High level of local endemism under high risk of extinction: Systematics, evolution, autecology, population and conservation status of Tiger geckos (*Goniurosaurus*) in Vietnam

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CONTENTS

SUMMARY

Tiger geckos of the genus *Goniurosaurus* currently consist of 24 species in Southeast and East Asia, all of which have been found in allopatry with a high level of local endemism. Five species are currently known from Vietnam, viz. *G. araneus, G. catbaensis, G. huuliensis, G. lichtenfelderi* and *G. luii*. Although new species of *Goniurosaurus* are still being discovered, the genus has become threatened by extinction through anthropogenic impacts. Unfortunately, once the thesis started, none of *Goniurosaurus* species were yet included in any wildlife protection laws, neither in China nor in Vietnam. In spite of the urgent requirement for protection, *Goniurosaurus* species have been omitted from the prioritization of conservation schemes due to a lack of biological background. In this study, I employed integrative methods of morphology, genetics, ecology, demography, and species distribution models in order to provide insights into the systematics, evolution, autecology, population status and threats of tiger geckos in Vietnam for conservation implications.

Based on data collected during recent field work by our team, we expanded morphological descriptions of Vietnamese tiger geckos, evaluated variations among all *Goniurosaurus* species and provided an identification key. Our phylogenetic analyses showed that all recorded populations of *Goniurosaurus* from Vietnam are monophyletic with low intra-specific divergences (0 – 1.9 %), which are assigned to one of the four known species, namely *G. catbaensis, G. huuliensis, G. lichtenfelderi* and *G. luii*. Together with genetic data of remaining species from China and Japan, we evaluated the phylogeny of 23 species of *Goniurosaurus*. The analysis strongly supported the recognition of four monophyletic species groups (namely *G. kuroiwae, G. lichtenfelderi, G. luii* and *G. yingdeensis*). We further calibrated the divergence date of generic origin in the Eocene (approximately ~45.3 mya), and the diversification within four monophyletic groups began in the mid-Miocene ~13.4 – 7.7 mya and continued to at least the mid-Pliocene ~2 mya among species. The cladogenetic events of *Goniurosaurus* were ergo assumed relative to past orogeny processes following the vicariance pattern, adapted habitat segregation (e.g. karst and granite) and altitudinal transition. Given the Grinnellian niche evolution, the speciation among *Goniurosaurus* species was further explained with both climatic niche conservatism and divergence patterns.

We studied the habitat preference of four Vietnamese tiger gecko species. The ecological analyses confirmed that three *Goniurosaurus* species (*G. catbaensis, G. huuliensis* and *G. luii*) are limestone karst-dwelling specialists, while the habitat preference of *G. lichtenfelderi* is significantly different, viz. adapted to granite-stream forest.

Being aware of the importance of demographical information for species conservation efforts, population monitoring was carried out to estimate the population size and density of two Vietnamese *Goniurosaurus* species (namely, *G. catbaensis* and *G. huuliensis*). Consequently, their population size and density were found to be exceedingly small and negatively correlated to the increasing severity of human impacts.

In this study, an extensive attempt was implemented to outline domestic and international trade activities in *Goniurosaurus* species. As a result, a large number of wild *Goniurosaurus* individuals from Vietnam and China have been over-harvested for international trade, although our records only reflected snapshots. Given potential impacts of climate change, we applied species distribution models (SDMs) to project the potential distribution of three Vietnamese *Goniurosaurus* species (namely, *G. catbaensis, G. huuliensis* and *G. lichtenfelderi*). Under climate change scenarios, their suitable habitats were predicted to shift towards higher latitudes, shrink significantly and even vanish in their entire distribution within the next decades. Furthermore, the increasing rate of habitat degradation and forest conversions dramatically imperils wild populations of *Goniurosaurus* species in Vietnam.

Under severe human impacts, all species of *Goniurosaurus* from China and Vietnam were listed in CITES Appendix II, and Vietnamese species were assessed as globally threatened in the IUCN Red List and were protected by national laws as well. In this context, we identified priority areas – especially in protected areas, islands and transboundary regions – for conservation actions, and highly recommended the establishment of species and habitat conservation areas for tiger geckos in Vietnam.

In conclusion, this study clarified issues related to the systematics and proved evolutionary hypotheses of geographical origin and relevant abiotic factors for the diversification of *Goniurosaurus* species and their allopatric distribution. Together with assessments of ecology, demography and anthropogenic threats, the thesis emphasized the urgent requirement of conservation actions. In this context, conservation programs and solutions were proposed to protect *Goniurosaurus* species in Vietnam and their habitats in the future.

I. INTRODUCTION

1.1. Global biodiversity and threats.

1.1.1. Global biodiversity crisis.

Through measures of variation at the level of genetics, species and ecosystems, understanding of biodiversity is much greater than heretofore (Collen et al. 2014; Roll et al. 2017; Schluter & Pennell 2017). There is no evidence for an ending in the increase of global biodiversity in the next decades due to extensive taxonomic reassessments of species complexes and discoveries from remote areas (Moura & Jetz 2021; Liu et al. 2022). Although they just constitute 3% of known species, vertebrates play vital roles to maintain the sustainability and basic functions of global ecosystems (Hoffmann et al. 2010). A huge number of new species have been discovered in the five vertebrate groups during the last three decades, in particular, 2,553 amphibians, 4,386 fishes, 711 mammals and 1,905 reptiles (Liu et al. 2022). In terms of herpetofaunal diversity, nearly 8,500 amphibian species and over 11,700 species of reptiles are known to occur in the world (Frost 2022; Uetz et al. 2022).

In contrast to the increase of new species, the rate of biodiversity loss has been exceptionally rapid over the last centuries (Monastersky 2014). Against the background of the ongoing sixth mass extinction event, a current extinction rate much higher than natural extinction events in the past has been estimated for global biomes (Ceballos et al. 2015; Pereira et al. 2010; Urban et al. 2012). Whilst causes of previous extinction events were commonly acknowledged relevant to the natural catastrophes, principal drivers of the current crisis are anthropogenic impacts mainly concerning habitat destruction, over-exploitation, pollution, invasive species, diseases and climate change (Butchart et al. 2010; Böhm et al. 2013; Monastersky 2014; Cox et al. 2022). Their overwhelmingly detrimental effects are predicted to be even more severe in the next decades. In particular, on average nearly one-fifth of vertebrates are classified at the global risk of extinction, ranging from 13% of birds to 41% of amphibians (Hoffmann et al. 2010; Cox et al. 2022). Allan et al. (2019) estimated one-quarter of vertebrate species to be impacted across >90% of their distribution under multiple anthropogenic stressors, and approximately 7% envelop their entire range. In tropical regions, the concentration of irreplaceable vertebrate diversity coincides with the increase of human pressure. Consequently, rather than the outstanding value of biodiversity almost global "hotspots" have been recently known as the top regions of biodiversity loss (Mittermeier et al. 2004; Bellard et al. 2014).

1.1.2. The fate of reptiles.

Over a fifth of all reptile species are classified at the global risk of extinction (Böhm et al. 2013; Cox et al. 2022). Across the tetrapod groups, while the proportion of threatened reptiles is higher than that of birds (13.6%), the number of reptiles is only fewer than amphibians in terms of endangerment (Hoffmann et al. 2010; Cox et al. 2022). Among reptiles, turtles, crocodiles, iguanid lizards, xenosaurids, and uropeltid and tropidophiid snakes (at least 50%

of the threatened proportion) are comparable to the most-threatened tetrapod groups (Cox et al. 2022). Notably, the tropical regions harbor a high concentration of reptiles, of which a large number are threatened species (Fig. 1; Böhm et al. 2013; Roll et al. 2017; Cox et al. 2022). The Indo-Burma hotspot region, for example, which contains top developing countries in Southeast Asia (e.g. Cambodia, Laos, Myanmar, Thailand and Vietnam; Fig. 2) in terms of biodiversity, yet harbors 127 reptile species (21.5%) of 590 globally threatened tetrapods in the IUCN Red List, mostly impacted by anthropogenic threats (CEPF 2020; IUCN 2021; Cox et al. 2022).

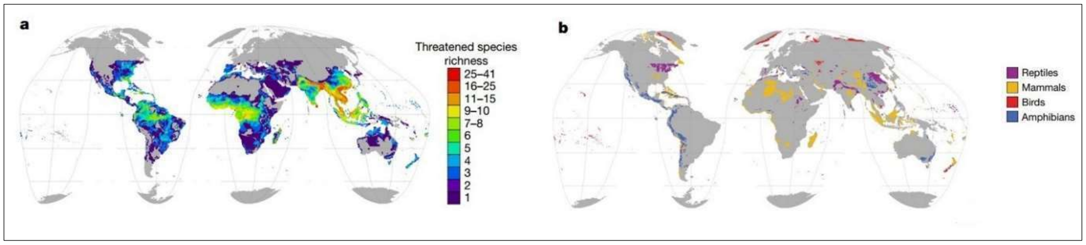

Figure 1. a) Global distribution of threatened reptiles; b) Regions with disproportionate numbers of threatened species for each tetrapod class (from Cox et al. 2022)

Agriculture expansion, urban development and logging leading to habitat destruction are the main anthropogenic factors for the increasing extinction risk in reptiles. Other important threats are identified as invasive species and hunting for commercial harvest and trade. In addition to the direct effects, climate change is known as a looming threat to the fate of reptiles in the future (Cox et al. 2022). Despite being globally threatened by extinction through these human impacts, most reptiles have been overlooked with conservation schemes due to a lack of biological knowledge (Böhm et al. 2013).

1.2. Vietnam: a threatened hotspot of biodiversity.

Covering about 330,000 km^2, Vietnam has boundaries with China in the north, with Laos and Cambodia in the west, and contains a coastline of 3,260 km in length with thousands of offshore islands (Fig. 2). The topography is subdivided into three principal categories, including mountainous areas (e.g. the Northwest Region, the Northeast Region, the Truong Son – Annamite range), Central Highlands (e.g. Tay Nguyen plateaus), and lowland areas (e.g. the Red River Delta, the Mekong Delta and coastal plains) (Sterling et al. 2006; Nguyen et al. 2009; Queiroz et al. 2013). A total of 14 typical ecoregions are recognized in Vietnam by WWF (Fig. 2; Queiroz et al. 2013). Bain & Hurley (2011) identified 19 sub-regions for the herpetofauna within the Indochina region, whereof 9 sub-regions are known in Vietnam. A wide range of elevations as well as the complexity of landforms and vegetation types have given the country a great level of genetic diversity (Fritz & Rahbek 2012).

In terms of climate, Vietnam is mainly enveloped by a stable tropical monsoon, yet various micro-climates are shaped by its complex topography and diverse ecosystems (Clements et al. 2006; Sterling et al. 2006; Queiroz et al. 2013). For example, temperatures decrease and may drop below freezing towards the mountain top, mean annual precipitation varies at the elevation of 800 m and disjunct karst outcrops offer multiple micro-ecological niches. Moreover, due to its geographic position in the transition zone from cold weather in Tibetan mountains of China to subtropical mountains in Southeast Asia, Vietnam is likely to harbor a high level of biodiversity, particularly in terms of herpetofauna (Sterling et al. 2006).

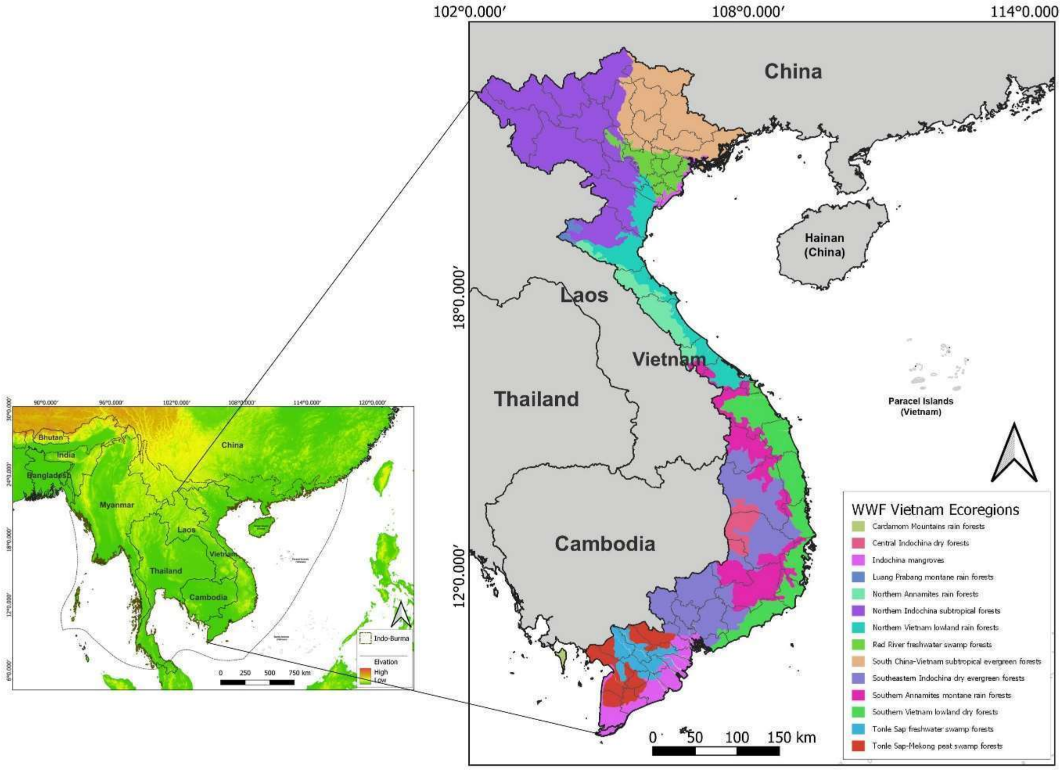

Figure 2. Maps of the Indo-Burma region (Left) and Vietnam ecoregions from WWF (Right).

In terms of reptile diversity, Vietnam is recognized as one of the most well-known countries in the world and many new species are still discovered annually (Nguyen et al. 2009; Uetz et al. 2022). Compared to neighboring countries in the Indo-Burma region, a total of 519 reptile species have been recorded from Vietnam so far, whereof 88 newly described taxa in the last decade, followed by Thailand – 475 species (73 new species), Myanmar – 368 species (54), Laos – 208 species (44) and Cambodia – 199 species (26) (Fig. 3; Uetz et al. 2022). However, the reptile fauna of the country is still imperfectly studied. For example, only three species of the genus *Cyrtodactylus* were recorded from Vietnam until 1997. Up to date, *Cyrtodactylus* became one of the most diverse genera of reptiles in Vietnam, with a total of 49 species recorded from the country and most of them are endemic (Nguyen et al. 2009; Uetz et al. 2022). Besides extensive efforts of new species discoveries, taxonomic revisions of many cryptic species or complex taxa have been clarified on the basis of morphological and molecular data.

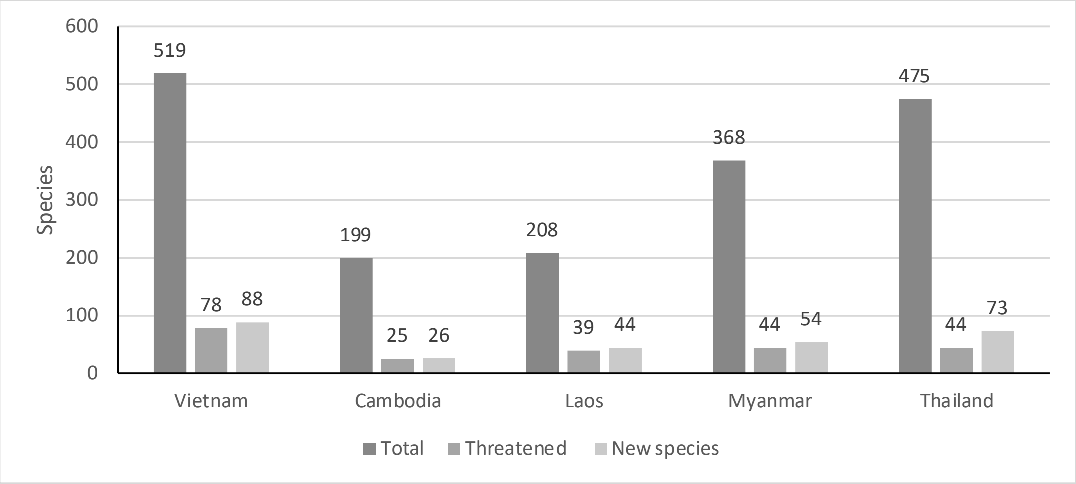

Figure 3. The reptile diversity of Vietnam in comparison with other countries in Southeast Asia, with the number of globally threatened species and newly described species in the last decade (IUCN 2021 and Uetz et al. 2022).

In terms of conservation concerns, a total of 78 species of reptiles (15%; Fig. 3) have been assessed as globally threatened in the IUCN Red List (CR – 21 species, EN – 22 species, and VU – 35 species), whereof 31 species (40%) are endemic to Vietnam. The number of threatened reptiles is relatively lower in other adjacent countries, such as Thailand and Myanmar (44 species), Laos (39 species) and Cambodia (25 species) (Fig. 3; CEPF 2020; IUCN 2021). In this context, anthropogenic stressors are undoubtedly known as principal threats that imperil the biodiversity in Vietnam, including habitat loss and forest land conversion (for agriculture, grazing land, mining, timber logging and infrastructure development), illegal wildlife trade, pollution, invasive species and climate change (Queiroz et al. 2013; CEPF 2020).

More than 13 million ha. of Vietnam's territory is covered by forests including naturally regenerated (74%) and planted forests (35%), whereas primary forests account for only 1%. However, most of Vietnam's biodiversity (85 %) is harbored in the small area of mature forests (Queiroz et al. 2013). Thus, a small loss of the forest has a significant impact on local biodiversity in general. In addition, Vietnam is one of the earth's five countries, which are most vulnerable to climate change with increasing frequency and severity of typhoons, changing seasonal rainfall and temperature regimes or sea-level rise (US Forest Service 2011). The impacts of climate change are more widespread and worse once forests are gradually being destroyed and replaced with suboptimal conditions.

Vietnam is globally known as a "hub" destination supplying wildlife products to domestic and international markets for the pet trade, food consumption and traditional medicine (Mott 2006; WWF 2012; Janssen & Indenbaum 2019). For instance, the globally vulnerable lizard, *Physignathus cocincinus,* has been used for food consumption and pet trade in China, Laos and Vietnam. Over the last 20 years, more than 1.4 million – mainly wild originating from Vietnam – *P. cocincinus* individuals were imported for the main purpose of pet trade into the

EU and US (Gewiss et al. 2020). Janssen & Indenbaum (2019) raised concerns that endemic reptiles from Vietnam may be especially vulnerable to overexploitation because of restricted ranges and small population sizes. *Cnemaspis psychedelica*, a new gecko species described from southern Vietnam in 2010, was observed in European fairs and on online markets with very high prices of up to 3,000 EUR/pair (Auliya et al. 2016; Ngo et al. 2016a). To safeguard its wild populations, the species was recently listed in CITES appendix I (Ngo et al. 2016a; 2018).

Under anthropogenic impacts, many threatened and endemic reptile species in Vietnam have been not protected by conservation measures yet due to the lack of comprehensive knowledge on species distribution, ecological traits and population status. Such information is crucial to evaluate the adaptive tolerance and resistance of species to cope with human impacts. Thus, basic research on biological traits and threat evaluations are urgently required to propose conservation schemes and kick off practical activities to safeguard wild populations.

1.3. Tiger geckos (Goniurosaurus): still poorly understood but already at risk.

Due to morphological and genetic distinctiveness to other eublepharid genera, Borner (1981) erected *Goniurosaurus* as a distinct genus in the subfamily Eublepharinae of the family Gekkonidae, comprising two species *G. kuroiwae* (Namiye, 1912) and *G. lichtenfelderi* (Mocquard, 1897) at that time. Grismer (1988) confirmed the monophyly of eublepharid geckos that have been introduced for the family Eublepharidae so far. *Goniurosaurus* (i.e. Tiger geckos) currently consists of 24 species, which are associated with evergreen forests on granitic or karst formations in Southeast and East Asia. The transboundary region between China and Vietnam (together with isolated islands) is considered a "center" of *Goniurosaurus* species richness, where 13 species (over 50%) have been discovered since 2008 (Uetz et al. 2022). Recent studies showed that all members of *Goniurosaurus* were split into four monophyletic groups, namely the *G. kuroiwae* group containing six species only from the Ryukyu Archipelago, Japan; the *G. lichtenfelderi* group with five species from both mainland and islands in China and Vietnam; the *G. luii* group comprising nine species distributed throughout islands and mainland in Vietnam and China; and the *G. yingdeensis* group consisting of four species from China (Nguyen et al. 2009; Nguyen 2011; Honda & Ota 2017; Liang et al. 2018; Qi et al. 2020a, 2020b; Zhu et al. 2020a, 2020b, 2021). In Vietnam, five species of *Goniurosaurus* have been recorded so far, viz. *G. araneus* Grismer, Viet & Boyle, 1999, *G. catbaensis* Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008, *G. huuliensis* Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008, *G. lichtenfelderi* (Mocquard, 1897) and *G. luii* Grismer, Viet & Boyle, 1999 (Grismer et al. 1999; Vu et al. 2006; Orlov et al. 2008; Ziegler et al. 2008; Nguyen et al. 2009; Nguyen 2011).

This genus is characterized by a high level of local endemism and most tiger gecko species have been only recorded from a single locality or an isolated island. Although most of their distributions are adjacent to each other, none of them have been recorded to be sympatric yet (Fig. 4; Orlov et al. 2008; Yang & Chan 2015; Honda & Ota 2017; Zhu et al. 2020a, 2020b, 2021; Ngo pers. obs). Among Vietnamese tiger geckos, for example, found in Cao Bang Province, *G. araneus* and *G. luii* have been never observed in the same habitat (Grismer et al. 1999; Vu et al. 2006; Ngo et al. 2016b). The habitats of *G. luii* extend to the north of Lang Son Province (Ngo et al. 2016b), while in the south of Lang Son Province it is replaced by *G. huuliensis* (Orlov et al. 2008). According to Orlov et al. (2008) the type locality of *G. lichtenfelderi* is on an offshore island in Bai Tu Long Archipelago, which is contiguous with Ha Long Bay in the Gulf of Tonkin. However, previous extensive field surveys failed to record any individuals of *G. catbaensis* – a karstic dweller, in the same habitat with *G. lichtenfelderi* – a non-karstic specialist (Orlov et al. 2008; Nguyen et al. 2011; Gawor et al. 2016).

Regarding the natural history, with an exception of three tiger geckos of the *G. lichtenfelderi* group (namely *G. bawanglingensis, G. hainanensis and G. lichtenfelderi*) in granitic habitats, all remaining species are found in karst formations. All tiger geckos are exclusively nocturnal species and only active after sunset. They usually hide in crevices of limestone rocks or caves or granitic-stream rocks during the day. At night, they climb on exposed surfaces or rocks under bushes, or on walls inside or outside of caves (Grismer et al. 1999; Orlov et al. 2008; Ziegler et al. 2008; Nguyen et al. 2009; Nguyen 2011; Zhou et al. 2018; Zhu et al. 2020a, 2020b, 2021).

Since the 1990s, tiger geckos have become very popular in the pet market due to their beautiful color patterns and high commercial revenues (Grismer et al. 1999; Stuart et al. 2006; Ngo et al. 2016b). For example, wild-caught animals of *G. luii* were recorded to fetch very high prices (e.g., US$2000 / individual). In addition, some tiger geckos including *G. araneus*, *G. luii* (Grismer et al. 1999), *G. catbaensis* (Bauer 2009) and *G. lichtenfelderi* (Liu 1993) were collected for the traditional medicine. Consequently, the unsustainable exploitation of *G. luii* and *G. araneus* – for example, up to 10,000 individuals during summer in 1996 – shortly after their formal description in 1999, led to the local extirpation at their type localities (Grismer et al. 1999; Yang & Chan 2015, Lindenmayer & Scheele 2017).

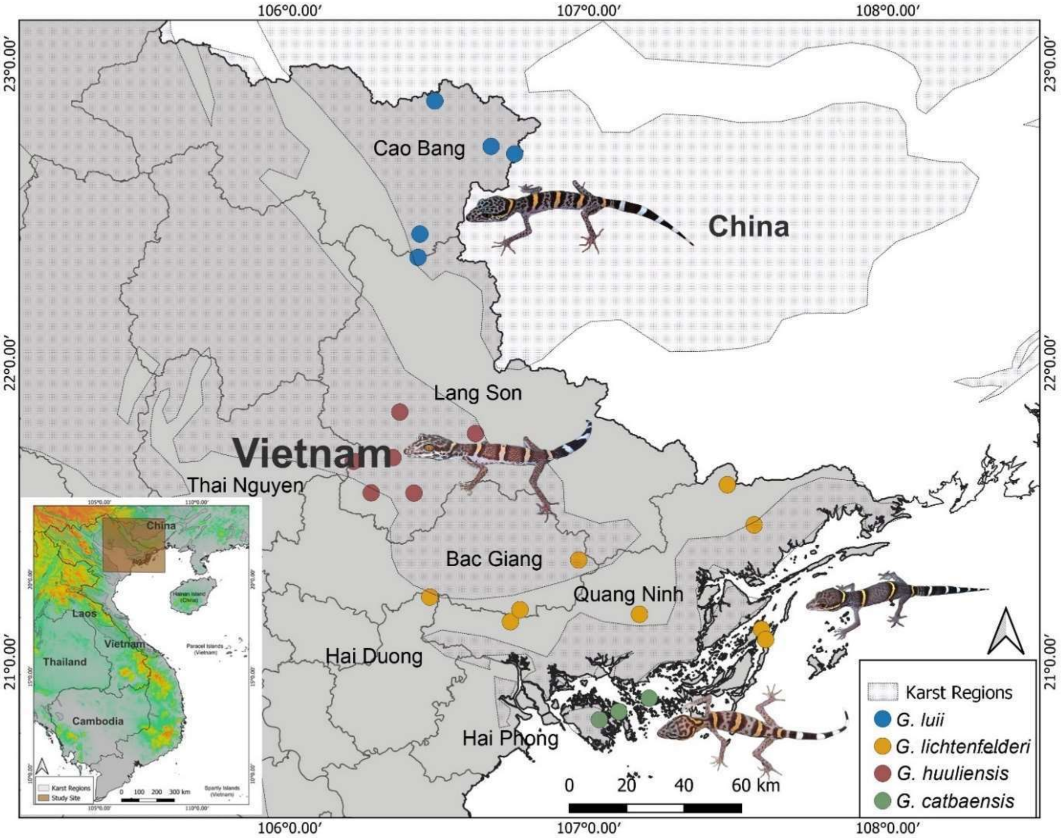

Figure 4. Study sites of *Goniurosaurus* species in Vietnam.

Under human impacts, all *Goniurosaurus* species are at a high risk of extinction. Once the thesis at hand started, no member of the genus *Goniurosaurus* was included in any wildlife protection laws yet, neither in China nor in Vietnam. Resembling 96% of the worldwide known species, knowledge gaps on *Goniurosaurus* distribution, population status, ecology and threats delay conservation efforts and limit the efficacy once the measures were implemented (Monastersky 2014). Given existing conservation assessments, only research on the Cat Ba Tiger Gecko (*G. catbaensis*) – an endemic species in Vietnam, showed that its population status is exceedingly smaller than the threshold value for minimal viable populations (Ngo et al. 2016b). The Cat Ba Tiger Gecko was recorded on European pet markets, such as the reptile fair in Hamm, Germany, as well as was offered on internet platforms (Ngo et al. 2016b). Besides the illegal collection of wild animals in the Cat Ba National Park, human impacts on their habitat have dramatically increased by expanding touristic activities (Ngo et al. 2016b).

II. OBJECTIVES

This study aimed to compare extended morphology data among Vietnamese tiger gecko species and with those from China and Japan, in order to identify paramount characteristics for an identification key. Our team further assessed the phylogenetic relationships across all recorded populations/ species of *Goniurosaurus* from Vietnam along with other species from China and Japan. Based on the phylogeny together with abiotic factors (e.g. habitat preference, altitudinal gradient, climate conditions), we aimed to reconstruct relevant evolutionary models that shaped the extraordinary richness in allopatry with a high level of endemism in *Goniurosaurus*.

Understandings of ecology and demography are highly necessary for species conservation efforts. The present study ergo intended to identify microhabitat characteristics of Vietnamese tiger gecko species and assess intraspecific and interspecific differences in their habitat use. Furthermore, this study had the goal to provide a detailed assessment of the current demographic status of Vietnamese tiger gecko species by calculations of the population size and density.

Although tiger geckos have been commonly recorded in the international pet market for a long time, the scale of the international demand for tiger gecko species, as well as the local trade level and shipping processes were unknown. This study herein implemented an attempt to outline domestic and international trade activities of *Goniurosaurus* species in order to inform legislation makers and develop conservation strategies. Furthermore, extensive surveys were conducted to document other threats causing the forest fragmentation and loss in Vietnam, and to assess their impacts on *Goniurosaurus* species. Besides direct anthropogenic impacts, *Goniurosaurus* species were assumed to be particularly susceptible to climate change. By using species distribution models (SDMs) to train environmental data (e.g. climate and vegetation), the present study aimed to predict the potential distribution of Vietnamese tiger gecko species and thereby record unknown populations at forecasted sites. We also used prediction models to assess future impacts of climate change on their suitable habitats under different scenarios. In an effort to protect wild populations of tiger gecko species and their habitats, the biological understandings and threat assessments are the background in order to propose and enhance the effectiveness of conservation measures.

In summary, the main objectives of this study are:

(1)*Systematics, evolution and zoogeography*:
- To review the taxonomy of *Goniurosaurus*;
- To identify origin and niche evolution patterns.

(2)*Autecology and population status*:
- To provide micro-habitat characteristics of *Goniurosaurus* species in Vietnam;
- To assess the population status of *Goniurosaurus* species in Vietnam.

(3)*Threats and implications for conservation*:
- To evaluate human impacts on *Goniurosaurus* species in Vietnam;

- To provide recommendations for conservation measures.

Based on stated objectives, related hypotheses were formulated:

(1) Extant Vietnamese populations are only assigned to one of the recorded *Goniurosaurus* species.
(2) Karst habitats support the most probable ancestral condition for *Goniurosaurus* species.
(3) The speciation process of *Goniurosaurus* followed the evolution pattern of climatic niche divergence intermixed with conservatism.
(4) *Goniurosaurus* species thrive in particular micro-habitats as specialists.
(5) Population densities and sizes of *Goniurosaurus* species are estimated exceedingly small, declining and prone to extinction under human impacts.
(6) All *Goniurosaurus* species in Vietnam are strongly imperiled due to over-exploitation for pet trade.
(7) Suitable habitats of *Goniurosaurus* species will shrink and even vanish under future impacts of climate change.

III. RESULTS

Chapter 1. Systematics, evolution and zoogeography

1.1. A morphological and molecular review of the genus *Goniurosaurus*, including an identification key.

Based on the results of our fieldwork in northern Vietnam and data compiled from literature, we herein provided a taxonomic review of the genus *Goniurosaurus*. Our phylogenetic analyses showed that all recorded populations of tiger geckos from Vietnam, which were found to be monophyletic with low intra-specific genetic divergences, were assigned to one of the four species: *G. catbaensis* (0.7–1.6 %), *G. huuliensis* (0.4– 1.2 %), *G. lichtenfelderi* (0–0.7 %) and *G. luii* (0–1.9 %). The members of *Goniurosaurus* from China and Vietnam were split into three major groups, viz. *G. lichtenfelderi, G. luii* and *G. yingdeensis* groups. Based on the newly collected data, we provided extended morphological descriptions for five *Goniurosaurus* species in Vietnam. In addition, an identification key for all *Goniurosaurus* species from China, Japan and Vietnam was provided.

1.2. Phylogeny and evolution of habitat preference in *Goniurosaurus* (Squamata: Eublepharidae) and their correlation with karst and granite-stream-adapted ecomorphologies in species groups from Vietnam.

Using two mitochondrial (16S and Cytb) and two nuclear (CMOS and RAG1) genes, we recovered the first phylogeny of all 23 extant *Goniurosaurus* species. The analyses strongly supported the recognition of four monophyletic species groups with identical inter-specific relationships within the *G. kuroiwae*, *G. lichtenfelderi*, and *G. yingdeensis* groups but discordant topologies at some nodes within the *G. luii* group. A stochastic character mapping analysis of karst versus non-karst habitats suggested that the karst habitat preference was identified as the most probable ancestral condition for *Goniurosaurus* (57 % probability), the *G. kuroiwae* group (62.7 %), the *G. luii* group (90 %), the *G. yingdeensis* group (95.7 %). Whilst the probable ancestral condition for the *G. lichtenfelderi* group was predicted as the non-karst habitat (55.4%). Ecomorphological analyses further demonstrated an adaptive response of morphology (e.g. longer head and snout-vent lengths, larger eyes, longer trunk and limbs) to the karst habitat in *Goniurosaurus* species.

1.3. Extraordinary species richness of *Goniurosaurus* genus in allopatry: Understanding niche evolution and the need of conservation measures.

Using phylogenetic analyses, we calibrated the divergence date of *Goniurosaurus* origin during the Eocene (approximately ~45.3 mya). The diversification within four monophyletic species groups began the mid-Miocene between ~13.4–7.7 Mya and continued to at least the mid-Pliocene (~2 mya). *Goniurosaurus* ancestor was predicted to originate somewhere in the contiguous continental Eastern Asia, including Ryukyu Archipelago and Hainan Island before the Eocene. On the other hand, the current regions in which each monophyletic *Goniurosaurus* group radiated are respectively their ancestral regions, such as *G. kuroiwae* group from Ryukyu Archipelago, Japan, *G. lichtenfelderi* group from Hainan Island, China, *G. luii* group from the transboundary region between China and Vietnam, and *G. yingdeensis* from southern China.

Low elevations were predicted to be the most probable ancestral state for *Goniurosaurus* and all four species groups as well. We further reconstructed the history of niche occupancy and calculated the rate of climatic niche evolution among *Goniurosaurus* species from China and Vietnam. Our results provided signals of both climatic niche conservatism and divergence among *Goniurosaurus* species. In particular, only *G. lichtenfelderi* of *G. lichtenfelderi* group, and *G. catbaensis, G. kwangsiensis* and *G. liboensis* of *G. luii* group followed the evolutionary pattern of climatic niche divergence, whereas the remaining species followed the conservatism pattern.

Chapter 2. Autecology and population status

2.1. First record of the Cat Ba Tiger Gecko, *Goniurosaurus catbaensis*, from Ha Long Bay, Quang Ninh Province, Vietnam: microhabitat selection, potential distribution, and evidence of threats.

2.2. First ecological assessment of the endangered Lichtenfelder's Tiger Gecko (*Goniurosaurus lichtenfelderi*) from northern Vietnam: Micro-habitat and macro-climatic niche comparisons between island and mainland populations.

2.3. Ecological niche overlap of two allopatric karst-adapted tiger geckos (*Goniurosaurus*) from northern Vietnam: Microhabitat use and implications for conservation.

As a result of our field surveys, the micro-habitat use of four recorded *Goniurosaurus* species in Vietnam were assessed. Three karst-adapted species (e.g. *G. catbaensis, G. huuliensis* and *G. luii*) were recorded in the forest on karst formations, covered with evergreen broad-leaved woody trees, intermixed with ferns, shrubs and vines. Their microhabitats were relatively similar in other typical traits, such as high vegetation coverage, high humidity, stable ambient temperature and dry-rock substrates. Whilst a granitic stream-adapted species, *G. lichtenfelderi*, was only found in evergreen forests with a high percentage of vegetation coverage and in close proximity to small stream sections with rocky shelters on granitic formations. We documented the similarity in ecological characteristics among intra-specific populations (e.g. island and mainland of *G. lichtenfelderi*) and among species (e.g. *G. huuliensis* and *G. luii*).

2.4. Living under the risk of extinction: Population status and conservation needs assessment of a micro-endemic tiger gecko in Vietnam.

A "capture mark – recapture" method was applied to estimate the population size of *G. huuliensis* and calculate the population density as well. Consequently, the total population size of *G. huuliensis* was estimated to comprise a maximum of 1,447 individuals in integrated suitable habitats (e.g. climate, vegetation cover, elevation) and achieve a maximum up to 2,855 individuals in only karst habitats within the whole extent of occurrence. However, this is exceedingly smaller than the threshold for a minimum viable population. Furthermore, its populations were also documented to occur in extremely small densities on average of 0.64 ind./ 100 m. Due to vulnerable population status, ongoing severe human impacts (e.g. wildlife exploitation and limestone quarrying) can force *G. huuliensis* to the brink of extinction.

Chapter 3. Threats, modeling refugia and implications for conservation

3.1. A case study on trade in threatened tiger geckos (*Goniurosaurus*) in Vietnam including updated information on the abundance of the endangered *G. catbaensis.*

This study provided an overview of international trade in *Goniurosaurus* based on available data from 1999 to 2018 in the U.S. as well as data from online surveys and interviews in Europe and Vietnam. A total of 16,714 *Goniurosaurus* specimens have been imported into the U.S. (mean of 835 ± 1082 individuals annually) between 1999 and 2018, whereof 68.9 % were wild-caught. Most imported specimens were traded for commercial purposes with sales fetching prices between US$35–200. All five tiger gecko species known from Vietnam were found in the national and international markets (in the U.S. and Europe). We found that entire trade chains of Vietnamese tiger geckos are very long (including several transfers and dealers involved) and that keeping and transport happen under poor conditions. Based on our findings, *Goniurosaurus* species are at high risk of extinction due to unsustainable trade. In this context, Vietnamese *Goniurosaurus* species were assessed in national and international laws (Group IIB of Vietnamese Governmental Decree No. 06/2019/ND-CP as well as the CITES Appendix II, respectively).

We further calculated the population size of *G. catbaensis* on newly discovered islands in Ha Long Bay. Its populations were found to be relatively stable, consisting of about 124 and 129 individuals in July 2017 and April 2018. Compared to disturbed areas (such as type locality), the abundance of *G. catbaensis* yet was found to be exceedingly low. Its densities in Ha Long Bay were on average 6 ind./ 100 m, but the mean density was much lower than that of other threatened reptile species.

3.2. Modeling the environmental refugia of the endangered Lichtenfelder's Tiger Gecko (*Goniurosaurus lichtenfelderi*) towards implementation of transboundary conservation.

3.3. Vulnerability of an endemic Tiger Gecko (*Goniurosaurus huuliensis*) to climate change: modeling environmental refugia and implications for *in-situ* conservation.

Species distribution modeling techniques were employed, trained with climate and vegetation cover conditions, to identify the contemporary potential distribution of two *Goniurosaurus* species in Vietnam (*G. huuliensis* and *G. lichtenfelderi*) and assess their alterations under different climate change scenarios. Based on the prediction, we discovered some new populations of *G. lichtenfelderi* at forecasted sites (e.g. border areas in Quang Ninh Province, Vietnam). Our predictions suggested that the potential distribution of two species mostly cover their respectively known sites of occurrence and surroundings. These areas will narrow significantly and/or shift towards higher latitudes under future scenarios of climate change.

Suitable habitats of the two species were even predicted to be entirely replaced by novel climatic conditions under the most severe scenarios by the 1970s. We further provided forecasted maps with identified core refugia for priorities of conservation measures to mitigate synergistic impacts from climate change and other human impacts. In this context, the border areas between China and Vietnam, Yen Tu Mountain Range, Bai Tu Long National Park, and their surroundings should be considered core refugia for *G. lichtenfelderi,* and evergreen forests on karst formations in Lang Son Province for *G. huuliensis.*

Chapter 1. Systematics, evolution and zoogeography

1.1. A morphological and molecular review of the genus *Goniurosaurus*, including an identification key

The author of the thesis, Prof. Dr. Thomas Ziegler and Prof. Dr. Truong Quang Nguyen mainly contributed to the planning of the study. Field surveys and data collection, morphological analyses, preparation of graphics as well as the writing of the paper were majorly carried out by the author of the thesis. Molecular analyses were implemented by Prof. Dr. Minh Duc Le and Hanh Ngo.

