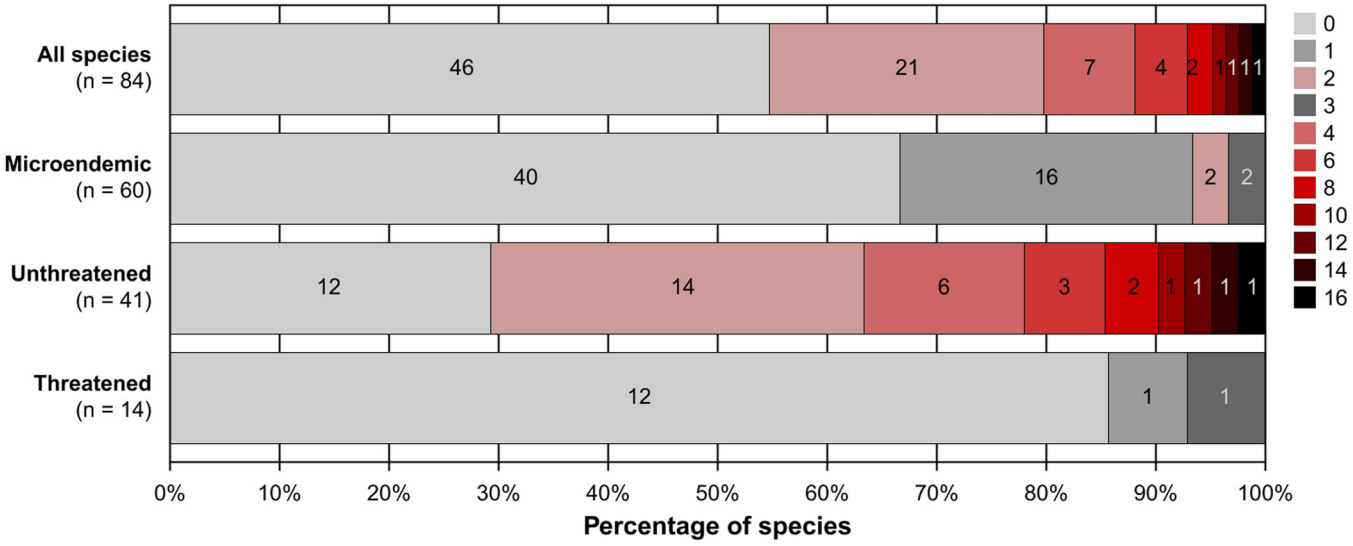

FIGURE 4 | Number of protected areas for all *Cyrtodactylus* ($n = 84$), for all ME *Cyrtodactylus* ($n = 60$) species, for all *Cyrtodactylus* of the unthreatened IUCN category ($n = 41$), and for all *Cyrtodactylus* of the threatened IUCN category ($n = 14$).

3.33% ($n = 2$) are found in two protected areas, and the remaining 3.33% ($n = 2$) species, *C. bobrovi* and *C. phongnhakebangensis*, are protected in two of the areas (Figure 4).

Out of all the examined *Cyrtodactylus* species in one of the unthreatened categories ($n = 41$), 12 (29.27%) species are not covered by any of the protected areas. 14 (34.15%) species are present in one of the protected areas, six (14.63%) species occur in two areas, three (7.32%) species are found in three, and two (4.88%) species are found in five of the protected areas. One (2.44%) species is distributed throughout seven, one (2.44%) throughout 11, one (2.44%) throughout 15, and one (2.44%) throughout 17 different PAs (Figure 4).

Of all the examined *Cyrtodactylus* species in one of the threatened categories ($n = 14$), 85.71% ($n = 12$) species are not protected by any of the PAs. The remaining two species, *C. nigriocularis* (7.14%) and *C. phuquocensis* (7.14%) are found in one and three Pas, respectively (Figure 4).

There are 29 species, that are not classified by the IUCN Red List, and, therefore, no information regarding their threat status is available. As many as 22 (75.86%) unassessed species are unprotected. Six (20.69%) species are recorded in one of the PAs, and *C. hangvaensis* is the only species (3.45%) found in two PAs.

4 | Discussion

There has been a significant increase of recognized *Cyrtodactylus* species in Indochina, particularly after 2008. The rise in species discovery coincides with a broader trend in biodiversity research, in which new species were uncovered at a higher rate (Hending 2024). In-depth molecular and morphological analyses can reveal cryptic species that were previously thought to be part of a broader species complex (Cerca et al. 2020). These findings suggest that the full extent of diversity within this genus may be far greater than the current taxonomy reveals, which in turn can affect conservation priorities and strategies (Ngo et al. 2022; Cerca et al. 2020).

However, despite the increasing taxonomic knowledge, 34.52% of the examined species, of which many are newly discovered species, especially those described since 2018, remain unassessed by the IUCN Red List. This results in gaps in conservation priorities of unknown severity.

The Vietnam Red Data Book has been released in an updated version shortly before the submission of this publication (Nguyen 2024). Four *Cyrtodactylus* species were assessed by the Vietnam Red Data Book. The species *Cyrtodactylus badenensis (VU)*, *Cyrtodactylus gialaiensis* (CR), and *Cyrtodactylus takouensis* (CR) are classified as EN, and *Cyrtodactylus nigriocularis* as CR. These species are also assessed by the IUCN Red list, however, only *Cyrtodactylus nigriocularis* has the same classification. Both *Cyrtodactylus gialaiensis* and *Cyrtodactylus takouensis* are considered to be CR, while *Cyrtodactylus badenensis* is a VU species.

The overwhelming majority of species (90.48%) are endemic, with a significant proportion of these being microendemic. The elevated levels of endemism observed within this group are indicative of the complex topography and habitat diversity of Indochina, where isolated mountain ranges and distinct ecological niches might have increased the speciation rates in the genus (Bain and Hurley 2011; Steinbauer et al. 2016). The rapid changes in climate patterns have also been identified as a significant threat to the reptiles of Southeast Asia, including the *Cyrtodactylus* (Bickford et al. 2010). This especially affects species with restricted ranges, such as those found in the karst formations and isolated mountain ranges of Cambodia and Vietnam, which are vulnerable to both climate shifts and human encroachment (Grismer et al. 2021; Murdoch et al. 2019).

The majority of microendemic species are not located within protected areas, which increases their vulnerability to other direct threats such as deforestation or habitat fragmentation. Species with smaller distribution ranges are more susceptible to extinction, as their survival is linked to the conservation of their specific microhabitats (Chichorro et al. 2019). Out of the 60 microendemic *Cyrtodactylus*, roughly 66.67% ($n = 40$) species occur outside of the PAs. In addition, karst regions are known as centers of speciation and micro-endemism (Luu et al. 2016b).

This finding demonstrates the lack of habitat protection, as many of the endemic species, especially the microendemic species, are at risk due to their limited ranges and are not found in the assigned conservation areas.

One of the key aspects of conservation of threatened species is the implementation of ex-situ conservation measures, including breeding programs, which can serve as an important safety net for species at risk (Kasso and Balakrishnan 2013). The data reveal, that, despite the increasing number of threatened *Cyrtodactylus* species identified in the IUCN Red List categories, there are no active ex-situ conservation programs for these species within ZIMS institutions. Additionally, it appears that no formal breeding programs have been established for these *Cyrtodactylus* in any zoological facilities or research institutions. These programs would not only have ensured that threatened species maintain viable populations in conservation breeding programmes but also provided resources for potential future reintroductions into protected areas or restored habitats (Bhaskar Mahanayak 2024).

Hence, our findings demonstrate that a large number of species have not been given the right amount of support, as there is a lack of ex-situ conservation, breeding programs or focused efforts for threatened *Cyrtodactylus* species.

Although there is a considerable number of protected areas across Vietnam, Laos, and Cambodia (284 in total), the spatial overlap between *Cyrtodactylus* species distributions and these areas is relatively low. Over half of the species (54.76%) do not occur in any of the existing protected areas, and even among the threatened species, the majority lack the safety of habitat protection. Additionally, the species not assessed by the IUCN Red List are only known from their type localities, with an artificial 20 km buffer applied around these points, potentially overestimating their true distribution, and thus alleviating the urgency for comprehensive assessments.

Furthermore, the data reveals a concerning trend: 85.71% of threatened species are not protected by any of the designated PAs. This finding strongly contrasts with the 70.73% of unthreatened species that do have some degree of protection. This suggests that there is a significant mismatch between the conservation status of *Cyrtodactylus* species and the actual protections in place for their habitats. The Hin Nam No National Park in Laos, which harbors the largest number of *Cyrtodactylus* with seven species, is an exception. The park demonstrates the potential for targeted conservation areas to protect multiple species and contribute to broader biodiversity goals. However, the current system of protected areas is insufficient for safeguarding the more localized or threatened species (Jenkins et al. 2013).