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A morphological and molecular review of the genus *Goniurosaurus*, including an identification key

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[8]urn:lsid:zoobank.org:author:822872A6-1C40-461F-AA0B-6A20EE06ADBA
[9]urn:lsid:zoobank.org:author:5716DB92-5FF8-4776-ACC5-BF6FA8C2E1BB

Abstract. The genus *Goniurosaurus* (tiger geckos) currently consists of 23 species distributed in China, Japan and Vietnam. Several species complexes and recent discoveries of cryptic species pose challenges to the species identification, which is crucial to effectively implement the recent listing of the species from China and Vietnam in CITES Appendix II and the species from Japan in CITES Appendix III. Based on the results of our field work in northern Vietnam and data compiled from literature, we herein provide a taxonomic review of the genus *Goniurosaurus*. Our phylogenetic analyses showed that all recorded populations of tiger geckos from Vietnam, which were found to be monophyletic with low intra-specific genetic divergences, are assigned to one of the four species: *G. catbaensis*, *G. huuliensis*, *G. lichtenfelderi* or *G. luii*. Both genetic and morphological analyses confirm that the species from China and Vietnam can be split into three major groups. Based on the newly collected data, we provide an extended morphological description of the Vietnamese species. In addition, we provide an identification key for all *Goniurosaurus* species from China, Japan and Vietnam in order to assist authorities in the enforcement of the recent CITES listing.

Keywords. CITES enforcement, morphology, molecular phylogeny, taxonomy, tiger geckos.

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Introduction

The eublepharid genus *Goniurosaurus* Grismer, Viets & Boyle, 1999 (tiger geckos) currently consists of 23 species associated with granitic or karst formations in Southeast and East Asia. This genus shows a high level of local endemism and most tiger gecko species have been only recorded from a single locality, within unique ecosystems or an isolated archipelago (Orlov *et al.* 2008; Ziegler *et al.* 2008; Yang & Chan 2015; Honda & Ota 2017; Zhou *et al.* 2018; Ngo *et al.* 2019a; Qi *et al.* 2020a, 2020b; Zhu *et al.* 2020a, 2020b). The complex topography and variable climatic conditions throughout the distribution range of the genus may account for the disjunct distribution and occupation of different ecological niches by distinct populations. These factors may have driven natural selection, morphological diversification and phylogenetic evolution within this genus (Vitt *et al.* 1997; Clements *et al*. 2006; Sexton *et al.* 2009; Gomes *et al.* 2016; Liang *et al.* 2018; Qi *et al.* 2020a, 2020b). According to recent morphological and molecular analyses, the genus *Goniurosaurus* is split into four species groups, namely the *G. lichtenfelderi* group with five species from both mainland and islands in China and Vietnam; the *G. kuroiwae* group containing six species from the Ryukyu Archipelago, Japan; the *G. luii* group comprising eight species distributed throughout islands and mainland in Vietnam and China; and the *G. yingdeensis* group consisting of four species from China (Nguyen *et al.* 2009; Nguyen 2011; Wang *et al.* 2013; Honda & Ota 2017; Liang *et al.* 2018; Qi *et al.* 2020a, 2020b; Zhu *et al.* 2020a, 2020b). However, the systematics of the genus *Goniurosaurus* remains challenging due to ongoing discoveries of further cryptic species and the fact that there is not a single genetic marker that covers all 23 species, precluding a complete generic phylogeny of the group. Five subspecies of the *G. kuroiwae* group from Japan were reinstated at full species status and a new species, *G. sengokui* (Honda & Ota, 2017), was recently discovered from the Ryukyu Archipelago (Honda & Ota 2017). In China, two taxa, previously identified as *G. luii* Grismer, Viets & Boyle, 1999, were described as distinct species, namely *G. kadoorieorum* Yang & Chan, 2015 and *G. kwangsiensis* Yang & Chan, 2015 (Yang & Chan 2015), and a sister species of *G. araneus* Grismer, Viets & Boyle, 1999, namely *G. gezhi* Zhu, He & Li, 2020, was recently discovered (Zhu *et al.* 2020a). These three species occur in Southwest Guangxi Province (Zhu *et al.* 2020a). Four additional species, namely *G. kwanghua* Zhu & He, 2020 and *G. zhoui* Zhou, Wang, Chen & Liang, 2018, belonging to the *G. lichtenfelderi* group, as well as *G. gollum* Qi, Wang, Grismer, Chen, Lyu & Wang, 2020 and *G. varius* Qi, Grismer, Lyu, Zhang, Li & Wang, 2020 of the *G. yingdeensis* group, were recently described from China (Zhou *et al.* 2018; Qi *et al.* 2020a, 2020b; Zhu *et al.* 2020b).

To date, five species of *Goniurosaurus* are reported from Vietnam, viz. *G. araneus*, *G. catbaensis* Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008, *G. huuliensis* Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008, *G. lichtenfelderi* (Mocquard, 1897) and *G. luii* (Grismer *et al.* 1999; Vu *et al.* 2006; Orlov *et al.* 2008; Ziegler *et al.* 2008; Nguyen *et al.* 2009; Nguyen 2011). All species were described based only on a few specimens and a small set of diagnostic characters. As such, phenotypic variability among these species may lead to the misidentification of taxa. For example, Orlov & Darevsky (1999) described a new species (*G. murphyi*) based on a juvenile specimen, which was subsequently synonymized with *G. lichtenfelderi* by Grismer (2000). Ngo *et al.* (2016) documented another case with indistinct morphological differences between the newly described *G. kadoorieorum* and *G. luii*.

Due to habitat degradation and over-harvesting for the pet trade, wild populations of species of *Goniurosaurus* from China and Vietnam have been subject to severe declines (Stuart *et al.* 2006; Yang & Chan 2015; Ngo *et al.* 2019b). Therefore, all Chinese and Vietnamese tiger gecko species have recently been included in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix II (CITES 2019; Ngo *et al.* 2019b). To effectively enforce relevant national and international regulations and to enable sustainable trade in CITES listed species, a detailed guideline for species identification is an essential prerequisite, especially as trade in the morphologically similar species from Japan is currently regulated under a lower protection level, namely CITES Appendix III (CITES Notification No. 2020/068). A proper identification guidance will help to prevent certain taxa from being traded under a wrong name to circumvent trade restrictions.

In this study, phylogenetic analyses were performed across all recorded populations of *Goniurosaurus* from Vietnam and along with other species from China and Japan using a fragment of the mitochondrial Cytochrome b gene. Based on newly collected data from Vietnam, we expanded morphological descriptions of each species and evaluated intra- and inter-specific morphological variations among the species of *Goniurosaurus*. We further compared morphological data of Vietnamese tiger geckos with those from China and identified characters that can distinguish the Japanese species. We thereby provide an identification key for all *Goniurosaurus* species from China, Japan and Vietnam, in order to assist CITES enforcement authorities in species identification.

Material and methods

Field surveys

Field surveys were conducted in June and August 2014, May 2015, July 2016, June and July 2017, April 2018, from April to September 2019, as well as in June and July 2020 in Vietnam. Surveys were conducted for *G. catbaensis* in Hai Phong City and Quang Ninh Province, for *G. huuliensis* in Lang Son Province, for *G. lichtenfelderi* in Hai Duong, Bac Giang and Quang Ninh provinces, and for *G. luii* in Cao Bang and Lang Son provinces. Animals were captured by hand and subsequently released at the same sites after taking measurements and photos in life. Coordinates of each captured individual were recorded using a GPS device (Garmin GPSmap64, WGS84 datum) and can be shared upon request to the authors.

In addition, several vouchered specimens and small tissue samples of *Goniurosaurus* deposited in collections of the Institute of Ecology and Biological Resources (IEBR), Vietnam National Museum of Nature (VNMN) and Hanoi University of Science (HUS), Hanoi, Vietnam, were examined for morphological characters and molecular phylogeny.

Molecular data and phylogenetic analyses

All taxa of the genus *Goniurosaurus* from China, Japan, and Vietnam, for which Cytochrome b data were available in GenBank, were included in the study. Three species, *Gekko chinensis* (Gray, 1842) (NC027191), *Coleonyx mitratus* Peters, 1863 (AB853481) and *Holodactylus africanus* Boettger, 1893 (AB853482), were used as outgroups following Honda *et al.* (2014).

Small tissue samples were collected from the tail tip of 18 wild specimens from representative localities in Vietnam and preserved separately in 70% ethanol (Merck, Germany). The mitochondrial DNA Cytochrome b (cytb) gene was selected for DNA sequencing. The total genomic DNA was extracted using GeneJet Genomic DNA Purification (ThermoFisher Scientific, Lithuania), following protocols by the manufacturer's instructions. PCR reactions were performed using HotStar Taq Mastermix (Qiagen, Germany) to amplify a fragment of approximately 1000 bps of cytb. The primer pair used for this study was L14731 (5'–TGGTCTGAAAAACCATTGTTG–3'; Honda *et al.* 2014) and GoniR1 (5'–CTACGGGCTGTCCTCCGATTCAGGTT–3'; this study). The PCR volume consisted of 21 µl: 2 µl of each primer, 5 µl water, 10 µl of Taq mastermix and 1–2 µl DNA template depending on the quality of DNA in the final extraction solution. The PCR was performed at 95°C for 15 minutes, followed by 35 cycles, for 30 s at 95°C, 45 s at 48°C and 60 s at 72°C with a final elongation step for 6 minutes at 72°C. Negative and positive controls were used for all DNA extractions and PCR reactions.

PCR products were visualized using electrophoresis through a 1% agarose gel, marker 1kb, 1X TBE, stained with ethidium bromide and photographed under UV light. Successful amplifications were purified using GeneJet PCR Purification Kit (ThermoFisher Scientific, Lithuania). Cleaned PCR products were sent to 1st Base (Malaysia) for sequencing in both directions.

The obtained sequences were aligned in Sequencher ver. 5.4 (Gene Codes Corp, Ann Arbor, MI, USA) and afterwards aligned using ClustalX ver. 2.1 (Thompson *et al.* 1997) with default settings. Data were analyzed using maximum parsimony (MP) as implemented in PAUP*4.0b10 (Swofford 2001) and Bayesian inference (BI) as implemented in MrBayes ver. 3.2 (Ronquist *et al.* 2012). Settings for these analyses followed Le *et al.* (2006), except that the number of generations in the Bayesian analysis was increased to 1×10^7. For the maximum likelihood (ML) analysis, we used IQ-TREE ver. 1.6.7.1 (Nguyen *et al.* 2015) with a single model and 10 000 ultrafast bootstrap replications. The optimal model for nucleotide evolution was set to TPM1uf+I+G as selected by Jmodeltest ver. 2.1.4 (Posada *et al.* 1998). As the phylogenetic relationships between species and species groups were well resolved and virtually every important node received high statistical support from all analyses, we opted not to partition our data by codon positions.

For the Bayesian analysis, we used the optimal model determined by Jmodeltest with parameters estimated by MrBayes ver. 3.2.7. 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 50 in the BI analysis, as -lnL scores reached stationarity after 50 000 generations in both runs. Nodal support was evaluated using Bootstrap replication (BP) as estimated in PAUP*4.0b10 and ultrafast BP in IQ-TREE ver. 1.6.7.1 and posterior probability (PP) in MrBayes ver. 3.2. BP ≥ 70 (Hillis & Bull 1993) and ultrafast BP (UBP) and PP ≥ 95% are regarded as strong support for a clade (Ronquist *et al.* 2012; Nguyen *et al.* 2015). The uncorrected pairwise distance (p) were calculated in PAUP*4.0b10.

Morphological analyses

A total of 486 live individuals and 54 museum specimens of four species from Vietnam were examined for morphological data, comprising 194 individuals of *G. catbaensis* (21 juveniles, 93 females and 80 males), 80 individuals of *G. huuliensis* (02 juveniles, 46 females and 32 males), 178 individuals of

G. lichtenfelderi (14 juveniles, 72 females and 92 males), and 88 individuals of *G. luii* (11 juveniles, 43 females and 34 males). Detailed descriptions of each species were based on newly collected data combined with previous literature for the morphological variation, such as for *G. catbaensis* from Ziegler *et al.* (2008) and Nguyen (2011), for *G. huuliensis* from Orlov *et al.* (2008) and Nguyen (2011), for *G. lichtenfelderi* from Grismer (2000), Grismer *et al.* (2002) and Nguyen (2011), and for *G. luii* from Grismer *et al.* (1999), Vu *et al.* (2006) and Nguyen (2011). No specimen of *G. araneus* was investigated in this study, however, we included morphological data of eight specimens from the type locality in Cao Bang Province, Vietnam and Guangxi Province, China from the literature (Grismer *et al.* 1999; Chen *et al.* 2014). The sex of each collected specimen was determined based on the presence (in males) or absence (in females) of large swollen hemipenial bulges. Lizards were categorized into two age classes based on the snout-vent length (*G. lichtenfelderi*: SVL < 80 mm = juveniles, SVL ≥ 80 mm = adults, while three other species of *Goniurosaurus* were sorted with SVL < 85 mm = juveniles, SVL ≥ 85 mm = adults).

Measurements were taken with dial calipers to the nearest 0.1 mm at the right side of each individual.

Abbreviations

AD = diameter of auditory meatus
AG = axilla to groin length, from posterior edge of forelimb insertion to anterior edge of hindlimb insertion
BH = maximum body height, from top of dorsal body to belly
BW = maximum body width, greatest width of torso, taken at level of midbody
CH = cheek height, from posterior edge of labial to top of head at parietal region
ED = diameter of eye, greatest diameter of orbit
EE = eye to ear distance, from posterior margin of eye to posterior margin of ear
FLL = forelimb length, from axilla to the tip of the fourth finger
HH = maximum head height
HL = head length, from the tip of snout to posterior edge of occiput
HLL = hindlimb length, from groin to the tip of the fourth toe
HW = maximum head width
IO1 = interorbital distance, distance between anteriormost points of eyes
IO2 = interorbital distance, distance between posteriormost points of eyes
ML = mouth length, from tip of snout to last posterior labial edge
MW = mouth width, distance between last posterior labial edges on each side
ND = supranasal distance, distance between nares
SE = snout to eye distance, measured from tip of snout to anteriormost point of eye
SVL = snout-vent length, from tip of snout to vent
TaL = tail length, from vent to tip of tail
WT = maximum tail width

Scale counts

CIL = eyelid fringe scales or ciliaria
DTR = dorsal tubercle rows at midbody
GP = gular scales bordering the postmentals
GST = granular scales surrounding dorsal tubercles
IFL = infralabials
IN = postrostrals or internasals
LD1 = subdigital lamellae under the first finger
LD4 = subdigital lamellae under the fourth finger
LT1 = subdigital lamellae under the first toe

LT4 = subdigital lamellae under the fourth toe
MB = scales around midbody
N = nasal scales surrounding nare
P-IN = post-internasals
PAT = postcloacal tubercles
PM = postmentals
PO = preorbital scales
PP = precloacal pores
SPL = supralabials
TL = paravertebral tubercles between limb insertions

Statistical analyses were performed by using the software environment R.3.1.2 (RStudio Team 2018). Shapiro-Wilk's test was used to test the assumption of normality. Kruskal-Wallis's test was performed to determine differences concerning the SVL of sex and age classes among species, as well as the extracted PC1 and PC2 values (mentioned below) of a principal component analysis among four tiger gecko species in Vietnam except of *G. araneus*. For both tests we applied $p = 0.05$. The SVL variable was excluded from subsequent analyses due to high collinearity with other dimensions. We further performed a Principal Component Analysis (PCA) of $\log_{10}$-transformed raw data of 18 remaining morphometric characters (except TaL and WT variables, due to many regenerated tails), using the packages "factoextra" and "FactoMinerR" to detect variances among the four selected Vietnamese species via contribution percentage of the PC1 and other PCs scores (Kassambara 2017). Morphometric variation of each species was illustrated by representatively clustered ellipse spaces with different coded-color within a spatial coordinate of the first two most important dimensions (PC1 and PC2) in the PCA analysis, to visually evaluate the overlap among the four species. In addition, we identified the overall difference in meristic characters among the 17 tiger gecko species native to China and Vietnam assigned to three groups (*G. lichtenfelderi*, *G. luii* and *G. yingdeensis*) by using a Multiple Correspondence Analysis (MCA). The meristic variation among the three groups was also visualized by convex ellipses within a spatial coordinate of the first two most important dimensions (Dim1 and Dim2) in the MCA analysis.

Results

Molecular phylogeny

The final matrix consisted of 949 bp aligned characters with 48 sequences of 14 ingroup and three outgroup taxa. The alignment contained no gaps. In total, 404 characters were found to be parsimony informative. The MP analysis produced 624 most parsimonious trees with 1404 steps (CI=0.54, RI = 0.85). Our phylogenetic analyses recovered a generally similar topology to those reported by Liang *et al.* (2018) and Zhu *et al.* (2020a, 2020b). Specifically, the *Goniurosaurus lichtenfelderi* group and the *G. luii* group form a monophyletic group with high nodal values from all analyses and the *G. kuroiwae* group is sister to the other two. In addition, each species group was also strongly corroborated as monophyletic from all analyses (Fig. 1). Within each species group, the results supported by this study are more similar to those generated by Liang *et al.* (2018), because Zhu *et al.* (2020a) used a different mitochondrial marker, the 16S ribosomal RNA gene. Nonetheless, we found several noted discrepancies between the two studies. Specifically, our phylogenetic analyses showed that *G. kuroiwa*e (Namiye, 1912) is polyphyletic with high nodal support for each of the clades. In addition, *G. luii* was strongly corroborated as monophyletic in our Bayesian and MP analyses (BP=93%; PP=98%), but only weakly recovered as monophyletic in Liang *et al.* (2018) (BP=67%; PP=64%). *Goniurosaurus lichtenfelderi* was recovered as monophyletic with perfect nodal support from all analyses in our study, but *G. hainanensis* Barbour, 1908 is polyphyletic. On the other hand, both species are not monophyletic in Liang *et al.* (2018). The positions of *G. zhoui* and *G. bawanglingensis* Grismer, Haitao, Orlov & Anajeva, 2002 are interchanged in the trees supported by the two studies with strong nodal support from Liang *et al.* (2018) and weak corroboration from ours. All surveyed sub-populations of *Goniurosaurus* from Vietnam were placed in four separate clades corresponding to four distinct species, *G. catbaensis*, *G. huuliensis*, *G. luii* and *G. lichtenfelderi* with significantly statistical support values, except for the ML analysis of *G. luii* (UBP = 88%). Intra-specific genetic divergences of these species were relatively low: *G. catbaensis* (0.7–1.6%), *G. huuliensis* (0.4–1.2%), *G. lichtenfelderi* (0–0.7%), and *G. luii* (0–1.9%) (Fig. 1; Supp. file 1: Table S1).

Taxonomic accounts

Class Squamata Oppel, 1811
Order Gekkota Cuvier, 1817
Family Eublepharidae Boulenger, 1883
Genus *Goniurosaurus* Grismer, Viets & Boyle, 1999

Goniurosaurus araneus Grismer, Viets & Boyle, 1999

Diagnosis

Body splayed and gracile, SVL 108.9–124.0 mm; external nares bordered by 6–8 nasal scales; supraorbital region with a row of enlarged tubercles; outer surface of upper eyelid composed of small granular

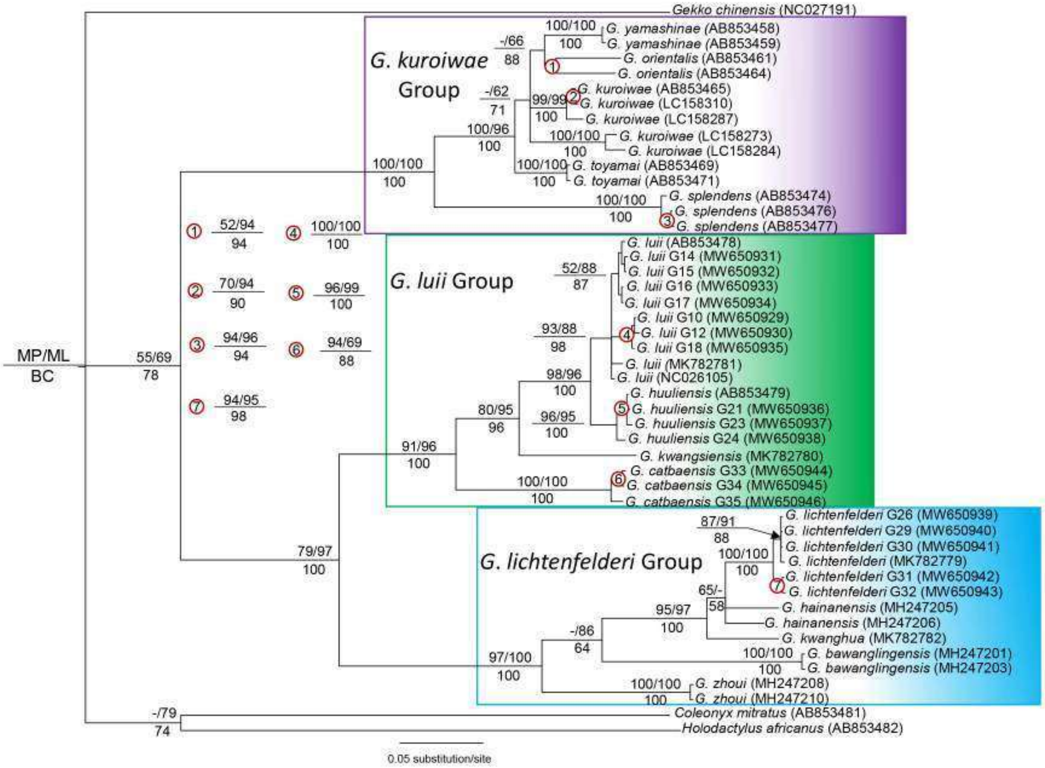

Fig. 1. Phylogram containing *Goniurosaurus* Grismer, Viets & Boyle, 1999 representatives from China, Japan and Vietnam based on the Bayesian analysis. Numbers above and below branches are MP/ML ultrafast bootstrap values and combined Bayesian posterior probabilities, respectively. Dash represents < 50% value.

scales, one-half the size of those on top of head; internasal single; supralabials 8–10; infralabials 8 or 9; preorbital scales 13–18; eyelid fringe scales 52–67; postmentals 4–6; dorsal body scales elongate; paravertebral tubercles 29–38; scales around midbody 129–147; scales surrounding dorsal tubercles 10–14; axillary pockets deep; subdigital lamellae under fourth toe 23–25; precloacal pores in males 18–23; iris dark brown; dorsal ground color of head, body and limbs immaculate dull yellow-gray; nuchal loop posteriorly protracted (in V-shape); dorsal body bands between limb insertions 3, wide, edged anteriorly and posteriorly by wide dark brown bands; light band on tail base in width of 15 or 16 granular scales; ground color of tail black, caudal bands 5, white, completed ventrally; ventral surface of head, body and limbs dull white and immaculate (Grismer *et al.* 1999; Nguyen 2011; Chen *et al.* 2014).

Remarks

During our field work in northern Vietnam, including the type locality in Cao Bang Province, no specimen of *Goniurosaurus araneus* was seen. Therefore, the diagnosis was solely based on the descriptions of Grismer *et al.* (1999) and Chen *et al.* (2014).

Goniurosaurus catbaensis Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008

Fig. 2

Diagnosis

Body splayed and gracile, SVL 89.3–125.3 mm; external nares bordered by 6–8 nasal scales; supraorbital region with a row of enlarged tubercles; outer surface of upper eyelid composed of granular scales, about the same size of those on top of head and with a row of 6–10 enlarged tubercles; internasals absent; supralabials 8–11; infralabials 7–10; eyelid fringe scales 45–56; postmentals 2–5; gular region below lower jaws without enlarged tubercles; paravertebral tubercles 31–38; scale rows around midbody 112–127, granular scales surrounding tubercles 8–11; axillary pockets deep; subdigital lamellae under fourth toe 22–25; precloacal pores in males 16–23; iris orange-brown; dorsal ground color of head, body and limbs gray-brown to pale brown and mottled with dark brown blotches; nuchal loop thin, posteriorly protracted (in V-shape); dorsal body bands between limb insertions 3–4, thin, yellow, without dark spots; light band on tail base in width of 8–9 scales; ground color of tail black, caudal bands 5, white, completed ventrally; ventral surface of head, body and limbs dull white and immaculate, gular region with brown spots (modified after Ziegler *et al.* 2008; Nguyen 2011).

Description (Supp. file 1: Table S2)

Body splayed and gracile, adult males: SVL 92.9–125.3 mm (mean ± SE: 112.3 ± 0.8 mm, n = 80), TaL 7.2–97.9 mm (75.2 ± 1.9 mm); adult females: SVL 89.3–122.1 mm (111.8 ± 0.8 mm, n = 93), TaL 17.5–98.3 mm (70.3 ± 1.7 mm); juveniles: SVL 53.4–78.8 mm (68.8 ± 1.8 mm, n = 21), TaL 11.8–61.2 mm (48.1 ± 2.6 mm) (Supp. file 1: Table S2); head triangular, wider than neck, covered by uniform granular scales interspersed with tubercles in temporal and occipital regions; scales on rostrum slightly larger and flatter; enlarged supraorbital tubercles in a conspicuous row; middorsal portion of rostral partially sutured dorsomedially, bordered laterally by first supralabial on each side, dorsolaterally by prenasal on each side, and dorsally by two supranasals; internasal (postrostral) scales absent; external nares bordered by 6–8 nasals: anteriorly by prenasal and supranasal, dorsally by supranasal, posteriorly by two slightly enlarged postnasals and 1 or 2 smaller granular scales, and ventrally by prenasal; prenasals with long recurved ventral portion; supranasals triangular, meeting in midline behind rostral suture; preorbital scales 10–13; supralabials 8–11; infralabials 7–10; eyes relatively large, pupils vertical; eyelid fringe scales 45–56, those of upper eyelid slightly enlarged; outer surface of upper eyelid composed of granular scales of about the same size of those on top of head, including a row of 6–10 enlarged tubercles; fold of skin originating from suborbital region extends posteroventrally across angle of jaw; external auditory meatus elliptical with long axis directed dorsoventrally; tympanum deeply recessed; mental triangular,

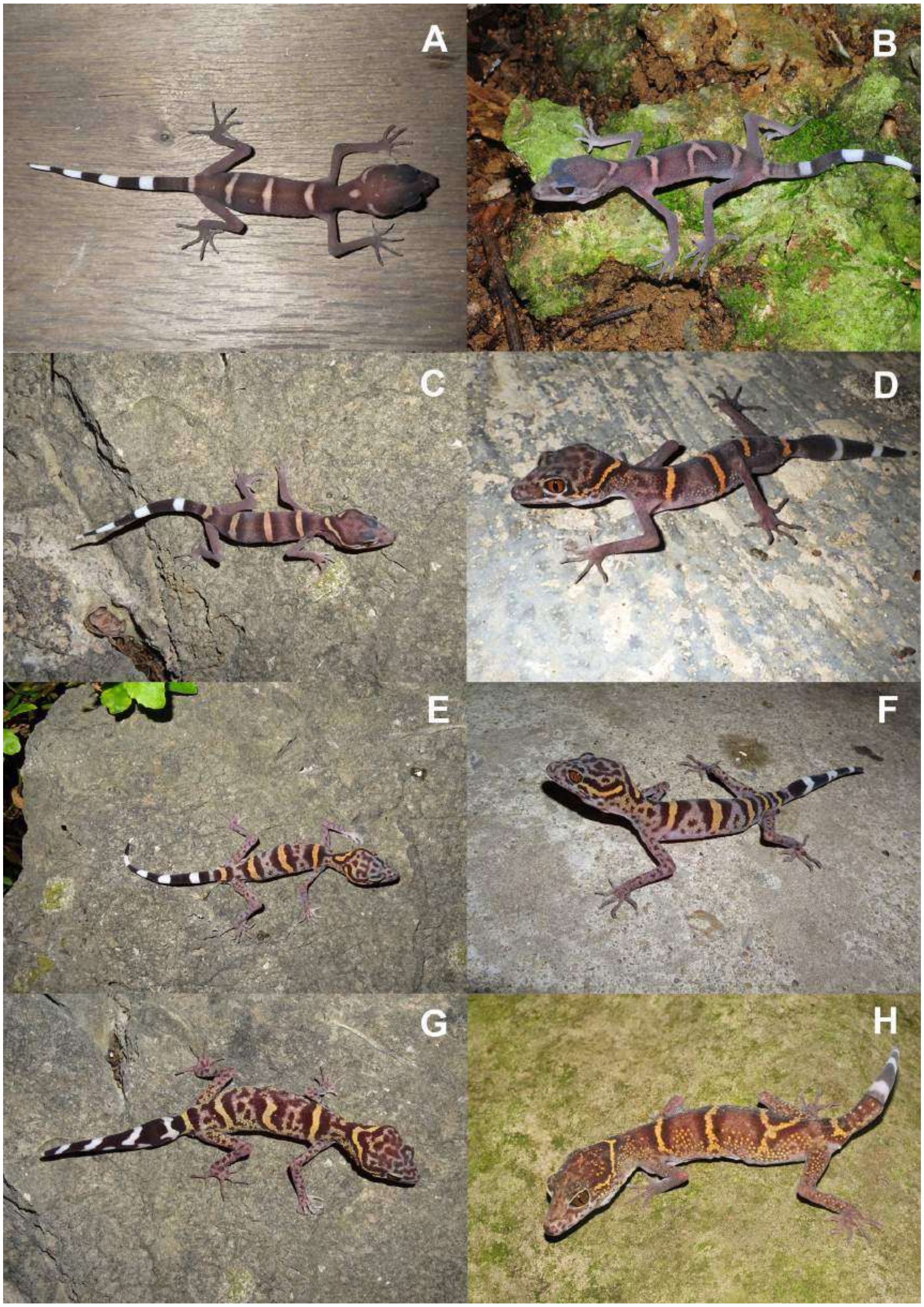

Fig. 2. *Goniurosaurus catbaensis* Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008. **A**. Juvenile (SVL = 55 mm). **B**. Juvenile (SVL = 69 mm). **C**. Juvenile (SVL = 75 mm). **D**. Adult (SVL = 91 mm). **E**. Adult (SVL = 99 mm). **F**. Adult (SVL = 105 mm). **G**. Adult (SVL = 116 mm). **H**. Adult (SVL = 125 mm).

bordered laterally by first infralabial on each side and posteriorly by 2–5 postmentals; postmentals bordered by 6–10 gular scales; gular region below lower jaws without enlarged tubercles; gular scales juxtaposed and granular, abruptly grading posteriorly into flat hexagonal pectoral scales and even larger ventral scales.

Neck narrower than body, covered with uniform granular scales interspersed with several sharply pointed conical tubercles on nape; tubercles on flanks conical, those of vertebral region somewhat lower in profile; dorsal body tubercles surrounded by 9–11 granular scales; dorsal tubercle rows at midbody 19–24; paravertebral tubercles between limb insertions 31–38, distinct vertebral row of tubercles absent; scales around midbody 112–127; larger ventral scales grade abruptly into smaller granular scales immediately anterior to vent at level of preanal pores; males with 18–23 precloacal pores in a transverse continuous series; region posterior to vent covered by flat juxtaposed scales and great hemipenial bulges, containing 2 or 3 enlarged postcloacal tubercles laterally on each side at level of vent; tail long and thin, thick at base, anteriorly with whorls; dorsal tail scales flat, smooth, up to 1.5 times the size of dorsal scales, arranged in more or less regular transverse rows; light band on tail base in width of 8–9 granular scales and with 7–8 tubercles in a transversal series; subcaudals larger than those on dorsal surface of tail.

Limbs relatively long and thin, covered dorsally with granular scales interspersed with several tubercles and ventrally with flat juxtaposed to subimbricate scales; dorsal granular scales grade into slightly flattened subimbricate scales on top of pes and manus; hind limbs slightly larger than forelimbs; larger granular scales on ventral surface of pes and manus; axillary pockets deep; subdigital lamellae wide, 9–11 under first finger, 18–21 under fourth finger, 9–12 under first toe, 22–25 under fourth toe; digits laterally compressed, increasing in length from first to fourth, fifth shorter than fourth (modified after Ziegler *et al.* 2008; Nguyen 2011).

Coloration in life (Fig. 2)

Dorsal ground color of head, body and limbs grey brown (in animals with SVL 53.4–105 mm) and blotches chestnut brown (in animals with SVL 110–125.3 mm), juveniles without small blotches, adults mottled with few circular blotches on body and limbs and long dark brown blotches on head; few yellow conical tubercles on franks of neck and body, and limbs in adults; iris light orange or red brown; five bands on the dorsal ground, thin, immaculate without dark spots, cream in juveniles and light orange or yellow in adults, all edged anteriorly and posteriorly by thin dark brown bands, including one thin nuchal loop extending from posterior corners of eyes and posteriorly protracted (in V-shape), three body bands between limb insertions, and another one on tail base; ground color of tail dark brown, and grey brown at mottled tail base; 3–5 immaculate white caudal bands, edged anteriorly and posteriorly in black; ventral surface of head, body and limbs dull white, juveniles absolutely immaculate, but adults with few dark spots on limbs, weak brown lateral spotting in gular region, venter and limbs.

Goniurosaurus huuliensis Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008

Fig. 3

Diagnosis

Body splayed and gracile, SVL 97.2–134.6 mm; external nares bordered by 6–8 nasal scales; supraorbital region with a row of enlarged tubercles; outer surface of upper eyelid composed of granular scales, about one half the size of those on top of head and without enlarged tubercles; internasal 1 or 2 (rarely absent); supralabials 9–12; infralabials 9–12; preorbital scales 14–20; eyelid fringe scales 51–59; postmentals 2–4; gular region below lower jaws with enlarged tubercles; paravertebral tubercles 31–37; scale rows around midbody 118–130, granular scales surrounding tubercles 11–13; axillary pockets deep; subdigital lamellae under fourth toe 21–25; precloacal pores in males 25–30; iris reddish brown; dorsal ground color of head, body and limbs dark brown, without small dark brown blotches (dark blotches present only on lower zone of flanks); nuchal loop thin, posteriorly protracted (in V-shape); dorsal body bands between limb insertions 3, thin, immaculate yellow; gular region with brown spots (modified after Orlov *et al.* 2008; Nguyen 2011).

Description (Supp. file 1: Table S2)

Body splayed and gracile, adult males SVL 97.2–132.2 mm (mean ± SE: 118.9 ± 1.4 mm, n = 32), TaL 36.7–108.6 mm (72.7 ± 3.7 mm), adult females SVL 97.4–134.6 mm (121.1 ± 1.2 mm, n = 46),

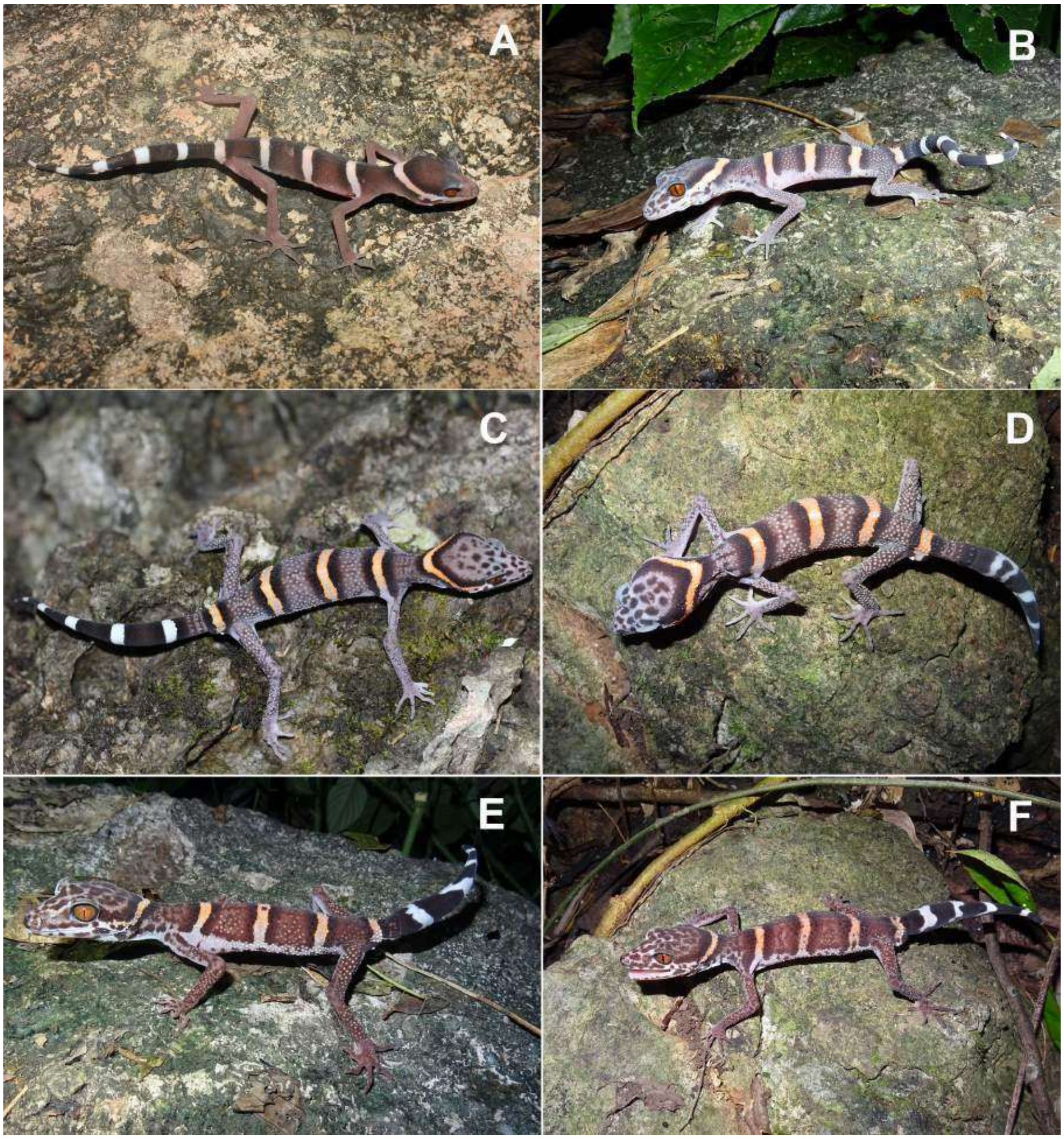

Fig. 3. *Goniurosaurus huuliensis* Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008. **A**. Juvenile (SVL = 74.4 mm). **B**. Adult (SVL = 97 mm). **C**. Adult (SVL = 108 mm). **D**. Adult (SVL = 116 mm). **E**. Adult (SVL = 125 mm). **F**. Adult (SVL = 132 mm).

TaL 48.4–106.7 mm (78.2±2.7 mm), juveniles SVL 73.5–74.4 mm (n=2), TaL 62.5–62.7 mm (Supp. file 1: Table S2); head triangular, wider than neck, covered by uniform granular scales interspersed with tubercles in temporal and occipital regions; scales on rostrum slightly larger and flatter; enlarged supraorbital tubercles in a conspicuous row; middorsal portion of rostral partially sutured dorsomedially, bordered laterally by first supralabial on each side, dorsolaterally by prenasal on each side, and dorsally by internasal and two supranasals; internasal 1–3 (rarely absent); external nares bordered by 5–7 nasals (prenasal, anterior and posterior supranasals, 2 slightly enlarged postnasal, and 1 or 2 granular scales); preorbital scales 14–20; supralabials 9–12, grading into granular scales posteriorly; infralabials 9–12; eyes large, pupils vertical; eyelid fringe scales 51–59, those of upper eyelid slightly enlarged; outer surface of upper eyelid composed of granular scales of about one half the size of those on top of head, without enlarged tubercles; fold of skin originating from suborbital region extends posteroventrally across angle of jaw; external auditory meatus elliptical with long axis directed dorsoventrally; tympanum deeply recessed; mental triangular, bordered laterally by first infralabial on each side and posteriorly by 2–4 postmentals; postmentals bordered by 7–10 gular scales; gular region below lower jaws with enlarged tubercles; gular scales juxtaposed and granular, grading posteriorly into flat hexagonal pectoral scales and even larger ventral scales.

Neck narrower than body, covered with uniform granular scales interspersed with several sharply pointed conical tubercles on nape; tubercles on flanks conical, those of vertebral region somewhat flatter; dorsal body tubercles surrounded by 11–13 granular scales; dorsal tubercle rows at midbody 19–24; paravertebral tubercles between limb insertions 31–37, distinct vertebral row of tubercles absent; scales around midbody 118–130; ventral scales large; males with 25–30 precloacal pores in continuous series, females without precloacal pores (but pitted scales present); region posterior to vent covered by flat juxtaposed scales and greatly swollen, containing 1 or 2 enlarged tubercles on each side at level of vent; tail thick at base; light band on tail base in width of 9–12 granular scales and with 8–10 tubercles in transversal series.

Limbs relatively long and thin, covered dorsally with granular scales interspersed with several tubercles and ventrally with flat juxtaposed to subimbricate scales; dorsal granular scales grade into slightly flattened subimbricate scales on top of pes and manus; hind limbs larger than forelimbs; axillary pockets deep; subdigital lamellae wide, 10 or 11 under first finger, 18–21 under fourth finger, 11 or 12 under first toe, 21–25 under fourth toe; digits laterally compressed, increasing in length from first to fourth, fifth shorter than fourth (modified after Orlov *et al.* 2008; Nguyen 2011).

Coloration in life (Fig. 3)

Dorsal ground color of head, body and limbs signal brown (juveniles), grey-brown (young adults, SVL: 97.2–116 mm) and signal brown (adults), without small dark brown blotches (dark blotches present only on lower zone of flanks); dull white tubercles on dorsal body, limbs in juveniles, few orange tubercles on limbs in adults; iris orange or red brown; five bands on the dorsal ground, thin, immaculate without dark spots, slight bisque in juveniles, orange brown or yellow in adults, all edged anteriorly and posteriorly by thin dark brown bands, including one thin nuchal loop extending from posterior corners of eyes and posteriorly protracted (in V-shape), three body bands between limb insertions, and another one on tail base; ground color of tail dark brown, and signal brown at mottled tail base; 3–6 immaculate white caudal bands; ventral surfaces of head, body and limbs dull white and immaculate except for few dark brown spots on margin regions of gular and limbs.

Goniurosaurus luii Grismer, Viets & Boyle, 1999
Fig. 4

Diagnosis

Body splayed and gracile, SVL 86.5–126.5 mm; external nares bordered by 5–8 nasal scales; supraorbital region with a row of enlarged tubercles; outer surface of upper eyelid composed of granular scales, about one half the size of those on top of head and without enlarged tubercles; internasals 1 or 2; supralabials 8–12; infralabials 8–11; preorbital scales 13–16; eyelid fringe scales 46–61; postmentals 2–6; gular region below lower jaws with enlarged tubercles; paravertebral tubercles 29–38; scale rows around midbody 119– 144, granular scales surrounding tubercles 9–14; axillary pockets deep; subdigital lamellae under fourth toe 20–26; precloacal pores in males 23–32; iris brown or bright orange; dorsal ground color of head, body and limbs pale brown to grey brown, mottled with small dark brown blotched; nuchal loop thin, posteriorly protracted (in V-shape); dorsal body bands between limb insertions 3, thin, immaculate yellow; gular region, belly, and ventral surface of limbs with brown spots (Grismer *et al.* 1999; Vu *et al.* 2006; Nguyen 2011).

Description (Supp. file 1: Table S2)

Body splayed and gracile, males: SVL 88.8–123.0 mm (mean ± SE: 109.3 ± 1.4 mm, n = 34), TaL 2.8–96.8 mm (72.5 ± 3.8 mm); adult females: SVL 86.5–126.5 mm (112.4 ± 1.3 mm, n = 43), TaL 43.3–102.2 mm (72.2 ± 2.1 mm); juveniles: SVL 55.0–84.8 mm (74.8 ± 3.1 mm, n = 11), TaL 44.2–68.9 (58.7 ± 2.7 mm) (Supp. file 1: Table S2); head triangular, wider than neck, covered by uniform granular scales interspersed with tubercles in temporal and occipital regions; scales on rostrum slightly larger and flatter; enlarged supraorbital tubercles in a conspicuous row; middorsal portion of rostral partially sutured dorsomedially, bordered laterally by first supralabial on each side, dorsolaterally by prenasal on each side, and dorsally by 1 or 2 internasal and two supranasals; internasals 1 or 2; external nares bordered by 5–8 nasal scales (prenasal, anterior and posterior supranasals, 2 slightly enlarged postnasal, and 1–3 granular scales); preorbital scales 13–16; supralabials 8–12, grading into granular scales posteriorly; infralabials 8–11; eyes large, pupils vertical; eyelid fringe scales 46–56, those of upper eyelid slightly enlarged; outer surface of upper eyelid composed of granular scales, about one half the size of those on top of head and without enlarged tubercles; fold of skin originating in suborbital region extends posteroventrally across angle of jaw; external auditory meatus elliptical with long axis directed dorsoventrally; tympanum deeply recessed; mental triangular, bordered laterally by first infralabial on each side and posteriorly by 2–6 postmentals; postmentals bordered by 6–11 gular scales; gular region below lower jaws with enlarged tubercles; gular scales juxtaposed and granular, abruptly grading posteriorly into flat hexagonal pectoral scales and even larger ventral scales.

Neck narrower than body, covered with uniform granular scales interspersed with several sharply pointed conical tubercles on nape; tubercles on flanks conical, those of vertebral region somewhat more flat; dorsal body tubercles surrounded by 11–13 granular scales; dorsal tubercle rows at midbody 20–24; paravertebral tubercles between limb insertions 29–38, distinct vertebral row of tubercles absent; scale rows around midbody 119–144; ventral scales large; males with 24–32 precloacal pores in a transverse continuous series, females without precloacal pores (but pitted scales present); region posterior to vent covered by flat juxtaposed scales and greatly swollen, containing 1–3 enlarged tubercles on each side at level of vent; tail thick at base, light band on tail base in width of 9–13 granular scales with 9–10 tubercles in transversal series; ventral caudals of tail base larger than dorsal caudals.

Limbs relatively long and thin, covered dorsally with granular scales interspersed with several tubercles and ventrally with flat juxtaposed to subimbricate scales; dorsal granular scales grade into slightly flattened subimbricate scales on top of pes and manus; hind limbs larger than forelimbs; axillary pockets deep; subdigital lamellae wide, 9–12 under first finger, 17–22 under fourth finger, 10–12 under first toe,

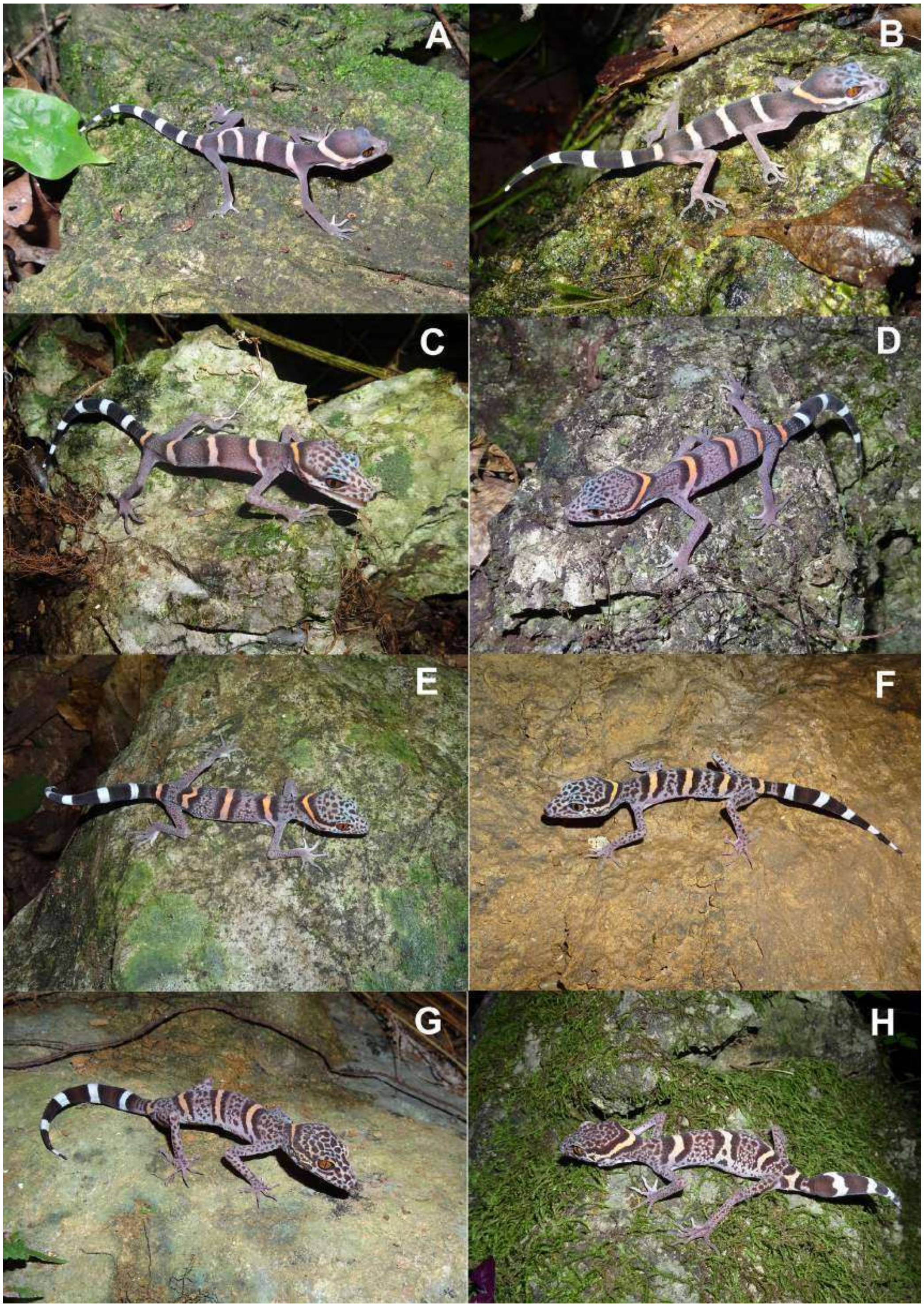

Fig. 4. *Goniurosaurus luii* Grismer, Viets & Boyle, 1999. **A**. Juvenile (SVL=61 mm). **B**. Juvenile (SVL=70 mm). **C**. Juvenile (SVL=80 mm). **D**. Adult (SVL=89 mm). **E**. Adult (SVL=96 mm). **F**. Adult (SVL70=104 mm). **G**. Adult (SVL=116 mm). **H**. Adult (SVL=126 mm).

20–26 under fourth toe; digits laterally compressed, increasing in length from first to fourth, fifth shorter than fourth (Grismer *et al.* 1999; Vu *et al.* 2006; Nguyen 2011).

Coloration in life (Fig. 4)

Dorsal ground color of head, body and limbs pale brown to grey brown, mottled with small dark brown circle blotches, juveniles without small dark brown blotches (except juveniles SVL ≥ 72 mm initially present on head); iris red brown or bright orange; five bands on dorsal ground, thin, immaculate without dark spots, slight bisque in juveniles, orange brown or yellow in adults, all edged anteriorly and posteriorly by thin dark brown bands, including one thin nuchal loop extending from posterior corners of eyes and posteriorly protracted (in V-shape), three body bands between limb insertions, and another one on tail base; ground color of tail nearly solid black, and grey brown at mottled tail base; 3–6 immaculate white caudal bands; ventral surfaces of head, body and limbs dull white with a few dark brown spots in gular region, on belly and limbs.

Goniurosaurus lichtenfelderi (Mocquard, 1897)

Fig. 5

Diagnosis

Body robust; SVL 80.6−113.5 mm; external nares bordered by 5–10 nasal scales; supraorbital region with a row of slightly enlarged tubercles; outer surface of upper eyelid composed of granular scales, about one-half the size of those on top of head and without enlarged tubercles; internasals 1–5 (rarely 1:2; 2:2 or 2:3); supralabials 7–10; infralabials 6–9; preorbital scales 12–19; eyelid fringe scales 43–58; postmentals 2–6; paravertebral tubercles 22−33; scale rows around midbody 117–131, granular scales surrounding tubercles 10−13; axillary pockets shallow; subdigital lamellae under fourth toe 17−21; precloacal pores in males 25–33, in females 17−21; dorsal ground color of head, body and limbs dark purple-brown, without small dark brown blotches; transverse body bands 4, nuchal loop thin, posteriorly rounded, in U-shape; dorsal body bands between limb insertions 2, thin, light yellow; gular region and without dark spots; ventral surfaces of head, body and limbs dull white with a few dark dots on margin regions (modified after Grismer 2000; Grismer *et al.* 2002; Nguyen 2011).

Description (Supp. file 1: Table S2)

Body robust, adult males: SVL 80.6–113.5 mm (mean ± SE: 97.8 ± 0.7 mm, n = 92), TaL 6.2–84.8 mm (61.5 ± 1.7 mm); adult females: SVL 81.0–105.5 mm (96.0 ± 0.7 mm, n = 72), TaL 22.7–81.2 mm (58.4 ± 1.4 mm); juveniles: SVL 41.1–77.1 mm (65.4 ± 2.9 mm, n = 14), TaL 20.9–64.7 mm (48.3 ± 3.4 mm) (Supp. file 1: Table S2); head triangular, wider than neck, covered by uniform granular scales interspersed with tubercles in temporal and occipital regions; scales on rostrum slightly larger and flatter; enlarged supraorbital tubercles in a conspicuous row; middorsal portion of rostral partially sutured dorsomedially, bordered laterally by first supralabial on each side, dorsolaterally by prenasal on each side, and dorsally by 1 or 2 internasals and two supranasals; internasals 1–5 (rarely 1:2; 2:2 or 2:3); external nares bordered by 5–10 nasals; preorbital scales 12–18; supralabials 7–10, grading into granular scales posteriorly; infralabials 6–9; eyes large, pupils vertical; eyelid fringe scales 47–58, those of upper eyelid slightly enlarged; outer surface of upper eyelid composed of granular scales of about one-half the size of those on top of head, without enlarged tubercles; fold of skin originating in the suborbital region extends posteroventrally across angle of jaw; external auditory meatus elliptical; tympanum deeply recessed; mental triangular, bordered laterally by first infralabial on each side and posteriorly by 2–5 postmentals; postmentals bordered by 7–10 gular scales; gular region below lower jaws without enlarged tubercles; gular scales juxtaposed and granular, abruptly grading posteriorly into flat hexagonal scales and even larger ventral scales.

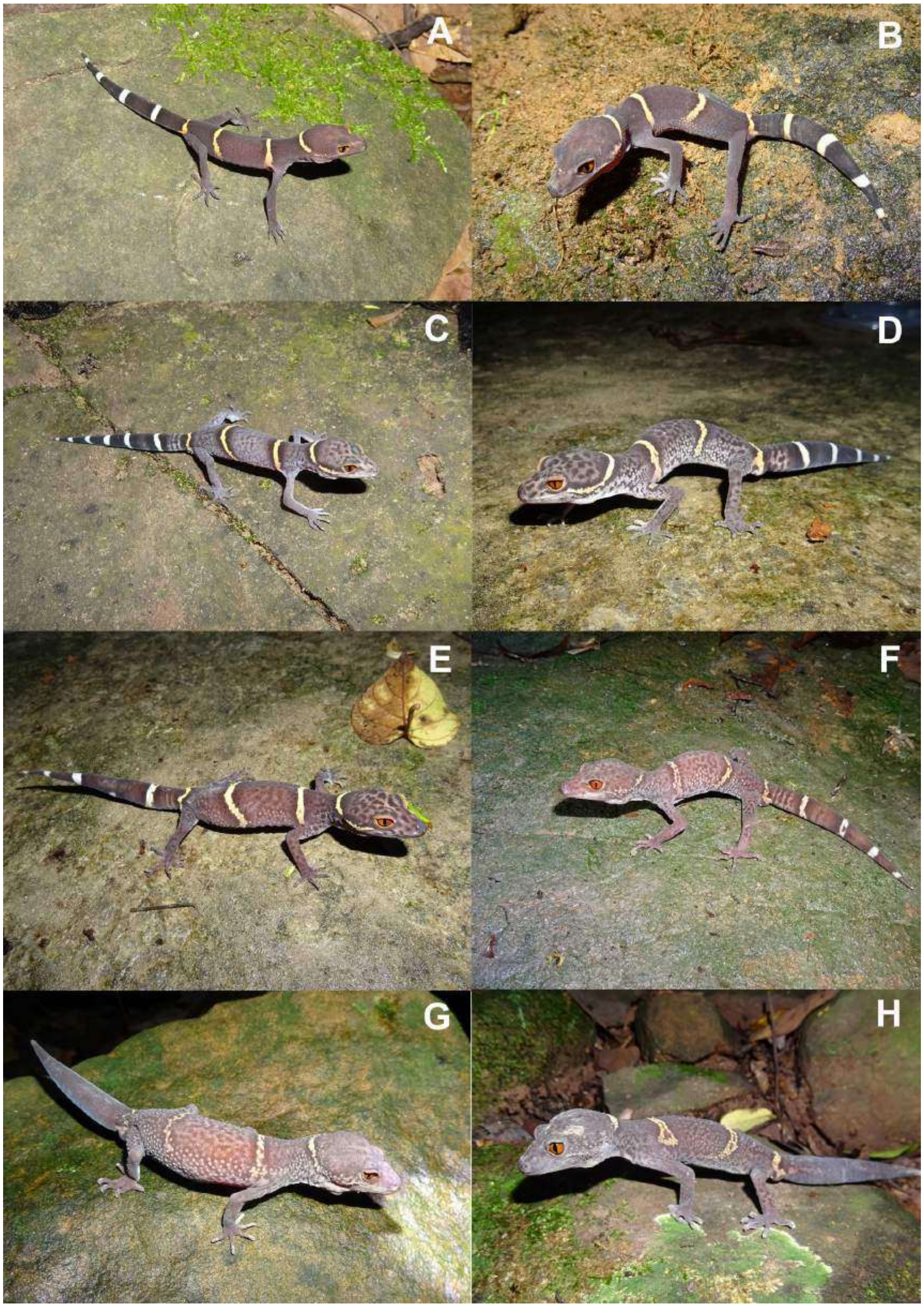

Fig. 5. *Goniurosaurus lichtenfelderi* (Mocquard, 1897). **A**. Juvenile (SVL=55 mm). **B**. Juvenile (SVL=63 mm). **C**. Juvenile (SVL=72 mm). **D**. Adult (SVL=80 mm). **E**. Adult (SVL=91 mm). **F**. Adult (SVL=97 mm). **G**. Adult (SVL=105 mm). **H**. Adult (SVL=113 mm).

Neck narrower than body, covered with uniform granular scales interspersed with several sharply conical tubercles on nape; tubercles on flanks conical, those of vertebral region somewhat more flat; dorsal body tubercles surrounded by 10–13 granular scales; dorsal tubercle rows at midbody 15–22; paravertebral tubercles between limb insertions 22–33, distinct vertebral row of tubercles absent; scales around midbody 117–130; ventral scales large; males with 24–33 precloacal pores in a transverse continuous series, females without distinct precloacal pores (but 25–33 pitted scales present); region posterior to vent covered by flat juxtaposed scales and greatly swollen, containing 1 (rarely 2) enlarged tubercles on each side at the level of vent; tail thick at base; light band on tail base in width of 4–7 scales and with 6–10 tubercles in a transversal series.

Limbs robust, covered dorsally with granular scales interspersed with several tubercles and ventrally with flat juxtaposed to subimbricate scales; dorsal granular scales grade into slightly flattened subimbricate scales on top of pes and manus; hind limbs larger than forelimbs; axillary pockets shallow; subdigital lamellae wide, 6–9 under first finger, 13–17 under fourth finger, 6–10 under first toe, 16–20 under fourth toe; digits laterally compressed, increasing in length from first to fourth, fifth shorter than fourth (modified after Grismer 2000; Grismer *et al.* 2002; Nguyen 2011).

Coloration in life (Fig. 5)

Dorsal ground color of head, body and limbs grey and grey brown in juveniles without blotches and chestnut brown in adults with dark brown blotches (in animals with SVL > 80 mm); iris orange or redish brown; four bands on the dorsal ground, thin, yellow in both juveniles and adults, immaculate without dark spots (few dark brown spots present in some animals), all edged anteriorly and posteriorly by thin dark brown bands, including one thin nuchal loop extending from posterior corners of eyes and posteriorly rounded (in U-shape), two body bands between limb insertions, and another one on tail base; ground color of tail dark brown, and grey brown at mottled tail base; 3–5 immaculate white caudal bands except first band slightly yellow, and some animals with regenerated tail present immaculate grey brown without white caudal bands; ventral surfaces of head, body and limbs dull white with a few dark dots on margin regions of belly, limbs, gular region, but immaculate dull white in juveniles (SVL ≤ 65 mm) without dark spots.

Morphological comparisons

Our morphological comparisons of the tiger gecko species (except for *G. araneus*) from Vietnam revealed an overall significant difference in the snout-vent length (SVL). As the result, *G. huuliensis* had the largest SVL, while *G. lichtenfelderi* had the shortest SVL in both adult males and females, compared to the other tiger geckos ($P < 0.05$). However, the SVL of *G. catbaensis* was not significantly different from that of *G. luii* ($P > 0.05$, Supp. file 2: Fig. S1).

The PCA of 18 selected morphometric characters estimated the first (PC1) and second principal (PC2) components to explain 82.5% (74.5% and 8.0%, respectively) of the variance among the four investigated species of *Goniurosaurus* in Vietnam (Supp. file 2: Fig. S2). The extracted PC1 and PC2 scores of the PCA differ significantly among the four species (Kruskal-Wallis's test, $P < 0.05$), of which the values and morphometric spaces of *G. lichtenfelderi* are not concordant with the three remainders (Fig. S2). Three morphological factors, namely head length (HL), head width (HW), and mouth width (MW) highly account for the overall difference in the PC1 score, while the PC2 score is mainly explained by the body height (BH) and body width (BW) (Supp. file 2: Fig. S2).

Regarding the comparisons of the 17 species of *Goniurosaurus* from China and Vietnam, the multiple correspondence analysis (MCA) clustered them separately into three groups (*G. lichtenfelderi* group, *G. luii* group and *G. yingdeensis* group) represented with different meristic spaces on the spatial coordinate of Dim 1 and Dim 2 (Fig. 6A, Table 1). The characters of fourth toe (LT4), body limb shape

Table 1 (continued on the next two pages). Meristic characters (minimum–maximum) of 17 species of *Goniurosaurus* Grismer, Viets & Boyle, 1999 in China and Vietnam. Data of *G. araneus* Grismer, Viets & Boyle, 1999 from Grismer *et al.* (1999) and Chen *et al.* (2014); *G. bawanglingensis* Grismer, Haitao, Orlov & Anajeva, 2002 from Grismer *et al.* (2002); *G. catbaensis* Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008, *G. huuliensis* Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008, *G. luii* Grismer, Viets & Boyle, 1999 and *G. lichtenfelderi* (Mocquard, 1897) from this study; *G. gezhi* Zhu, He & Li, 2020 from Zhu *et al.* (2020a); *G. gollum* Qi, Wang, Grismer, Chen, Lyu & Wang, 2020 from Qi *et al.* (2020b); *G. hainanensis* Barbour, 1908 from Grismer *et al.* (1999, 2002); *G. kadoorieorum* Yang & Chan, 2015 and *G. kwangsiensis* Yang & Chan, 2015 from Yang & Chan (2015); *G. kwanghua* Zhu & He, 2020 from Zhu *et al.* (2020b); *G. liboensis* Wang, Yang & Grismer, 2013 from Wang *et al.* (2013); *G. varius* Qi, Grismer, Lyu, Zhang, Li & Wang, 2020 from Qi *et al.* (2020a); *G. yingdeensis* Wang, Yang & Cui, 2010 from Wang *et al.* (2010) and Qi *et al.* (2020b); *G. zhelongi* Wang, Jin, Li & Grismer, 2014 from Wang *et al.* (2014) and Qi *et al.* (2020b); and *G. zhoui* Zhou, Wang, Chen & Liang, 2018 from Zhou *et al.* (2018).

Goniurosaurus luii **group**								
	G. araneus	*G. catbaensis*	*G. gezhi*	*G. huuliensis*	*G. kadoorieorum*	*G. kwangsiensis*	*G. liboensis*	*G. luii*
Enlarged row of supraorbital tubercles (0) absent. (1) present	1	1	1	1	1	1	1	1
Scales of upper eyelid to top of head ½ size (1) of those on the top of the head or equal in size (2)	1	2	–	1	2	2	2	1
Deep axillary pockets (0) absent. (1) present	1	1	1	1	1	1	1	1
Body and limb (1) splayed–gracile (2) compact–robust	1	1	1	1	1	1	1	1
Posterior nuchal loop (1) protracted (2) rounded	1	1	1	1	1	1	1	1
Number of Body bands	4	4	4	4	4	4	4	4
Dorsal body bands (1) immaculate (2) maculate	1	1	1	1	1	1	1	1
Dark borders of body band (1) wide (2) narrow	1	1	1	1	1	1	1	1
Adult ground color (1) mottled (2) immaculate	2	1	1	2	1	1	1	1
Lateral spotting on belly (0) absent (1) present	0	1	–	1	1	1	0	1
IN	1–2	0–1	0–1	0–3	2	1–2	2–3	1–2
P–IN	–	0–2	3–5	0–7	3–9	1–2	3	2–6
SPL	8–10	8–11	9–10	9–12	10–11	8–10	9–11	8–12
IFL	8–9	7–10	8–10	9–12	9	7–9	9–12	8–11
N	6–8	6–8	6–7	5–7	6–7	6–7	8–9	5–8
PM	4–6	2–5	3–5	2–4	4–5	3–6	3–5	2–6

Table 1 (continued).

***Goniurosaurus luii* group**								
	G. araneus	*G. catbaensis*	*G. gezhi*	*G. huuliensis*	*G. kadoorieorum*	*G. kwangsiensis*	*G. liboensis*	*G. luii*
GP	7–9	6–10	7	7–10	8–11	7–9	9–14	6–11
PO	13–18	10–1	15–19	14–20	15–19	15–19	16–18	13–16
CIL	52–67	45–56	44–52	51–59	47–55	52–58	52–59	46–56
MB	129–147	112–127	123–151	118–130	124–132	122–128	127–129	119–144
GST	10–14	9–11	10–12	11–13	11–13	10–13	10–13	11–13
TL	32–38	31–38	32–39	31–37	30–34	27–32	27–28	29–38
DTR	21–22	19–24	20–21	19–24	22–24	20–22	23–24	20–24
LD1	9–12	9–11	9–11	10–11	10–11	10–12	9–10	9–12
LD4	19–21	18–21	20–21	18–21	17–19	18–21	17–19	17–22
LT1	9–14	9–12	9–12	11–12	10–11	11–13	11–12	10–12
LT4	23–24	22–25	21–25	21–25	21–24	22–27	23–26	20–26
PP	18–23	18–23	18–20	25–30	26–28	31–33	23	24–32
PAT	3–6	2–3	2	1–2	1–2	1–2	2–4	1–3

	***Goniurosaurus lichtenfelderi* group**					***Goniurosaurus yingdeensis* group**			
	G. bawanglingensis	*G. hainanensis*	*G. kwanghua*	*G. lichtenfelderi*	*G. zhoui*	*G. gollum*	*G. varius*	*G. yingdeensis*	*G. zhelongi*
Enlarged row of supraorbital tubercles (0) absent. (1) present	0	0	1	1	1	0	0	1	1
Scales of upper eyelid to top of head ½ size (1) of those on the top of the head or equal in size (2)	1	2	1	1	2	2	2	1	1
Deep axillary pockets (0) absent. (1) present	1	0	0	0	1	1	1	1	1
Body and limb (1) splayed–gracile (2) compact–robust	2	2	2	2	2	1	1	1	1
Posterior nuchal loop (1) protracted (2) rounded	2	2	2	2	1	2	2	2	2
Number of body bands	4	3	3	3	4	4	4	4	4

Table 1 (continued).

	***Goniurosaurus lichtenfelderi* group**					***Goniurosaurus yingdeensis* group**			
	G. bawanglingensis	*G. hainanensis*	*G. kwanghua*	*G. lichtenfelderi*	*G. zhoui*	*G. gollum*	*G. varius*	*G. yingdeensis*	*G. zhelongi*
Dorsal body bands (1) immaculate (2) maculate	2	2	2	1	2	1	1	1	1
Dark borders of body band (1) wide (2) narrow	2	2	2	2	1	1	1	1	1
Adult ground color (1) mottled (2) immaculate	1	1	1	1	1	1	1	1	1
Lateral spotting on belly (0) absent (1) present	0	0	0	1	0	1	1	1	1
IN	–	–	0–1	1–5	1	1	1–2	2–3	1–2
P–IN	–	–	0–2	2	2–4	2	3–4	2–6	3
SPL	8–10	7–10	7–9	7–10	8–9	10	7–10	8–10	7–10
IFL	7–11	6–9	6–8	6	7–9	10	8–9	8–10	6–9
N	–	–	9–10	5–10	8	8–9	7–9	7–11	6–8
PM	2–3	2–5	3–5	2–5	3–4	2–3	3–4	2–4	4–6
GP	–	–	4–8	7–10	5–7	7–8	6–8	5–7	7–9
PO	12–18	14–19	18–21	12–18	15–20	15–17	11–16	16–20	13–17
CIL	56–67	55–70	47–49	47–58	49–62	59–63	50–56	46–64	42–53
MB	104–133	95–125	109–118	117–130	130–140	121–128	101–110	102–115	99–109
GST	9–13	11–15	11–12	10–13	11	9–11	8–12	8–12	9–12
TL	32–36	23–32	25–26	22–33	24–32	25–26	27–29	25–33	28–33
DTR	–	–	20–21	15–22	19–22	16–17	21–24	20–25	23–28
LD1	–	–	9–10	6–9	9–10	10	7	8	7–8
LD4	–	–	18–19	13–17	14–16	13/14	15–17	18–19	15–17
LT1	–	–	9–10	6–10	9–11	15/16	8	11–12	7–9
LT4	18–22	18–23	18–19	17–20	19–22	22/23	18/21	19–24	17–22
PP	37–46	24–31	28	24–33	36–38	10–11	10	10–13	9–12
PAT	–	–	1–2	1–2	2–3	2	2	2	2

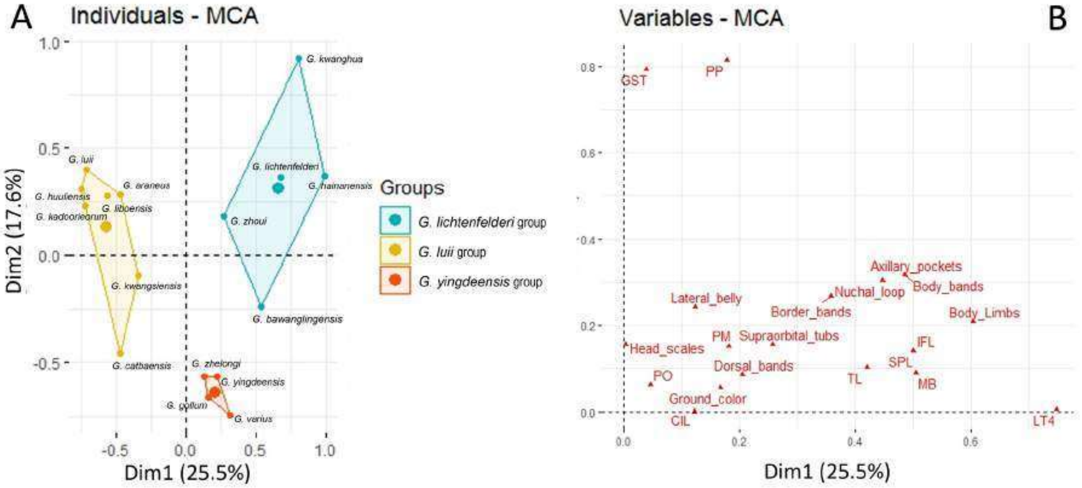

Fig. 6. A. Multiple correspondence analysis (MCA) on meristic variation among 17 recorded species of *Goniurosaurus* Grismer, Viets & Boyle, 1999 in China and Vietnam. **B**. Correlation between meristic variables and principal dimensions (Dim1 and Dim2).

and body bands highly account for the Dim1 score, whereas the characters of precloacal pores (PP) and granular scales surrounding dorsal tubercles (GST) are the most correlated with the Dim 2 score (Fig. 6B).

Key to the species of Goniurosaurus (Fig. 7, Table 1)

Modified from Grismer *et al.* (1999, 2002), Vu *et al.* (2006), Orlov *et al.* (2008), Ziegler *et al.* (2008), Wang *et al.* (2010, 2013, 2014), Nguyen (2011), Chen *et al.* (2014), Yang & Chan (2015), Honda & Ota (2017), Zhou *et al.* (2018, 2020a, 2020b), Qi *et al.* (2020a, 2020b) and the present study.

1. Precloacal pores in males present, claws are sheathed by scales 7
– Precloacal pores absent, unsheathed claws (*G. kuroiwae* group) 2

2. Yellow brown to gold iris; a single scale at the base of each digit which is occasionally slightly enlarged .... ***G. yamashinae*** (Okada, 1936)
– Blood-red iris; one to three enlarged scales at the base of each digit on the limbs 3

3. Adults without interspace mottling 4
– Adults with interspace mottling 5

4. Robust body stature; dorsal body tubercles between the limb insertions in cross-section are triangular to elliptical and sharply keeled anteriorly; 34–42 paravertebral tubercles; ventral scales are juxtaposed and sharply raised .... ***G. toyamai*** Grismer, Ota & Tanaka, 1994
– Slender body stature; dorsal body tubercles between the limb insertions in cross-section are smooth or very weakly keeled; 22–29 paravertebral tubercles; ventrals are flat, wide, and subimbricate to imbricate .... ***G. splendens*** (Nakamura & Uéno, 1959)

5. Dorsal banding absent or incomplete .... ***G. kuroiwae*** (Namiye, 1912)
– Prominent dorsal pattern consisting of transverse bands between the nape of the neck and the caudal constriction 6

6. No such reddish or pinkish tint in dorsal pattern and iris .... ***G. orientalis*** (Maki, 1931)
– Reddish or pinkish tint to some extent in dorsal pattern and iris .... ***G. sengokui*** (Honda & Ota, 2017)

7. Precloacal pores in males less than 16 (*G. yingdeensis* group) 8
– Precloacal pores in males more than 16 11

8. Scales around midbody 121–128; longitudinal dorsal tubercle rows at midbody 16–17 .... ***G. gollum*** Qi, Wang, Grismer, Chen, Lyu & Wang, 2020
– Scales around midbody 99–115; longitudinal dorsal tubercle rows at midbody 20–28 9

9. Nuchal loop and body bands with small dark blotches; enlarged row of supraorbital tubercles absent; trunk of body usually with a longitudinal light vertebral stripe .... ***G. varius*** Qi, Grismer, Lyu, Zhang, Li & Wang, 2020
– Nuchal loop and body bands without dark blotches; enlarged row of supraorbital tubercles present; trunk of body without a longitudinal light vertebral stripe 10

10. Tubercles between orbits present; gular scales bordering the postmentals 2–4; preorbital scales 5–7 .... ***G. yingdeensis*** Wang, Yang & Cui, 2010
– Tubercles between orbits absent; gular scales bordering the postmentals 4–6; preorbital scales 7–9 .... ***G. zhelongi*** Wang, Jin, Li & Grismer, 2014

11. Body and limbs robust (*G. lichtenfelderi* group) 12
– Body and limbs splayed gracile (*G. luii* group) 16

12. Precloacal pores in males 37–46 .... ***G. bawanglingensis*** Grismer, Haitao, Orlov & Anajeva, 2002
– Precloacal pores in males fewer than 37 13

13. Nuchal loop protracted posteriorly (in V-shape); number of body bands 4; axillary pockets deep; precloacal pores in males more than 33 .... ***G. zhoui*** Zhou, Wang, Chen & Liang, 2018
– Nuchal loop rounded posteriorly (in U-shape); number of body bands 3; axillary pockets shallow; precloacal pores in males fewer than 33 14

14. Eyelid fringe scales 55–70; enlarged row of supraorbital tubercles absent; scales of upper eyelid to top of head equal of those on the top of the head in size .... ***G. hainanensis*** Barbour, 1908
– Eyelid fringe scales 47–58; enlarged row of supraorbital tubercles present; scales of upper eyelid to top of head ½ of those on the top of the head in size 15

15. Lateral spotting on belly absent; preorbital scales 18–21; scales around midbody 109–118; subdigital lamellae under the fourth finger 18–19 .... ***G. kwanghua*** Zhu & He, 2020
– Lateral spotting on belly present; preorbital scales 12–18; scales around midbody 117–130; subdigital lamellae under the fourth finger 13–17 .... ***G. lichtenfelderi*** (Mocquard, 1897)

16. Internasal absent .... ***G. catbaensis*** Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008
– Internasal present 17

17. Postcloacal tubercles 3–6 .... ***G. araneus*** Grismer, Viets & Boyle, 1999
– Postcloacal tubercles 1–3 18

18. Adult body length (SVL) > 126 mm; dorsum without dark blotches .... ***G. huuliensis*** Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008
– Maximum body length (SVL) ≤ 126 mm; dorsum with dark blotches 19

19\. Dorsum with many small dark blotches .... ***G. luii*** Grismer, Viets & Boyle, 1999
– Dorsum with rarely scattered dark blotches 20

20\. Outer surface of upper eyelid composed of granular scales, about the same size of those on top of head and with enlarged tubercles .... ***G. gezhi*** Zhu, He & Li, 2020
– Enlarged row of supraorbital tubercles present; scales of upper eyelid to top of head ½ of those on the top of the head in size 21

21\. Precloacal pores in males 31–33 .... ***G. kwangsiensis*** Yang & Chan, 2015
– Precloacal pores in males fewer than 31 22

22\. Lateral spotting on belly present; nasal scales 6–7; paravertebral tubercles between limb insertions 30–34; precloacal pores 26–28 ....***G. kadoorieorum*** Yang & Chan, 2015
– Lateral spotting on belly absent, nasal scales 8–9; paravertebral tubercles between limb insertions 27–28; precloacal pores 23 ....***G. liboensis*** Wang, Yang & Grismer, 2013

Discussion

Taxonomic review

Our phylogenetic analyses strongly suggest that populations recorded from Vietnam indeed belong to four known species, namely *G. catbaensis*, *G. huuliensis*, *G. lichtenfelderi* and *G. luii* (Grismer *et al.* 1999; Vu *et al.* 2006; Orlov *et al.* 2008; Ziegler *et al.* 2008; Nguyen *et al.* 2009; Nguyen 2011). Despite extensive surveys over the last two decades, *G. araneus* has not been recorded in Vietnam, leaving its accurate type locality in Cao Bang Province, northern Vietnam ambiguous (Grismer *et al.* 1999; Ngo *et al.* 2016). It is possible that the species has been extirpated from Vietnam as a result of over-exploitation to supply the international pet trade or that the species has never occurred at the documented type locality (Grismer *et al.* 1999; Ngo *et al.* 2016). Similarly, *G. luii* has not been recorded again at its type locality in China (Grismer *et al.* 1999; Stuart *et al.* 2006; Yang & Chan 2015).

Molecular results supported by our study confirm that *G. murphyi*, described by Orlov & Darevsky (1999), is a junior synonym of *G. lichtenfelderi* (Grismer 2000). All mainland populations of *G. lichtenfelderi* found in granitic forests are conspecific with those from the granitic offshore islands in Bai Tu Long National Park, Quang Ninh Province, northern Vietnam (type locality), with an intraspecific genetic distance of less than 0.7% (Supp. file 1: Table S1). However, a few issues need to be resolved in the future. In particular, *G. hainanensis* was recovered as polyphyletic by Liang *et al.* (2018) and our phylogenetic analyses. Moreover, Zhu *et al.* (2020a) suggested that *G. luii* and *G. kadoorieorum* are polyphyletic. We highly recommend that they may be synonymized. Samples assigned to *G. kuroiwae* were not recovered as monophyletic in our phylogenetic analysis. It is possible that more cryptic species from the Japanese group will be discovered. Further studies with additional samples, especially from type localities, should be undertaken to clarify these taxonomic problems.

Regarding morphological analyses, six morphometric characters, namely snout-vent length, head length, head width, mouth width, body height and body width strongly supported the overall difference among the four species in Vietnam and five characteristics, namely fourth toe, body and limb shapes, body bands, precloacal pores and granular scales surrounding dorsal tubercles, mainly accounted for the variation among the 17 tiger geckos from China and Vietnam. Six species of the *G. kuroiwae* group from Japan were not included in this study. However, the Japanese *G. kuroiwae* group can be distinguished from Chinese and Vietnamese species by the absence of precloacal pores and unsheathed claws (Wang *et al.* 2014; Yang & Chan 2015; Honda & Ota 2017). We highly recommend that all these traits be considered as diagnostic characters for species of *Goniurosaurus*.