Our results highlight several areas where additional measures are necessary to prevent extinction within the genus *Cyrtodactylus*. The key recommendations include:

Enhancing taxonomic and ecological research. As many species remain unassessed by the IUCN Red List, it is crucial that taxonomists and ecologists work together to gather data on these species' populations, trends, and habitat requirements (Bickford et al. 2010).

Prioritizing species that have already been affected by climate change and species that are particularly vulnerable to habitat loss is also essential (Bhaskar Mahanayak 2024). This measure includes species that are already in the threatened categories as well as range-restricted species. Microendemic species are the most vulnerable due to their restricted ranges, and conservation efforts should prioritize habitat preservation in areas where these species are most concentrated (Mcneely 2020). This approach addresses current threats, but also works to prevent future declines, ensuring the survival and resilience of *Cyrtodactylus* biodiversity.

The expansion of the PA system is another preventive and active measure against the extinction risk of *Cyrtodactylus* (Mcneely 2020). Existing protected areas should be expanded, especially in regions where threatened *Cyrtodactylus* species are known to occur. Additionally, connectivity between PAs should be improved to allow for a better gene flow and ensure the stability of various populations (Chichorro et al. 2019).

For species with rapidly declining populations or limited range, ex-situ conservation efforts, such as breeding programs in zoos, should be initiated. Ex-situ conservation is essential when in-situ measures alone are insufficient to prevent extinction (Kasso and Balakrishnan 2013). They ensure the survival and genetic diversity of species in human holdings, stabilize, or even increase population numbers and help reduce the pressures of overexploitation of *Cyrtodactylus* in the wild (Bhaskar Mahanayak 2024). Furthermore, conservation institutions are important and effective platforms for raising public awareness about endangered species (Bhaskar Mahanayak 2024).

The success of ex-situ conservation is likely to be enhanced when combined with in-situ conservation efforts (Bhaskar Mahanayak 2024). A way of combining them would be to implement the One Plan Approach, which integrates both in-situ and ex-situ conservation strategies into a single, unified conservation plan that addresses the needs of a species both in the wild and in human hands (Traylor-Holzer et al. 2019). It enables the development of integrated recovery plans that focus on both the long-term viability of wild populations and the role of managed care in preventing extinction (Gusset 2019). Although breeding programmes provide a safety net, the ultimate goal is to restore the populations in their natural habitats (Traylor-Holzer et al. 2019). Moreover, the One Plan Approach encourages collaboration between institutions that manage both in-situ and ex-situ populations, ensuring that conservation actions are coordinated and based on the latest ecological and genetic data (Gusset 2019).

Cyrtodactylus, which are often highly endemic to specific regions, require extensive protection based on accurate research and an integrated conservation plan that includes measures for species both in the wild and in human hands. In conclusion, the study calls for urgent action in enhancing taxonomic research, expanding protected areas, and initiating ex-situ conservation programmes. It also advocates for a more comprehensive assessment of species for the IUCN Red List to better conservation programs and strategies in the three countries.

Author Contributions

Matilda Julia Lasota: writing – original draft, data curation, formal analysis, visualization, investigation, methodology, conceptualization. **Hanh Thi Ngo:** data curation, validation. **Cuong The Pham:** data curation, validation. **Truong Quang Nguyen:** data curation, validation. **Minh Duc Le:** data curation, writing – review and editing. **Dennis Rödder:** writing – review and editing, software, formal analysis, validation. **Thomas Ziegler:** writing – review and editing, supervision, conceptualization, project administration.

Acknowledgements

We thank Morris Flecks for his help regarding Figure 1 and the graphical abstract, as well as the reviewers for their comments, which greatly improved previous drafts.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in IUCN Red List at https://www.iucnredlist.org/.

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Note: References marked with an asterisk (*) are cited in Supplementary File.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Appendix I: Species list that includes information on whether a *Cyrtodactylus'* spatial information is derived from the IUCN Red List or if a polygon with a buffer of approximately 20 km has been manually incorporated into the map, utilizing coordinates from the species' type locality (Figure 1a).

Chapter 14. Diet of Critically Endangered Black-eyed bent-toed gecko, Cyrtodactylus nigriocularis, Nguyen, Orlov & Darevsky, 2006 from Vietnam

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Diet of Critically Endangered Black-Eyed Bent-Toed Gecko, *Cyrtodactylus nigriocularis*, Nguyen, Orlov & Darevsky, 2006 From Vietnam

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Received: 15 February 2025 | **Revised:** 9 May 2025 | **Accepted:** 11 June 2025

Funding: This work was supported by Zoologischen Gesellschaft für Arten und Populationsschutz, VNM-2024; Critical Ecosystem Partnership Fund, CEPF-111890 and National Foundation for Science and Technology Development (NAFOSTED), 106.05-2020.24.

Keywords: Ba Den Mountain | invertebrates | prey items | stomach contents | Tay Ninh Province

ABSTRACT

The Black-eyed Bent-toed Gecko, *Cyrtodactylus nigriocularis*, a species endemic to Ba Den Mountain Cultural and Historical Complex, Tay Ninh Province, Vietnam, has been classified as Critically Endangered in the IUCN Red List since 2018. However, knowledge of its natural history is virtually non-existent. To fill this gap, the diet of the gecko was studied by stomach flushing. We identified a total of 22 prey categories with 407 items in the stomachs of *C. nigriocularis*. The most important (IRI) groups among its prey were Araneae (24.33%), followed by Opiliones (16.59%), Achatinidae (10.67%), Blattidae (8.77%), Scolopendridae (7.59%), and Acrididae (4.20%), similar to food items consumed by tropical geckos as reported in previous studies. There was no relationship between body mass and mouth width of the species and length/volume of prey consumed, but there were significant differences in the diet composition between sexes and between age groups. Despite the discrepancies, spiders are important prey of all groups. In addition to furthering our knowledge of this poorly studied lizard, the research results can help design ex situ conservation measures for the species in case the wild population continues to decline as a result of local anthropogenic threats.

1 | Introduction

Diet plays an important role in the daily life of animals, as it serves as an essential source of energy for growth, maintenance, and reproduction (Huey and Pianka 1981; Dunham et al. 1989; Zug et al. 2001). Animals usually specialize in different prey items and develop complex feeding behaviors based on dietary requirements or their anatomy (Schwenk 2002). Studying food composition can thus provide crucial information on ecological roles and the relative importance of each prey item in their diet (Losos and Greene 1988; Ortega-Rubio et al. 1995; Znari and El Mouden 1997; Norval et al. 2012; Tan et al. 2020). In addition, dietary analysis in the context of interspecific and intraspecific interactions may shed light on niche overlap and how species partition their resources (Ortega-Rubio et al. 1995; Rocha and Anjos 2007; Viieira and Port 2006; Bulté et al. 2008; Norval et al. 2012). Moreover,

understanding their diet could help inform ex situ conservation efforts by determining the nutritional needs in captivity and develop more effective conservation measures for threatened species (Antwis et al. 2014; Chatpongcharoen et al. 2021; Gao et al. 2023).

The genus *Cyrtodactylus* Gray, 1827 is the most diverse radiation of the family Gekkonidae with more than 380 recognized species (Uetz et al. 2025). This widely distributed group occurs from West India through Bangladesh, southern China southwards to Malaysia, Indonesia, Sundas, Bali, the Philippines, and the Solomon Islands (Wood et al. 2012; Grismer et al. 2021, 2022; Uetz et al. 2025). Vietnam has long been recognized as a hotspot for new bent-toed gecko discoveries (Grismer et al. 2021; Ngo et al. 2022). Since 1997, 52 new species of *Cyrtodactylus* have been described, making it a total of 55 known species (Uetz et al. 2025).

However, most of its members have been only found at their type localities within their specialized microhabitats or isolated islands. For example, *C. takouensis* is restricted to a few small granite caves in Ta Kou Nature Reserve; *C. phuquocensis* is only recorded in Phu Quoc Island; and *C. culaochamensis* only occurs in Cu Lao Cham Island (Ngo and Bauer 2008; Ngo et al. 2010; Ngo et al. 2020; Uetz et al. 2025). Consequently, many of them are exceptionally vulnerable to anthropogenic threats, such as habitat loss and degradation. Several species in Vietnam are facing exceedingly high extinction risks, including three taxa categorized as Critically Endangered, three as Endangered, and six as Vulnerable in the IUCN Red List (2025). To date, data on their ecology and population biology are scant. Until now, no study has been conducted to investigate the diet of the threatened species with a view to better understanding their trophic niche and designing appropriate *ex situ* conservation measures.