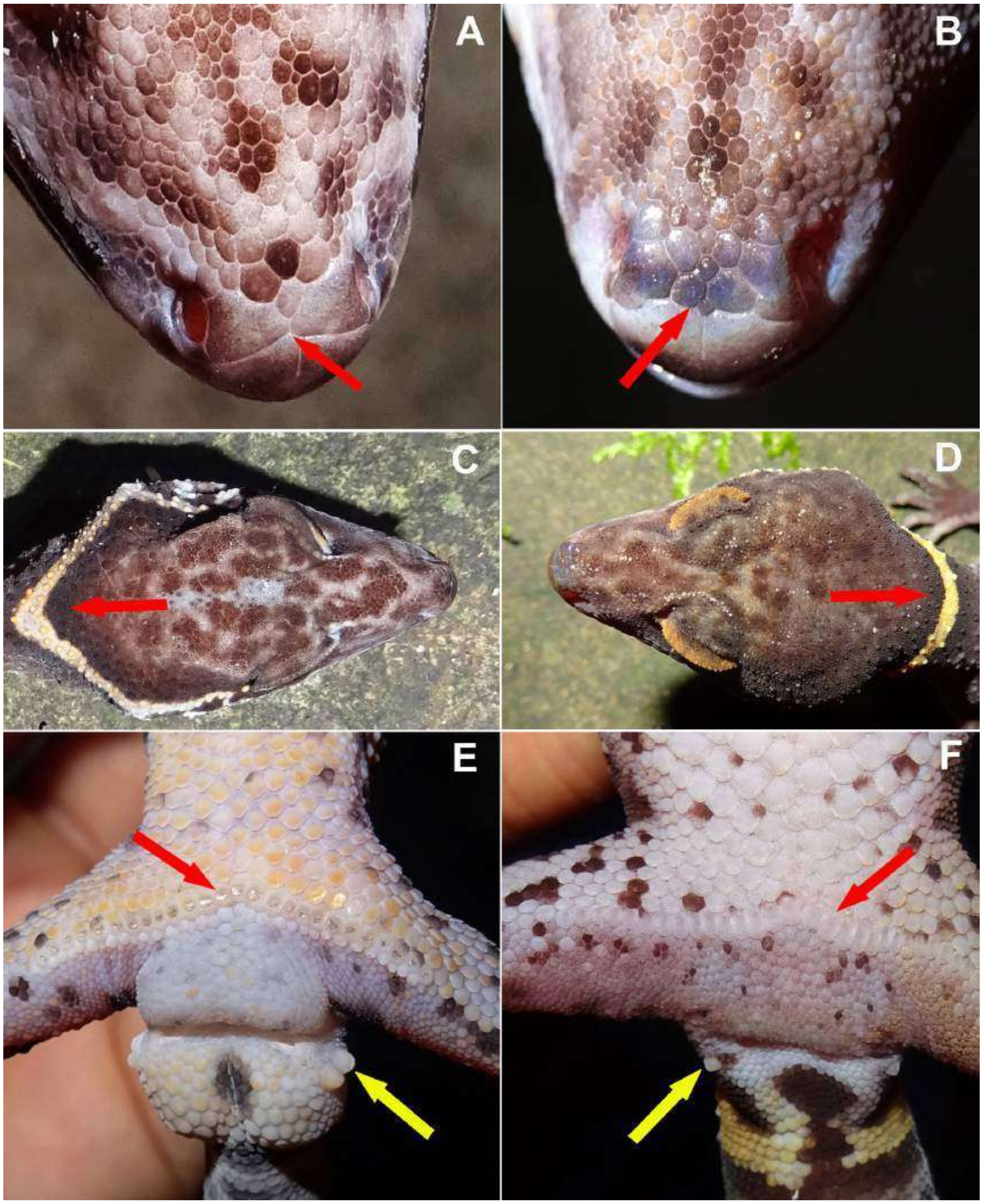

Fig. 7. A few key characters for species identification in the genus *Goniurosaurus* Grismer, Viets & Boyle, 1999. **A**. Snout tip of *G. catbaensis* Ziegler, Nguyen, Schmitz, Stenke & Rösler, 2008 with lacking postrostral (internasal) and two supranasals are in contact with each other. **B**. Snout tip of *G. lichtenfelderi* (Mocquard, 1897) with few internasals. **C**. Nuchal loop of *G. huuliensis* Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008 protracted posteriorly (in V-shape). **D**. Nuchal loop of *G. lichtenfelderi* rounded posteriorly (in U-shape). **E**. Precloacal pores and large swollen hemipenial bulges of *G. luii* Grismer, Viets & Boyle, 1999 in males, and two postcloacal tubercles. **F**. Indistinct precloacal pores of *G. lichtenfelderi* in females and only one postcloacal tubercle on each site. Yellow arrows point to postcloacal tubercles, and red arrows to other scales.

Based on our identification key in accordance with the morphological and phylogenetic analyses, all tiger gecko species were grouped into four separate species groups, in agreement with the previous findings (Liang *et al.* 2018; Qi *et al.* 2020a, 2020b; Zhu *et al.* 2020a, 2020b). Liang *et al.* (2018), Qi *et al.* (2020a, 2020b) and our genetic analyses revealed that *G. bawanglingensis* and *G. zhoui* are embedded within the *G. lichtenfelderi* group, while they were previously placed as sister taxa in the *G. luii* group due to superficial morphological similarities as a result of adaptation to karst habitat (Grismer *et al.* 2002; Zhou *et al.* 2018). In accordance with these genetic findings, we found that the two karst-adapted species are morphologically most similar to three remaining granite-stream-adapted species of the *G. lichtenfelderi* group. Further comparative studies in southern China and Japan may help to resolve the phylogenetic relations of the genus *Goniurosaurus*.

Due to the high degrees of adaptation to specific microhabitats and local endemism, the genus *Goniurosaurus* could serve as a model system to study the evolution in lizards. In Vietnam, all species of *Goniurosaurus* have been found in isolated geographic ranges and none of them occurs in sympatry. For example, the distribution of *G. luii* stretches to the north of the Lang Son and Cao Bang provinces (Ngo *et al.* 2016), while *G. huuliensis* has been recorded in karst forests in the south of Lang Son Province. *Goniurosaurus catbaensis* is currently known only from offshore islands in Cat Ba National Park and the Ha Long Bay. *Goniurosaurus lichtenfelderi* can only be found in granitic habitats at several mainland localities and on some offshore islands in the Bai Tu Long Archipelago, which is contiguous with Ha Long Bay in the Gulf of Tonkin (Orlov *et al.* 2008; Gawor *et al.* 2016; Ngo *et al.* 2019a). *Goniurosaurus araneus* has not yet been re-discovered at its type locality in Cao Bang Province, and we only recorded *G. luii* inhabiting limestone karst forests of Cao Bang Province (Grismer *et al.* 1999; Vu *et al.* 2006; Ngo *et al.* 2016). The allopatric distribution has also been documented among species of *Goniurosaurus* from China. To date, neither the four species from the Hainan Archipelago, nor the five species of the *G. luii* group from Guangxi Province, China, nor the four known species of the *G. yingdeensis* group from Guangdong Province were recorded to occur in sympatry (Grismer *et al.* 2002; Zhou *et al.* 2018; Zhu *et al.* 2020a, 2020b). A large river (Zuojiang River) is regarded as the potential geographic barrier between the two species *G. luii* and *G. araneus* within Nonggang Nature Reserve, China (Chen *et al.* 2014). Likewise, geographic barriers (e.g., rivers and canyons) are considered to prevent a genetic exchange between members of the *G. yingdeensis* group (Qi *et al.* 2020a). The global rising of the sea level during the last melting period of glacial ice has shaped the myriad of archipelagoes (Clements *et al*. 2006; Ziegler *et al.* 2008; Liang *et al.* 2018; Ngo *et al.* 2019a). Thus, the oceanic barrier known as an important geographic feature has constrained current distributions and limits the genetic exchange of insular tiger gecko species, such as *G. catbaensis*, species from the Hainan Archipelago and *G. kuroiwae* group (Ziegler *et al.* 2008; Honda & Ota 2017; Liang *et al.* 2018; Zhu *et al.* 2020b). However, the last glacial maximum period occurring around 10 000–50 000 years ago was not sufficiently long for speciation events within *Goniurosaurus* (Clements *et al.* 2006; Sterling *et al.* 2006; Ziegler *et al.* 2008). Indeed, by employing a molecular dating method, Liang *et al.* (2018) estimated that the latest speciation events among tiger geckos took place approximately 2.8–2.9 Ma, in the late Pliocene, between *G. yamashinae*–*G. orientalis*, and *G. huuliensis*–*G. luii*. It is likely that the global warming during the Pliocene promoted the diversification in the genus, as also reported in the crocodile newts (genus *Tylototriton* Anderson, 1871) in Asia (Bernardes *et al.* 2020). Thus, the genus *Goniurosaurus* might offer a unique opportunity to investigate mechanisms of speciation and evolutionary adaptation, that are expected to highly correlate with a pre-dominantly allopatric mode of diversification.

Implications for conservation

The taxonomy of several existing species complexes requires further investigation and new species are still being discovered. Nevertheless, wild populations of the genus *Goniurosaurus* are under high risks of extinction, due to habitat destruction and exploitation for national and international trade. Of the 23 described tiger gecko species, 12 have been considered threatened (listed as VU, EN and CR) and one data deficient (DD) in the IUCN Red List (Ngo *et al.* 2019b). Assessments for the remaining species are still lacking. In order to better regulate the international trade in the species, all species of *Goniurosaurus* from China and Vietnam were recently listed in CITES Appendix II and the Vietnamese species were also protected under the Governmental Decree 06/2019/ND–CP (Group IIB). Trade data in the US showed that about half of the number of traded tiger geckos were only assigned to the genus level (Ngo *et al.* 2019b). This fact could be a consequence of the highly similar morphology among tiger gecko species and lacking guidance to distinguish the species (Ngo *et al.* 2019b). Besides, the conservation status and national protection status are not assessed equally among species. Therefore, the present identification guide will aid to prevent tiger geckos from being traded under a wrong name to circumvent legislations. The detailed descriptions of morphology provided in this study can assist local, national, and international authorities, such as local rangers, CITES authorities, custom officers, in enforcing international regulations. The identification key can also be useful for scientists and breeders to accurately identify tiger gecko species. However, morphological identification of captive lineages should be treated with reservation, as some breeders have produced hybrids (L. Grismer, pers. comm.), and thus molecular identification tools should be consulted as well in these cases.

After the CITES listing, four new species, namely *G. gezhi*, *G. gollum*, *G. kwanghua* and *G. varius* were recently described from China, which are automatically listed in Appendix II of the Convention (Qi *et al.* 2020a, 2020b; Zhu *et al.* 2020a, 2020b). As the Japanese species appear to be similarly threatened and to prevent a shift towards international trade, Janssen & Shepherd (2019) and Ngo *et al.* (2019b) highly recommended that the Japanese species be also included in the CITES Appendices. During the time of writing, the Japanese Ministry for Environment announced to list all six endemic species of *Goniurosaurus* from Japan in CITES Appendix III to prevent over-harvesting of wild animals for trafficking activities. As a result, the listing of all Japanese species in Appendix III has come into force since 14 February 2021 (CITES Notification No. 2020/068).

Acknowledgments

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Supplementary files

Supp. file 1. Supplementary tables. https://doi.org/10.5852/ejt.2021.751.1379.4325

Table S1. Pair-wise genetic divergence between species included in this study.
Table S2. Morphological (minimum–maximum (mean ± standard deviation)) and meristic characters (minimum–maximum (number of specimens)) of four tiger geckos in Vietnam (except *Goniurosaurus araneus*). Length given in mm.

Supp. file 2. Supplementary figures. https://doi.org/10.5852/ejt.2021.751.1379.4327

Fig. S1. Snout-vent length (SVL) of four tiger geckos in Vietnam including *Goniurosaurus catbaensis*, *Goniurosaurus huuliensis*, *Goniurosaurus lichtenfelderi*, *Goniurosaurus luii.* A. Juveniles. B. Adult males. C. Adult females.
Fig. S2. A. Principal component analysis (PCA) on morphological variation amongst four recorded tiger geckos in Vietnam. B. Scatterplots of principal component scores for the first and second principal axes.

1.2. Phylogeny and evolution of habitat preference in *Goniurosaurus* (Squamata: Eublepharidae) and their correlation with karst and granite-stream-adapted ecomorphologies in species groups from Vietnam

The author of the thesis provided genetic and morphological data, prepared some figures and corrected the content of the paper. Phylogenetic analyses and writing the paper were mostly implemented by the first author (Prof. Dr. Lee L. Grismer).

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Phylogeny and evolution of habitat preference in *Goniurosaurus* (Squamata: Eublepharidae) and their correlation with karst and granite-stream-adapted ecomorphologies in species groups from Vietnam

L. Lee Grismer, Hai Ngoc Ngo, Shuo Qi, Ying-Yong Wang, Minh Duc Le, Thomas Ziegler

Abstract

Maximum likelihood (ML) and Bayesian inference (BI) analyses using two mitochondrial (16S and cyt b) and two nuclear (CMOS and RAG1) genes and 103 specimens recovered the first phylogenies of all 23 extant species of *Goniurosaurus*. The analyses strongly supported the recognition of four monophyletic species groups with identical inter-specific relationships within the kuroiwae, lichtenfelderi, and yingdeensis groups but discordant topologies at some nodes within the luii group. Both analyses recovered a polyphyletic *G. luii* with respect to *G. kadoorieorum*, and owing to the lack of diagnostic characters in the latter, it is considered a junior synonym of *G. luii*. A stochastic character mapping analysis of karst versus non-karst habitat preference suggested that karstic landscapes may have played a major role in the evolution and diversification of *Goniurosaurus*. A karst habitat preference is marginally supported as the most probable ancestral condition for *Goniurosaurus* as well as for the kuroiwae, luii, and yingdeensis groups. However, a non-karst habitat preference is marginally supported as the most probable ancestral condition for the lichtenfelderi group. Multivariate and univariate ecomorphological analyses of the karst-adapted *G. catbaensis, G. huuliensis*, and *G. luii* of the luii group and the granite-stream-adapted *G. lichtenfelderi* of the lichtenfelderi group demonstrated that their markedly statistically different body shapes may be an adaptive response that contributes to habitat partitioning in areas of northern Vietnam where they are nearly sympatric.

Keywords. Asia, stochastic character mapping, systematics, synonymy, tiger geckos.

1.3. Extraordinary species richness of *Goniurosaurus* genus in allopatry: Understanding niche evolution and the need of conservation measures

The author of the thesis, Dr. Dennis Rödder and Prof. Dr. Lee L. Grismer mainly contributed to the planning of the study. Data was collected by the author and Shuo Qi. Scripts for models in the R software were provided by Dr. Dennis Rödder and Prof. Dr. Lee Grismer. Data analyses, preparation of graphics and the writing of the manuscript draft were mainly carried out by the author of the thesis.

Ngo N.H., Grismer L., Nguyen Q.T., Le D.M., Qi S., Rödder D. and Ziegler T. (Manuscript) Extraordinary species richness of *Goniurosaurus* genus in allopatry: Understanding niche evolution and the need of conservation measures.

Extraordinary species richness of *Goniurosaurus* genus in allopatry: Understanding niche evolution and the need of conservation measures.

Hai Ngoc Ngo, Lee Grismer, Truong Quang Nguyen, Minh Duc Le, Shuo Qi, Dennis Rödder, Thomas Ziegler

Abstract

Global biodiversity crisis is identified with a dominant cause of extremely high rates of extinction, especially for reptiles due to anthropogenic impacts. In addition to typical criteria in the IUCN Red List to evaluate the status of threatened species, the rate of niche evolution can help to estimate the level of ecological flexibility and adaptive capacity of species to environmental shifts. Highly restricted-range endemism in disjunct karst ecosystems, tiger geckos of the genus *Goniurosaurus* offer a unique opportunity to investigate mechanisms of species radiation and allopatric evolution. Dated phylogenetic reconstruction revealed that the first cladogenesis of *Goniurosaurus* ancestor took place during the Eocene (~ 45.3 mya) and continued to radiate across East Asia until the Pliocene. Using ecological niche modeling (ENM) approaches, we reconstructed the history of niche occupancy and calculated the rate of climatic niche evolution by estimations of the phylogenetic signal during cladogenesis within the genus *Goniurosaurus* from China and Vietnam. Our results provide evidence of both phylogenetic niche conservatism and divergence among Chinese and Vietnamese *Goniurosaurus* species. Coupled with estimations of ancestral ranges and habitats, we thereby suggest related hypotheses that shaped the extraordinary richness of *Goniurosaurus* and their allopatric distribution. Following the pattern of niche conservatism, range-restricted *Goniurosaurus* species, which are identified to be likely more susceptible to environmental alteration, should be received higher priorities of conservation attention and protection.

Keywords. Cladogenesis, conservatism, divergence, environmental shifts, phylogenetic signal, radiation, Tiger geckos.

Chapter 2. Autecology and population status

2.1. First record of the Cat Ba Tiger Gecko, *Goniurosaurus catbaensis*, from Ha Long Bay, Quang Ninh Province, Vietnam: microhabitat selection, potential distribution, and evidence of threats

The author of the thesis, Prof. Dr. Thomas Ziegler and Prof. Dr. Truong Q. Nguyen mainly contributed to the planning of the study. Data collection, analyses, preparation of graphics and the writing of the paper were mainly carried out by the author of the thesis. Species distribution models were implemented by Tuan Quang Le.

Ngo N.H., Le Q.T., Nguyen Q.T., Pham L.M., Le D.M., van Schingen M. and Ziegler T. (2019) First record of the Cat Ba Tiger Gecko, *Goniurosaurus catbaensis*, from Ha Long Bay, Quang Ninh Province, Vietnam: Microhabitat selection, potential distribution, and evidence of threats. Amphibian & Reptile Conservation 13(2): 1–13.

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First record of the Cat Ba Tiger Gecko, *Goniurosaurus catbaensis*, from Ha Long Bay, Quang Ninh Province, Vietnam: microhabitat selection, potential distribution, and evidence of threats

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***Abstract.*—The Cat Ba Tiger Gecko (*Goniurosaurus catbaensis*) was described from Cat Ba Island, Hai Phong, northern Vietnam in 2008, while a presumed congener was recently spotted from another offshore island in the Ha Long Bay. During the field surveys reported here, new *Goniurosaurus* occurrences were discovered for the first time on small offshore islands in the Ha Long Bay, Quang Ninh Province. These were identified and confirmed as *G. catbaensis* based on morphological and molecular data. However, these newly found populations are very small and exposed to increasing anthropogenic pressures. Since knowledge about the species ecology remains poor, the first microhabitat characterization for *G. catbaensis* is provided herein, which is essential for conservation of the species as well as its natural habitats. Sex- and age-related differences in selection of perch height are herein presented. In addition, we present evidence for various anthropogenic threats such as regular trade in living tiger geckos (including *G. catbaensis*) on local markets in Hai Phong and Ho Chi Minh cities, Vietnam. These findings highlight the need for more stringent conservation measures to reduce human impacts on the extremely small, insular populations of the Cat Ba Tiger Gecko.**

Key words. Anthropogenic pressure, conservation, ecology, offshore islands, phylogram, trade

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Introduction

The genus *Goniurosaurus* currently comprises 19 species with a disjunct distribution in southern East Asia. Most *Goniurosaurus* species are endemic with restricted distribution ranges (Chen et al. 2014; Grismer et al. 1994, 1999; Honda and Ota 2017; Seufer et al. 2005; Yang and Chan 2015; Zhou et al. 2018; Ziegler et al. 2008). Habitat degradation and overharvesting for the pet trade were identified as major threats to wild populations of tiger geckos (Yang and Chan 2015). At present, five species of *Goniurosaurus* are known from Vietnam, namely *G. araneus*, *G. catbaensis*, *G. huuliensis*, *G. lichtenfelderi*, and *G. luii* (Nguyen et al. 2009). Among these species, the insular Cat Ba Tiger Gecko (*Goniurosaurus catbaensis*) was discovered on Cat Ba Island in Cat Hai District, Hai Phong City, northeastern Vietnam, where it was assumed to be endemic (Ziegler et al. 2008). Preliminary population assessments of *G. catbaensis* revealed that its effective population size, defined as number of mature individuals, is much smaller than the suggested threshold values for minimal viable populations to maintain a stable population in the long term (Ngo et al. 2016; Nguyen et al. 2016, 2018; Reed et al. 2003; Traill et al. 2007).

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Fig. 1. New population. **(A)** Habitat of *Goniurosaurus catbaensis* on one offshore island in Ha Long Bay, Quang Ninh Province; **(B)** Microhabitat of *G. catbaensis* in Ha Long Bay; **(C)** Adult male; and **(D)** Adult female from Ha Long Bay. *Photos: H.N. Ngo.*

Even in undisturbed habitats, *G. catbaensis* occurs at low densities (Ngo et al. 2016; Nguyen et al. 2016, 2018).

The insular Cat Ba Tiger Gecko was found to be vulnerable to anthropogenic disturbances, and of high demand in pet markets as well as on Internet platforms (Ngo et al. 2016; Nguyen et al. 2018). In addition to poaching, habitat destruction for touristic purposes has dramatically increased the pressure on the wild *G. catbaensis* population. Consequently, the need for protection of the Cat Ba Tiger Gecko has received growing attention. Based on the first international population and trade investigations, this species has recently been listed in the IUCN Red List of Threatened Species as "Endangered" (Nguyen et al. 2016). The wild population is probably in peril due to its restricted distribution range, rising anthropogenic threats, and the lack of appropriate conservation measures. For the latter, detailed information on habitat requirements and the exact distribution of this species is essential, but such data are currently lacking. Ngo et al. (2016) recently suggested the potential occurrence of *G. catbaensis* on at least one more offshore island in Ha Long Bay.

To confirm this possibility, we investigated other small offshore islands in Ha Long Bay, Quang Ninh Province to locate populations of *G. catbaensis*, and predicted the overall availability of suitable habitats for the species in northeast Vietnam. In addition, the present study aimed to provide the first data on microhabitat selection of *G. catbaensis*. We assumed that differences in habitat use would occur between age classes and sexes, as they have been observed in other lizards (Snyder et al. 2010; van Schingen et al. 2015).

Materials and Methods

Study areas: Study sites were selected based on our previous surveys on Cat Ba Island, Hai Phong City, and on photo documentation which gave evidence for the possible occurrence of *Goniurosaurus* on a small island in Ha Long Bay, Quang Ninh Province (Ngo et al. 2016). Cat Ba Island and adjacent islands comprise isolated limestone karst formations, which provide diverse habitats for a unique flora and fauna (Clements et al. 2006). Cat Ba Archipelago was recognized as the "Cat Ba Archipelago Biosphere Reserve" (CBBR) by the United Nations Educational, Scientific and Cultural Organization (UNESCO) in 2004 due to its significant ecosystem and biodiversity values (CBBR Authority 2013). Ha Long Bay was also twice recognized (in 1994 and 2000) by UNESCO as a World Heritage Site for the outstanding universal value of its landscape, geology, and geomorphology (The Management Department of Ha Long Bay 2014). Both areas are among the most popular tourist destinations in Vietnam, and face challenges from rapid tourism development.

Field surveys: Field surveys were conducted on Cat Ba Island between June and August 2014, May 2015, and during a short time in June 2016, which fell in the non-hibernation season of *Goniurosaurus* (Grismer et al. 1999; Ngo et al. 2016). Furthermore, six offshore islands in Ha Long Bay, situated in close proximity to Cat Ba Archipelago, were surveyed in July 2016. Night excursions were conducted between 7:30 and 11:30 PM, when the lizards were found to be active (Ngo et al. 2016; Ziegler et al. 2008). To measure morphological characters, the animals were captured by hand and subsequently released at the same spot after checking and taking measurements.

Ecological analyses: Microhabitat data were recorded for each sighted *G. catbaensis*, including substrate types (classified as cliff, rock, branch, sand, or forest floor), perch height (vertical distance between captured animal and ground, in cm), percentage of vegetation or cave coverage, position (resting outside or inside cave), substrate surface condition (dry or wet), and activity (resting, feeding, or foraging). Air temperature and relative humidity were measured with a digital thermo-hygrometer (TFA Dostmann/Wertheim Kat. Nr. 30.5015), and substrate temperature and body surface temperature of animals were measured with an infrared thermometer (Measupro IRT20).

To identify intraspecific differences in microhabitat selection by *G. catbaensis*, individuals were classified into different age classes according to their snout-vent lengths (SVL): SVL < 85 mm = juvenile, SVL ≥ 85 mm and < 105 mm = sub-adult, and SVL ≥ 105 mm = adult (Ngo et al. 2016). Adults were differentiated between the sexes, as well as between gravid and non-gravid individuals. Sex of specimens was determined by the presence of the large swollen hemipenal bulges in males, while non-swollen in females.

A *t*-test, with $\alpha = 0.05$, was performed to determine differences in microhabitat parameters between age classes and sexes. Statistical analyses were performed with the program PAST, Version 2.17c (Hammer et al. 2001).

Morphological analyses: Morphometric measurements of captured individuals were taken with a digital caliper to the nearest 0.1 mm. In addition, two voucher specimens of the newly discovered populations in Ha Long Bay were collected, euthanized with ethylacetate, preserved in 70% ethanol, and deposited in the collections of the Vietnam National Museum of Nature (VNMN), Hanoi, Vietnam (VNMN 05423, VNMN 05424). Morphological characters were taken according to Ngo et al. (2016), Orlov et al. (2008), Yang and Chan (2015), and Ziegler et al. (2008).

Abbreviations of measurements are as follows: snout vent length (SVL) from tip of snout to vent; tail length (TaL) from vent to tip of tail; distance between axilla and groin (AG) from posterior edge of forelimb insertion to anterior edge of hind limb insertion; forelimb length (FoL) from axilla to tip of longest finger; hindlimb length (HiL) from groin to tip of longest finger; snout to eye distance (SE) from tip of snout to anterior-most point of eye; eye to ear distance (EE) from posterior margin of eye to posterior margin of ear; orbital diameter (OD) greatest diameter of orbit; ear diameter (ED) longest dimension of ear; internarial distance (IND) as distance between nares; anterior eye distance (AED) as distance between anterior corners of eyelids; posterior eye distance (PED) as distance between posterior corners of eyelids; maximum head width (HW); maximum head height (HH); head length (HL) from tip of snout to posterior edge of occiput; pileus length (PL) from tip of snout to posterior scale of the head; and jaw length (JL).

Abbreviations of scalation are as follows: supralabials (SPL); infralabials (IFL); nasal scales surrounding nare (N); internasals (IN); gular scales bordering the internasals (PostIN); postmentals (PM); gular scales bordering the postmentals (GP); eyelid fringe scales or ciliaria (CIL); granular scales surrounding dorsal tubercles (GST); dorsal tubercle rows at midbody (DTR); paravertebral tubercles between limb insertions (TL); scales around midbody (MB); subdigital lamellae under the first finger (LF1) and the fourth finger (LF4); subdigital lamellae under the first toe (LT1) and the fourth toe (LT4); precloacal pores (PP); and postcloacal tubercles (PAT).

Molecular analyses: To confirm the taxonomic status of the newly collected *Goniurosaurus* from Ha Long Bay, Quang Ninh Province, a fragment of the mitochondrial 16S ribosomal gene was amplified, using the primer pair 16Sar and 16Sbr (Palumbi et al. 1991), for three samples (VNMN 05424 plus two small tissue samples from two released individuals, field numbers G8 and G12). Tissue samples were taken from the tail tips, which were disinfected before immediate release of the animals at the site of capture. DNA was extracted from tissue samples using the DNeasy blood and tissue kit, Qiagen (Redwood City, CA). The extracted DNA from the fresh tissue samples were amplified by PCR, with the PCR volume (21µl) consisting of 10 µl of mastermix (Fermentas, Canada), 5 µl of water, 2 µl of each primer at 10 pmol/µl, and 2 µl of DNA. The PCR conditions were: 95 °C for five minutes to activate the taq; with 40 cycles at 95 °C for 30s, 50 °C for 45s, 72 °C for 60s; and the final extension at 72 °C for six minutes (Ngo et al. 2016).

PCR products were subjected to electrophoresis through a 1% agarose gel (UltraPure™, Invitrogen). Gels were stained for 10 minutes in 1x TBE buffer at 2 pg/ml of ethidium-bromide, and visualized under UV light. Successful amplifications were purified to eliminate PCR components using GeneJET™ PCR Purifcation Kit (Fermentas, Canada). Purified PCR products were sent to 1st Base (Selangor, Malaysia) for sequencing. Sequences were edited using the program Geneious v.7.1.8 (Kearse et al. 2012). After sequences were aligned using Clustal X v2 (Thompson et al. 1997), data were analyzed by Bayesian inference as implemented in MrBayes v3.2 (Ronquist et al. 2012). Settings for these analyses followed Le et al. (2006), except that the number of generations in the Bayesian analysis was increased to 1×10^7. The optimal model for nucleotide evolution was set to GTR+I+G as selected by Modeltest v3.7 (Posada and Crandall 1998). The cutoff point for the burn-in function was set to 13 in the Bayesian analysis, as -lnL scores reached stationarity after 13,000 generations in both runs. Nodal support was evaluated using posterior probability in MrBayes v3.2. Uncorrected pairwise divergences were calculated in PAUP*4.0b10 (Swofford 2001).

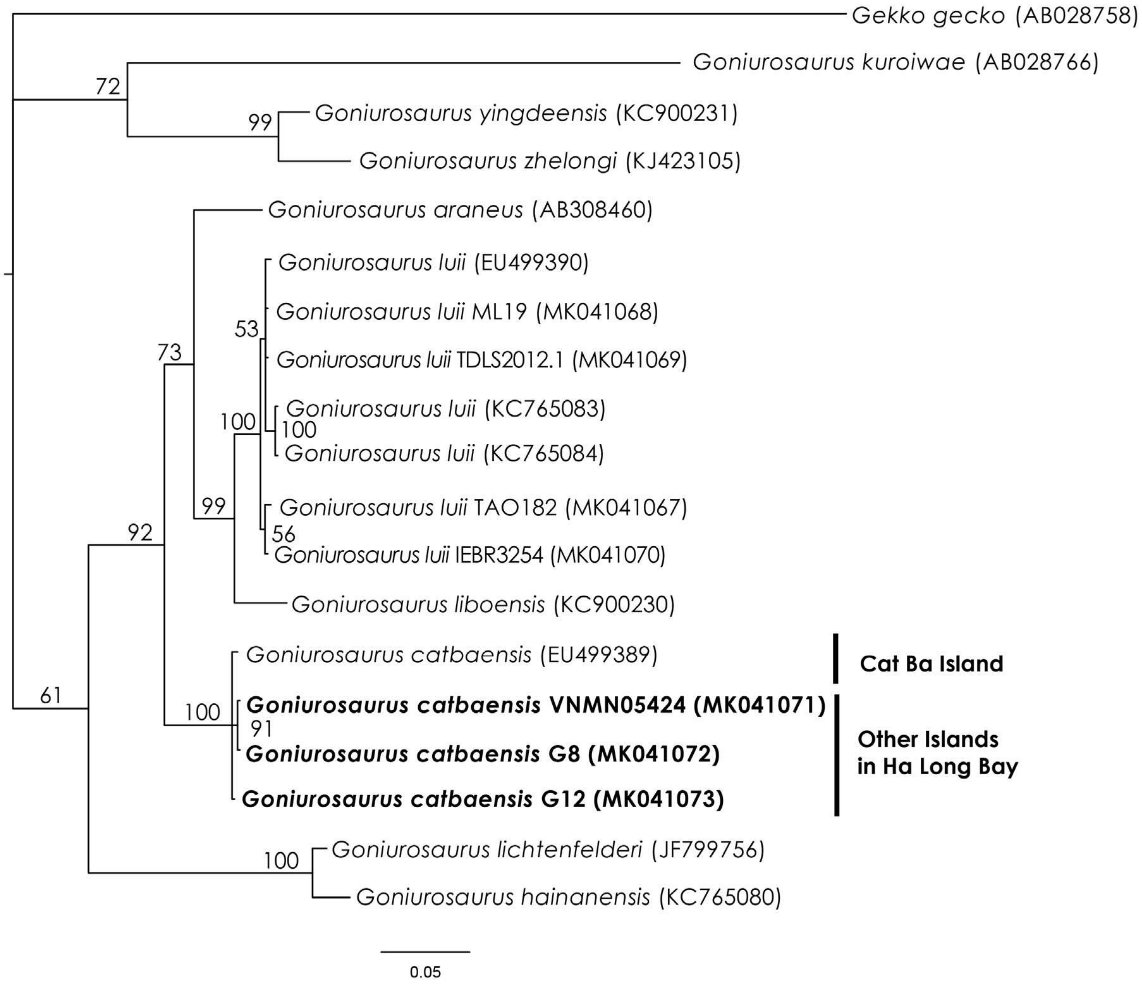

Fig. 2. Phylogram of *Goniurosaurus* based on the Bayesian analysis of a 16S ribosomal fragment. Numbers next to nodes are Bayesian posterior probabilities. Voucher numbers of new samples and GenBank accession numbers are placed after species names and in parentheses, respectively.

Species distribution models (SDMs): Based on occurrence records and a set of 19 environmental factors, the current overall availability of suitable habitats for *G. catbaensis* were predicted using the program Maxent Version 3.3.3.e (Beaumont et al. 2005; Phillips et al. 2006). Only the most distant occurrences of each site were included in the analyses to minimize effects of spatial autocorrelation and to ensure the independence of the records (Jennings and Veron 2011; Jennings et al. 2013). As a result, 11 records were filtered from a total of 60 localities of *G. catbaensis* on Cat Ba Island and Ha Long Bay. Nineteen bioclimatic variables that were obtained from the WorldClim global climate database (http://www.worldclim.org, accessed September 2016; Hijmans et al. 2005; Table 1) were used as environmental predictors.

Threat records: To get a first impression of trade in *Goniurosaurus* species in Vietnam, local pet markets were visited in Hai Phong and Ho Chi Minh cities, the two most important trade centers in the country, and different Internet platforms were investigated. Two local dealers from Ho Chi Minh City offering *Goniurosaurus* online were interviewed in September 2016, in order to trace the source of the traded *Goniurosaurus* species in Vietnam. Additionally, five fishermen from the Ha Long Bay were interviewed to identify caves used by tourism companies for night parties, and determine the general attitude and use of the species in Ha Long Bay. Those sites located within the World Heritage Site were subsequently surveyed in July 2016 to evaluate potential threats from tourism activities. The names of interviewees are kept anonymous to ensure data privacy rights and Internet links are not disclosed to prevent misuse. Accurate locality data, cave names, and prices are also not presented to prevent targeted poaching for the wildlife trade.

Results

New records of *Goniurosaurus catbaensis*: During the present study, new *Goniurosaurus* occurrences were discovered on four small offshore islands, including two tourism caves in Ha Long Bay, Quang Ninh Province. The distances between these islands ranged from 1.4 km to 13 km, while the shortest distance between Cat Ba Island and one surveyed island in Ha Long Bay was 1.2 km. A total of 14 individuals (eight males, four females, one juvenile, and one unsexed individual which was only photographed) were recorded on these islands, which

Table 1. Bioclimatic variables from environmental data (Source: http://www.worldclim.org, accessed September 2016).

No.	Bioclimatic variables from the WorldClim dataset
1	BIO1 = “Annual Mean Temperature”
2	BIO2 = “Mean Diurnal Range" (Mean of monthly [max temp - min temp])
3	BIO3 = "Isothermality" (P2/P7) (*100)
4	BIO4 = "Temperature Seasonality" (standard deviation *100)
5	BIO5 = "Max Temperature of Warmest Month"
6	BIO6 = "Min Temperature of Coldest Month"
7	BIO7 = "Temperature Annual Range" (P5–P6)
8	BIO8 = "Mean Temperature of Wettest Quarter"
9	BIO9 = "Mean Temperature of Driest Quarter"
10	BIO10 = "Mean Temperature of Warmest Quarter"
11	BIO11 = "Mean Temperature of Coldest Quarter"
12	BIO12 = "Annual Precipitation (year)"
13	BIO13 = "Precipitation of Wettest Month"
14	BIO14 = "Precipitation of Driest Month"
15	BIO15 = "Precipitation Seasonality" (Coefficient of Variation)
16	BIO16 = "Precipitation of Wettest Quarter"
17	BIO17 = "Precipitation of Driest Quarter"
18	BIO18 = "Precipitation of Warmest Quarter"
19	BIO19 = "Precipitation of Coldest Quarter"

ranged between 0.34 and 2.94 km^2 in size.

Molecular analysis using Bayesian inference of the obtained matrix containing 613 aligned characters showed that all samples from Cat Ba Island (n = 1) and from the most distant other islands in Ha Long Bay (n = 3) clustered in a single clade with strong statistical support (posterior probability = 100%, Fig. 2). Genetic analyses revealed that sequences of the new records from Ha Long Bay, Quang Ninh Province, were identical to each other and virtually the same (99% to 100%) as that of the holotype of *G. catbaensis* from Cat Ba Island (GenBank accession number: EU499389). The maximum genetic divergence between the samples is approximately 0.3%, whereas the lowest divergence between two species of this genus, i.e., *G. hainanensis* and *G. lichtenfelderi*, is approximately 2.3% (Table 2). These results confirmed the newly recorded *Goniurosaurus* populations in Ha Long Bay are conspecific with *G. catbaensis* from Cat Ba Island (Fig. 2).

In addition, the morphological characters of the newly recorded *G. catbaensis* from Ha Long Bay accorded well with the population from Cat Ba Island, except that three of six individuals from a single site in Ha Long Bay showed a postrostral (internasal) scale. This character is consistently lacking in individuals recorded so far from Cat Ba Island (Ziegler et al. 2008) [Fig. 3A, 3B; Table 3].

Microhabitat selection: A total of 61 sightings took place (13 from smaller islands in the Ha Long Bay, and 48 from Cat Ba Island). *Goniurosaurus catbaensis* was active in the surroundings of large limestone caves covered in part by primary forest vegetation and in the vicinity of primary shrub vegetation on limestone. Mean air temperatures were 28.1 ± 1.7 °C (21.5–31.3 °C, n =

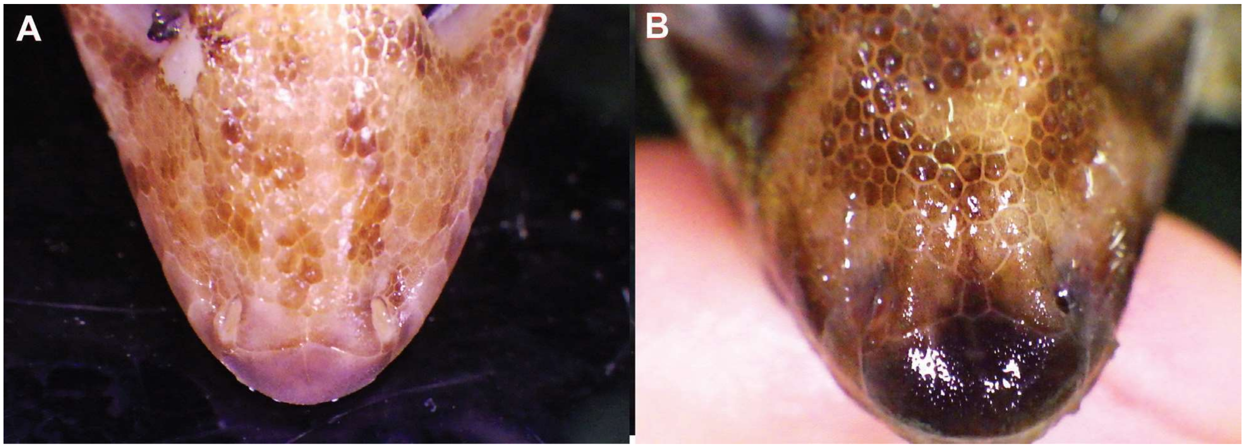

Fig. 3. Absence versus occasional presence of internasal scales of *Goniurosaurus catbaensis* from **(A)** Cat Ba Island and **(B)** Ha Long Bay. *Photos H.N. Ngo.*

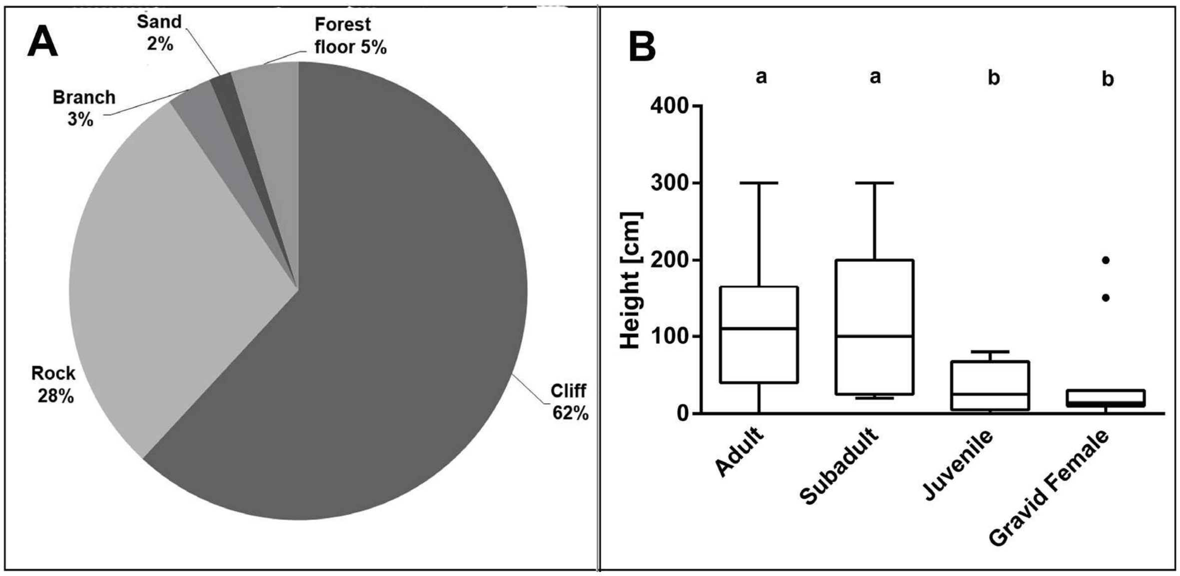

Fig. 4. (A) Substrate selection of *Goniurosaurus catbaensis*. **(B)** Box plots of perch heights of different age classes and sexes.