C. nigriocularis was described in 2006 based on the type series collected in Ma Thien Lanh Valley of Ba Den Mountain Cultural and Historical Complex (MCHC), Tay Ninh Province, southern Vietnam (Nguyen et al. 2006; IUCN Red List 2025). This species has been listed as Critically Endangered (CR) in the IUCN Red List since 2018 (Nguyen et al. 2018; Nguyen et al. 2019). Previous studies at its type locality in Tay Ninh Province only detected a handful of individuals, including ten during the first survey in 2005, two in 2007, one in 2011, and four individuals in 2017 (Nguyen et al. 2019). To better understand the dietary ecology of the poorly studied black-eyed bent-toed gecko, this study aims to provide novel data on food selection of *C. nigriocularis* using the stomach flushing method and compare diet between different sex and age groups. Besides adding to our limited knowledge of this lizard, the results of the study can be used to support future conservation breeding programs of this Critically Endangered taxon, in case anthropogenic threats still pose significant harm to its population.

2 | Material and Methods

2.1 | Field Surveys and Sampling

Field surveys were conducted in Ba Den MCHC in September 2022 by H.T Ngo, Q.H Do, H.Q Nguyen, and one local person. Ba Den MCHC, located in Tay Ninh Province, southern Vietnam, was established by Decision No. 1351/QD-TTg of the Prime Minister, dated 11 July 2016, with an area of 1762.76 ha (The People's Committee of Tay Ninh Province 2021). In terms of climatic conditions, the national park is located in the subtropical climate region of northern Vietnam, with an annual average rainfall of 1.800 mm an annual average temperature of 27°C, and an annual average humidity of 78% (The People's Committee of Tay Ninh Province 2021). A total of three transects were set up based on previous surveys, literature reviews, and interviews with local people and rangers. Coordinates of each sampling locality were taken using a Garmin GPSMap 64SC (WGS 84 datum) and can be shared upon request with the authors. Animals were captured by hand and subsequently released at the collecting site after being photographed, recorded for habitat characteristics, and measured. SVL (snout-vent length) and MW (mouth width) were taken with a digital caliper to the nearest 0.1 mm, and weight was measured using electronic scales to the nearest 0.1 g. Surveys were undertaken after sunset between 19:00 and 01:00 to guarantee the highest detection probability. During the study, we followed the guidelines approved by the American Society of Ichthyologists and Herpetologists for animal care (Beaupre et al. 2004). The surrounding habitat was granite outcrops with medium hardwoods, shrubs, and arrowroot.

Individuals of *C. nigriocularis* were classified as adult, subadult, or juvenile based on their snout-vent lengths (SVL) according to the original description and our unpublished data (Nguyen et al. 2006). Sex of adults was determined based on the presence of large swollen hemipenial bulges in males, whereas females' parts were un-swollen (H. S. Fitch 1987; McDiarmid et al. 2012). A stomach-flushing technique was used to obtain stomach contents without sacrificing them (Griffiths 1986; Solé et al. 2005; Norval et al. 2012). Forceps, a threaded syringe (60 mL), and infusion tubes of soft material (silicon) were used to collect prey items in the stomach of the species, in particular for small individuals to avoid perforations of the esophagus and stomach. Each individual was stomach-flushed only once, following the guidelines approved by Beaupre et al. (2004). The water for flushing was taken from bottled drinking water. After stomach-flushing, animals were monitored for vigor and body conditions and released within 30 min at the place of capture. Prey items were preserved in 70% ethanol (Merck, Germany).

For taxonomic identification, three tail tissue samples were collected and preserved in 70% ethanol (Merck, Germany) for DNA extraction. Total DNA was then extracted using GenJET Genomic DNA Purification kit (Thermo Fisher Scientific, Lithuania) following the manufacturer's instructions. Extraction DNA was amplified by DreamTaq Mastermix (Thermo Fisher Scientific, Lithuania) with 21 μL volume (10 μL of mastermix, 5 μL of water, 2 μL of each primer and 2 μL of total DNA). PCR conditions were 95°C for 5 min to active the taq; with 35 cycles at 95°C for 30s, 48°C for 45 s, 72°C for 60s; and a final extension at 72°C for 6 min. A fragment of the mitochondrial gene, cytochrome c oxidase subunit I (COI), was amplified using the primer pair VF1d (5′-TTCTCAACCAACCACAARGAYATYG G-3′) and VR1d (5′-TAGACTTCTGGGTGGCCRAARAAYCA -3′) (Ivanova et al. 2006). PCR products were visualized using electrophoresis through a 2% agarose gel stained with ethidium bromide. Successful amplifications were purified to eliminate PCR components using GenJET PCR Purification kit (Thermo Fisher Scientific, Lithuania). Purified PCR products were sent

to FirstBase (Malaysia) for sequencing in both directions. Afterward, sequences were validated with Sequencher v4.10 (Gene Codes, Ann Arbor, MI) with default setting and compared with data available on Genbank using BLAST Tool as implemented in the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov).

After sequences were aligned by Clustal X v2.1 (Thompson et al. 1997), data were analyzed using maximum likelihood (ML) as implemented in IQ-TREE v1.6.12 (Nguyen et al. 2015), maximum parsimony (MP) as implemented in PAUP*4.0b10 (Swofford 2001) and Bayesian inference (BI) as implemented in MrBayes v3.2.7 (Ronquist et al. 2012). For the MP analysis, heuristic analysis was conducted with 100 random taxon addition replicates using the tree-bisection and reconnection (TBR) branch-swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) was calculated using 1000 pseudo-replicates and 100 random taxon addition replicates. All characters were equally weighted and unordered. For the ML analysis, 10,000 ultrafast bootstrap replications (UFB) were used. The optimal model for nucleotide evolution was determined using jModeltest v1.2.10 (Darriba et al. 2012).

For the BI analysis, we used the optimal model determined by jModeltest with parameters estimated by MrBayes v3.2.7. Two independent analyses with four Markov chains (one cold and three heated) were run simultaneously for 10^7 generations with a random starting tree and sampled every 1000 generations. Loglikelihood scores of sample points were plotted against generation time to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function. The posterior probability values (PP) for all nodes in the final majority rule consensus tree were provided. We regard BP $\geq$70% and UFB $\geq$95 and PP >= 0.95 as strong support and values of <70%, <95 and < 0.95, respectively, as weak support (Hillis and Bull 1993; Ronquist et al. 2012; Minh et al. 2013). The optimal model for nucleotide evolution was set to GTR + I + G for BI and analyses. The cut-off point for the burnin function was set to 25% of the total number of trees generated in Bayesian analysis. Uncorrected pair-wise divergences were calculated in PAUP*4.0b10.

2.2 | Stomach Content Analysis

In the laboratory, prey items were identified under a microscope (Olympus SZ 700) following the taxonomic literature of invertebrates (i.e., Naumann et al. 1991; Johnson and Triplehorn 2005; Brusca et al. 2016; Thai 2022). The maximum length (L) and width (W) of each prey item were measured to the nearest 0.1 mm using either a caliper or a calibrated ocular micrometer fitted to a microscope (Hirai and Matsui 2001). The volume (V, mm^3) of the prey item was calculated using the formula for a prolate spheroid ($\pi = 3.14$; Magnusson et al. 2003):

$$V = \frac{4\pi}{3} * \left(\frac{L}{2}\right) * \left(\frac{W}{2}\right)^2$$

The index of relative importance (IRI) was used to determine the importance of each food category. This index provides a more informed estimation of prey item consumption than any of the three components alone by using the following formula (Norval et al. 2012):

$$\text{IRI} = \frac{\%F + \%N + \%V}{3}$$

Where F is the frequency of prey occurrence in stomachs and N is the total number of prey items concerning all prey items.

We used linear regression to examine the relationship between mouth width (MW), snout-vent length (SVL), body mass (BM), and prey size.

Statistical analyses were performed with the SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA) and with the significance level set to $p < 0.05$ for all analyses. Data are presented as mean ± standard deviation (SD) unless otherwise noted. We used Kendall's tau b statistics to examine the relationship between MW, SVL, BM and the prey volume. Wilcoxon's rank sum test (W) was used to examine the size of prey items, the number of prey items, and prey volume from frogs of different sexes.

3 | Results

3.1 | Species Identification

The matrix of molecular data contained 657 aligned characters, of which 380 were constant, 260 characters were parsimony-informative. The MP analysis produced a single most parsimonious tree (tree length = 1551; consistency index = 0.31; retention index 0.70). Tree topologies from three analyses, ML, MP and BI, were similar and the *Cyrtodactylus* from Ba Den MCHC, Tay Ninh Province were recovered with strong statistical support in all analyses as *C. nigriocularis* (Figure 1). In terms of genetic divergences, all samples of *C. nigriocularis* showed a maximum 0.46% based on a fragment of the mitochondrial COI gene, suggesting they all belong to the same species.

Morphological characteristics of the individuals collected in Tay Ninh Province resemble the diagnosis of *C. nigriocularis* (Figure 2; Nguyen et al. 2006) with SVL min–max: 79.77–112.31 mm; mean and SD: 102.26 ± 10.39 mm, $n = 15$, MW 15.13–23.65 mm (20.34 ± 2.35, $n = 15$), and body mass (BM 8.41–26.89 g, 19.93 ± 5.68 g, $n = 15$) in males and SVL 83.20–111.38 mm (102.76 ± 6.23, $n = 33$); MW 16.61–22.10 mm (19.71 ± 1.18, $n = 33$); BM 10.28–28.49 g (20.51 ± 4.60 g, $n = 33$) in females; and SVL: 72.13–76.74 mm; mean and SD: 74.82 ± 2.40 mm, $n = 3$, MW 14.08–17.75 mm (15.61 ± 1.91 mm, $n = 3$), and body mass (BM 5.0–7.20 g, 6.37 ± 1.20 g, $n = 3$) in subadults (Table 1). There were strong positive correlations between the morphological measurements (SVL and MW: $r = 0.860$, $p < 0.001$; SVL and BM: $r = 0.948$; $p < 0.001$; MW and BM: $r = 0.833$, $p < 0.001$; Figure 3a–c).

3.2 | Food Items and Prey Dimension

A total of 51 individuals, including 15 males, 33 females, and 3 subadults, of *C. nigriocularis* were captured in Tay Ninh Province for stomach flushing. Overall, 407 prey items

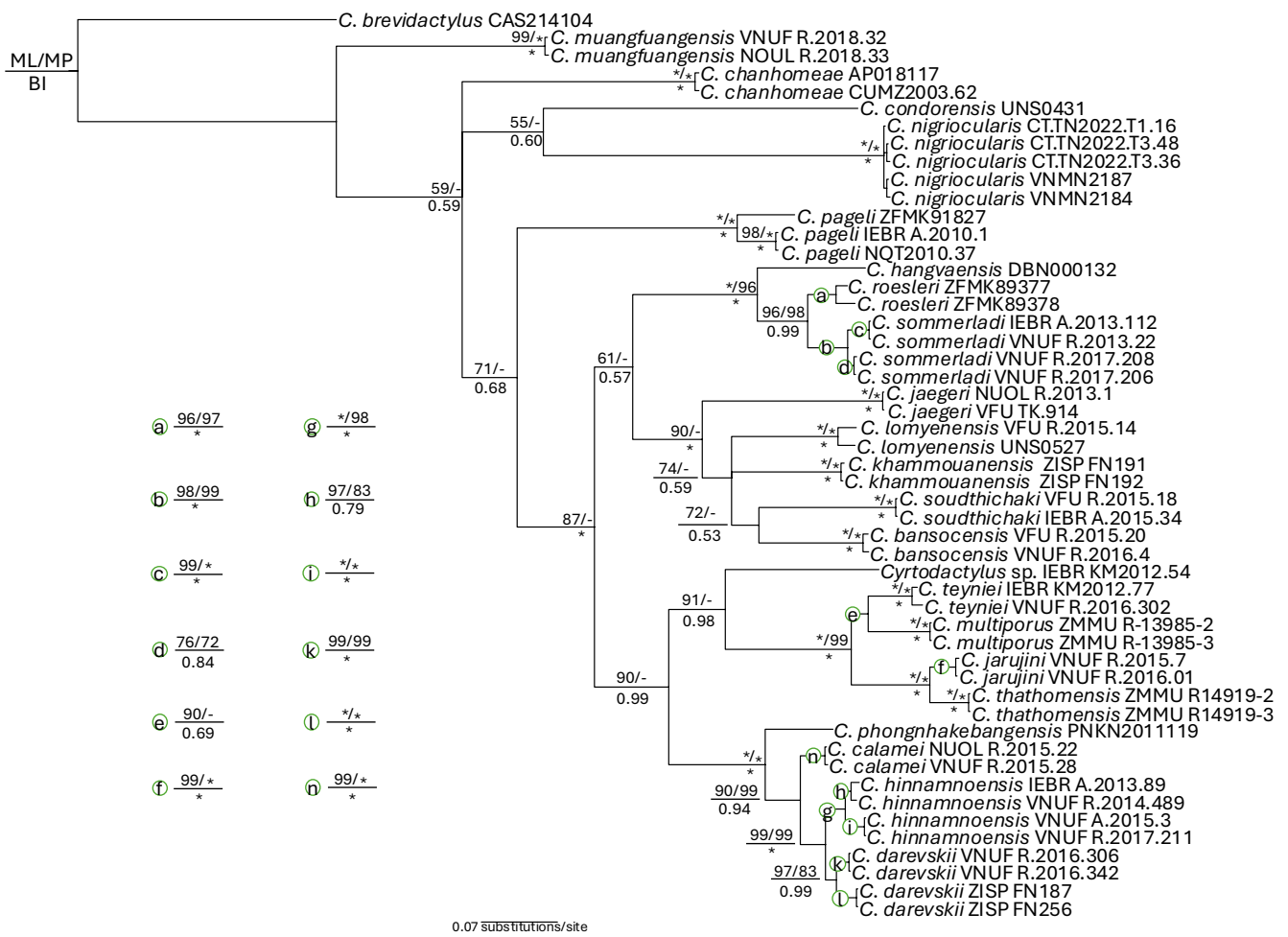

FIGURE 1 | Phylogram based on the Bayesian analysis. Number above and below branches are ML/MP bootstrap and ultrafast bootstrap values and Bayesian posterior probabilities, respectively. Asterisk and hyphen denote 100% in ML and MP or 1 in BI and < 50% values in ML and MP or < 0.5 in BI, respectively.

FIGURE 2 | Adult male (A) and adult female (B) of *Cyrtodactylus nigriocularis* in Tay Ninh Province, Vietnam.

TABLE 1 | Measurements (in mm) of *Cyrtodactylus nigriocularis* in Tay Ninh Province, Vietnam.

	SVL	MW	BM
Male	102.26 ± 10.39	20.34 ± 2.35	19.93 ± 5.68
(n = 15)	79.77–112.31	15.13–23.65	8.41–26.89
Female	102.76 ± 6.23	19.71 ± 1.18	20.51 ± 4.60
(n = 33)	83.20–111.38	16.61–22.10	10.28–28.49
Subadult	74.82 ± 2.40	15.61 ± 1.91	6.37 ± 1.20
(n = 3)	72.13–76.74	14.08–17.75	5.0–7.20

(164 items from 15 males, 217 items from 33 females, and 26 items from three subadult) were found. The range number of prey items per individual was 1.0–43.0 (mean and SD: 7.98 ± 8.42 items, n = 51). Mean and SD prey item length was 7.53 ± 6.55 mm (min–max: 0.5–61.0 mm, n = 407); mean and SD prey item width was 1.44 ± 1.10 mm (min–max: 0.2–8.0 mm, n = 407). The average dietary volume per individual was 186.13 ± 343.07 mm^3 (min–max 0.85–1590.12 mm^3, n = 51).

There was no positive correlation between the bent-toed gecko SVL and the minimum prey volume (Kendall's tau b: tau = −0.023, p = 0.818), mean prey item volume (tau = 0.002, p = 0.987), maximum prey item volume (tau = −0.015, p = 0.879) and the total prey volume (tau = 0.006, p = 0.959) (Figure 4a–d).

The median number of prey among individuals with food in their stomachs was 10.93 ± 12.24 for males and 6.58 ± 6.19 for females with no statistically significant intersexual difference (Wilcoxon's rank sum test, W = 402.5, p = 0.434). Mean prey item length in males was 6.81 ± 6.97 mm (min–max: 1.0–61.0 mm, n = 164); ranged from 0.5 to 50.0 mm in females (7.96 ± 6.42 mm, n = 217); and 2.0–20.0 mm in subadults (8.44 ± 4.83 mm, n = 26). Mean prey item width in males was 1.43 ± 1.07 mm (0.20–7.0 mm, n = 164) ranging from 0.5 to 8.0 mm in females (1.48 ± 1.17 mm, n = 217); and 0.5–2.0 mm in subadults (1.18 ± 0.49 mm, n = 26). The length, width, and volume of prey items were significantly different between females and males (Wilcoxon's rank sum test; length: W = 110, p < 0.001; width: W = 22584.5, p < 0.001; volume: W = 3490, p < 0.001). The average dietary volume per individual in males was 273.36 ± 447.06 mm^3 (0.85–1590.12 mm^3, n = 15); 155.66 ± 301.42 mm^3 (1.11–1514.66 mm^3, n = 33) in females (Wilcoxon's rank sum test, sex: W = 406.50, p = 0.386).