59) slightly higher than mean substrate temperatures of 26.02 ± 1.5 °C (22.2–28.2 °C, n = 28, Table 4). Recorded relative humidity at microsites ranged between 70–99% (mean 84.9 ± 6.99%, n = 52).

A vast majority of lizards was found on limestone cliffs (62%), followed by rocks (28%), while only a few specimens were found on the forest floor (5%), branches (3%), or sand (2%) [Fig. 4A]. A significantly lower number of lizards was encountered inside compared to outside of limestone caves (26.9% vs. 73.1%, respectively). *Goniurosaurus catbaensis* selected spots with a mean canopy coverage of 95.2 ± 9.6% (n = 63, Table 4). Adult specimens (non-gravid) were found at average heights of 1.15 m (n = 38), while juveniles and gravid females resided at significantly lower heights of 0.28 m (n = 4) and 0.41 m (n = 12), respectively ($t = 2.82$, df = 48, $P < 0.05$; $t = 2.06$, df = 40, $P < 0.05$, Fig. 4B). A majority (about 77.4%, n = 48) of lizards was resting during the surveys, while only a few individuals (n = 14) were found actively foraging.

Suitable habitats for *G. catbaensis* were predicted to encompass a majority of small islands belonging to Cat Ba Island and Ha Long Bay, and include a wider area on the coastal mainland of Quang Ninh Province, where no surveys have been conducted so far (Fig. 5).

Trade: Trade in living tiger geckos has been frequently recorded by our team in local pet markets from Hai Phong and Ho Chi Minh cities, as well as on Facebook since 2015. Interviews with two local traders in Ho Chi Minh City revealed that they pay for local villagers living within the species' distribution range to collect live tiger geckos during the non-hibernation season, confirming the wild (rather than captive-bred) source of traded animals. The dealers reportedly received individuals of three tiger gecko species, namely *G. huuliensis*, *G. luii*, and *G. catbaensis,* collected in April 2015. Among those, three individuals of *G. huuliensis* (one male and two females) were allegedly collected by a local hunter from Huu Lien Nature Reserve, Lang Son Province. Two local collectors from Cao Bang Province reportedly collected six individuals (three males and three females) of *G. luii* in northern Vietnam and another local hunter collected two couples of *G. catbaensis*. These 13 wild caught tiger geckos were transferred to pet markets in Ho Chi Minh City, southern Vietnam, in April 2015.

Human impacts on the habitat: Tourism activities in the region have dramatically increased in the past, and likely exerted enormous pressure on wild *G. catbaensis* populations. Events organized by tourism companies

Table 2. Uncorrected ("p") distance matrix showing percentage pairwise genetic divergence (16S) between members of *Goniurosaurus.*

Species name	1	2	3	4	5	6	7	8	9
1. *G. araneus*	–								
2. *G. catbaensis*	6.4–6.7	–							
3. *G. hainanensis*	13.7	12.4–12.8	–						
4. *G. kuroiwae*	20.4	19.5–19.8	19.3	–					
5. *G. liboensis*	6.3	6.6-6.8	12.8	21.9	–				
6. *G. lichtenfelderi*	12.9	11.2–11.6	2.3	18.8	13.3	–			
7. *G. luii*	5.6–6.2	6.2–7.1	12.2–12.9	20.0–20.4	3.4–3.8	11.5–13.5	–		
8. *G. yingdeensis*	14.8	13.4–13.5	15.2	18.8	13.0	15.2	13.3–13.5	–	
9. *G. zhelongi*	15.3	14.2–14.4	16.9	21.4	13.4	16.2	14.8–15.4	4.8	–

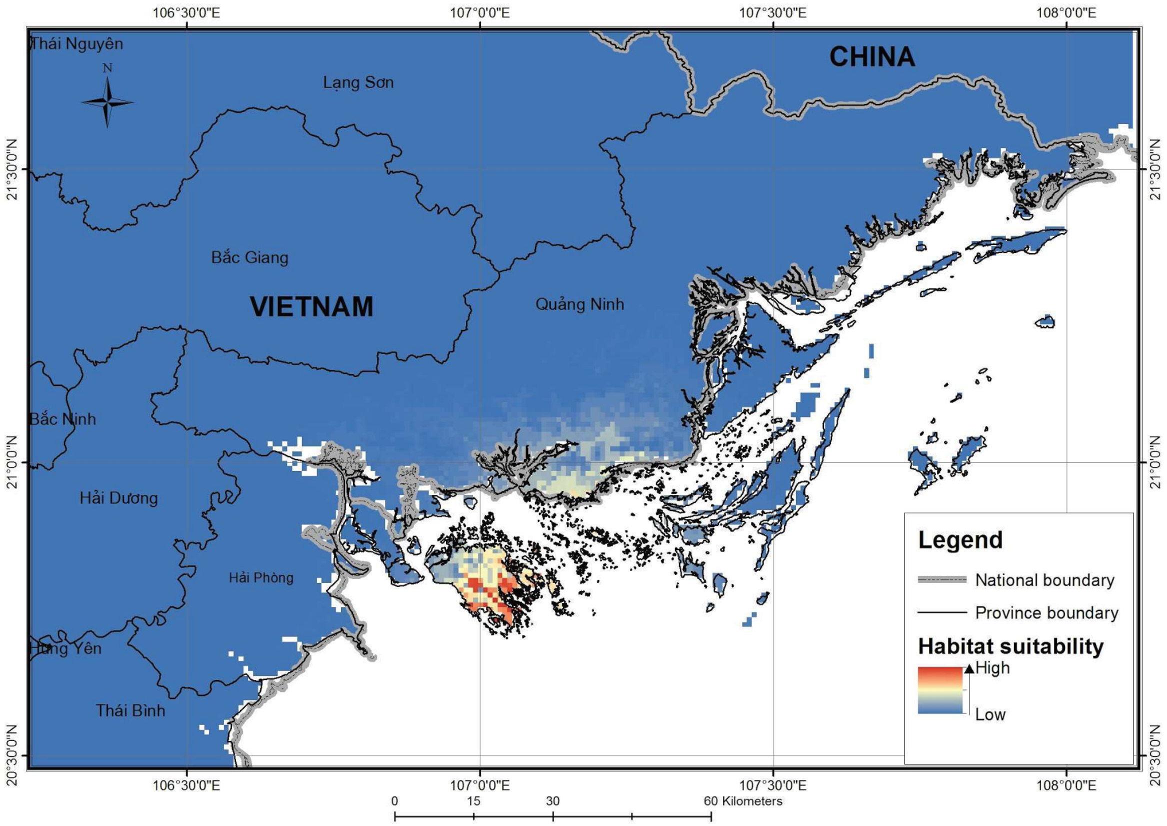

Fig. 5. Predicted habitat suitability for *Goniurosaurus catbaensis* in Vietnam.

regularly took place in at least two caves located within the UNESCO World Heritage Site. According to interviews with fishermen, daily excursions to the caves are scheduled to start at 7:30 PM and end around 11:00 PM. On these occasions, tourists dine in brightly lit caves before returning to their tour boats (Fig. 6B). As a consequence, wildlife is likely to be disturbed by the candle light, noisy sounds, and waste left by the tourists.

Discussion

New population records: Since its discovery in 2008, the Cat Ba Tiger Gecko was thought to be endemic to Cat Ba Island (Ziegler et al. 2008). These new records of *G.* cf. *catbaensis* on further offshore islands in Ha Long Bay confirmed for the first time the occurrence of the species outside its type locality. The newly recorded specimens showed an insignificant genetic divergence from the type series from Cat Ba Island and could be assigned to *G. catbaensis* (Table 2). Accordingly, the newly collected specimens from Ha Long Bay were also almost identical to the type series of *G. catbaensis* in morphology, except for the presence of a single internasal scale (which is absent in the type series from Cat Ba, see Ziegler et al. 2008) in a few individuals from a single site in Ha Long Bay. These findings indicated a slightly broader distribution range of the species than previously expected.

According to Li et al. (2010), the islands of Ha Long Bay and Cat Ba Archipelago were shaped by the erosion of limestone karst formations within the Gulf of Tonkin at the northern east coast of Vietnam after the coastal shelf region became inundated by marine waters about 13,000 years ago. Repeated falls (> 50 m) of the sea level during glaciations periodically connected various islands and the mainland, which allowed exchanges between island and mainland populations, as well as colonization and re-colonization between island and mainland populations (Li et al. 2010; Liang et al. 2018). Thus, past recurrent gene flow is assumed to have occurred between (sub)populations, which helped to maintain a classical island-mainland metapopulation—in accordance with the high genetic similarity between *G. catbaensis* (sub)populations from different islands with identical habitats (Hanski 1991; Harrison and Taylor 1997; Levins 1969). Orlov et al. (2008) confirmed that *G. lichtenfelderi* was found from both continental mainland and islands. On the other hand, Liang et al. (2018) suggested that *G. lichtenfelderi* diverged from *G. hainanensis* of Hainan Island to Vietnam (including both mainland and island populations), which might have occurred during the glacial periods with past dispersal events. The speciation in the diversification process of *Goniurosaurus* was probably promoted by the adaption to different microhabitats. Populations of *G. lichtenfelderi* were found on granite beds of valley streams, while the closely related *G. hainaensis* is found on igneous rocks and *G. catbaensis* occurs in karst forests (Orlov et al. 2008; Liang et al. 2018; Ziegler et al. 2008; Nguyen et al. 2018).

To avoid the misuse of distribution data for targeted harvesting of the species (e.g., Lindenmayer and Scheele

Table 3. Morphological characters of *Goniurosaurus* from Ha Long Bay, Quang Ninh Province, compared with *G. catbaensis* from Cat Ba Island, Hai Phong Province. Measurements are given in mm. Note: (*) n = 6; (a) n = 2.

Specimens	Ha Long Bay (current study, n = 13)	Cat Ba Island (current study, n = 48)	Cat Ba Island (Ziegler et al. 2008) [n = 4]
SVL	74.54–122.7 (111.2 ± 11.9)	69.2–130.4 (108.9 ± 12.6)	84.7–111.5 (102.4 ± 14.5)
TaL	10.1–97.6 (69.9 ± 27)	28.9–104.02 (78 ± 17.7)	52.5–101.5 (68.1 ± 27.6)
AG	33.9–60.2 (52.9 ± 6.5)	43.07–58.43 (48.4 ± 5.4)*	-
HL	21.3–33.8 (30.2 ± 2.9)	17.8–34.2 (29.8 ± 3.5)	23.1–30.6 (27.7 ± 4.1)
HW	14.4–24.56 (22.1 ± 2.5)	13.9–28.2 (21.9 ± 2.5)	16.2–21.6 (19.5 ± 2.9)
HH	7.1–14.9 (12.8 ± 1.9)	8.2–16.9 (12.4 ± 1.9)	10.1–14.3 (12.2 ± 2.0)
FoL	32.2–53.8 (50.4 ± 5.6)	29.7–54 (47.8 ± 4.7)	-
HiL	42–67.47 (60.1 ± 6.2)	36.2–65 (57.9 ± 5.99)	-
SE	8.7–13.4 (11.9 ± 1.1)	10.45–13.4 (12.1 ± 1.0)*	9.8–12.6 (11.5 ± 1.6)
EE	9.4–13.2 (10.8 ± 1.5)*	9.78–12.13 (11.1 ± 0.88)*	8.5–12.3 (10.6 ± 2.1)
OD	5.6–8.3 (7.5 ± 0.7)	6.1–8.95 (7.6 ± 1.1)*	-
ED	2.8–5.3 (4.01 ± 0.8)	2.8–4.3 (3.5 ± 0.5)*	-
IND	3.39–4.33 (3.9 ± 0.34)*	2.9–4.2 (3.7 ± 0.5)*	-
IOD	6.78–8.62 (7.98 ± 0.67)*	6.9–8.47 (7.5 ± 0.6)*	-
ION	11.8–15.03 (13.9 ± 1.23)*	11.9–15.1 (13.1 ± 1.3)*	-
JL	12.3–10.8 (18.1 ± 2.1)	15.5–19.5 (17.2 ± 1.4)*	-
PL	27.6–32.5 (29.9 ± 1.7)*	26.6–32.8 (29.2 ± 2.5)*	-
SVL:HL	3.5–3.8 (3.68 ± 0.1)	3.3–4.3 (3.7 ± 0.2)	3.61–3.67 (3.7 ± 0.05)
SVL:AG	1.9–2.3 (2.1 ± 0.1)	2.04–2.45 (2.3 ± 0.14)*	-
HL:HW	1.28–1.48 (1.37 ± 0.05)	1.1–1.5 (1.36 ± 0.09)	1.43–2.11 (1.6 ± 0.4)
HL:HH	2.3–3.01 (2.35 ± 1.6)	1.79–3.3 (2.4 ± 0.4)	2.29–2.43 (2.33 ± 0.07)
SE:EE	0.9–1.2 (1.1 ± 0.1)*	1.07–1.1 (1.09 ± 1.14)*	1.02–1.15 (1.09 ± 0.07)
SPL	9–10 (9.4 ± 0.5)*	8–11 (10.08 ± 1.1)*	8–9 (8.7 ± 0.5)
IFL	8–9 (9.75 ± 0.45)*	8–10 (8.8 ± 0.7)*	6–8 (7.8 ± 0.6)
N	5–6 (5.25 ± 0.5)[a]	6–8 (7 ± 0.47)*	5–6 (5.1 ± 0.4)
IN	0–1 (0.23 ± 0.4)	0	0
PostIN	0–2 (0.4 ± 0.77)	0	0
PM	2–3 (2.5 ± 0.7) [a]	2–3 (2.83 ± 0.41)*	2–3 (2.8 ± 0.5)
GP	7[a]	6–9 (7.8 ± 1.2)*	6–7 (7.22 ± 0.6)
CIL	45–49 (46.75 ± 1.7)[a]	41–56 (47.8 ± 4.4)*	52–55 (54.0 ± 1.1)
MB	104–109 (106.5 ± 3.5)[a]	102–109 (103.8 ± 3.8)*	112–127 (119.2 ± 7.6)
GST	9–12 (10.5 ± 1.3)[a]	9–14 (10.3 ± 1.6)*	8–11 (9.8 ± 1.6)
TL	35–37 (36 ± 1.4)[a]	27–34 (31.5 ± 3.0)*	33–34 (33.7 ± 0.6)
DTR	23[a]	19–25 (22.3 ± 1.97)*	23–25 (24.0 ± 1.2)
LF1	9–12 (10.25 ± 1.3)[a]	9–11 (10 ± 0.7)*	11–12 (11.75 ± 0.5)
LF4	18–19 (18.75 ± 0.5)[a]	19–20 (19.3 ± 0.5)*	18–19 (18.1 ± 0.5)
LT1	9–10 (9.75 ± 0.5)[a]	9–10 (9.91 ± 0.3)*	11–12 (11.4 ± 0.6)
LT4	24[a]	22–24 (23.4 ± 0.8)*	22–24 (23.4 ± 0.7)
PP	20–24 (22.5 ± 1.4)*	21*	5–21 (15.3 ± 2.5)
PAT	1–3 (2.25 ± 0.6)*	2–3 (2.5 ± 0.5)*	2–3 (2.8 ± 0.5)

2017; Stuart et al. 2006; Yang and Chan 2015), detailed locality information of the new records is being withheld. According to the SDMs *G. catbaensis* is predicted to occur on other, similar islands in the Gulf of Tonkin, but is still endemic to Ha Long Bay and Cat Ba Archipelago. According to Orlov et al. (2008) the type locality of *G. lichtenfelderi* is an offshore island in Bai Tu Long Archipelago, which is contiguous with Ha Long Bay

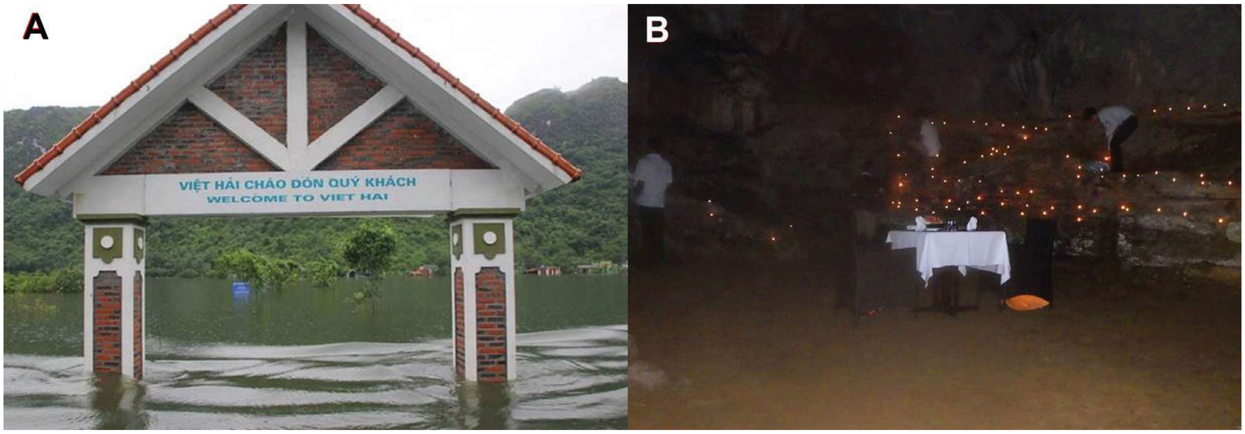

Fig. 6. Potential threats to *Goniurosaurus*. **(A)** Flooding of Viet Hai Commune in August 2015. **(B)** Tourist event in a cave within the UNESCO World Heritage Site on Ha Long Bay. *Photos H.N. Ngo.*

in the Gulf of Tonkin. However, extensive field surveys have failed to record any individual of *G. catbaensis*, occurring in syntopy with *G. lichtenfelderi* (Gawor et al. 2016; Nguyen et al. 2011; Orlov et al. 2008). The habitat of *G. lichtenfelderi* in Bai Tu Long was described as valleys of forest streams on granite rocks within mixed forests of bamboo and broad-leaved trees (Gawor et al. 2016; Nguyen et al. 2009; Nguyen et al. 2011; Orlov et al. 2008; Ziegler et al. 2008), while *G. catbaensis* was found only in limestone karst ecosystems present in Ha Long and Cat Ba archipelagos. Accordingly, our SDMs predicted the potential distribution of *G. catbaensis* to encompass Ha Long Bay and Cat Ba Archipelago, but excluding Bai Tu Long Archipelago (Fig. 5). However, the present SDMs also predicted the mainland area including limestone formations around Ha Long City to be suitable for *G. catbaensis.* Thus, it will be important to search for further occurrences at these predicted sites in order to determine the exact distribution boundaries, and to assess genetic diversity of potentially new populations.

Microhabitat selection: Both sex- and age-related perch selection were found in *G. catbaensis*, namely differences in perch heights. Specifically, juveniles and gravid females occurred at significantly lower heights than subadults and adults. Similar habitat divergences between juveniles and adult individuals have been reported for Crocodile Lizards in Vietnam (van Schingen et al. 2015), and gekkonids in New Caledonia (Snyder et al. 2010).

This study also revealed that the body surface temperature of *G. catbaensis* showed a highly positive correlation with the air temperature ($r_s = 0.56$; $P < 0.05$, n = 23) and substrate temperature ($r_s = 0.66$; $P < 0.001$, n = 26). Thus, as in other ectotherms, basic physiological functions of *G. catbaensis*, such as locomotion, growth, and reproduction are determined by the environmental temperature. Since tropical lizards are considered to have narrow temperature optima, and only few options for behavioral and physiological compensation, they are assumed to be especially vulnerable to extinction by climate warming (Deutsch et al. 2008; Doody and Moore 2010; Huey et al. 2009; Vié et al. 2009). In particular, body surface temperatures of *G. catbaensis* ranged from between 23.6 and 30.6 °C (mean = 27.2 ± 1.6 °C, n = 26) and were comparably higher than those of *G. kuroiwae* with average skin surface temperatures of 16.6 °C in the humid subtropical Oriental forest (Werner et al. 2005).

Potential threats and recommendations for conservation: Due to the restricted distribution range, low densities, and estimated global population being much lower than suggested threshold values for minimal viable populations, the Cat Ba Tiger Gecko is expected to be especially endangered to unsustainable for harvest (Ngo et al. 2016). Consequently, the species was recently assessed and ranked by the IUCN Red List of Threatened Species as "Endangered" (Nguyen et al. 2016). Other members of the genus *Goniurosaurus* from Vietnam have not been considered for inclusion on the IUCN Red List yet, as data on their population statuses are currently lacking. The findings reported here indicate that not only *G. catbaensis,* but also *G. huuliensis* and *G. luii*, are subject to intensive collection for local trade and provide concrete evidence for the wild source of the respective specimens. It is likely that the reported

Table 4. Environmental parameters characterizing the microhabitat selection of *Goniurosaurus catbaensis.*

Parameter	Number of sightings (n)	Min	Max	Mean ± SD
Canopy cover [%]	63	50	100	95.2 ± 9.6
Height [m]	54	0	3	0.97 ± 0.86
Elevation [m asl]	60	4	132	46.2 ± 32.9
Air Temperature [°C]	59	21.5	31.3	28.1 ± 1.7
Substrate Temperature [°C]	28	22.2	28.2	26.02 ± 1.5
Relative air Humidity [%]	52	70	99	84.9 ± 6.99

cases only reflect a small proportion of illegal harvesting activities. Since over-exploitation of local populations of range-restricted lizard species has been repeatedly found to rapidly cause extinction (e.g., Auliya et al. 2016; Stuart et al. 2006; Yang and Chang 2015), further research on the population status, distribution, ecology, and availability of suitable microhabitat sites is critically needed. The results of such studies may lead to the elevation or determination of the conservation status of other tiger gecko species and provide critical scientific data for future captive breeding programs. To reduce poaching and to control the trade in wild *Goniurosaurus*, we recommend continued monitoring of the scales and patterns of trade in combination with aforementioned population assessments. We also strongly advise against providing exact locality information for new *Goniurosaurus* populations in future publications, as this action might increase poaching activities at respective sites (Lindenmayer and Scheele 2017; Stuart et al. 2006; Yang and Chan 2015).

In addition to the illegal collection of animals, human impacts on habitats have dramatically increased by means of expanding tourism activities (see also Ngo et al. 2016). Tourism events in caves, causing disturbance by candle light, noisy sounds, and waste might result in the extirpation of *G. catbaensis* within these limestone caves. We suggest that tourism companies should hold such events only on their boats to reduce disturbances in the cave habitats of *G. catbaensis*, or at least restrict tourist access to only limited, selected islands.

Following Ngo et al. (2016), the sites in Viet Hai Village on Cat Ba Island had been recommended as a priority conservation zone for species conservation, since *G. catbaensis* was found to be most abundant at those sites. However, during the most recent survey in July 2016, no specimens of *G. catbaensis* were observed in Viet Hai Commune. We assume that an extensive flood in August 2015 might have killed a large amount of the local wildlife, including the Cat Ba Tiger Gecko, at this site. Viet Hai Commune was isolated for a week after torrential rains brought the water level up to the roofs of local houses. Since *G. catbaensis* was found to generally occur at low elevation ranges (4–132 m asl), and Viet Hai is situated only up to 36 m asl (see Fig. 6B), this species is particularly vulnerable to natural catastrophes such as storms, floods, and sea level rises, throughout its distribution range (see Dessler 2016; Saunders et al. 1991). Since local populations are extremely small, they are especially prone to extinction by catastrophic events. The devastating consequences of such natural disasters underline the importance of maintaining numerous independent subpopulations in order to compensate for such events.

In summary, the insular (sub)populations of *G. catbaensis* are threatened by harvest for the pet trade, human activities within its habitats, and natural catastrophes such as increasingly extreme floods and storms in northeastern Vietnam, probably triggered by climate change (The Governmental Committee on Flood and Storm Prevention 2016). Thus, we herewith emphasize the importance of setting aside priority conservation zones for this species, in order to establish a connected and buffered system that allows (sub) populations to recover from catastrophes. We also recommend the establishment of an assurance population, i.e., an *ex situ* conservation breeding program for the species. Although such an effort has been started at the Me Linh Station for Biodiversity (see Ziegler et al. 2016) in Northern Vietnam, more resources need to be allocated to enhance the effort to conserve the species.

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2.2. First ecological assessment of the endangered Lichtenfelder's Tiger Gecko (*Goniurosaurus lichtenfelderi*) from northern Vietnam: Micro-habitat and macro-climatic niche comparisons between island and mainland populations

The author of the thesis, Prof. Dr. Thomas Ziegler and Prof. Dr. Truong Q. Nguyen mainly contributed to the planning of the study. Data collection, analyses, preparation of graphics and the writing of the paper were mainly carried out by the author of the thesis. Macro-climatic niche comparison was corrected by Dr. Dennis Rödder. Field surveys in 2020 were carried out by Tien Quang Phan and Huy Quoc Nguyen.

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First ecological assessment of the endangered Lichtenfelder's Tiger Gecko (*Goniurosaurus lichtenfelderi*) from northern Vietnam: micro-habitat and macro-climatic niche comparisons between island and mainland populations

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Abstract. The Lichtenfelder's Tiger Gecko, *Goniurosaurus lichtenfelderi* is currently known only from northern Vietnam and southern China. Because of its restricted distribution, the species is potentially threatened by extinction due to anthropogenic impacts. Recently, the species has been listed in the IUCN Red List of Threatened Species as "Vulnerable" and included in CITES Appendix II and the Vietnam Governmental Decree – Group IIB. However, conservation activities to safeguard wild populations of *G. lichtenfelderi* have not yet been implemented due to a lack of detailed information about the population status and its ecological requirements. In this study, the micro-habitat use of *G. lichtenfelderi* was assessed. As a result of our field surveys, we found this species in evergreen forest areas with a high percentage of vegetation coverage and in close proximity to medium or small stream sections with rocky shelters on granitic formations. Canopy coverage, three micro-climatic variables (air, substrate temperature and humidity), weather condition and substrate type were the most important characteristics explaining the variation in the micro-habitat use of *G. lichtenfelderi*. Coupled with a macro-ecological (climate niche) approach, the complex niches of *G. lichtenfelderi* were defined by comparing them between two geographically distant populations in island and mainland sites. We found high similarities in macro-climatic and micro-habitat niches between the island and mainland populations of *G. lichtenfelderi*. Based on the ecological information, we recommend conservation actions to protect the core refugia of *G. lichtenfelderi* and reduce negative influences of anthropogenic impacts on wild populations in the future.

Keywords: conservation, ecological niches, granitic mountains, Grinnellian niche, micro-habitat use, stream.

Introduction

The Lichtenfelder's Tiger Gecko, *Goniurosaurus lichtenfelderi* (Mocquard, 1897), was originally discovered on a small island (Hon Norway) of the Bai Tu Long Archipelago in the Gulf of Tonkin, Vietnam (Mocquard, 1897). The species was subsequently found

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on the mainland in northern Vietnam and in Chongzuo, southern China (Orlov et al., 2008; Nguyen, Ho and Nguyen, 2009; Orlov, Ananjeva and Nguyen, 2020; Zhu et al., 2020b). *Goniurosaurus lichtenfelderi* is well-known as a forest-dwelling species occurring in granitic montane habitats. On the other hand, the four remaining *Goniurosaurus* species from northern Vietnam are adapted to limestone karst ecosystems either on islands or in mainland areas (Grismer, Viets and Boyle, 1999; Orlov et al., 2008; Nguyen, Ho and Nguyen, 2009; Nguyen, 2011; Ngo et al., 2019a, Ngo et al., 2021).

Even though *G. lichtenfelderi* was the first tiger gecko to be discovered in northern Vietnam (Mocquard, 1897; Orlov et al., 2008), the taxonomic status of the species remained unclear for a long time. In particular, Orlov and Darevsky (1999) described a new granitic-adapted species, namely *Goniurosaurus murphyi*, from a mainland area in northern Vietnam based on a juvenile specimen. However, *G. murphyi* was afterwards identified as a junior synonym of *G. lichtenfelderi* by Grismer (2000). In another case, a sister species, namely *G. hainanensis* from Hainan Island, China, was assigned as a subspecies of *G. lichtenfelderi* (Orlov et al., 2008). These misidentifications took place because only a few specimens and a small set of diagnostic characters were used for the descriptions without convincing evidence of molecular analyses. Moreover, an unquestionable recognition of the ocean as a geographic barrier, which might prevent genetic exchange between island and mainland populations, supported the initial description of *G. murphyi* (Orlov and Darevsky, 1999; Grismer, 2000). However, these taxonomic issues were recently clarified by Ngo et al. (2021), based on integrating morphological and molecular approaches. As a result, all mainland populations of *Goniurosaurus* found in granitic forests in northern Vietnam were revealed to be conspecifics of island populations of *G. lichtenfelderi* in Bai Tu Long National Park, including the type locality (Ngo et al., 2021). Furthermore, Zhu et al. (2020b) confirmed that a granite-stream-adapted tiger gecko from Chongzuo, southern China is a conspecific of *G. lichtenfelderi* as well.

Under ongoing human impacts, the given species is at the brink of extinction (Ngo et al., 2019a, 2021, 2022). In particular, *G. lichtenfelderi* was documented as the most common tiger gecko species in the international pet trade with more than 7,200 imported individuals into the United States (US) from 1999 to 2018 (Ngo et al., 2019b). The target species was predicted to be potentially severely impacted by climate change in the future (Ngo et al., 2022). Recently, *G. lichtenfelderi* has been listed in the IUCN Red List (Vulnerable), CITES Appendix II and the Vietnam Governmental Decree No. 06/2019/ND-CP (Group IIB) (Nguyen, 2018; Ngo et al., 2019a). These legal regulations have recently come into force to stop the illegal harvest and prevent the decline of wild populations of *G. lichtenfelderi*. Using distribution records and macro-environmental conditions, Ngo et al. (2022) projected the potential distribution of *G. lichtenfelderi* and identified core refugia as prioritized areas for conservation plans. However, conservation activities to safeguard wild populations of *G. lichtenfelderi* and their habitats have not yet been implemented so far. Knowledge gaps in ecology and micro-habitat requirements of this threatened tiger gecko are limiting the implementation of conservation plans.

It has long been acknowledged that ecological conditions can constrain species distributions and determine range limits (Orr and Smith, 1998; Schluter, 2009). Species that are widely distributed across divergent ecological gradients may respond quickly to changes in environmental conditions with adaptive phenotypic plasticity or even colonization (Deutsch et al., 2008; Pyron et al., 2015; Seebacher, White and Franklin, 2015). Alternatively, as habitat specialists, *Goniurosaurus* species occur within restricted areas, even at single localities, in which they have likely adapted to unique environmental conditions (Clements et al., 2006; Sterling, Hurley and Le, 2006; Yang and Chan, 2015; Honda and Ota, 2017; Liang et al., 2018; Ngo et al., 2019a, b; Qi et al., 2020a, b; Zhu et al., 2020a, b). Furthermore, as an ectothermic group of vertebrates, tiger geckos might be susceptible to prevailing climatic conditions that may constrain their distributions, influence their dispersal abilities, and prevent genetic exchange among populations (Araújo, Thuillerand and Pearson, 2006; López-Alcaide and Macip-Ríos, 2011; Powers and Jetz, 2019; Vicente et al., 2019). Using broad-scale climate information together with spatial coordinates, macro-ecological approaches have been applied by herpetologists to define the Grinnellian niche and species' ecological tolerance (Grinnell, 1917; Pyron and Burbrink, 2009; Rödder and Engler, 2011; Zhang et al., 2014; Rato et al., 2015; Ficetola et al., 2018; Dinis et al., 2019; Heidari, 2019; Nogueira et al., 2019; Sheu et al., 2020). However, the use of coarse-scale climate variables still has limitations. Typically, the actual distribution of an ectothermic species is much smaller than the potential distribution projected by species distribution models (SDMs) (Rödder and Engler, 2011; Kearney, Isaac and Porter, 2014; Ficetola et al., 2018). Furthermore, physiological limits of ectotherms strongly influence the micro-habitat it can occupy (Sunday et al., 2014; Ficetola et al., 2018; Taylor et al., 2020). Thus, adequate information about the micro-habitat use, coupled with a macro-climate approach, is expected to identify limiting ecological factors and provide a more complete understanding of species distribution.

The aim of this study is to provide detailed data on the micro-habitat selection of *G. lichtenfelderi*. Given that differences in micro-habitat use can mitigate competitive interactions within a species (Schoener, 1968; Irschick et al., 2005; van Schingen et al., 2015a), we further consider intra-specific variation in the micro-habitat selection between age classes and sex. By employing micro-habitat and macro-climatic approaches, we test whether the realized niches at fine- and broad-scales, respectively, are different or similar between geographically distant populations in island and mainland sites. Based on those results, we aim to assist conservationists by providing recommendations and sustainable conservation measures to safeguard wild populations of the threatened species and conserve its natural habitats.

Materials and methods

Study sites

Study sites were selected based on previous observations, literature and interviews with local people and rangers in northern Vietnam. In particular, insular populations of *G. lichtenfelderi* are known from two islands in the area of the Bai Tu Long National Park (NP), Quang Ninh Province (Site-1). The Bai Tu Long Archipelago is located in close proximity to Ha Long Bay and belongs to the Gulf of Tonkin. The Gulf of Tonkin comprises spectacular landscapes of more than a hundred isolated limestone karst islands intermixed with granitic formations, harbouring an exceptionally unique flora and fauna (Clements et al., 2006; Gawor et al., 2016). Three localities were selected to survey mainland populations, namely Chi Linh District, Hai Duong Province (Site-2), Yen Tu mountains, Quang Ninh Province (Site-3) and Tay Yen Tu Nature Reserve (NR), Bac Giang Province (Site-4), northern Vietnam (fig. 1). These areas are a part of contiguous tropical broad-leaved evergreen rainforest on granitic mountains in northeast Vietnam (Orlov and Darevsky, 1999; Orlov et al., 2008; Nguyen, 2011).

Field surveys

Field surveys were conducted along six transects (0.05 km to 1.65 km in length) on the islands of Bai Tu Long NP (Site-1) in June 2017, April 2018, May and September 2019, and five transects (0.5 km to 1.37 km in length) in the mainland in April (sites 2 and 3), June (Site-2), August (Site-4) and September (Site-2) in 2019, to collect data on the micro-habitat characteristics of *G. lichtenfelderi*. As a granitic-adapted species, we spent survey periods searching for animals and collecting data on the granite formations (Nguyen et al., 2009; Ngo et al., 2021, 2022). We further took three days in April 2018 and May 2019 to conduct surveys on the interspersed karstic formations in the island site to consider whether *G. lichtenfelderi* can be found there. Night excursions were carried out after sunset between 19:00 and 05:00 of the next day, since *G. lichtenfelderi* is nocturnal and spends the daytime hidden in rocky crevices (Orlov et al., 2008; Nguyen, 2011).

In total, 186 individuals of *G. lichtenfelderi* were observed along eleven transects during the field surveys, of which 75 individuals were recorded on two islands of Bai Tu Long NP, and 111 individuals were recorded from

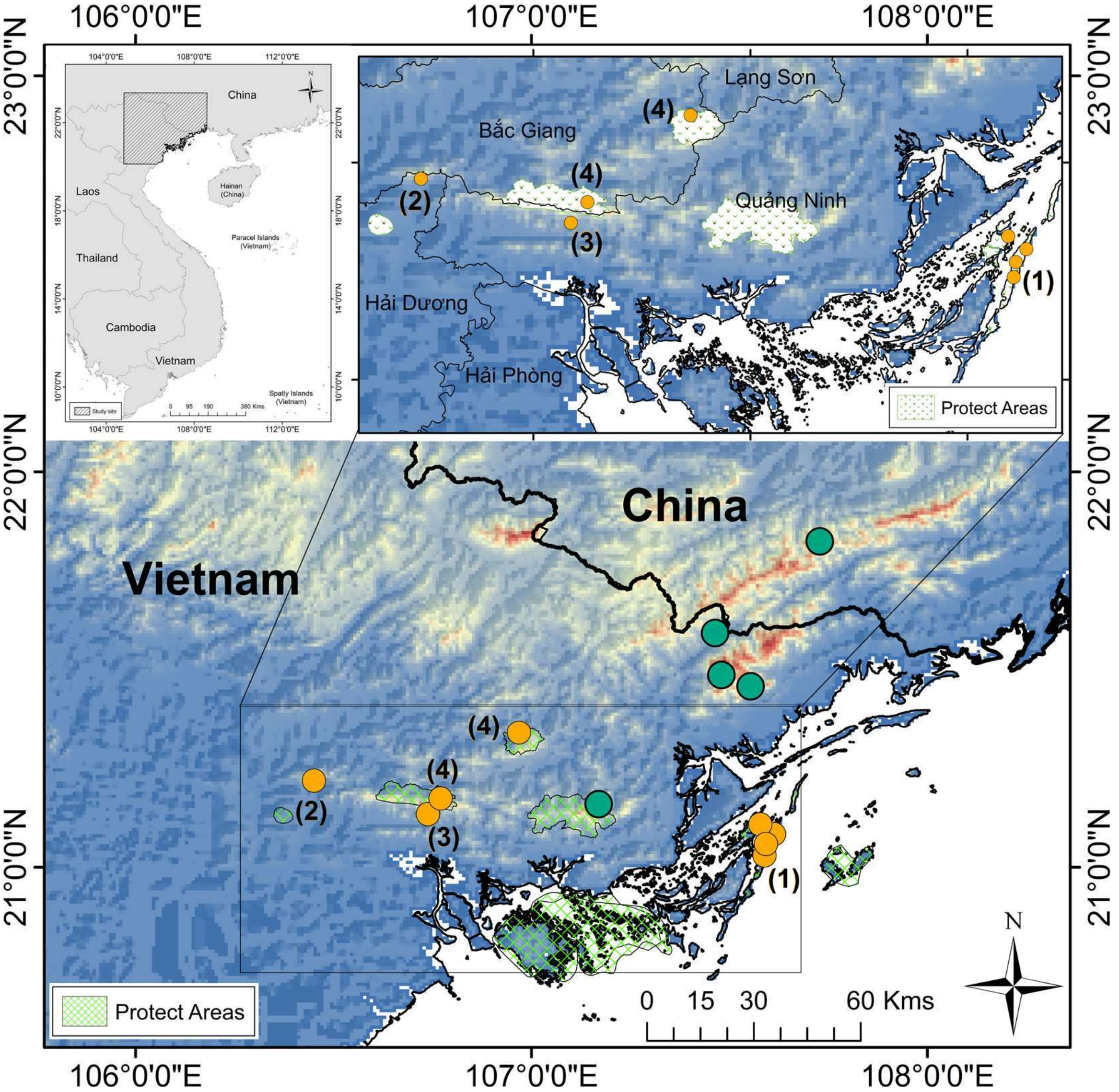

Figure 1. Records of *Goniurosaurus lichtenfelderi* (orange circles – surveyed locations; blue green circles – other recorded occurrences); (1): Bai Tu Long National Park, Quang Ninh Province (2): Chi Linh District, Hai Duong Province, (3): Yen Tu Mountain, Quang Ninh Province, (4): Tay Yen Tu Nature Reserve, Bac Giang Province. The background depicts elevation in northern Vietnam and southern China (from dark blue to red indicating higher elevation).

the mainland localities (85, 23 and 3 individuals in Chi Linh District, Tay Yen Tu NR and Yen Tu, respectively). All lizards were captured by hand to identify the sex of each captured individual based on the presence (in males) or absence (in females) of the large swollen hemipenal bulges. Each individual was assigned to an age class by measuring the Snout-vent Length (SVL < 80 mm = juveniles, SVL $\geqslant 80$ mm = adults) (Ngo et al. 2021). All captured animals were subsequently released at their collecting site.