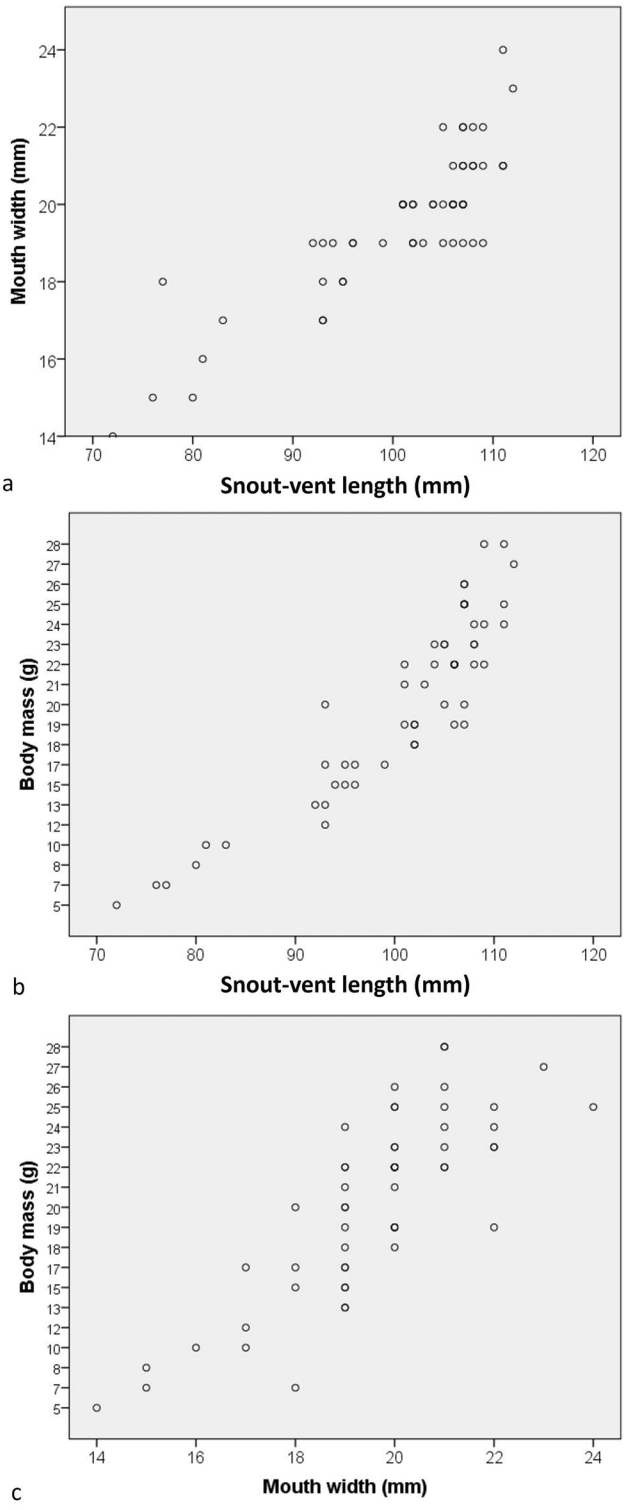

FIGURE 3 | Dispersion diagrams from Pearson's correlations between (a) snout-vent length and mouth width, (b) snout-vent length and body mass, and (c) mouth width and body mass of *Cyrtodactylus nigriocularis* in Tay Ninh Province, Vietnam.

3.3 | Dietary Diversity

We identified 22 prey categories in the stomachs of *C. nigriocularis*. Insects formed the main food component of *C. nigriocularis*, with 14 prey categories (Apidae, Formicidae, Cicadellidae, Blattidae, Byrrhidae, Elateroidea, Culicidae, Hemiptera, Termitidae, Hepialidae, Lepidoptera other, Mantidae, Acrididae, Tetrigidae). Other categories were other invertebrate groups (Araneae, Opiliones, Uropygi, Lumbricidae, Scolopendridae, Armadillidiidae, Achatinidae, Stylommatophora representatives) (Table 2). The highest frequency of occurrence (%F) of prey items identified was Araneae (26.67%), followed by Opiliones (17.33%), Scolopendridae (8.0%), Achatinidae and Blattidae (6.67%), Acrididae (5.33%), while the most proportion (%N) prey group was Araneae (35.87%), followed by Opiliones (23.59%), Blattidae (11.06%), Achatinidae (5.65%), Uropygi and

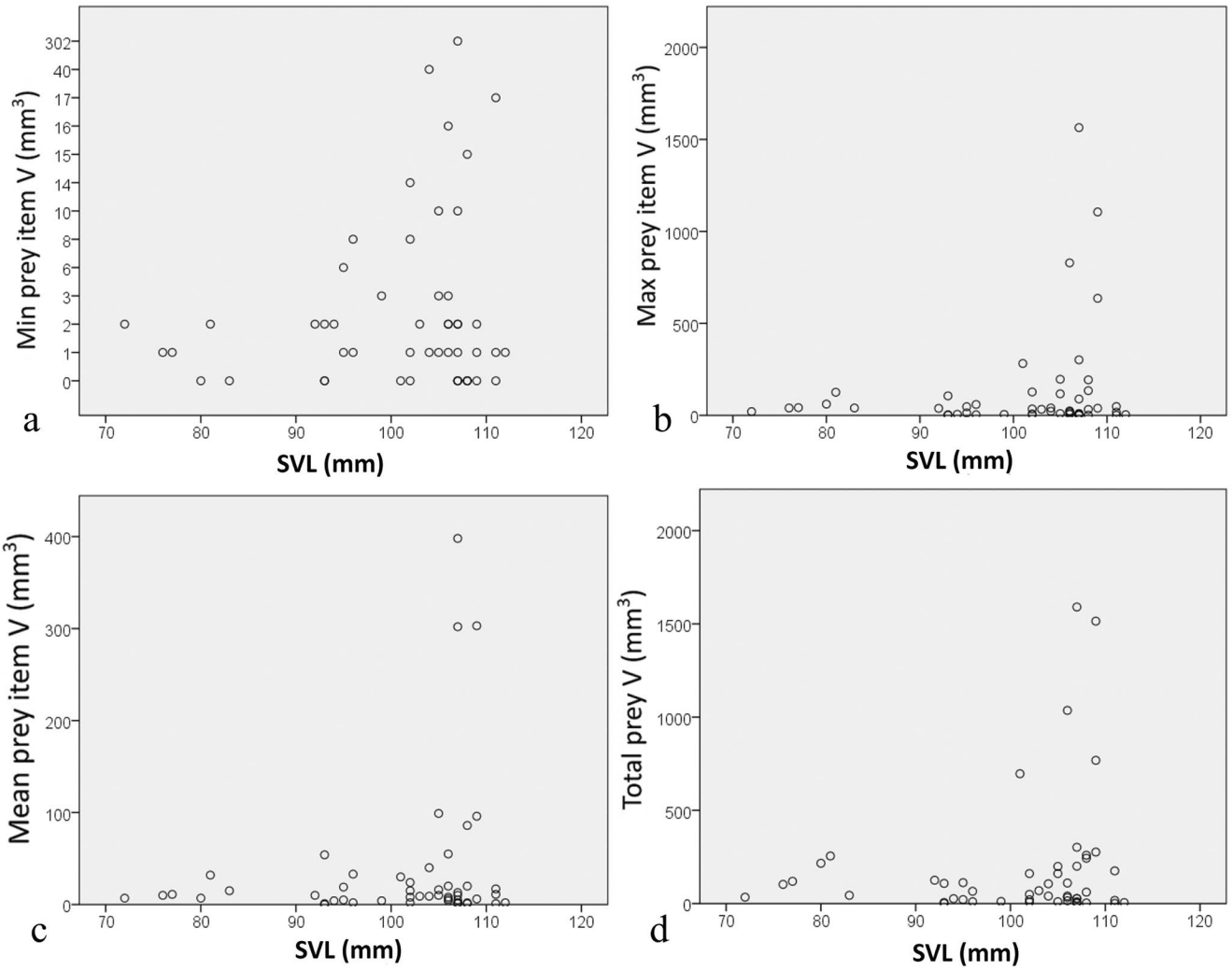

FIGURE 4 | Relationships between the prey volume per stomach (a) snout-vent length, (b) snout-vent length, and (c) body mass of *Cyrtodactylus nigriocularis* in Tay Ninh Province, Vietnam.

Acrididae (4.67%), Scolopendridae (3.44%). In the comparisons by the IRI (%), Araneae (24.33%), followed by Opiliones (16.59%), Achatinidae (10.67%), Blattidae (8.77%), Scolopendridae (7.59%), and Acrididae (4.20%), were found to be the most important prey groups (Figure 5).

4 | Discussion

The stomach flushing method we used in this study focuses primarily on identifying invertebrates in the dietary items of *C. nigriocularis*. Although this method has some limitations, as it is quite invasive (Luiselli et al. 2011) and cannot account for smaller food items, in the absence of more advanced microscopic and molecular equipment, we opted to employ the practice. Other techniques might include microscopic analysis of pollen grains and other vegetation components in lizards' diet or using more advanced approaches, for example, environmental DNA (eDNA), to determine their food composition (Alemany et al. 2023; Pekár et al. 2023; Pinho et al. 2023; Deso et al. 2024). Using a combination of different methods, especially less invasive ones such as eDNA, can provide more comprehensive and accurate data as well as insights into the trophic niche of the species. Furthermore, because the data collection in this study was limited to a single sampling period (September 2022), seasonal variations in the species diet have not been captured. Future works should clarify the outstanding issues.

Most lizards have often been reported feeding on spiders, beetles, grasshoppers, termites, and ants (Bonfiglio et al. 2006; Rocha and Anjos 2007; Iturriaga and Marrero 2013; Tan et al. 2020). In natural environments, the number and frequency of non-winged groups (such as spiders, orthopterans, ants, Lepidoptera larvae and termites) account for a comparatively higher volume (Vitt and Zani 1997; Vitt et al. 1997; Zamprogno and Teixeira 1998; Colli et al. 2003; Iturriaga and Marrero 2013). Similarly, in this study, the diet of *C. nigriocularis* was found to mainly consist of arthropods (non-winged groups), most categorically represented by Araneae, Opiliones, Scolopendridae, and Blattidae. These invertebrates were quite abundant in the study area in Ba Den Mountain MCHC, as we often encountered them during our field surveys. Other studies show that *Cyrtodactylus* can feed on annelid worms, cricket, and insect larvae in both natural and captive conditions (Ellis and Pauwels 2012; Kane et al. 2022). It is thus likely that *Cyrtodactylus* species are opportunistic predators.

TABLE 2 | Prey categories consumed by *Cyrtodactylus nigriocularis* in Tay Ninh Province, Vietnam ($n = 51$), (F) total frequency, (%F) relative frequency, (N) total abundance, (%N) relative abundance, (V) total volume (mm^3), (%V) relative volume; (IRI) importance index.

Prey taxa	F	%F	N	%N	V	%V	IRI
Annelida							
Opisthopora							
Lumbricidae	1	1.33	3	0.74	1493.33	15.73	5.93
Mollusca							
Stylommatophora							
Achatinidae	5	6.67	23	5.65	1869.09	19.69	10.67
Stylommatophora other	1	1.33	1	0.25	1.67	0.02	0.53
Arthropoda							
Arachnida							
Araneae	20	26.67	146	35.87	990.90	10.44	24.33
Opiliones	13	17.33	96	23.59	841.44	8.86	16.59
Uropigi	1	1.33	19	4.67	1035.68	10.91	5.64
Scolopendromorpha							
Scolopendridae	6	8.00	14	3.44	1074.99	11.32	7.59
Isopoda							
Armadillidiidae	1	1.33	2	0.49	39.90	0.42	0.75
Insecta							
Blattodea							
Blattidae	5	6.67	45	11.06	815.22	8.59	8.77
Coleoptera							
Byrrhidae	1	1.33	1	0.25	9.81	0.10	0.56
Elateroidea	1	1.33	1	0.25	302.03	3.18	1.59
Diptera							
Culicidae	1	1.33	1	0.25	14.65	0.15	0.58
Hemiptera							
Cicadellidae	1	1.33	1	0.25	10.47	0.11	0.56
Pentatomidae	2	2.67	2	0.49	159.15	1.68	1.61
Hymenoptera							
Apidae	1	1.33	2	0.49	198.87	2.09	1.31
Formicidae	4	5.33	9	2.21	28.78	0.30	2.62
Isoptera							
Termitidae	3	4.00	10	2.46	63.52	0.67	2.38
Lepidoptera							
Hepialidae	1	1.33	1	0.25	133.97	1.41	1.00
Lepidoptera other	1	1.33	1	0.25	70.52	0.74	0.77
Mantodea							
Mantidae	1	1.33	6	1.47	85.17	0.90	1.23

(Continues)

TABLE 2 | (Continued)

Prey taxa	F	%F	N	%N	V	%V	IRI
Orthoptera							
Acrididae	4	5.33	19	4.67	247.76	2.61	4.20
Tetrigidae	1	1.33	4	0.98	5.63	0.06	0.79

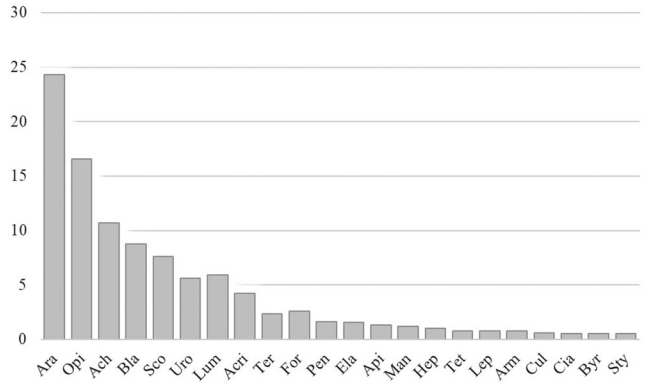

FIGURE 5 | Importance index (IRI) for prey categories of *Cyrtodactylus nigriocularis* in Vietnam. Araneae = Ara, Opiliones = Opi, Blattidae = Bla, Acrididae = Acr, Scolopendridae = Sco, Pentatomidae = Pen, Achatinidae = Ach, Lumbricidae = Lum, Uropigi = Uro, Tetrigidae = Tet, Termitidae = Ter, Formicidae = For, Elateroidea = Ela, Armadillidiidae = Arm, Apidae = Api, Mantidae = Man, Hepialidae = Hep, Lepidoptera other = Lep, Stylommatophora other = Sty, Culicidae = Cul, Cicadellidae = Cia, Byrrhidae = Byr.

Our results are consistent with those reported in previous studies, which show bent-toed geckos are typical sit-and-wait foragers (Huey and Pianka 1981; Ananjeva and Tsellarius 1986). However, there is a significant difference between the diet composition of *C. nigriocularis* and those from other geckos. For example, while the diet of *Coleodactylus natalensis* from the Brazilian Atlantic Forest ($n = 49$) is dominated by arthropods (Isopoda, Araneae, Pseudoscorpiones, Coleoptera, Homoptera, and Collembola) (Lisboa et al. 2012) the most important prey in the diet of *C. nigriocularis* include Araneae, Opiliones, Scolopendridae, Blattidae, and Stylommatophora. Despite the divergence, Araneae makes up the most important prey category for the two species. With regard to *Mediodactylus kotschyi* (Steindachner) in the Mediterranean Insular Ecosystems of the Aegean region, Erstratios and Polyme (1990) found that the diets of one population ($n = 68$) was composed predominantly of Araneida, Thysanura, Coleoptera, Isopoda, Insects larvae, and Pseudoscorpions. Similarly, despite differences in diets between *C. nigriocularis* and *M. kotschyi*, Araneae also constitutes the most important prey category for the two species.

Because the number of individuals in this study is small, it is difficult to support comparisons of the diet by sex and age group. Nonetheless, our preliminary results show that the diets of males and females were slightly different in terms of number of prey items ingested and prey size. In addition, females had a broader trophic spectrum than males (females: 18 prey categories, males: 11 prey categories), possibly because females require more energy for reproduction (Vitt and Caldwell 2009; Iturriaga and Marrero 2013).

Despite the differences in diets between the sexes, all of their main food categories comprise non-flying invertebrates. Furthermore, Araneae and Opiliones represent the most important prey categories for all groups. The findings need to be confirmed in more in-depth studies with a larger sample size. Some studies on lizards have shown that morphological differences between males and females might result in differences in diet composition (H. S. Fitch 1978; Schoener et al. 1982; Preest 1994). Furthermore, in this study, we did not find a positive correlation between body size and prey volume, which may indicate that the sample size was not large enough and further research is needed to investigate this issue in more detail. *Cyrtodactylus nigriocularis* inhabits Tay Ninh Province's Ba Den Mountain Cultural and Historical Complex, which receives a lower level of protection compared to a nature reserve. *C. nigriocularis* is a highly specialized granite cave-dwelling species known only from just a few small caves in Ma Thien Lanh Valley. It occurs in very low numbers and is extremely susceptible to habitat degradation by tourism activities, road construction, and illegal collecting (Nguyen et al. 2019). Ba Den Mountain has become a popular tourist destination, and a new cable car line was launched a few years ago. Our observation confirms that a new road to the top of the mountain is being constructed, further threatening the species. To our knowledge, there has been no ex situ conservation program established for this or any endangered Indochinese bent-toed geckos anywhere in the world. The data from this study will therefore help inform future captive breeding conservation efforts to safeguard the microendemic and Critically Endangered species from the risk of extinction, especially because feeding a diverse and natural diet can maintain the physical fitness of captive populations (Antwis et al. 2014; Chatpongcharoen et al. 2021; Gao et al. 2023).