Micro-habitat assessment

Habitat descriptions of *G. lichtenfelderi* were recorded from direct observations. Regarding micro-climatic characteristics, we measured the air temperature [°C] and relative air humidity [%] at each location of captured individuals with a digital thermometer (TFA Dostmann/Wertheim Kat. Nr.30.5015). Animal and substrate surface temperatures [°C] were also measured with an infrared thermometer (Measupro IRT20). We documented the weather condition of each surveyed day (raining or not).

We recorded further micro-habitat characteristics, including substrate type (classified as Rock (bare rock), Rock – Moss (rock covered with moss), Rock – Vines – Leaves (rock covered with vines and leaves), Rock – Soil (rock covered with soil), Wood, Fern, Bamboo, Soil-Sand on Ground, Leaves – Vines on Ground), perch height [m] (vertical distance from the ground to the animal position), perch position (outside or inside – under a rock or in a crevice), canopy – vegetation coverage [%] (estimated by direct observation), surface condition of the substrate (dry or wet), horizontal distance from the animal to the nearest shore of stream [m] (D), horizontal distance from the animal to the middle of stream [m] (W). The stream section next to the location of each animal was classified as dry stream, pool, riffle, run or waterfall. Furthermore, we measured other stream characteristics, e.g., water depth [m], stream width [m], water temperature [°C] (using a HHC201 thermocouple thermometer and a hermetically sealed thermocouple OMEGA). The activity status of each animal was also recorded as resting, feeding or moving. To evaluate human impacts on the species and its habitat, we classified the habitat types into either "undisturbed" in the core forest or "disturbed" when in close proximity to or within residential/agricultural/tourist areas.

Statistical analyses

Statistical analyses were performed by using the software environment R.3.1.2 (R Core Team, 2018). The Shapiro–Wilk test was used to check the assumption of normality of all continuous micro-habitat variables. The Kruskal–Wallis test combined with the Mann–Whitney pairwise test was performed to determine differences of those continuous variables among age and sex classes, survey sites, and the Wilcoxon test between island and mainland populations. Additionally, we used the Chi-square test to examine the differences of all categorical variables. A Linear Regression (LR) was computed to test for the linear relationship between animal and substrate temperatures. For all of these tests, we applied a significance level of $P = 0.05$. We further performed a Multiple Factor Analysis (MFA) for the micro-habitat dataset, comprising two qualitative groups – "Location" and "Habitat" (e.g., activity status, perch position, stream section, substrate type, surface condition and weather condition), and three quantitative groups – "Canopy coverage", "Climate" (e.g., air and substrate temperatures, humidity) and "Micro-position" (e.g., distance to the middle (D), distance to the shore of stream (W), occupied height). This multiple test was applied to identify active groups, variables that account for the variation in the dataset and measure their contribution. In addition, we used a Multiple Correspondence Analysis (MCA) to identify the most important variables in the active qualitative groups of the MFA test. Each micro-habitat niche of island and mainland populations was visually illustrated by clustering with a specific coded color within a spatial coordinate of the Dimension 1 (Dim1) and Dim2 axes in the MFA analysis, to evaluate the possibility of overlap. These multiple tests were performed using the packages "factoextra" (Kassambara and Mundt, 2020) and "FactoMinerR" (Le, Josse and Husson, 2008) for R.

Macro-climatic niche comparison

A total of 19 climatic variables (namely, Bio1: Annual mean temperature, Bio2: Mean diurnal range, Bio3: Isothermality, Bio4: Temperature seasonality, Bio5: Max temperature of warmest month, Bio6: Min temperature of coldest month, Bio7: Temperature annual range, and mean temperatures of Bio8: wettest quarter, Bio9: driest quarter, Bio10: warmest quarter, Bio11: coldest quarter, Bio12: Annual precipitation, precipitations of Bio13: wettest month, Bio14: driest month, Bio15: seasonality, Bio16: wettest quarter, Bio 17: driest quarter, Bio 18: warmest quarter and Bio19: coldest quarter) were obtained for the current climate condition with a high resolution (30 arc sec) from the official website of Global Climate Data (https://www.worldclim.org; version 1.4; Hijmans et al., 2005). Occurrence data of *G. lichtenfelderi* were compiled from literature and direct observations from 2014 to 2019. An extensive survey was further conducted in Quang Ninh Province in July 2020 to find new populations of *G. lichtenfelderi* by interviewing local people. However, several records were removed by using the packages "dismo" and "sp" in the R software due to duplicates of the same locality, obvious georeferencing errors and too dense records within a grid cell. Thus, we only used representative records that are at least 1 km apart to reduce geographic bias. Finally, we used 36 coordinates as representative records of *G. lichtenfelderi*, comprising 10 records from the island population and 26 records from the mainland population (fig. 1).

To compare the macro-climatic niche between island and mainland populations of *G. lichtenfelderi*, a pairwise niche overlap test was computed by using the package "ecospat" (Di Cola et al., 2017). The degree of climatic-niche space overlap between both populations of *G. lichtenfelderi* was evaluated using Schoener's D, which was first introduced by Renkonen (1938) to measure the proportional similarity of two niches making it comparable to a percentage overlap (Kohn and Riggs, 1982). The metric varies from 0 (no overlap) to 1 (complete overlap) (Schoener, 1970; Warren, Glor and Turelli, 2008; Rödder and Engler, 2011).

The macro-climatic niche and occurrence density were calculated and visually illustrated in a coordinate space defined by the first two principal components in an environmental principal component analysis (PCA-env), which was recently proposed by Broennimann et al. (2012). The result was used to visually evaluate the overlap of macro-climatic niche between island and mainland populations of *G. lichtenfelderi*. To define the available macro-climatic space of each population, we constructed a buffer of 60 km radius enclosing the mainland records and another buffer of 20 km radius for the island records (suggested by Anderson and Raza, 2010), using the packages "dismo" and "raster" in R. These buffer areas are expected to cover the entire potential refugia of *G. lichtenfelderi* from the mainland and islands, respectively.

Results

Micro-habitat characteristics

All individuals of *G. lichtenfelderi* were exclusively recorded in evergreen forests on granitic mountains at various elevations from 0 up to

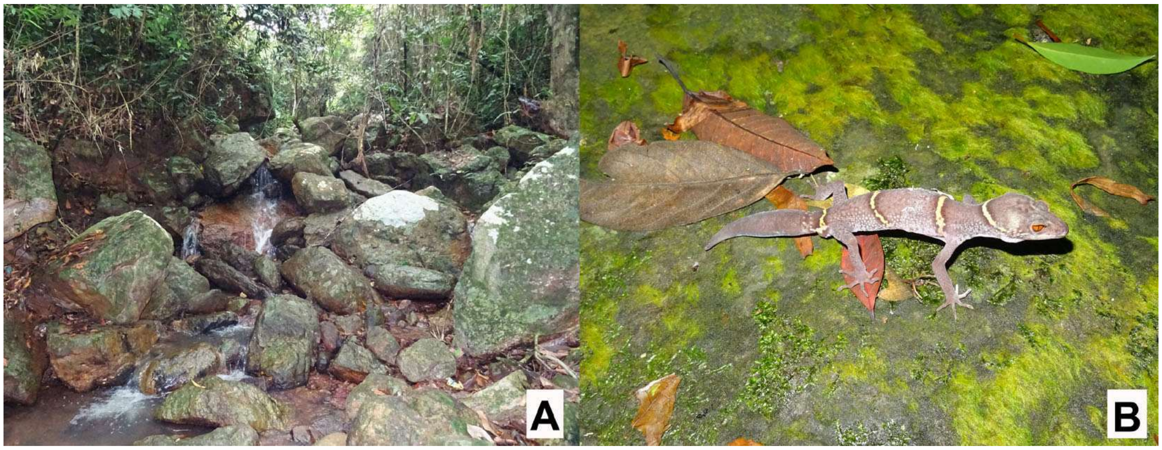

Figure 2. (A) Natural micro-habitat of *Goniurosaurus lichtenfelderi*; (B) An adult male resting on a moss-rock substrate.

Table 1. Micro-habitat characteristics of *Goniurosaurus lichtenfelderi* in island and mainland sites.

Parameter	Total		Island mean	Mainland mean
	Numbers	Min – Max (Mean ± SE)		
Elevation [m]	179	0–301 (134.1 ± 6.8)	37.4	193.4
Humidity [%]	178	59–92 (78.1 ± 0.6)	80.6	76.6
Air Temp [°C]	177	19–30.8 (26.3 ± 0.2)	25.9	26.5
Substrate Temp [°C]	149	18.8–28.6 (24.7 ± 0.1)	25.5	24.4
Stream Temp [°C]	34	22.8–25.7 (24.4 ± 0.1)	23.1	24.9
Canopy coverage [%]	176	0–100 (91.1 ± 1.3)	92.1	90.4
Height to ground [m]	176	0–2.5 (0.7 ± 0.1)	0.55	0.78
Height to stream [m]	55	0–5 (1.13 ± 0.2)	1.1	1.15
Stream depth [m]	45	0–2 (0.15 ± 0.03)	0.17	0.13
Distance to stream [m]	176	0–10 (1.25 ± 0.2)	0.9	1.58
Distance to middle of stream [m]	176	0–12 (2.15 ± 0.3)	1.6	2.48

301 m a.s.l. Animals were found in close proximity to the shore areas along stream sections on rocky cliffs, and in areas covered with evergreen broad-leaved trees and densely intermixed with bamboo, ferns, shrubs and vines (fig. 2A, B). No animal was found in the forest on karstic mountains at either the island or mainland sites, or along stream sections in proximity to residential areas and agricultural regions of the "disturbed" areas.

Ambient parameters of micro-sites of *G. lichtenfelderi* were described with a mean air temperature of 26.3 ± 0.2°C (19.0–30.8°C, n = 177), relative humidity ranging between 59–92% (78.1 ± 0.6%, n = 178) and a mean surface stream temperature of 24.4 ± 0.1°C (22.8–25.7°C, n = 34) (table 1). Animals were found on substrates with a mean temperature of 24.7 ± 0.1°C (18.8 – 28.6°C, n = 149; fig. 3A, table 1). We further measured temperatures of captured animals, with values ranging from 19.7 up to 28.9°C (24.9 ± 0.1°C, n = 153).

Canopy coverage at lizard capture locations were estimated to be 91.1 ± 1.3 % (0–100%, n = 176; fig. 3B, table 1). The average height of occupied spots of *G. lichtenfelderi* were approximately 0.7 ± 0.1 m (0–2.5 m, n = 176; fig. 3C, table 1) to the ground and 1.1 ± 0.2 m (0–5 m, n = 55; table 1) to the water surface. A majority of individuals were found on rock substrates covered with moss (33.5%, n = 59), followed by bare rocky surfaces, rocks with soil and rocks with vegetation (29.0%, 19.9%, 7.0%, respectively). We observed only a few lizards on tree trunks (6.2%), soil substrates (4%) and leaves – vines on the forest floor (3.4%) (fig.

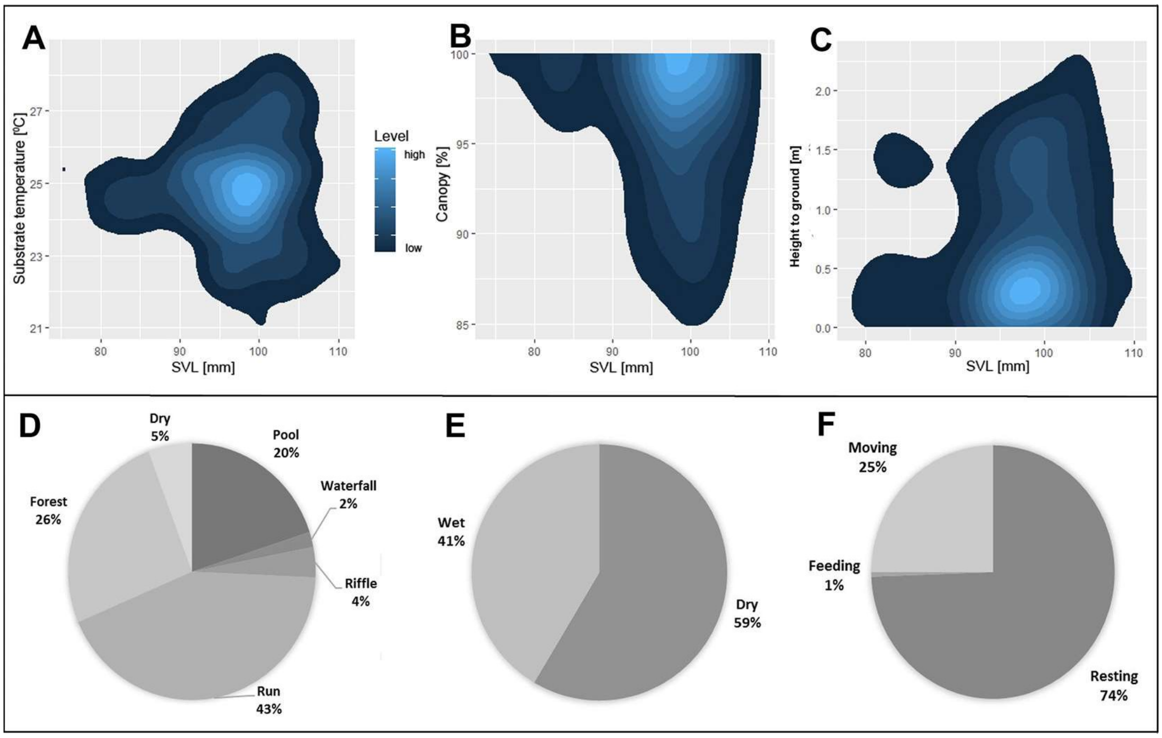

Figure 3. Micro-habitat characteristics of *Goniurosaurus lichtenfelderi* (A) Substrate temperature; (B) Canopy coverage; (C) Height above the ground in relation to the Snout-Vent Length (From low to high levels mentioned the frequency of captured animals); (D) Stream section type; (E) Surface substrate condition; (F) Activity status.

4A). A slightly lower number of lizards were encountered on wet substrates (41%, n = 73), compared to on dry substrates (59%; fig. 3E). We mainly observed animals residing outside their rock shelters (67.8%, n = 120; fig. 4B).

Inhabited stream areas were generally characterized with a slow water-flow and shallow water-depth, even dryness in a few sections of the four surveyed streams during April. The width of these streams was estimated to range from 1 to 12 m. We encountered animals resting near the shore of surveyed streams with distances from 0–10 m (n = 176) and to the middle with distances from 0–12 m (n = 176) (table 1). The stream areas at the resting sites were mostly running – slow water-flow (43%, n = 45), followed by forest, water-pool, dry stream, riffle and waterfall (26%, 20%, 5%, 4% and 2%, respectively) (fig. 3D). The stream depth at those positions ranged from 0 to 2 m (n = 73; table 1).

With regard to the daily activity pattern, *G. lichtenfelderi* was found to be active at night from approximately 19:00 h to 05:00 h. The species was most frequently observed between 21:00 h and 24:00 h (98 animals – 60% of captured lizards). We mainly observed animals in rock shelters from 19:00 h to 21:00 h, while most animals were encountered outside their shelters after 21:00 h (fig. 4B). A majority of animals were resting during the survey period (74%, n = 131; fig. 3F).

In general, there were no significant differences in the habitat use among age and sex classes (Chi–square tests, $P > 0.05$, and Kruskal–Wallis tests, $P > 0.05$). However, comparing among surveyed locations, the farthest distance from animals to the near stream shore was measured with a mean value of 5.8 ± 0.38 m (2.5–10 m, n = 23) from the Tay Yen Tu subpopulation (Site-4), which is significantly different to remaining subpopulations (0.53 ± 0.07, 0–5 m, n = 84; Mann-Whitney pairwise test, $P < 0.05$) (fig. 4D). With respect to the substrate, all types were recorded in both the island and mainland sites (fig. 4A). However, *G.*

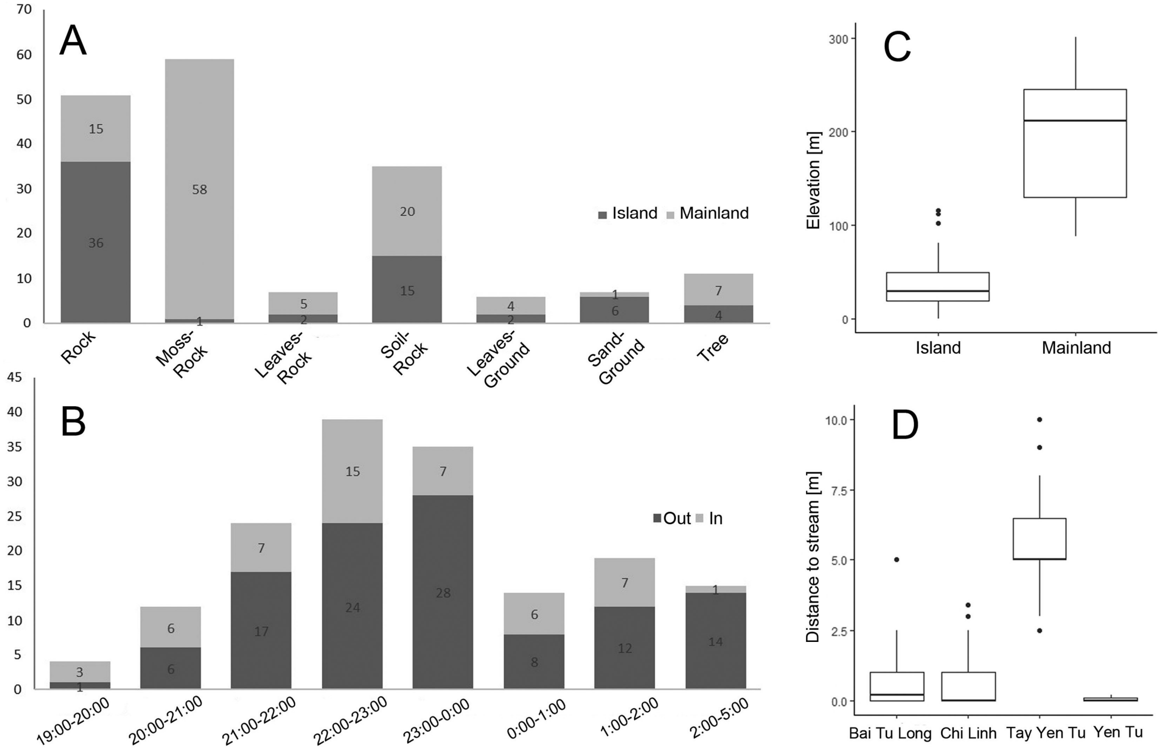

Figure 4. (A) Substrate types between island and mainland populations of *Goniurosaurus lichtenfelderi*; (B) Number of observed individuals at positions (in or out) in different time intervals; (C) Elevations resided by island and mainland populations; (D) Distances from the observed animal to the near stream shore among four study areas.

lichtenfelderi was mainly found on moss-rock (52.7%, n = 58) in the mainland, while island animals mostly resided on bare rock (54.5%, n = 36) and only one individual was found on moss-rock (Chi-square test, $P < 0.05$; fig. 4A). We further recorded a significant difference in the resided elevation between mainland and island populations (Wilcoxon test, $P < 0.05$; fig. 4C). With the exceptions of elevation and substrate type, there was no difference in other variables between both populations (Chi–square tests, $P > 0.05$ and Wilcoxon tests, $P > 0.05$).

The MFA analysis identified the data sets of micro-climate, canopy and habitat as active groups (fig. 5A). Together with the MCA analysis, we found that canopy coverage, three climatic variables (air, substrate temperature and humidity), weather condition and substrate type are the most important variables explaining the variation in the micro-habitat use of *G. lichtenfelderi* (fig. 5B, C, D). The MFA further showed that the space presenting the micro-habitat niche of the island population almost entirely overlays with the mainland population's niche (fig. 5E).

Macro-climatic niche overlap

The first axis (PC1) accounted for 39.01% and the second axis (PC2) accounted for an additional 34.01% of the overall variation (fig. 6). Macro-climatic spaces of the mainland and island populations were most dominantly influenced by Bio11 (Mean Temperature of Coldest Quarter), Bio6 (Mean Temperature of Coldest Month) and Bio1 (Annual Mean Temperature) in the PC1 axis. However, the island space was much more positive to Bio7 (Temperature Annual Range). In the PC2 axis, all negative variables influenced the position of both macroclimatic spaces. However, these variables had a greater influence on the island space, and Bio3 (Isothermality) and Bio2 (Mean Diurnal Range) had a strong effect on the mainland space (fig. 6C).

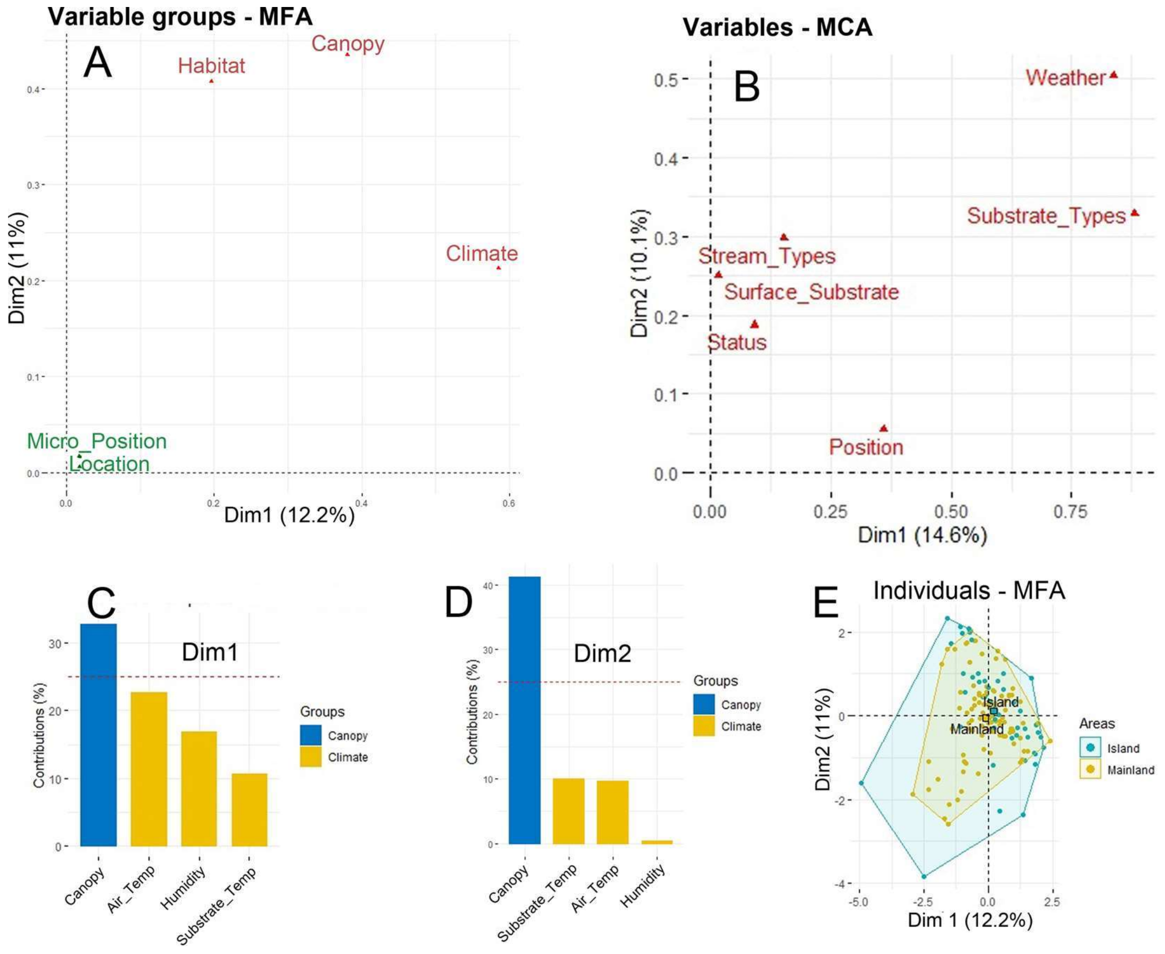

Figure 5. (A) Scatterplot of all variable groups for the first (Dim1) and second (Dim2) axes in the Multiple factor analysis (MFA) (green triangles as inactive groups, red triangles as active groups or variables); (B) Scatterplot of all qualitative variables in the Multiple correspondence analysis (MCA); (C) The first four important variables of the Dim1; and (D) The Dim2; (E) Scatter diagram illustrating the micro-habitat niche space of island and mainland populations.

The macro-climatic niche overlap between island and mainland populations of *G. lichtenfelderi* was very low with only 1.25% (Schoener's D = 0.0125). However, we found that the island population has a narrow macro-climatic niche space, which is nearly encompassed by the mainland population niche (fig. 6A, B).

Discussion

Micro-habitat characteristics

In the MFA and MCA analyses, the vegetation (canopy coverage), ambient parameters (temperature, humidity, weather) and substrate type were identified as the most important characteristics explaining the variation in the habitat selection of *G. lichtenfelderi*. Resembling previous researches, we also identified the potential micro-habitat of *G. lichtenfelderi* as shore areas along medium or small stream sections comprising rocky shelters on the soil floor, covered with high micro-vegetation coverage in evergreen forests on granitic mountains (Grismer, 2000; Orlov et al., 2008; Nguyen, 2011). However, *Goniurosaurus* geckos were exclusively recorded on rocky cliffs in the forest in Tay Yen Tu NR (Site-4). No animal was found along shore areas next to large stream sections (8–10 m of width) in Site-4, characterized with fast flow-velocity and shores on the sand –

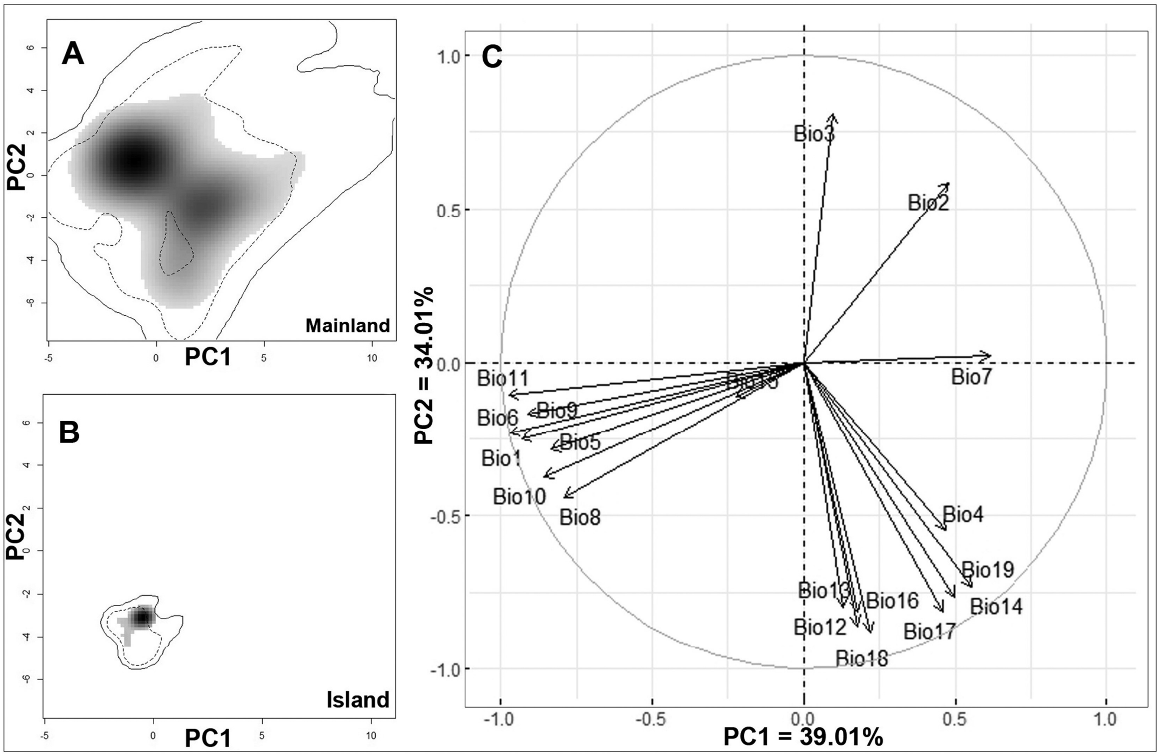

Figure 6. Comparisons of macro-climatic niches of *Goniurosaurus lichtenfelderi* between island and mainland populations. (A) Climate niche space of the mainland population; (B) Climate niche space of the mainland population along the first two axes of the PCA-env (The solid (100%) and dashed contour (50%) lines illustrate the available macro-climate space); (C) The contribution of 19 climatic variables for loading PCA-env axes and the percentage of inertia explained by axes one and two.

gravel floor without vegetation coverage. These stream characteristics are significantly different to the recorded streams of *G. lichtenfelderi*.

Our study revealed that the body temperature of *G. lichtenfelderi* was positively correlated with the substrate temperature (LR test, $r = 0.7$, $P < 0.001$, $n = 149$). As an ectothermic lizard, basic physiological functions of *G. lichtenfelderi*, such as locomotion, growth and reproduction are influenced by environmental conditions (van Schingen et al., 2015a; Ngo et al., 2018; Ngo et al., 2019a; Vicente et al., 2019).

Realized niche comparisons

Intra-specific micro-habitat partitioning has been recorded from a congeneric tiger gecko species, *G. catbaensis* (Ngo et al., 2019a), and other Vietnamese lizard species (e.g., *Cnemaspis psychedelica*, *Shinisaurus crocodilurus*) (van Schingen et al., 2015a; Ngo et al., 2018). However, in this study, we did not record any significant differences in the micro-habitat use among age and sex classes of *G. lichtenfelderi*. Resembling other tiger gecko species, *G. lichtenfelderi* is assumed to occur in low population densities, which likely reduce competitive interactions among conspecifics for shared resources within a restricted micro-habitat (Irschick et al., 2005; van Schingen et al., 2015a; Ngo et al., 2016, 2019a). Regarding the occupied substrate type, moss-rock was used the most by mainland animals, while the bare rock was the most common substrate used in the island population. As an ectotherm whose body temperature correlates positively with the substrate, the variation in occupied substrate types between populations of *G. lichtenfelderi* could be a behavior to maintain optimal body temperatures for development (Taylor et al., 2020). However, the temperature of moss-rock used by the mainland population (the mean value of 23.9°C, $n = 58$)

is relatively different to that of the bare rock in the island site (25.8°C, n = 15, Kruskal–Wallis tests, $P < 0.05$). We also took the substrate condition (wet and dry) into account, which is assumed to be related to the substrate humidity. There was no difference in the substrate condition between the most common substrate type of island and mainland populations (Chi-square tests, $P > 0.05$). Thus, this variation should be further studied to identify whether the main cause is the substrate humidity in relation to hydration and water loss of animals (Sannolo and Carretero, 2019) or different availability of local substrate types between island and mainland sites.

Integrating macro- and micro-habitat information, we confirmed that the species' ecological niches are comparatively similar across localized distributions, including island and mainland populations. With only 1.25% (Schoener's D = 0.0125), the overlap of the macro-climatic niche space between the island and mainland populations of *G. lichtenfelderi* is considered to be very low (Warren, Glor and Turelli, 2008; Rödder and Engler, 2011). However, the macro-climatic niche of the island population was very narrow and nested within the niche of mainland population (fig. 6A, B). The ordination analysis revealed that both macro-climatic spaces are positively related to variables of the mean temperature of coldest month and quarter (Bio6 and Bio11, respectively) and annual mean temperature (Bio1) along the PC1 axis, and some negative variables along the PC2 axis (fig. 6C). Therefore, the concordance between the realized macro-niche space of the island and mainland populations is high. Given their distributions, *G. lichtenfelderi* inhabits across latitudes from 20 to 24°N on the mainland, while the insular population is only known from a small area on two islands (Nguyen, Ho and Nguyen, 2009; Orlov, Ananjeva and Nguyen, 2020; Ngo et al., 2022). The ocean barrier has likely constrained the realized macro-climate niche of the island population within a small geographic area, while the mainland population's niche varies across a large granitic ecosystem nesting the island population's niche (Sterling, Hurley and Le, 2006; Whittaker, Triantis and Ladle, 2008; Lomolino, Brown and Sax, 2010).

In contrast to the high overlap of macro-climatic spaces, the micro-niche space of the mainland population was nested within the island population's space (fig. 5E). The relatively larger micro-niche space of island population could be a result of the variation in habitat characteristics among surveyed islands, and the different fieldwork times between the two populations causing the variation of micro-climate variables. Long-term surveys should be simultaneously conducted by at least two field teams in both mainland and island sites in the future, to minimize the seasonal or yearly variation in the micro-climate.

Conservation

Our study confirms that *G. lichtenfelderi* is restricted to a specific micro-habitat within its distribution range. Thus, the actual distribution could be much smaller than the whole extent of occurrence and the target species might be susceptible to any alterations in its natural habitats (Ngo et al., 2022). In fact, *G. lichtenfelderi* individuals were not found along some stream sections in close proximity to residential, agricultural and tourist regions. Furthermore, *G. lichtenfelderi* is sympatric with another threatened lizard, *Shinisaurus crocodilurus*, in some surveyed streams across the Yen Tu Mountain range, where their habitats and wild populations have been severely impacted by anthropogenic activities, such as coal mining, timber logging and construction works for the tourism (van Schingen et al., 2015a, b; Ngo et al., pers. obs). These anthropogenic activities can negatively alter the micro-habitat, reduce the potential distribution and could even lead to the complete destruction of their natural habitats in the near future. Based on our findings of microhabitat use, we recommend that higher protection within core natural forests along occupied streams of *G. lichtenfelderi* should be undertaken to ensure the long-term persistence of the species. In fact, *G. lichtenfelderi* is found in four protected areas, namely Ba Vi National Park (NP), Bai Tu Long NP, Dong Son–Ky Thuong Nature Reserve (NR), Tay Yen Tu NR (Nguyen, Ho and Nguyen, 2009; Nguyen et al., 2011; Orlov, Ananjeva and Nguyen, 2020). Using Maxent species distribution models, Ngo et al. (2022) projected the potential distribution of *G. lichtenfelderi* and identified core environmental refugia including these protected areas as well and border areas between China and Vietnam. Nevertheless, deforestation activities have occurred there (van Schingen et al., 2015a, b; Ngo et al., pers. obs). Thus, we recommend better monitoring of forest protection by local rangers and relevant authorities, as well as enhancing community education for the biodiversity value. Establishing a species and habitat conservation area for the endangered species *G. lichtenfelderi* in national protected areas is necessary to conserve the integrity of natural forests and safeguard the wild populations. Due to the concordance in the macro- and micro-niches between geographically distant populations in island and mainland sites, as we documented, one-size-fits-all type of conservation measures can be applied for all populations of *G. lichtenfelderi*.

Recently, an ex-situ conservation breeding program for the target species has been established by our team at the Me Linh Station for Biodiversity in March 2019 (Pham et al., 2021) in concert with an ex-situ breeding program at the Cologne Zoo, Germany. The information on micro-habitat use of *G. lichtenfelderi* from this study will help to develop and better implement the ex-situ conservation activities in the future.

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2.3. Ecological niche overlap of two allopatric karst-adapted tiger geckos (*Goniurosaurus*) from northern Vietnam: Microhabitat use and implications for conservation

The author of the thesis, Prof. Dr. Thomas Ziegler and Prof. Dr. Truong Q. Nguyen mainly contributed to the planning of the study. Data collection, analyses, preparation of graphics and the writing of the manuscript mainly were carried out by the author. Ecological data from 2014 was provided by Dr. Mona van Schingen-Khan. Extend field surveys in 2020 and 2021 were carried out by Tien Quang Phan and Huy Quoc Nguyen.

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Ecological niche overlap of two allopatric karst-adapted tiger geckos (*Goniurosaurus*) from northern Vietnam: Microhabitat use and implications for conservation

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Abstract

Anthropogenic pressures have caused a substantial decline of global biodiversity and have been further reported to strongly affect the ecological performance of species in their habitat, especially reptiles. Understanding the ecology of species and how species respond to habitat alterations is basic knowledge to address biodiversity loss. All five tiger gecko (*Goniurosaurus*) species in northern Vietnam are known to be threatened by extinction due to anthropogenic impacts such as habitat degradation and over-harvesting for the international pet trade. However, conservation actions have only been implemented for the better-studied species. This study provides detailed data on microhabitat use of two allopatric sister taxa (*Goniurosaurus huuliensis* and *G. luii*). In total, 145 geckos (including 59 records of *G. huuliensis* and 86 records of *G. luii*) were observed during field surveys. All *Goniurosaurus* individuals were mostly recorded in the forest on karst formations, covered with evergreen broad-leaved woody trees, intermixed with ferns, shrubs and vines. Microhabitats of the two species were relatively similar in other traits, such as high vegetation coverage, high humidity, stable ambient temperature and dry-rock substrates. A multiple factor analysis (MFA) supported that the ecological niche space of the two species highly overlap, even though their distribution ranges are geographically separated. We further found no intra-specific niche segregation in both species. The present data provide baseline knowledge for both *in-situ* and *ex-situ* conservation measures to protect the *Goniurosaurus* species.

Keywords

Core refugia, *Goniurosaurus huuliensis, Goniurosaurus luii*, human impacts, karst-dwelling, niche conservatism.

2.4. Living under the risk of extinction: Population status and conservation needs assessment of a micro-endemic tiger gecko in Vietnam.

The author of the thesis and Prof. Dr. Truong Nguyen mainly contributed to the planning of the study. Data collection, analyses, preparation of graphics and the writing of the paper were carried out by the author of the thesis. Prediction models were provided by Dr. Dennis Rödder. An extended survey in 2021 was carried out by Huy Quoc Nguyen.

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Living under the risk of extinction: population status and conservation needs assessment of a micro–endemic tiger gecko in Vietnam

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Abstract

Living under the risk of extinction: population status and conservation needs assessment of a micro–endemic tiger gecko in Vietnam. Human impact is considered the major threat to the global decline of biodiversity, especially for threatened endemic species in karst ecosystems. Studies assessing a species' demography based on temporal and spatial indicators of population size, density and structure are expected to evaluate the level of impact of threats and are therefore becoming increasingly important for species conservation efforts. *Goniurosaurus huuliensis*, an endemic species in Vietnam, is one of the most threatened reptiles in the world. This karst–adapted species is classified by the IUCN Red List as Critically Endangered and listed under CITES Appendix II due to habitat loss and over–exploitation for the international pet trade. Here we provide the first evaluation of the population status of *G. huuliensis*. We applied a 'capture mark–recapture' method to estimate the population size and identify the population density and structure. The total population size was estimated to comprise a maximum of 1,447 individuals in integrated suitable habitats, possibly reaching up to 2,855 individuals exclusively in karst habitats within the total extension of occurrence. This is exceedingly lower than the threshold for a minimum viable population. Furthermore, *G. huuliensis* is documented to occur in extremely small mean population densities of only 6.4 indiv./km and 2.5 indiv./km/day along the surveyed transects. Based on the demographic information, the ongoing severe human impact (e.g. wildlife exploitation and limestone quarrying) is driving *G. huuliensis* to the brink of extinction. In situ conservation measures are therefore urgently required. We recommend that in-situ actions should be increased, and a plan should be developed to establish a species and habitat conservation area for *G. huuliensis*.

Key words: Density, *Goniurosaurus huuliensis*, Huu Lien Nature Reserve, Invisibility rate, Population size, Karst habitat

Resumen

Vivir bajo la amenaza de la extinción: estado de la población y evaluación de las necesidades de conservación de un gecko leopardo microendémico en Vietnam. La actividad humana se considera una de las principales causas de la disminución mundial de la biodiversidad, en especial de especies endémicas en peligro de extinción en ecosistemas kársticos. Se espera que los estudios realizados para evaluar la demografía de la especie a partir de indicadores temporales y espaciales del tamaño, la densidad y la estructura de la población permitan determinar la gravedad de las amenazas y que, por lo tanto, sean cada vez más importantes para las iniciativas de conservación de la especie. *Goniurosaurus huuliensis*, una especie endémica de Vietnam, es una de las especies de reptil más amenazadas del mundo. Esta especie adaptada al karst se considera en peligro crítico en la Lista Roja de Especies Amenazadas de la Unión Internacional para la Conservación de la Naturaleza, y se recoge en el Apéndice II de la Convención sobre el Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres (CITES) debido a la pérdida de hábitat y a la sobreexplotación a la que se ve sometida por el comercio internacional de mascotas. En este artículo presentamos la primera evaluación de la situación demográfica de *G. huuliensis*. Se utilizó un sistema de captura, marcaje y recuperación

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de marcas para estimar el tamaño de la población y determinar su densidad y estructura. Como resultado, se estima que el tamaño total de la población de *G. huuliensis* es de 1.447 individuos como máximo en hábitats adecuados integrados y que puede llegar a 2.855 individuos exclusivamente en hábitat kársticos dentro de toda la extensión de presencia. Estas cifras son muy inferiores al límite que determina la viabilidad de una población. Además, se ha documentado que las poblaciones de *G. huuliensis* tienen una densidad media extraordinariamente baja, de solo 6,4 individuos/km y de 2,5 individuos/km/día a lo largo de los transectos estudiados. A partir de la información demográfica, los graves efectos constantes de la actividad humana (por ejemplo, la explotación de la flora y fauna silvestres y la excavación de canteras de piedra caliza) empujan a *G. huuliensis* al borde de la extinción. Por consiguiente, es urgente adoptar medidas de conservación in situ. En el presente estudio recomendamos mejorar la eficacia de las medidas in situ, incluida la elaboración de un plan para establecer un área de conservación de *G. huuliensis* y del hábitat kárstico.