Author Contributions

Hanh Thi Ngo: conceptualization (equal), data curation (equal), formal analysis (equal), funding acquisition (equal), investigation (equal), resources (equal). **Minh Duc Le:** conceptualization (equal), data curation (equal), formal analysis (equal), investigation (equal). **Trang Thu Hoang:** data curation (equal), formal analysis (equal). **Ha Hoang Nguyen:** data curation (equal), formal analysis (equal). **Huy Quoc Nguyen:** investigation (equal). **Quyen Hanh Do:** investigation (equal). **Hanh Minh Vu Nguyen:** data curation (equal), formal analysis (equal). **Truong Quang Nguyen:** methodology (equal), writing – review and editing (equal). **Thomas Ziegler:** writing – review and editing (equal). **Minh Duc Le:** investigation (equal), methodology (equal), writing – review and editing (equal). **Anh Van Pham:** data curation (equal), formal analysis (equal), writing – original draft (equal), writing – review and editing (equal).

Acknowledgments

We are grateful to the directorates of Ba Den MCHC for their support of our fieldwork and for issuing relevant permits. We thank L.V. Pham (Tay Ninh) for his assistance in the field. We thank T.T. Nguyen, N.A.T. Ho, and M.H. Le for their support in the laboratory. Doctoral research of H.T. Ngo in Germany is funded by the German Academic Exchange Service (DAAD). This research is supported by the Critical Ecosystem Partnership Fund under Grant Number CEPF-111890, the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.05-2020.24 and Zoologischen Gesellschaft für Arten und Populationsschutz (ZGAP, Project code: 7. Ba Den Mountain.VNM-2024).

Disclosure

Benefit-Sharing Statement: This study complies with the principles of the Convention on Biological Diversity and the Nagoya Protocol. A stomach-flushing technique was used to obtain stomach contents without sacrificing animals. Each individual was stomach-flushed only once, following the guidelines approved by Beaupre et al. (2004). This research was undertaken in accordance with all national and international regulations on animal welfare. All researchers involved in this study are acknowledged as co-authors, ensuring fair recognition of their intellectual contributions.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data and analysis code are available at Zenodo (https://zenodo.org/records/14876916).

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4. Discussion

4.1. Investigating the taxonomic status of all described *Cyrtodactylus* species in Vietnam and discover new taxa

4.1.1. New discoveries

Over the past four years, seven new species of *Cyrtodactylus* have been described from the provinces of Binh Dinh, Dak Lak, Khanh Hoa, Lao Cai, Phu Yen, and Tay Ninh (**Chapters 1-6**). Of these, six belong to the *C. irregularis* species complex, while the last one is a member of the *C. chauquangensis* group (Grismer et al. 2021, 2022). The discoveries have increased the total number of *Cyrtodactylus* species in Vietnam from 46 to 55 (include *C. hangvaensis* and *C. borgattaorum* which were described by Duong et al. 2024 and Tran et al. 2024, respectively), establishing the country as a hotspot for new species within the *Cyrtodactylus* genus.

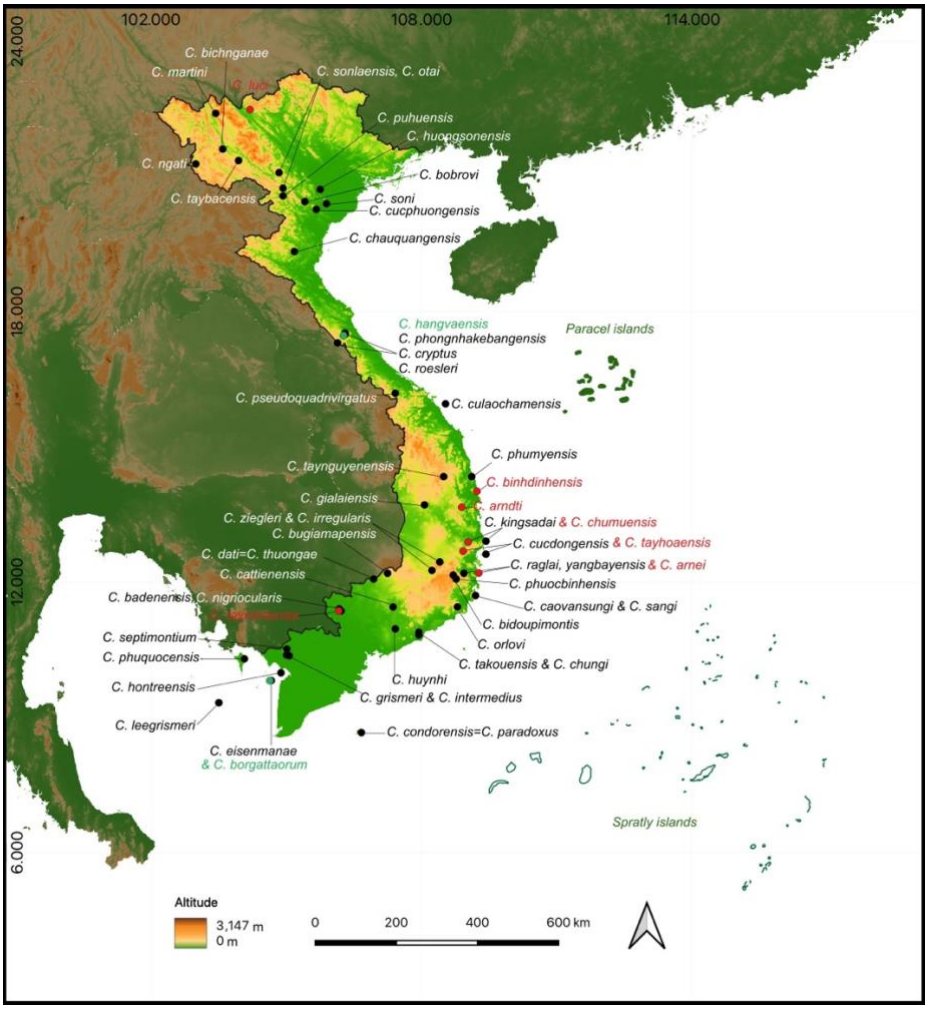

Fig. 5. Type localities of all *Cyrtodactylus* taxa occurring in Vietnam (Uetz et al. 2025). Black, red, and green dots represent species described up to 2021, as well as seven new species identified in this, and other studies conducted over the past four years.

Furthermore, more than 10 new forms have been discovered based on morphological and molecular data. All of the new forms will be officially described in the near future. Consequently, the study shows that the diversity of *Cyrtodactylus* in Vietnam has been significantly underestimated, confirming the **second hypothesis**.

4.1.2. Taxonomic revision

Using an integrative taxonomic approach that combines morphological and molecular analyses, it has been determined that *C. thuongae* is a junior synonym of *C. dati*, as detailed in **Chapter 7**. This finding indicates that *C. thuongae* is not a valid taxon. *C. thuongae* was described based on a holotype and a paratype collected from Tay Ninh Province, while *C. dati* was identified in Lam Dong and Binh Phuoc Provinces. Both species inhabit complex mountainous regions and were previously described solely based on morphological examinations (Ngo 2013; Phung et al. 2014). This result emphasizes that the high rate of species description has resulted in taxonomic confusion and that some species complexes cannot be easily distinguished through morphological analyses alone.

Additionally, it is discovered that *C. rufford* is a junior synonym of *C. lomyenensis* (also in **Chapter 7**). Both species were found in Khammouan, Laos, and they occur in karst areas (Ngo and Pauwels 2010; Luu et al. 2016). These results underscore the taxonomic challenges in biodiversity research in the region. In conclusion, the **first hypothesis**, which assumes the existence of synonyms within *Cyrtodactylus*, is validated.

4.2. Investigating phylogenetic relationships, evolutionary process and biogeographic history of *Cyrtodactylus*

4.2.1. Phylogenetic relationships

Considering the large lack of nuclear markers in previous phylogenetic studies, the research focused on small monophyletic groups within the genus *Cyrtodactylus*. Our investigation incorporates the most extensive dataset to date for all described species of *C. angularis* and *C. chauquangensis* groups, utilizing three mitochondrial and four nuclear markers. **Chapters 8** and **Chapter 9** of this work present the first comprehensive studies that include all described species of the *C. angularis* and *C. chauquangensis* groups. Similar to Grismer et al. (2021, 2022), both chapters confirm that the *C. angularis* and *C. chauquangensis* groups are monophyletic although the groups were rendered paraphyletic in the study by Chomdej et al. (2022) (*C. chauquangensis*). On the other

hand, the results reveal several discrepancies compared to previous hypotheses. For examples, according to the trees based on both mitochondrial and nuclear genes in our study in **Chapter 8**, *C. pageli* is placed as a sister taxon to *C. muafuangensis* and the two species together form a distinct lineage, while Grismer et al. (2021) and Duong et al. (2024) suggested that *C. pageli* clusters with *C. angularis* and *C. chanhomeae*. Additionally, molecular analyses suggest that while mitochondrial markers produce well-resolved topologies for more recent divergences, combining mitochondrial and nuclear data results in enhanced statistical nodal values, especially for the deep nodes due to their slower rate of evolution. Therefore, the results from **Chapters 8 and 9** affirm the **third hypothesis**.

4.2.2. Time calibration and biogeography

Our time-calibrated molecular results in **Chapter 8** and **Chapter 9** based on three mitochondrial genes and four nuclear genes resemble those reported by Grismer et al. (2022) based on one mitochondrial gene and show that diversification of most major lineages of *C. chauquangensis* and *C. angularis* occurred during the Miocene Episode and *C. nigriocularis* and other three clades of *C. angularis* emerged in the Oligocene. In addition, both studies (**Chapter 9** and Grismer et al. 2022) reveal that *C. bobrovi*, *C. houaphanensis*, *C. otai* and *C. puhuensis* are the youngest members of the group. However, the divergence time estimation results produce several discrepancies between the subgroups of *C. angularis* and *C. chauquangensis* compared to the study by Grismer et al. (2022) (please refer to the discussion sections in **Chapters 8 and 9**.). The differences might result from the increase in number of loci. In this study, we used three mitochondrial genes (COI, Cytb, ND2) and four nuclear genes (Cmos, PDC, Rag1 and Rpl35), while a study by Grismer et al. (2022) included only one mitochondrial gene (ND2).

Chapter 8 and **Chapter 9** represent the first investigations within the genus *Cyrtodactylus* with the incorporation of areas of endemism and tectonic history, as proposed by Bain and Hurley (2011), pertinent to the subregions in Indochina. In contrast, Grismer et al. (2022) have combined eastern Myanmar, Indochina, Yunnan, China, and the Burma-Thai-Malay Peninsula into a single region referred to as Indochina. Consequently, the findings from our studies in **Chapters 8 and 9** reveal discrepancies concerning the ancestors of the *C. angularis* and *C. chauquangensis* groups. In particular, the result of our study indicates that the *C. angularis* group began to disperse

out of the Mekong Lowland (MEK) to the northern Annamites (NAN) and eastern Thailand (West Indochina). Whereas Grismer et al. (2022) reported that the ancestor of the *C. angularis* group likely originated in karst habitats in central Indochina. Furthermore, our results suggest that the ancestor of the *C. chauquangensis* group most probably lived in the Northwest Uplands (area NWU) during the Oligocene – Miocene boundary (~23 Mya); some of their descendants speciated within this area, whereas others dispersed to North-western Thailand (area NWTL), South-central China (area SCC), Northern Annamites (area NAN), Northeast Lowlands (area NEL) and Northeast Uplands (area NEU) before their descendants gave rise to the extant taxa. In contrast, Grismer et al. (2022) proposed that the ancestor of the *C. chauquangensis* group radiated from the central Indochina region in the early Miocene to northern Vietnam, southern China, Laos and northern Thailand. The differences might result from the combination of nuclear loci. In this study, we used three mitochondrial genes (COI, Cytb, ND2) and four nuclear genes (Cmos, PDC, Rag1 and Rpl35), whereas a study by Grismer et al. (2022) included only one mitochondrial gene (ND2).

Interestingly, the results in **Chapter 8 and 9** show that dispersal of *C. angularis* and *C. chauquangensis* groups may have been enhanced by the development of the East Asian monsoon and the accompanying copious precipitation (especially the winter precipitation) around the Oligocene and Miocene boundary. During this period, a transition from broadleaf vegetation to evergreen broadleaf vegetation and plant diversity increased, which probably provided suitable habits and hosts for members of the *C. angularis* and *C. chauquangensis* groups. In addition, precipitation from the East Asia monsoon likely accelerated the dissolution of the limestone substrate and deeply influenced the development of the karst region, which possibly contributed to the expansion and divergence of these groups (Li et al. 2021, 2022; Chen et al. 2023).

Consequently, the combination of findings in **Chapter 8** and **Chapter 9** affirms the **fourth and fifth hypothesis**, suggesting Asia monsoons play a major role in the evolution of the *Cyrtodactylus* genus and karst promotes speciation rate of *Cyrtodactylus* in Vietnam.

4.3. Ecological features and population status of two endemic and critically endangered species

Preliminary population estimations for two endemic and critically endangered species of *Cyrtodactylus* (*C. gialaiensis* and *C. takouensis*) in Vietnam in **Chapters 11 and 12**, demonstrate that population size of *C. gialaiensis* in Chu Se District consists of fewer

than 100 individuals and *C. takouensis* in Ta Kou Mountain is composed of fewer than 600 individuals. These are much lower than the minimum viable size of at least 3,000 to 7,000 individuals required to maintain a stable over a longer period of time (Reed et al. 2003; Trail et al. 2007). Thus, the **sixth hypothesis** stating that natural populations of the most threatened species in Vietnam are extremely small could be herein affirmed, underscoring a critical conservation concern.

Furthermore, the first evaluations of threats to the two endemic and critically endangered species are presented in **Chapter 11** and **Chapter 12**. In **Chapter 11**, habitat loss and degradation from land use change and habitat degradation is identified the most critical threats to all populations of *C. gialaiensis*. All these populations face uncertainty because of land use changes, driven by the profit motives of landowners, who may convert coffee plantation into durian plantations or other agricultural uses, while tourism activities and parasites are regarded as major threats to the natural populations of *C. takouensis* in **Chapter 12**. In particular, infrastructure development associated with the cable car built a decade ago is still expanding to accommodate the growing tourism since Ta Kou NR has become a popular tourist destination in southern Vietnam in recent years. In fact, one survey transect was visited every day and flash lighted every night by tourists and another surveyed transect was visited at least two or three times a month. Along the transects, a large amount of plastic waste, which is likely to significantly reduce the habitat quality of this species, left by tourists were observed. Furthermore, many individuals were detected with ticks as ectoparasites and an unidentified parasite as endoparasites on the body surface. Although we couldn't identify the parasites so far, they were common in adult individuals of *C. takouensis*. Therefore, further studies are needed to provide more information on the parasites and how they affect the fitness of *C. takouensis* in the future. Accordingly, the present pilot studies largely affirm the **seventh hypothesis**, proposing that habitat loss and degradation are the main threats to two threatened species.

First investigations of microhabitat preferences of two endemic and critically endangered species are also presented in **Chapter 11 and Chapter 12**. **Chapter 11** confirms that *C. gialaiensis* was distributed in both basalt and adobe soil types and this species was observed in the hole cliff or the soil cliff, tree trunk and shrubs between the coffee plantation and the road or coffee plantation areas. The geographic distance between subpopulations also suggests that *C. gialaiensis* may have had a more extensive

distribution across the Gia Lai District in the past. However, the current land use may soon clear all of its suitable habitats. **Chapter 12** shows that *C. takouensis* was only found on granitic rocks. Microhabitat sites suitable for this species are thus limited and isolated from each other.

Regarding microhabitats of *C. takouensis* in Ta Kou Mountain, one sympatric and ecologically similar bent-toed gecko, namely *C. chungi*, is frequently observed to co-occur with *C. takouensis*. Thus, understanding the morphological characteristics, dietary habits, niche segregation, and potential interactions between the two species is essential for understanding speciation processes. This knowledge also plays a critical role in developing conservation measures. During the PhD period, I was also able to investigate the spatial and dietary niche segregation between these two sympatric species. Preliminary results indicate significant differences in their small-scale spatial distribution, especially in terms of substrate types and positioning. The results indicated that *C. takouensis* is a granite specialist, whereas *C. chungi* prefers semi-rocky or arboreal habitats. Furthermore, *C. takouensis* and *C. chungi* exhibit significant differences in 12 morphological characteristics. Unfortunately, the data were not yet published before this dissertation, but it is expected to be released in the near future.

5. General References

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