Palabras clave: Densidad, *Goniurosaurus huuliensis*, Reserva Natural de Huu Lien, Tasa de invisibilidad, Tamaño de población, Hábitat kárstico

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Introduction

The ongoing sixth mass extinction of wildlife is driven by severe anthropogenic impacts on a global scale (Gibbons et al., 2000; Ceballos et al., 2015; Marshall et al., 2020). The major factors accounting for 88 % of the decline in global biodiversity are exploitation of wildlife, habitat degradation, fragmentation and loss, and climate change (Monastersky, 2014). Reptiles are especially affected, with approximately 20 % of the total species number being threatened by extinction (Böhm et al., 2013). Marshall et al. (2020) reported the exploitation of 3,943 reptile species for wildlife trade, with more than 80 % of the species not being regulated by a listing in the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). A total of 540 of these exploited species were assessed as at least Vulnerable by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. However, most threatened reptile species were assessed following Criteria B and D2 relative to restricted geographic ranges, and very few data are available on population trends, needed for Criteria A and C (Böhm et al., 2013). Despite being globally threatened, these species have rarely been protected by conservation measures due to the lack of comprehensive knowledge concerning population status, ecology and threats. The gap in conservation assessments could bring threatened species to the brink of extinction (Gibbons et al., 2000; Böhm et al., 2013; Marshall et al., 2020).

Accounting for approximately only 10 % (460,000 km^2) in Southeast Asia, karst ecosystems are still considered an 'ark' of biodiversity, containing an extraordinarily high level of endemism (Day and Urich, 2000; Clements et al., 2006; Luo et al., 2016; Tolentino et al., 2020). Giving an example of the gekkonid genus *Cyrtodactylus*, comprising approximately 300 species mostly native to South East Asia, the karstic habitats (which far out–number all others) are not simply refugia but rather a mosaic of unique micro–niches forcing speciation in disjunct outcrops (Clements et al., 2006; Grismer et al., 2020). However, unsustainable human activities (particularly limestone quarrying) are endangering the biodiversity of karst ecosystems in Southeast Asia (Clements et al., 2006; Luo et al., 2016; Grismer et al., 2020).

The Huulien Tiger gecko, *Goniurosaurus huuliensis* Orlov, Ryabov, Nguyen, Nguyen and Ho 2008, was originally described from the Huu Lien Nature Reserve (NR) in Lang Son Province, northern Vietnam (Orlov et al., 2008; Nguyen et al., 2009; Nguyen, 2011). Being a habitat specialist, this endemic tiger gecko is only found in evergreen forests on karst formations (Orlov et al., 2008). Thus, *G. huuliensis* is particularly susceptible to the increase of anthropogenic threats that lead to habitat fragmentation and degradation. Additionally, Ngo et al. (2019a, 2019b) documented that a large number of wild tiger geckos have been illegally harvested for both local and international pet trade. Climate change may also have potentially negative impacts on wild populations of *G. huuliensis* in the future (Ngo et al., 2021a). Consequently, the species was recently listed as Critically Endangered (CR) in the IUCN Red List (Nguyen, 2018), and included in CITES Appendix II and the Vietnam Government's Decree No. 06/2019/ND–CP (Group IIB) in 2019 (Ngo et al., 2019b). Although the tiger gecko is dramatically affected by severe threats, a comprehensive assessment of population status of the target species is still lacking.

Research on the demography and viability of in–situ populations can quantify the impact level of anthropogenic threats and evaluate the explicit status of endangerment (Beissinger and Westphal, 1998; Coulson et al., 2001; Jones et al., 2017), and is therefore increasingly important for species conservation efforts (Selman and Jones, 2017; Maida et al., 2018). Based on this background, we aimed to provide a detailed description of the current demographic status of *G. huuliensis* by assessing the population size and density. Integrating results from previous studies on the species' ecological niche, we intended to estimate the global population size of *G. huuliensis* within the total extent of occurrence, covering suitable habitats in terms of elevation, karst formations and environmental characteristics (climate and vegetation cover). We further evaluated differences in the population structure between age and sex of *G. huuliensis*. Together with the evaluation of anthropogenic impacts, our main goal was to identify priorities and provide recommendations to improve the efficacy of in–situ conservation measures for the protection of *G. huuliensis* in the future.

Material and methods

Field surveys

The study site was selected within the known distribution range of *G. huuliensis* in the Huu Lien NR, Lang Son Province (S1) based on available literature (Orlov et al., 2008; Nguyen et al., 2009) and our direct observations, and in Thai Nguyen Province, northern Vietnam (S2) from interviews with local people (fig. 1). We carried out surveys along five transects (T1 to T5) in Lang Son Province and one transect (T6) in Thai Nguyen Province. Transects were set up along forest paths or patrol trails of forest rangers. These transects ranged from 0.4 to 2.6 km in length and were located at elevations from 176 up to 500 m a.s.l. (table 1). Their surrounding habitat is exclusively covered with the evergreen forest on karst formations, combining dominant characteristics of a high micro–vegetation coverage, high humidity, stable ambient temperature, dry–rock substrates and low height to forest ground (Orlov et al., 2008; Nguyen, 2011; Ngo et al., in press). We also checked rocks on two crop hills in close proximity to the survey transects (T1 and T5) for the presence of tiger geckos.

We conducted surveys during the non–hibernation season of *Goniurosaurus* in April and August 2019, June 2020 in Lang Son Province, and April 2021 in Thai Nguyen Province. To guarantee the highest detection probability for the nocturnal species (Orlov et al., 2008; Nguyen, 2011) and to limit the bias of observers, excursions were carried out between 8 p.m. and 5 a.m. of the next day. Each survey in each transect was conducted with at least two researchers, together with a ranger and a local person.

Population assessment

Due to its small body size and distribution within remote areas (Orlov et al., 2008; Ngo et al., 2021a), estimating population size of *G. huuliensis* based on direct records is deemed problematic. Huang et al. (2008) used statistical data from a 'capture mark–recapture' approach, developing the 'invisibility rate' method to estimate the population size. This method has been applied for other lizards in Vietnam, namely *Cnemaspis psychedelica*, *Cyrtodactylus gialaiensis*, *Goniurosaurus catbaensis*, *Physignathus cocincinus*, and *Shinisaurus crocodilurus* (van Schingen et al., 2014, 2016; Ngo et al., 2016a, 2019a; Nguyen et al., 2018; Gewiss et al., 2020; Luu et al., 2020). Accordingly, all encountered individuals of *G. huuliensis* were captured and individually marked with passive integrated transponder (PIT) tags (ISO FDX–B Glastransponder, 1.4x9 mm, Germany). In newly captured individuals, a microchip was injected under the skin on the left side of the body behind the shoulder. All captured and recaptured individuals were identified with a transponder–reader (Breeder Reader IC, Planet ID GmbH, Germany) (van Schingen et al., 2014; Ngo et al., 2019b). Each transect was repeatedly surveyed in intervals of at least two days. Coordinates and elevations of all capture localities were recorded using a GPS device (Garmin GPSmap62s, WGS84 datum) and are available on request to the authors. Geckos were captured by hand and subsequently released at the same sites after taking measurements and marking.

The method of Huang et al. (2008) includes the calculation of the invisibility rate using the formula:

$$i = [\Sigma(b_n - a_n)]/a_n$$

where a_n is the number of observed individuals along transect n during the first survey and b_n is the total number of observed individuals along transect n. The invisibility rate is a compensation for the undetected but present individuals of the population along the surveyed transect. In this study, we calculated an average value of i for all transects and survey periods for each survey site. The average i was employed in the formula to estimate the population size:

$$\check{N} = \Sigma\ [m^*(1 + (i/x))]$$

where $\check{N}$ is the estimated population size within the survey site, m is the total number of observed individuals along the transect including all surveys and x is the number of conducted surveys along the transect (Huang et al., 2008).

However, the estimated population size only refers to the study site and does not encompass the entire in–situ populations of the species, since it is difficult to detect and survey all occupied habitats within the extent of occurrence (EOO). By definition, the EOO range contains all the known occurrences of a taxon within the shortest continuous imaginary boundary (IUCN, 2019). The use of the species distribution modelling (SDM) method is expected to provide a reliable estimate of the total population size of a taxon within its suitable habitats. This technique is based on the hypothesis that the relationship between species abundance and environmental suitability is highly positive (Weber et al., 2016). In particular, environmental variables, to which a species has historically adapted to define the Grinnellian niche (Grinnell, 1917; Rödder and Engler, 2011), provide highly suitable conditions for larger populations within their optimal range with high birth and survival rates, and vice versa (Araújo et al., 2002; Morrison et al., 2006). Thus far, many organisms have been studied to test the relationship, such as 450 correlations of earthworms, mollusks, insects, reptiles, mammals, fishes, and plants in the research of Weber et al. (2016). Moreover, the projected habitat suitability has already been used to estimate the population size and density of some terrestrial species, such as *Panthera onca* (Tôrres et al., 2012); *Mergus squamatus* (Zeng et al., 2015), British mammals (Croft et al. 2017), and *Nomascus annamensis* in Vietnam (Tran and Vu, 2020).

For the extent of occurrence, we extracted the probability of environmental suitability. This varied from 0.27 to 0.88 in the climate–based model and from 0.08 to 0.90 in the vegetation–based model (fig. 1A, 1B). Values closer to 1 indicate higher suitability (Ngo et al., 2021a). However, the two probability values were not significantly different among survey routes, and they all provided conditions with very high suitability (from 0.66 to 0.90; table 1). A regression analysis was used to evaluate the relationship between species abundance and suitable probability per survey transects (Weber et al., 2016). However, the relationship was not statistically significant (Linear Regression Analysis, $P > 0.05$). Moreover, only six sample numbers for the statistics may lead to under– or over–fitting. In this study, we only employed the binary values of suitability and unsuitability to evaluate the presence and absence of the species, respectively. Thus, species abundance was considered equal among suitable sites within the extent of occurrence. The population size ($\check{N}_S$) within the suitable area restricted in the extent of occurrence was estimated based on an equivalent rate:

$$\check{N}_S/A_S = \check{N}/A_F$$

where A_F is the area of the field survey site, covering visited transects in each survey; A_S is the area of an overlapped layer of integrated suitable habitats. Given only in the karst layer within the EOO, the value:

$$\check{N}_{max} = (\check{N} \text{ x } A_K)/A_F$$

is considered a maximum that the population size can achieve (where A_K is the area of karst habitats),

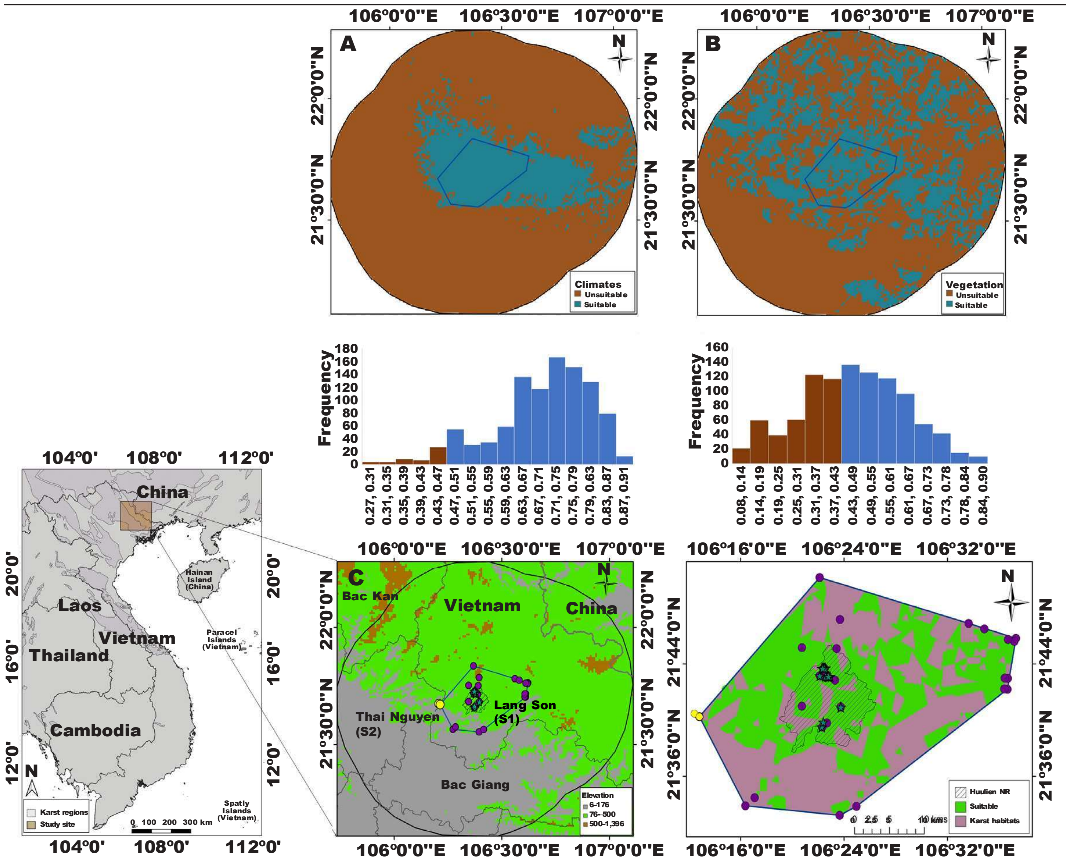

Fig. 1. Projected potential distribution of *Goniurosaurus huuliensis* in northern Vietnam: A, the climate model; B, the vegetation cover model, including histograms of suitable probability within the extent of occurrence (brown columns as unsuitable and blue columns as suitable); C, the suitable elevations within the selected buffer region (indicated by a black circle line); and D, the overlap of these three layers together with the karst layer within the extent of occurrence (marked with blue lines; teal stars, surveyed locations in Lang Son Province; yellow circle, surveyed location in Thai Nguyen Province; violet circles, other known occurrences). Projected data were extracted from the studies of Ngo et al. (2021a, in press).

Fig. 1. Posible distribución prevista de Goniurosaurus huuliensis *en el norte de Vietnam: A, el modelo climático; B, el modelo de la cobertura vegetal que incluye histogramas de la probabilidad adecuada dentro de la extensión de presencia (columnas marrones como no adecuada y columnas azules como adecuada); C, la elevación adecuada dentro de la región de transición seleccionada (indicada con un círculo negro), y D, la superposición de estas tres capas junto con la capa de karst dentro de la extensión de presencia (cubierta por líneas azules; estrellas azules, sitios estudiados en la provincia de Lang Son; círculo amarillo, sitio estudiado en la provincia de Thai Nguyen; círculos violeta, otros sitios con presencia conocida). Los datos previstos se extrajeron de los estudios de Ngo et al. (2021a, en prensa).*

because the target species is exclusively found in karst habitats (Orlov et al., 2008; Grismer et al., 2021; Ngo et al., 2021b, in press).

In terms of environmentally suitable habitats, we used the structural data layers of climate and vegetation cover variables from previous species distribution models, projected by Ngo et al. (2021a) (fig. 1A, 1B). The suitability layer was extracted from each binary map (climate and vegetation cover) containing values above the 10 % training presence Cloglog threshold within the selected buffer area (an area with a radius of 50 km enclosing all occurrence points; fig. 1A, 1B) (Ngo et al., 2021a). The localized elevation layer ranging from 176 m to 500 m a.s.l. (fig. 1C; Ngo et al., in press) and the karst layer from the World Karst Aquifer Map were extracted (downloaded from https://www.whymap.org/whymap/EN/Maps_Data/Wokam/wokam_node_en.html [Accessed on May

Table. 1. Number of individuals observed together with population densities and estimated population sizes (Ň) of *Goniurosaurus huuliensis* in Lang Son Province (S1) in April and August 2019, and June 2020, and in the Thai Nguyen Province (S2) in April 2021. Information on transect length, climate suitability, vegetation suitability and level of human impacts are also included. (* missing data).

Tabla. 1. Número de individuos observados, densidad de población y tamaño de la población estimado (Ň) de Goniurosaurus huuliensis *en la provincia de Lang Son (S1) en abril y agosto 2019 y junio de 2020, y en la provincia de Thai Nguyen (S2) en abril de 2021, incluida información sobre la longitud de los transectos, la idoneidad climática, la idoneidad de la vegetación y el grado de afectación de la actividad humana. (* datos que faltan).*

Transect	T1	T2	T3	T4	T5	T6	Total
Length (km)	0.9	2.6	1.5	0.4	0.9	1.5	
Climate suitability	0.80	0.79	0.87	0.87	0.87	0.66	
Vegetation suitability	0.81	0.74	0.83	0.83	0.9	0.77	
Human impact level	High	High	Medium	Medium	High	Low	
April 2019 / Lang Son (S1): survey area 15 km^2							
Total obs	5	5	12	*	*	*	22
Density	5.6	1.9	8	*	*	*	5.2
Density/day	2.8	0.96	2.7	*	*	*	2.1
Ň	11	11	27	*	*	*	49
Ň Huu Lien NR (85 km^2)							278
$\check{N}_S$ (408 km^2)							1,333
$\check{N}_{max}$ (805 km^2)							2,630
August 2019 / Lang Son (S1): survey area 21 km^2							
Total obs.	1	3	17	4	2	*	27
Density	1.1	1.15	11.3	10	2.2	*	6.5
Density/day	1.1	1.15	2.8	5	2.2	*	3.1
Ň	2	7	38	9	4	*	60
Ň Huu Lien NR (85 km^2)							242
$\check{N}_S$ (408 km^2)							1,166
$\check{N}_{max}$ (805 km^2)							2,300
June 2020 / Lang Son (S1): survey area 12 km^2							
Total obs.	1	*	9	*	1	*	11
Density	1.1	*	6	*	1.1	*	2.7
Density/day	1.1	*	1.5	*	1.1	*	1.2
Ň	2	*	16	*	2	*	20
Ň Huu Lien NR (85 km^2)							142
$\check{N}_S$ (408 km^2)							680
$\check{N}_{max}$ (805 km^2)							1,342
April 2021 / Thai Nguyen (S2): survey area 11 km^2							
Total obs.	*	*	*	*	*	14	14
Density	*	*	*	*	*	11.3	11.3
Density/day	*	*	*	*	*	3.77	3.77
Ň	*	*	*	*	*	39	39
$\check{N}_S$ (408 km^2)							1,447
$\check{N}_{max}$ (805 km^2)							2,855

Table. 1. (Cont.)

Transect	T1	T2	T3	T4	T5	T6	Total
Maximum abundance: survey area 32 km^2							
Total obs.	5	5	17	10	2	14	53
Ň	11	11	38	9	4	39	112
Ň Huu Lien NR (85 km^2)							298
$\check{N}_S$ (408 km^2)							1,428
$\check{N}_{max}$ (805 km^2)							2,818

2022]; Goldscheider et al., 2020). These layers were geographically restricted within the extent of occurrence and later overlapped to calculate the intersected area of suitable habitats (fig. 1D). We collected the Huu Lien NR's shape file from the website of https://www.protectedplanet.net/ [Accessed on June 2019] (fig. 1D). The related maps were generated, and the areas (including all study sites, karst habitats, suitable habitats and the extent of occurrence) and the length of survey transects were calculated using Quantum GIS software (QGIS Version 3.12.0, Development Team. 2020. Available online at http://qgis.osgeo.org [Accessed on 25 March 2020].

Population densities of *G. huuliensis* were calculated per kilometer (indiv./km) with reference to each surveyed transect, and per day (indiv./km/day). To assess the age structure of the *G. huuliensis* population, geckos were categorized into two classes based on their snout–vent length (juveniles with SVL < 85 mm and adults with SVL ≥ 85 mm) (Ngo et al., 2021b). The sex of each captured individual was determined based on the presence of large swollen hemipenal bulges and 25–30 precloacal pores in males, and the lack of these characteristics in females (Orlov et al., 2008; Ngo et al., 2021b). To test for significant differences in the age and sex structures between surveyed periods, we used a Chi–square test. For the test, we applied $\alpha = 0.05$. Statistical analyses were carried out in R v 3.1.2 (Rstudio Team, 2018).

Threat assessment

Day excursions were carried out to obtain evidence of anthropogenic activities within the Huu Lien NR and its surroundings, including impacts to the species habitat and hunting activities. We documented the information through our observations and interviews with local communities. The level of threats was classified for each surveyed transect depending on the frequency, extent and intensity of recorded human impacts (Ngo et al., 2019b). In particular, 'undisturbed', 'low', 'medium' and 'high' were respectively defined as never being observed, rarely observed, several times and frequently recorded in a high intensity (such as a large number of wild–caught individuals or extensive limestone quarrying).

Results

Population status

We observed a total of 74 individuals of *G. huuliensis:* 60 individuals in S1 (Lang Son Province) and 14 individuals in S2 (Thai Nguyen Province). None of the tiger geckos was observed in the two crop hills. We noted the highest number of individuals in August 2019 (27 indiv.), followed by April 2019 (22 indiv.), April 2021 (14 indiv.) and June 2020 (11 indiv.) (table 1). It is noteworthy that we observed only one juvenile in June 2020 and two other juveniles in April 2021 (fig. 2). We recorded a consistently imbalanced sex ratio among the four survey periods, with the number of observed females accounting for at least 54 % and at most 68 % (Chi–square test; $\chi^2 = 0.67$, df = 3, $P > 0.05$; fig. 2).

The average invisibility rates were 1.27 in S1 and 1.8 in S2. The population size of *G. huuliensis* at the study site fluctuated significantly between survey periods, depending on the number of visited transects. In particular, the population was estimated to consist of a minimum of 20 indiv. along two transects in S1 in June 2020 and a maximum of 60 indiv. along five transects in S1 in August 2019 (table 1). For transect T3, we estimated 16 to 38 indiv., while the remaining transects in S1 had only a maximum estimated number of 11 indiv. (table 1). A total of 39 indiv. was estimated for the population along the transect T6 in S2.

To estimate the total population size of *G. huuliensis*, we extracted respective layers of the survey sites and calculated their areas according to the survey periods (15 km^2 in April 2019; 21 km^2 in August 2019; 12 km^2 in June 2020 in S1; and 11 km^2 in April 2021 in S2), the area of the Huu Lien NR (85 km^2) and suitable habitats (408 km^2) within the whole extent of occurrence (EOO, 805 km^2) (table 1, fig. 1). The extracted karst area is equal to the EOO (table 1). The Huu Lien NR was estimated to harbor 142–289 indiv. (238 ± 67 indiv.). The population size ($\check{N}_S$) within the suitable habitats was estimated to range from 680 to 1,447 indiv. (1,157 ± 293 indiv.), whereas the maximum population size ($\check{N}_{Max}$) in the karst habitats can achieve up to 2,855 individuals (table 1). We further calculated a potential maximum population size based on the maximum number of animals within each

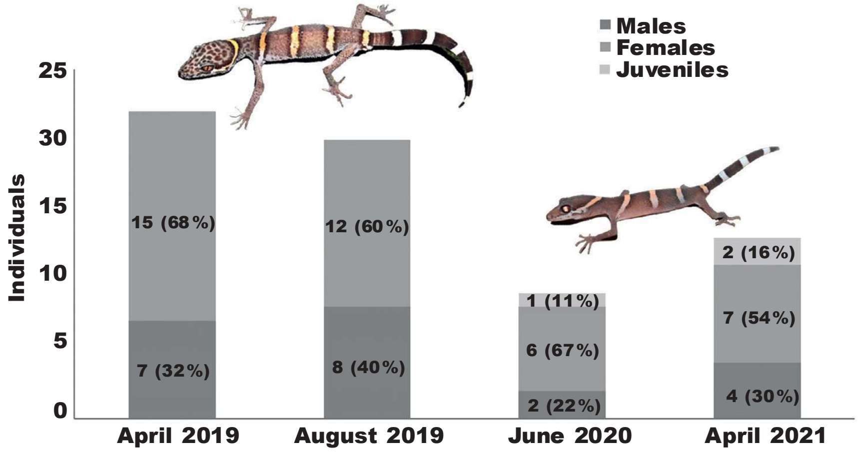

Fig. 2. Variations in the age and sex structures of *G. huuliensis* between survey periods in Lang Son (S1) and Thai Nguyen (S2) provinces, northern Vietnam (individuals observed but not caught were not included herein).

Fig. 2. Variaciones en las estructuras de edad y sexo de G. huuliensis *en los períodos de estudio en las provincias de Lang Son (S1) y Thai Nguyen (S2) en el norte de Vietnam (no se incluyeron los individuos observados pero no capturados).*

survey transect. Based on a maximum of 53 indiv. observed along all transects in the two study sites (S1 and S2), we estimated the potential total population size of 1,428 indiv. in the suitable habitats and 2,818 indiv. in the karst habitats (including 112 indiv. in the study site and 228 indiv. in the Huu Lien NR) (table 1).

The average population density of *G. huuliensis* was 6.4 indiv./km (1.1–11.3 indiv./km) along the surveyed transects. In particular, the lowest density was documented at T1 and T5 (1.1 indiv./km), whereas the highest density was recorded at T3 and T6 (11.3 ndiv./km) (table 1). The density per day was further calculated with an overall mean value of 2.5 indiv./km/day (max. 5 indiv./km/day at T4; table 1).

Anthropogenic impacts

We documented that human activities have severely degraded the habitats of *G. huuliensis*. More specifically, we observed many plastic products scattered on rocky shelters along survey transects (fig. 3A). Many large woody plants had been cut down, reducing forest cover (fig. 3B). Furthermore, evergreen of forests on karst formations have been gradually replaced by cultivated land or grassy hills; fig. 3C), and even completely destroyed so as to expand roads and for limestone quarrying (fig. 3D).

Regarding illegal collection activities, our interviews with local people and rangers revealed that wildlife dealers have paid 150,000–200,000 Vietnam Dong (7–9 USD) per adult of *G. huuliensis* to local hunters in the past. These geckos were transferred to Lang Son City, Vietnam, from where they were shipped to other cities in Vietnam. However, according to local hunters, no dealers have contacted them to collect wild tiger geckos in the last two years.

Due to the over–exploitation in the past, the ongoing deforestation, and the low number of documented tiger geckos, the level of human impact in transects T1, T2 and T5 is considered 'high' (table 1). Transects T3 and T4 are close to ranger stations and have not experienced exploitation (T3) or deforestation (T4), which is why the overall impact level is classified as 'medium'. Transect T6 in S2 currently remains undisturbed but as it is not protected, its impact level is considered 'low' rather than 'undisturbed'.

Discussion

Population status

This is the first study to evaluate the population status of the endemic tiger gecko *G. huuliensis* in northern Vietnam. Basic knowledge of the population status is crucial to establish conservation plans to protect threatened species in their natural distribution range (Huang et al., 2008; IUCN, 2019; Ngo et al., 2019a). Being aware of this importance, a few studies were recently carried out on other lizard species in Vietnam (Ngo et al., 2016a, 2016b, 2019a; Nguyen et al., 2018; Gewiss et al., 2020; Luu et al., 2020). However, these studies only estimated the population size within small representative sites. To overcome this limitation, we conducted large–scale investigations to provide the full extent of occurrence of *G. huuliensis* (Ngo et

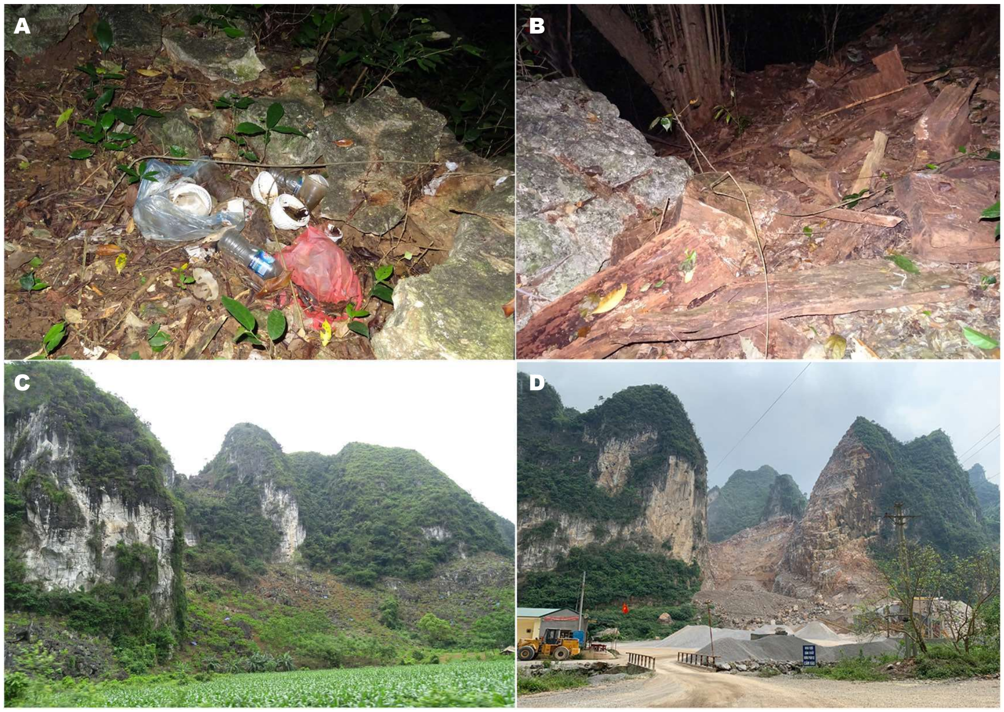

Fig. 3. Anthropogenic impacts in the study area: A, plastic trash; B, timber logging; C, cultivated hill; D, quarrying (Photographed by Hai N. Ngo).

Fig. 3. Efectos antrópicos en la zona del estudio: A, restos de plástico; B, tala de madera; C, colina cultivada; D, cantera (fotografías de Hai N. Ngo).

al., 2021a). Combined with the projected results of environmentally suitable habitats (climate and vegetation cover) (Ngo et al., 2021a), inhabited elevation ranges and karst formations (Ngo et al., in press), we were able to estimate the global population size of *G. huuliensis*. Similar to other tiger gecko species, the population size of *G. huuliensis* was predicted to be extremely small (Ngo et al., 2016b, 2019a, 2021b). In particular, we estimated that the global population size of *G. huuliensis* has a potential of 1,447 indiv. within the integrated suitable habitats. The maximum size can achieve up to 2,855 indiv. within the karst habitats in the whole extent of occurrence (table 1). However, the maximum population size seems to be much larger than the actual size since a large proportion of unsuitable habitats (such as crop hills, residential areas, roads) within the karst region were included in the estimate. Meanwhile, the minimum viable population was assessed to require at least 3,000–7,000 indiv. to ensure long–term persistence of a species (Reed et al., 2003; Traill et al., 2007).

The mean density of *G. huuliensis* (6.4 indiv./km) is slightly higher than that of an endangered relative (*G. catbaensis* in Cat Ba National Park, Hai Phong City with 1.2 indiv./km). However, it is considerably lower than the density of *G. catbaensis* populations on islands in Ha Long Bay, Quang Ninh Province with more than 60 indiv./km (Ngo et al., 2016b, 2019a). Moreover, the population density of *G. huuliensis* is also significantly lower than that of other globally threatened lizards in Vietnam (*Cnemaspis psychedelica*, *Physignathus cocincinus*) (Ngo et al., 2016a; Nguyen et al., 2018; Gewiss et al., 2020).

According to Ngo et al. (in press), the similar microhabitat selection among age and sex classes of *G. huuliensis* may be the main cause leading to competitive interactions among conspecifics. The occurrence in low densities likely mitigates the pressure of using shared resources within a restricted habitat (Irschick et al., 2005; Gilad, 2008; van Schingen et al., 2015). This is expected to occur in wild populations of *G. huuliensis* as well (Ngo et al., in press). Accordingly, we documented a small population density of *G. huuliensis* per day, with only 2.5 indiv./km/day (1.2–3.77 indiv./km/day, table 1).

Threats

The species has been illegally over–harvested by local hunters in the Huu Lien NR in the past (Ngo et al., 2019a, 2019b). Generally, the species abundance of in–situ populations is expected to be relatively balanced, with a large number of individuals under the same and eminently suitable environmental conditions (Araújo et al., 2002; Morrison et al., 2006). However, in this study, the abundance is uneven among survey transects. In particular, the number of estimated tiger geckos in each transect in the Huu Lien NR is relatively low and lower than that at the undisturbed transect (T6) in Thai Nguyen Province (table 1). We assume that the difference is due to the higher level of human impacts, especially exploitation, at the survey transects in S1 within the Huu Lien NR (Ngo et al., 2019a, 2019b). The decline of the population size due to the main anthropogenic impact of trade has been recorded in another insular Vietnamese tiger gecko, namely *Goniurosaurus catbaensis* (Ngo et al., 2019b). Long–term population assessments of *Shinisaurus crocodilurus* confirmed that its wild populations became extremely small in Vietnam and even declined up to 90 % in China due to the main threat of over–harvesting (Huang et al., 2008; van Schingen et al., 2014, 2016). Fetching alarmingly high prices of up to a thousand US dollars, the high commercial revenue provides a great incentive for poaching and excessive trade documented in other tiger gecko species (e.g. *G. luii* and *G. araneus*) and an insular Vietnamese gecko (*Cnemaspis psychedelica*) (Grismer et al., 1999; Stuart et al., 2006; Ngo et al., 2016a, 2019b). Due to the extremely small population size and occurrence in low densities, the exploitation severely imperils the existence of wild populations of *G. huuliensis*.

Additionally, Ngo et al. (2021a) predicted that the potential distribution of *G. huuliensis* will significantly shrink and even likely vanish under climate change within the next decades. The potential impact of climate change was also documented for other Vietnamese tiger gecko species (*G. catbaensis* and *G. lichtenfelderi*) (Le et al., 2017; Ngo et al., 2022). Given the sex structure of viviparous *G. huuliensis*, the predominance of female individuals could be explained due to the rate of female offspring being more common in the warm temperature gradient (Cunningham et al., 2017). Under climate change, global warming temperatures may increase the current imbalance in the sex structure of in–situ *G. huuliensis* populations in the future.

Besides serving as preferred habitat and refugia for almost tiger gecko species under impacts of climate change, the karstic ecosystem containing complex topographies and unique micro–environmental conditions is considered the ancestral habitat of *Goniurosaurus* species (Grismer et al., 2021; Ngo et al., 2021b). Karst formations indeed are identified as the exclusive habitat of *G. huuliensis* (Ngo et al., in press); as such, its existence depends entirely on the integrity of karstic habitats. Similar to what is happening all over Southeast Asia, as documented by Clemens et al. (2006), the high rate of limestone quarrying is a primary threat to *G. huuliensis,* which has been extensively recorded in Lang Son and Thai Nguyen provinces, including habitats of the species in Huu Lien NR. As such, the newly undisturbed but unprotected population of *G. huuliensis* in Thai Nguyen Province should be classified as at a low level of threat. To meet the local high demand for limestone products (e.g. cement), more cement factories could be built in Lang Son and Thai Nguyen provinces in the future, including the karst habitats of *G. huuliensis* and directly imperils untouched populations of *G. huuliensis*. Other human activities (namely logging activities, road construction, and pollution by plastic trash) have been observed within natural habitats of *G. huuliensis*, leading to forest fragmentation and degradation.

Conservation measures

In view of the ongoing severe human impacts, conservation measures to safeguard wild populations of the target species are urgently required. Recent studies on trade (Ngo et al., 2019b), taxonomy (Ngo et al., 2021b), ecology (Ngo et al., in press), impacts of climate change (Ngo et al., 2021a), and population status (this study) fill the gaps in basic knowledge on the biology of *G. huuliensis*. On the basis of findings from such research, in–situ and ex–situ conservation actions can be simultaneously implemented more effectively in the future. The target species was recently assessed as Critically Endangered (CR) in the IUCN Red List of Threatened Species (Nguyen, 2018), and was included in CITES Appendix II and the Vietnam Government's Decree No. 06/2019/ND–CP (Group IIB) in 2019 (Ngo et al., 2019b). As a result, any activity concerning domestic and international trade in wild individuals of *G. huuliensis* for commercial purposes is prohibited. Including the species in the international convention and the local decree may help to mitigate the illegal over–exploitation. Since the regulations came into effect, dealers have not yet contacted local hunters to collect wild animals of *G. huuliensis*. To completely stop illegal trafficking in relation to the target species, we highly recommend implementing community education to enhance awareness of the biodiversity value in the local communities within the Huu Lien NR and surrounding areas. Furthermore, these areas need to be increasingly patrolled by local rangers to improve the effectiveness of forest protection and to stop the illegal activities of exploitation.

Covering a total area of 85 km^2, the Huu Lien NR potentially harbors an estimated maximum number of 289 indiv. (accounting for approximately 15 % of the total population size). Based on this result, we strongly support a recommendation of Ngo et al. (2021a) that the Huu Lien NR should be considered as a 'center' for conservation programs to protect wild populations of *G. huuliensi*s. However, approximately 85 % of the total wild individuals of *G. huuliensis* may be located outside the boundaries of the nature reserve. Without proper law enforcement and forest ranger patrolling in there, a plan to establish a species and karst habitat conservation area within the EOO is an adequate conservation solution to safeguard the wild populations of *G. huuliensis.* As such, priorities for the local economic development relative to karst formations (limestone quarrying) can be reassessed together with priorities of biodiversity protection by relevant authorities. We highly recommend increasing research activities to promote the biodiversity value of karst ecosystems. Recently, many new species have been discovered from the karst habitats in the Indo–Burma biodiversity hotspot region, including tiger gecko species in China and Vietnam (Nguyen et al., 2009; CEPF, 2020; Ngo et al., 2021b). Globally threatened species, such as *G. huuliensis* should be highlighted as high–profile flagship species of karst ecosystems. The high value of biodiversity should be given with the priority of conservation policies to protect the karst landscape rather than favoring economic development.

Methodological limitations

The 'invisibility rate' method was developed by Huang et al. (2008) and it has been widely used to estimate the population size of lizards (van Schingen et al., 2014, 2016; Nguyen et al., 2018; Ngo et al., 2019a; Gewiss et al., 2020; Luu et al., 2020). Indeed, the advantage of this method is that the invisibility–rate index is calculated based on repeated surveys at one site and can then be employed for congeneric species or at other sites that are surveyed only once (Huang et al., 2008; Ngo et al., 2016b; Gewiss et al., 2020). However, since this method is based on direct records of observed individuals, the detection probability may have a strong influence on the observed and estimated number of individuals. In particular, the detectability of *G. huuliensis* is assumed to be rather low due to its small size, secretive lifestyle and association with habitats with high vegetation coverage in remote areas. In the present study, for example, based on one survey, the minimum population size was estimated at transect T1 in August 2019 and June 2020 (2 indiv.), while the value was significantly higher (9 indiv.) in April 2019 after the survey was repeated. These differences between surveys at each transect that mainly account for the variation in the total population size of *G. huuliensis*; a minimum of 1,342 indiv. compared to a maximum of 2,855 indiv. Further general impacts on the detectability are changes in environmental conditions, transect length, survey time, and human factors (Gewiss et al., 2020). For example, Ngo et al. (pers. obs.) recorded more than 40 indiv. of *Goniurosaurus lichtenfelderi* in Tay Yen Tu NR, northern Vietnam in August 2019, but failed to observe any individual at all after two repeated surveys in September 2019 due to temperature variations. We also recorded that the number of *G. huuliensis* individuals observed per day varied from 0.96 to 5 indiv./km/day. Therefore, to gain more complete knowledge of the real population status we highly recommend a long–term monitoring program in different habitat types. Furthermore, all selected sites should be surveyed at least three times rather than using representative invisibility–rate values of repeatedly surveyed sites. Thereby, other capture–mark–recapture methods such as Schnabel index could be applied to estimate the population size, which might improve the accuracy of the estimated values (Schlüpmann and Kupfer, 2009; Ngo et al., 2016a, 2016b).

As we mentioned above, the karst ecosystem is considered the prerequisite habitat of *G. huuliensis*. However, the inclusion of karst formations to estimate the maximum population size ($\check{N}_{Max}$) was limited by a low spatial resolution of the respective layer, including unsuitable habitats (crop hills, residential areas, roads). Given the total population size ($\check{N}_S$) within the integrated suitable habitats, the species abundance is expected to be highly correlated with the probability of environmental suitability. A regression ratio from the correlation can be applied to estimate the total population size of *G. huuliensis* more properly. However, under– or over–fitting can take place due to using only representative data of the six survey transects, leading to inaccuracy in the interpolating estimation. In this study, the population size was equally estimated in all sites of environmental suitability, whereas all unsuitable sites indicated the species absence within the extent of occurrence. We recommend that further surveys should be carried out along other natural transects within occurrence areas of *G. huuliensis* with high probabilities of environmental suitability according to our models. Based on such data, it would be possible to correlate and interpolate the total size more precisely.

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Chapter 3. Threats, modeling refugia and implications for conservation

3.1. A case study on trade in threatened Tiger Geckos (*Goniurosaurus*) in Vietnam including updated information on the abundance of the Endangered *G. catbaensis*

The author of the thesis, Prof. Dr. Thomas Ziegler and Dr. Mona van Schingen-Khan mainly contributed to the planning of the study. Data collection, analyses, preparation of graphics and the writing of the paper were mainly carried out by the author of the thesis. International trade data were provided by Dr. Mona van Schingen-Khan.

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A case study on trade in threatened Tiger Geckos (*Goniurosaurus*) in Vietnam including updated information on the abundance of the Endangered *G. catbaensis*

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Abstract

Tiger geckos of the genus *Goniurosaurus* are considered as a susceptible reptile group, due to their restricted distribution ranges, specialisation to specific microhabitats and generally low population densities. While still new species have been discovered recently, *Goniurosaurus* species are threatened by extinction through habitat loss and collection for the pet trade. Of the 19 described species, for only eight species, the conservation status has been assessed within the IUCN Red List between 2016 and 2018 and all have been classified in the threat categories VU (Vulnerable), EN (Endangered) and CR (Critically Endangered). *Goniurosaurus* spp. are popular in the international pet market at least since the 1990s and several species experienced local extirpations as a consequence of massive over-collection in the past. However, tiger geckos have not been paid much attention for conservation, amongst others, due to the lack of comprehensive knowledge on their conservation status and biology. This study provides an overview of international trade in *Goniurosaurus* based on available data from 1999 to 2018 in the U.S. as well as data from online surveys and interviews in Europe and Vietnam, with the main focus on species native to Vietnam. All five tiger gecko species known from Vietnam were found in the local trade as wild captures for the national and international market and / or in the U.S. and Europe for relatively higher prices. We found that entire trade chains are very long (including several transfers and dealers involved) and that keeping and transport happen under poor conditions. We herein provide updated information on the abundance of the Endangered Cat Ba Tiger Gecko, which was recently shown to not only occur at its type locality, Cat Ba Island, Hai Phong City, North Vietnam, but also to inhabit small offshore islands in the Ha Long Bay, Quang Ninh Province. While the wild effective population was found to be relatively stable on four islands in Ha Long Bay, consisting of about 124 and 129 individuals in July 2017 and April 2018, respectively, the abundance of other sub-populations, impacted by anthropogenic pressures were found to be very low (2–10 individuals). Based on our findings, we propose stringent conservation measures to more efficiently protect wild tiger gecko populations, such as the inclusion in the Governmental Decree in Vietnam, the assessment of remaining species in the IUCN Red List of Threatened Species and the inclusion in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Keywords

Pet markets, Vietnam, abundance, anthropogenic pressures, conservation

Introduction

Tiger geckos of the genus *Goniurosaurus* currently comprise 19 species with a disjunct distribution in Southeast Asia and East Asia (Chen et al. 2014; Grismer et al. 1994, 1999; Honda and Ota 2017; Liang et al. 2018; Yang and Chan 2015; Zhou et al. 2018; Ziegler et al. 2008). The genus contains a high level of local endemism and many species have been recorded only from a single locality, mountain range or archipelago of China, Japan and Vietnam. *Goniurosaurus* is also considered as one of the most susceptible reptile groups due to its generally low population densities and restricted distribution areas, which make tiger geckos particularly threatened by extinction through habitat loss and over-harvesting for the pet trade (Ngo et al. 2016b; Yang and Chan 2015).

Tiger geckos have been recorded in the international pet market at least since the 1990s (Rösler 1995; Stuart et al. 2006; Yang and Chan 2015). Some rare species are fetching high prices in relation to other gekkonids (e.g. US$2000 / per individual of *Goniurosaurus luii*), which provides great incentives for poaching and excessive collection (Grismer et al. 1999; Stuart et al. 2006). However, the scale of the international demand for tiger geckos, as well as trade levels and patterns, are unknown due to tiger geckos are not listed in CITES. Thus, we herein present an attempt to outline domestic and international trade activities in *Goniurosaurus* species in order to inform decision-makers and develop conservation strategies.

The insular Cat Ba Tiger Gecko (*Goniurosaurus catbaensis*) was originally discovered on Cat Ba Island in Hai Phong City, north-eastern Vietnam (Ziegler et al. 2008). A preliminary population assessment of *G. catbaensis* revealed that its effective population size at the type locality is extremely small with less than 24 individuals (Ngo et al. 2016b). The species are considered to be vulnerable to anthropogenic disturbances and ongoing demand has been recorded in pet markets, as well as on internet platforms for many years (Ngo et al. 2016b; Ngo et al. in press). Over-collection for the pet trade has probably led to local extirpation of *G. luii* and *G. araneus* from their respective type localities in the past (Stuart et al. 2006; Yang and Chan 2015). In addition, habitat destruction for touristic purposes may increase the pressure on the wild *G. catbaensis* population (Ngo et al. 2016b; Nguyen et al. 2018b).

Recently, Ngo et al. (in press) confirmed for the first time that *G. catbaensis* occurs outside its type locality, , by also providing a microhabitat characterisation of *G. catbaensis* throughout its distribution range in Cat Ba Ha Long archipelagos. The present study was conducted to further provide a first assessment on the population size of and threats impacting subpopulations of the Cat Ba Tiger Gecko from Ha Long Bay.

Methods

Analysis of trade

To obtain an overview on the availability and evidence for trade in *Goniurosaurus* spp. in Vietnam, we surveyed several pet markets in both southern and northern Vietnam (including Hai Phong City, Quang Ninh Province, Ha Noi City, Ho Chi Minh City and Dong Nai Province) in March 2018, based on public information on the internet. Five local dealers were questioned in order to determine the origin, availability, demand, price and use of traded species. To investigate the reptile market in the European Union (EU) for the availability of *Goniurosaurus* spp., we further screened online markets (online shops, internet platforms and forums, Facebook) and visited the largest reptile fair "Terraristika" in Hamm, Germany in March and June 2018. Names of interviewees were kept anonymous to ensure data privacy rights.

Furthermore, we analysed import volumes of *Goniurosaurus* spp. into the United States (U.S.). Data were obtained from the LEMIS database of the U.S. Fish & Wildlife Service, which included all recorded imports of *Goniurosaurus* spp. into the U.S. from 1999 to 2018. The purpose of trade in *Goniurosaurus* was categorised as (B) "breeding in captivity or artificial propagation", (H) "hunting trophies", (P) "personal", (S) "scientific", (T) "commercial" and (Z) "zoo". The source of specimens was differentiated between wild (W), captive bred (C), captive born (F) and ranched (R), animals following the CITES definition in Res. Conf. 12.3 (Rev. Cop17).

Field survey

Field surveys were conducted in Ha Long Bay in July 2017 and April 2018 during the non-hibernation season of *Goniurosaurus catbaensis* (Grismer et al. 1999; Ngo et al. 2016b). We conducted a total of 24 night excursions between 19:30 h and 05:00 h of the next day, when the lizards were found to be active (Ngo et al. 2016b; Ziegler et al.

Table 1. Study sites in Ha Long Bay, Quang Ninh Province, Vietnam.

Study sites (Transect)	Transect Length (m)	Area of survey sites (m^2)	Elevation a.s.l. (m)	Habitat types
Island 1 (T-1)	380	5 000	4–99	Shrub vegetation on limestone karst
Island 2 (T-2)	300	2 180	5–20	Shrub vegetation on limestone karst
Island 3 (T-3)	200	1 180	10–72	Shrub vegetation on limestone karst
Island 4 (T-4)	410	5 560	14–67	Shrub vegetation on limestone karst
Total	1290	13 920	4–99	Shrub vegetation on limestone karst

2008). Four survey transects (T-1 to T-4) were set up on four offshore islands, with length of 200 to 410 m each. The islands, covering a total area of 13,920 m^2 (Table 1). Thereof, two transects (T-3, T-4) were selected based on previous surveys by Ngo et al. (2016b) and two further sites were surveyed according to recommendations by interviewed staff of the Management Board of Ha Long Bay (T-1, T-2). Study sites were located within shrub vegetation on limestone outcrops at elevations between 4 and 99 m a.s.l (Table 1). Boat surveys were also conducted at night to search for animals on out-surfaces of limestone karsts close to the oceanic surface. Coordinates and elevations of each captured individual were recorded with a GPS Garmin 64. However, GPS data is not presented herein, to prevent the misuse of the data.

Population analysis

To estimate population, a "mark-recapture method" was applied. In case of one-time mark and recapture event at two sites (T1, T3), the "Lincoln-Peterson Index" and, in the case of several recapture events at two remaining sites (T2, T4) (e.g. Caughley 1980; Schlüpmann and Kupfer 2009; Smith and Smith 2009; Nguyen et al. 2018a), the "Schnabel Index" was applied. Therefore, all encountered individuals were captured and individually marked with passive integrated transponder (PIT) tags (ISO FDX-B Glastransponder, 1.4 × 9 mm). The microchip was injected under the skin on the left body side behind the shoulder. All captured and recaptured individuals were identified with a transponder reader and afterwards released immediately at the spot of capture (van Schingen et al. 2014; Smyth and Nebel 2013). Each transect was repeatedly surveyed in intervals of two days. Estimated population sizes only refer to the surveyed sites and do not encompass the entire wild population of the species. Since it is impossible to survey all potentially suitable habitats in the region, density estimates with reference to transect lines were used as relative abundances.

To assess the population structure of *G. catbaensis*, lizards were categorised into three age classes, based on the snout-vent length (juvenile with SVL < 85 mm, sub-adult with 85 mm ≤ SVL < 105 mm and adult with SVL ≥ 105 mm) (Ngo et al. 2016b). We also differentiated between sexes and between gravid and non-gravid females. Sexes could only be determined clearly for adult and sub-adult specimens as males have enlarged swollen cloacal and 16–21 precloacal pores, while those are lacking in females (Ziegler et al. 2008). Furthermore, the time was noted for each encounter to evaluate the activity pattern of the species. To test for differences in population structure between July 2017 and April 2018, as well as between islands and time of the day, a c^2 test with $P \leq 0.05$ was applied. Statistical analyses were performed with the SPSS software, version 16.0 (SPSS Inc., Chicago).

Anthropogenic Impacts

Potential threats to *G. catbaensis* were investigated in Ha Long Bay and Cat Ba Archipelago. Evidence for harvesting of *G. catbaensis* was obtained through interviews with local dealers, market surveys and a literature survey. Other potential anthropogenic impacts, namely "tourist activities" and "habitat degradation" were recorded by our own observations and interviews with local fishermen (Ngo et al. 2016b; Ngo et al. in press). Depending on the frequency and extent of recorded negative impacts, threats to each of the sub-populations were classified as "not recorded", defined as never being recorded, "low" as being rarely observed, "medium" as being recorded several times, or "high" as being recorded frequently or the extent of destruction was evaluated as too high.

Results

Trade

Most, if not all *Goniurosaurus* species were found being sold in the international pet market in Europe and the U.S. during the present study. According to the LEMIS database of the U.S. Fish & Wildlife Service, a total of 16,714 specimens of *Goniurosaurus* spp. have been imported into the U.S. (mean of 835 ± 1082 individuals annually) between 1999 and 2018 (Fig. 1A). The majority of specimens were imported on genus level as *Goniurosaurus* spp. (44.5%). Amongst the others, the most imported species was *G. lichtenfelderii* (43.6%, n = 7281 individuals), followed by *G. hainanensis* (6%), *G. luii* (3.6%), *G. orientalis* (1.3%) and *G. kuroiwae* (1%) (Fig. 1C). The vast majority of imported specimens were traded for commercial purposes (97%), while only 3% were imported for scientific and zoo purposes (Fig. 2B). A number of 11,515 specimens (68.9%) were wild caught and 5,086 animals (30.4%) were imported as bred in captivity (Fig. 2A). Thereby, most *Goniurosaurus* species imported into the U.S. were from Taiwan (40.5%) as a major exporter, followed by Hong Kong (32.2%), China (15.7%), Indonesia (5.2%) and other CITES Parties (6.4%) (Fig. 1B).

In March 2018, market surveys showed that the trade in *Goniurosaurus* in the EU mainly takes place online, but also in reptile fairs. It was recorded that specimens for sale fetch prices between US$35–200 on the international internet markets, e.g. price for two unsexed juveniles or one male of *G. araneus* was recorded for sale for US$150, *G. bawanglingensis* for €175 per pair (US$200), *G. catbaensis* for US$195–230 per

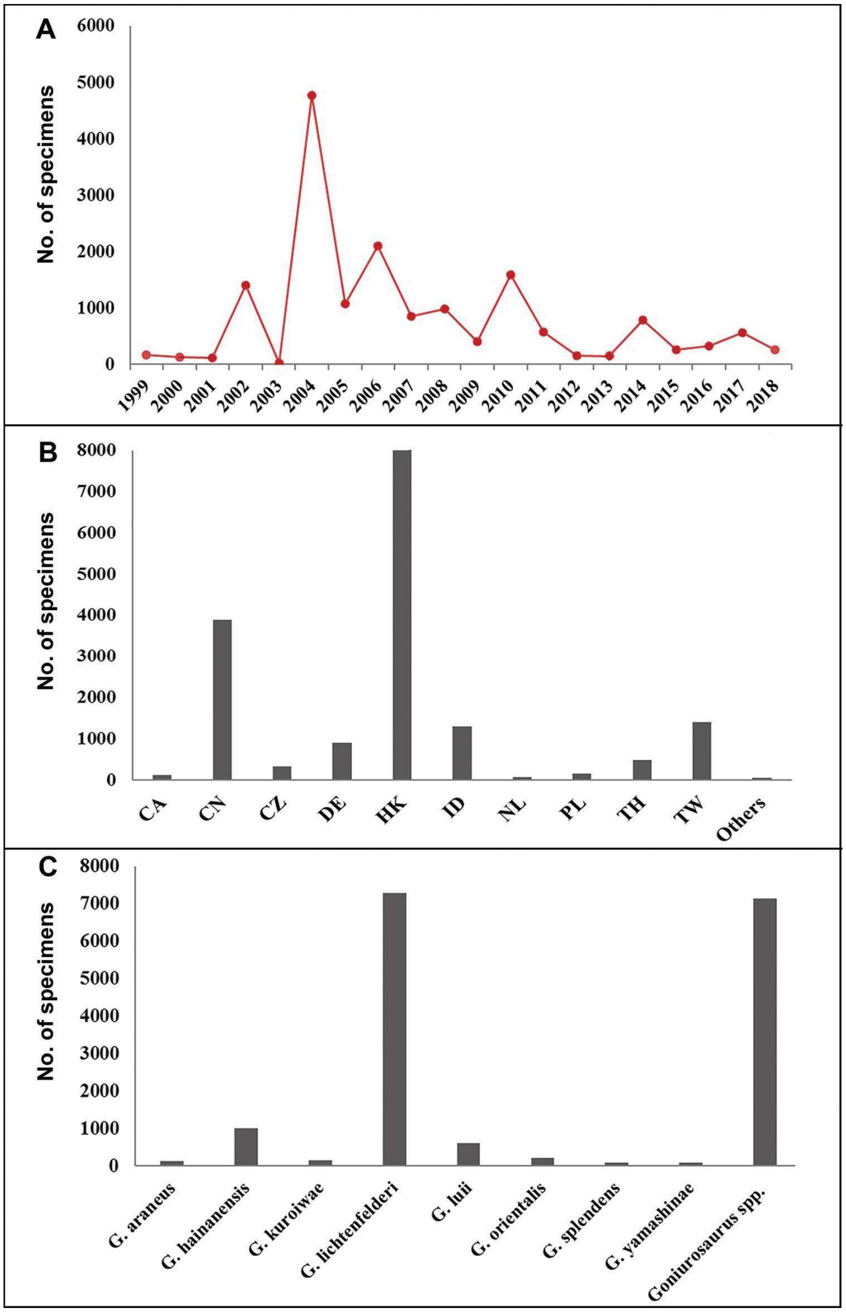

Figure 1. International trade in *Goniurosaurus* species between 1999 to 2018 to or from the U.S. **A** Annual volumes of import into the U.S. **B** number of exported specimens per exporting party (CA = Canada; CN = China; CZ = Czech Republic; DE = Germany; HK = Hong Kong; ID = Indonesia; NL = Netherlands; PL = Poland; TH = Thailand; TW = Taiwan) **C** number of imported specimens per species into US. Source: LEMIS database of the U.S. Fish & Wildlife Service (1999–2018).

specimen or for €300 (US$340) per pair, *G. hainanensis* for US$45–150 per specimen, *G. huuliensis* for US$400 per pair or US$150 for one male, *G. lichtenfelderii* for US$70–100 per specimen and *G. luii* for US$175 per two juveniles or for US$40–60 per pair or one (Table 2).

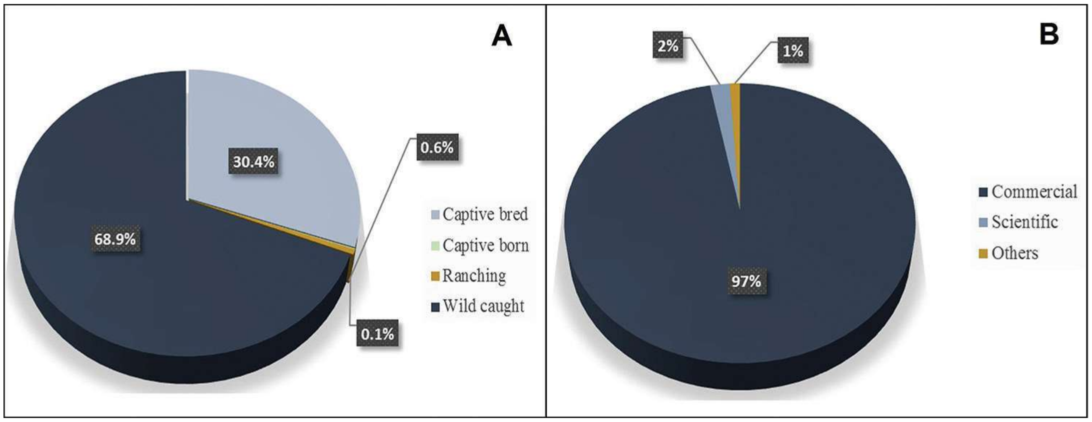

Figure 2. International trade in *Goniurosaurus* species from 1999 to 2018 to or from the U.S. **A** Source of animals **B** purpose of trade. Source: LEMIS database of the U.S. Fish & Wildlife Service (1999–2018).

Table 2. International trade in several *Goniurosaurus* species in Europe based on online investigations and interviews with dealers on reptile fairs (own surveys in 2018 and data from Altherr et al. *in lit.* 2019).

Species	Trade type	Place	Country	Price (USD) per specimen
G. araneus	Offer	Internet Shops	Europe	150–170 (€142)
G. bawanglingensis	Offer	Fair, Internet Shops	Germany, Europe	€67–250 (or €175 per pair)
G. catbaensis	Offer	Fair, Internet Shops	Germany, Europe	195–230 (or €300 per pair)
G. hainanensis	Demand and Offer	Internet Shops, Shops, and Private	Czech Republic, France, Germany	45–150
G. huuliensis	Demand and Offer	Internet Shops and Private	Europe	€160–220 (€400 per pair)
G. lichtenfelderii	Demand and Offer	Internet Shops and Private	Europe	70–100
G. luii	Demand and Offer	Fair, Internet Shops	France, Germany, Europe	40–170

Furthermore, Altherr et al. (*in lit.* 2019) reported a total of 835 specimens of *Goniurosaurus* spp. were observed for sale on 142 different online adverts with prices ranging from €35–300 (US$40–365) between September 2017 and September 2018, whereof, *G. araneus* (n = 56) were offered for sale of €142, *G. bawanglingensis* (n = 102) for €67–250 *G. catbaensis* (n = 29) for €170, *G. hainanensis* (n = 162) for €35–140, *G. huuliensis* (n = 41) for €160–220, *G. lichtenfelderii* (n = 97) and *G. luii* (n=150) for €35–142.

According to interviews with local dealers in Vietnam in March 2018, all five native *Goniurosaurus* species have been frequently recorded in local pet shops from Dong Nai Province and Ho Chi Minh City in northern Vietnam, on Social Media, e.g. Facebook, Zalo online and other internet platforms. These tiger geckos were usually wild-caught by local villagers who live within the species' distribution range and then sold for little money to dealers to be either offered in local pet shops or be sold to other traders. *Goniurosaurus* specimens were found to be locally offered for sale at US$7–25 per animal in pet shops in Vietnam (Table 3). According to dealers in pet shops, *Goniurosaurus* specimens have been regularly exported from Vietnam to Thailand and Indonesia without any permits for higher prices of US$100–150 per individual. Charges consist of at least 20–50 specimens per deal. Afterwards, specimens would allegedly be mainly exported further to Europe and the United States (Table 3).

Table 3. Information on local trade in and international trade pathways for *Goniurosaurus* species in Vietnam based on interviews with local dealers in 2018.

Species	Sources	Offered in pet shops	Country of destination	Minimum quantity per deal for export	Price (USD) per specimen*
G. araneus	China	Dong Nai Province	Thailand, Indonesia, EU and the U.S.	50	(2): 100–150
G. catbaensis	Cat Ba NP, Hai Phong city, Viet Nam	Dong Nai Province, Ho Chi Minh City	Thailand, Indonesia, EU and the U.S	>= 20	(1): 7–25 (2): 150
G. huuliensis	Huu Lien NR, Lang Son Province, Viet Nam	Dong Nai Province, Ho Chi Minh City	Thailand, Indonesia, EU and the U.S.	>= 20	(1): 20–25 (2): 100
G. lichtenfelderii	Yen Tu, Quang Ninh Province, Viet Nam	Dong Nai Province	Thailand, Indonesia, EU and the U.S.	>=20	(1): 20–25 (2): 100
G. luii	Cao Bang Province, Viet Nam	Dong Nai Province, Ho Chi Minh City	Thailand, Indonesia, EU and the U.S.	50	(1): 20–25 (2): 100–150

*(1): for locals; (2): for export

Population status of *Goniurosaurus catbaensis*

We observed a total of 73 animals (54 adults) in July 2017 on four islands and a total of 93 individuals (70 adults) in April 2018 within sites covered by the shrub vegetation on three islands in Ha Long Bay. We did not find any specimens of *Goniurosaurus* on out-surfaces of karst formations by boat surveys. The total population size at the survey areas was estimated at 175 individuals in July 2017 and 180 animals in April 2018. Regarding the effective population size – considering only mature animals – this estimated wild population was relatively similar to about 124 and 129 individuals in July 2017 and April 2018, respectively (Table 4). With respect to each sub-population, the number of estimated animals was highest on island 1 (86 individuals) (Table 4).

The mean density of *G. catbaensis*, along suitable habitat sites in Ha Long Bay, was estimated to be around 6 individuals and 4.5 adults per 100 m transect length during April and 9.1 individuals and 7 adults per 100 m transect length during July. By comparing different sites, the highest abundance was estimated at nearly 12 individuals per 100 m/ transect length at site 1 in April, while we found the lowest density of 2 animals per 100 m/ transect length at site 2 in July (Table 4).

In both investigated months, the sex ratio of adults was relatively balanced with a little higher percentage of females of *G. catbaensis* (57% and 55%, respectively). The percentage of gravid and non-gravid females significantly differed between both months ($c^2 = 21$; df = 2; $P < 0.001$), while 44% of females (n = 14) were gravid in July 2017 (Fig. 3A) and 95% of females (n = 41) were non-gravid in April 2018 (only two females were gravid). With respect to the presence of different age classes, the population structure was relatively similar between two months ($c^2 = 3.04$; df = 2; $P = 0.219$) with most of the observed animals being identified as adults (75% in July 2017 and 80% in April 2018 see Fig. 3B). Frequency histograms of SVL (Min–Max = 53.42–125.28 mm, n = 149) showed a slight shift in the presence of small juveniles between July and April ($c^2 = 19.88$; df = 7; $P = 0.006$). Individuals with the smallest SVL

Table 4. Summary of the population assessment of *Goniurosaurus catbaensis* including observed individuals, densities (D) and population size estimates (N) in Ha Long Bay in July 2017 and April 2018. Ind: individuals; Asterisks indicate missing data (no survey).

	Island 1 (T-1)	Island 2 (T-2)	Island 3 (T-3)	Island 4 (T-4)	Total
July-2017					
Total (observed)	27	7	20	19	73
Mature (obs.)	17	3	16	18	54
D observed [ind/100 m of route]	7.1	2.3	10	4.6	6
D [mature/ 100 m of route]	4.5	1	8	4.4	4.5
N_{mature}	36	4	33	50	**124**
N_{total}	56	16	49	54	**175**
April-2018					
Total (observed)	45	*	15	33	93
Mature (obs.)	28	*	12	30	70
D [ind/100 m of route]	11.8	*	7.5	8.1	9.1
D [mature/100 m of route]	7.3	*	6	7.5	7
N_{mature}	60	*	27	45	**129**
N_{total}	86	*	45	49	**180**

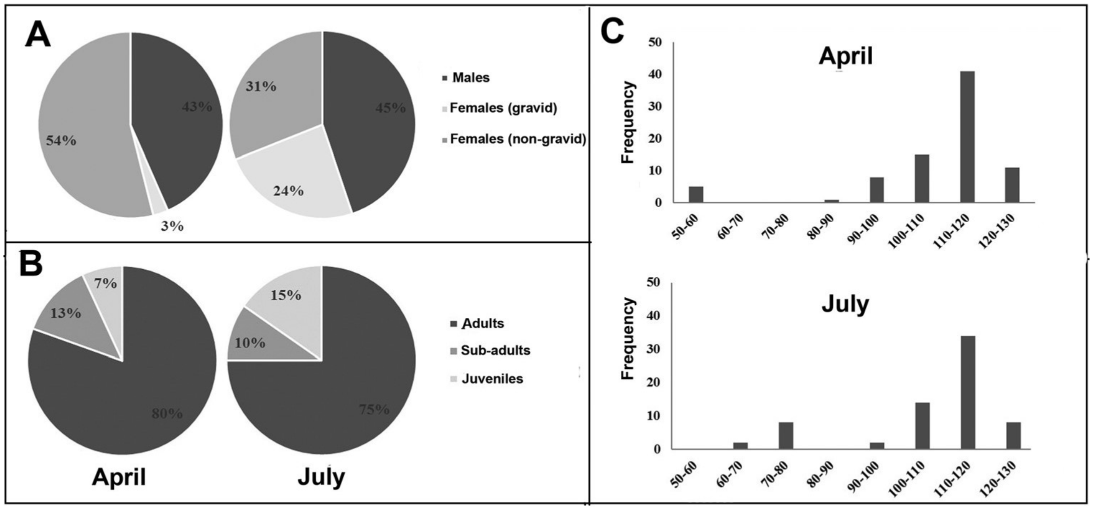

Figure 3. A, B Population structure of *Goniurosaurus catbaensis* from Ha Long Bay (July 2017 and April 2018, respectively) **C** Frequency histogram of snout-vent length of *G. catbaensis* for the months April and July.

(50–60 mm) were only found in April, while juveniles with longer SVL (60–80 mm) were exclusively found in July. The number of adults with SVL ranging between 110–120 mm accounted for the highest percentage in both months (Fig. 3C).

Daily activity

With regard to the daily activity pattern, *G. catbaensis* was found to be active at night from approximately 20:00 h to 04:00 h. The vast majority of lizards (98 animals or

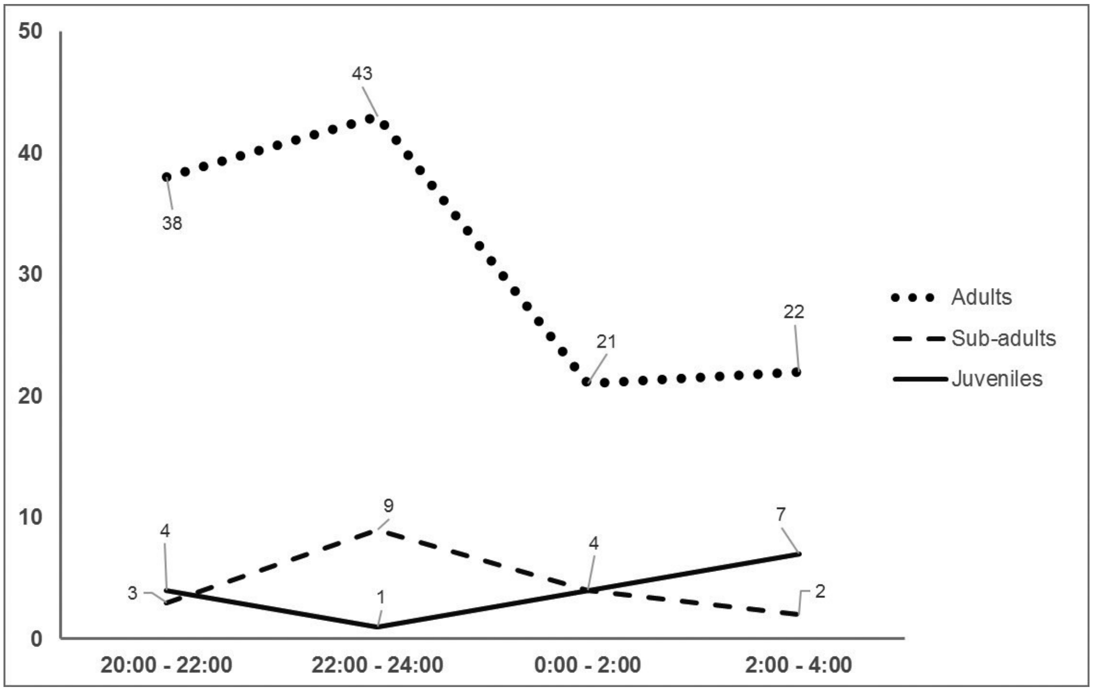

Figure 4. Number of observed *Goniurosaurus catbaensis* specimens at different time intervals in Ha Long Bay.

62% of the recorded lizards) were observed between 22:00 h and 24:00 h. We further found a difference in population structure amongst different times of the day (c^2 = 14.39; df = 6; P = 0.026 < 0.05). Particularly, we observed that more adult specimens were active during the first four hours between 20:00 h and 24:00 h (n = 81 account 65.3% of captured adults), while juveniles were frequently found either before 22:00 h or mainly between 00:00 h and 04:00 h. Most juveniles were found between 02:00 h and 04:00 h (Fig. 4).

Threat evaluation

The number of captured animals and the size of sub-populations of *G. catbaensis* from Ha Long Bay in Quang Ninh Province were compared with other differently affected sub-populations in Cat Ba Archipelago of Hai Phong City. In the Cat Ba National Park and Ha Long Bay's tourist caves, total anthropogenic impacts were considered to be "Medium" and local populations were relatively small (2–10 individuals). A strong flood event in 2015 appeared to have caused the local extirpation of *G. catbaensis* at one site in Viet Hai Commune on Cat Ba Island (Table 5). The mean population size of the species was highest within untouched sites on four islands in Ha Long Bay (Table 5), where total anthropogenic impacts were considered as "Low".

Table 5. Recorded impacts on *Goniurosaurus catbaensis* at known sites. Single impacts were ranked according to severity as "not recorded" defined as never being recorded, "low" as being rarely observed, "medium" as being recorded several times, or "high" as being recorded frequently, or the extent of destruction was evaluated too high.

Study sites	Population size (Mean)	Harvest	Tourist activities	Habitat degradation	Total impacts
Sites near the headquarters of Cat Ba National Park (NP) – Ngo et al. 2016b	5	Medium	High	Medium	Medium
Viet Hai Commune – Cat Ba NP – Ngo et al. 2016b	10	Medium	Medium	Low	Medium
One site in Viet Hai Commune – Cat Ba NP – Ngo et al. in press	0 (in August 2015)	Not recorded	Not recorded	High	High
Ha Long Bay (4 islands) – Current study	51	Not recorded	Not recorded	Low	Low
Ha Long Bay's Caves (4 caves) – Current study	2 (total animals)	Not recorded	High	Medium	Medium

Discussion

Trade

Ngo et al. (in press) provided evidence for the collection of at least three of five native species, namely *G. catbaensis, G. huuliensis* and *G. luii* in Vietnam for the domestic trade. During our recent market surveys in 2018, we further recorded that all five native tiger geckos are being collected for sale and also kept as pets in Vietnam, while some tiger geckos including *G. araneus, G. luii* (Grismer et al. 1999), *G. catbaensis* (Bauer 2009) and *G. lichtenfelderii* (Liu 1993) were reported to be used in traditional medicine in China. Our interviews identified commercial revenues in relation to the domestic and international pet trade as the most common incentive for domestic collection in Vietnam. Janssen and Shepherd (2019) documented that the *Goniurosaurus* is the most popular endemic genus offered for sale on the Nansei Islands in Japan.

All tiger geckos have a restricted distribution ranges and are – like many endemic reptiles – extremely vulnerable to exploitation, so that international trade can quickly become a significant threat for extinction (Janssen and Indenbaum 2019; Janssen and Shepherd 2018; Lyons and Natusch 2013). Accordingly, many endemic species have not been seen any more over a long period of time in recent years and some taxa are considered extinct at their type localities (Lindenmayer and Scheele 2017; Meiri et al. 2018). Evidence from extensive field works in recent years suggests that populations of *G. araneus* in Vietnam and *G. luii* in China have been extirpated at their respective type localities in the past due to over-harvesting for the pet trade (Stuart et al. 2006, Yang and Chan 2015, pers. obs.).

Goniurosaurus spp. have been popular in the international pet market at least since the 1990s (Stuart et al. 2006; Yang and Chan 2015). According to the LEMIS database, a total of 16,714 specimens of *Goniurosaurus* spp. have been imported into the U.S. between 1999 and 2018. The U.S., together with Japan and the European Union, are considered as three important destinations for the transaction of reptile species including tiger geckos (Auliya et al. 2016; Janssen and Indenbaum 2019; Sollund and Maher 2015). Several individuals of the Huu-Lien Tiger Gecko, endemic to Vietnam, were observed in some reptile shops in Japan (Janssen and Indenbaum 2019). Janssen and Shepherd (2019) found that all *Goniurosaurus* species, endemic to Japan and being nationally protected by law, are offered for sale in the EU and the U.S. in large quantities for prices reaching up to US$714 for single specimens.

We herein observed most *Goniurosaurus* species for sale in the EU online as well as in reptile fairs. Accordingly, Altherr et al. (*in lit.* 2019) spotted a total of 835 specimens of *Goniurosaurus* spp. for sale on different social media platforms between 2017 and 2018. These observations, during random physical as well as internet market surveys, only reflect snapshots of current EU trade in tiger geckos. Actual trade volumes remain unknown – as *Goniurosaurus* spp. are not as yet listed in the CITES Appendices – but are likely to be higher.

Stuart et al. (2006) supported the idea that captive breeding can reduce further demand on wild-caught animals. Our study indeed showed that a large quantity of animals offered for sale was labelled as captive bred in Europe and several reports on successful captive breeding in *Goniurosaurus* species exist (e.g. Einsfelder 2016; Kaverkin 2000). However, wild-caught animals are still being imported into the EU and the U.S. in large quantities. During the recent ten years, there were still about 4,000 imports of wild specimens to U.S. recorded. Endemic tiger geckos of China and Vietnam, which were imported into the U.S. as wild-caught specimens, originated from non-range states, likely without any related permits from countries of origin.

According to interviewees in Vietnam and Europe, captive-bred animals were not yet available in quantities to meet the global demand and wild animals were generally offered for much cheaper prices than captive-bred animals. Furthermore, wild-caught specimens are considered to have a high mortality rate during transport and stockpiling (Sollund and Maher 2015), even though no studies exist that assessed mortality rates between collection and export or between export and country of destination in *Goniurosaurus*. According to local dealers, wild animals are usually kept for a long time clumped together in small boxes and then transported with motorbikes, trains, ships or air freights under poor conditions without supply of food and water. It is likely, that many of the sensitive animals die before reaching their final destination. According to our interviews, local dealers from Vietnam nowadays usually contact local collectors who are living close to the habitats of *Goniurosaurus* species via online wildlife trade groups, for example, on Facebook and pay about US$4 – 5 per individual for collection during the active season of the species. We found *G. araneus* amongst the animals offered in Vietnam (likely imported from China), a species which has probably already been extirpated from its type locality in Vietnam (Ngo et al. 2016b). After collection, specimens are frequently transported via motorbike to Hanoi, northern Vietnam and then transported by train or motorbike to pet shops in Dong Nai and Ho Chi Minh City, southern Vietnam. Some specimens are sold in Vietnam, but the majority of animals are allegedly transported by train or boat to Thailand and Indonesia as intermediary countries without any permits, as they reach higher prices than on the national market. From there, these animals are mainly exported to Europe and the U.S. (Fig. 5). As such, the entire trade chains are rather long as they include numerous stations.

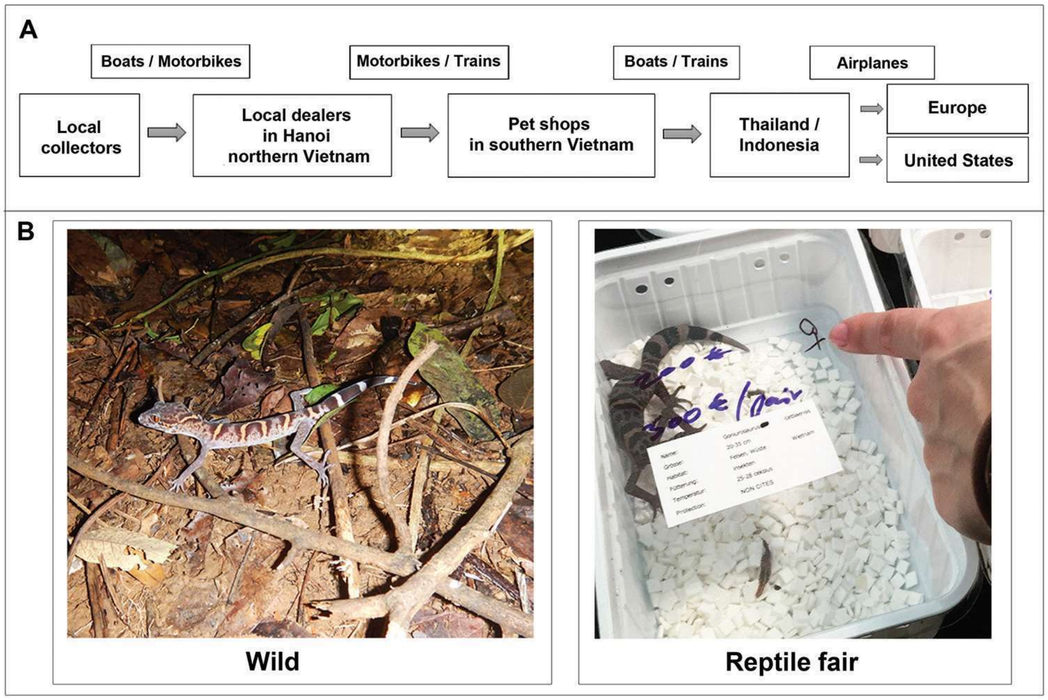

Figure 5. One case example showing the route of local and international trade in *Goniurosaurus catbaensis*.

Population status of *Goniurosaurus catbaensis*

Recent population estimates of the Cat Ba Tiger Gecko at its type locality on Cat Ba Island suggested extremely low population sizes (Ngo et al. 2016b; Nguyen et al. 2018b). Ngo et al. (in press) recently recorded new sub-populations of *G. catbaensis* on further small islands in the adjacent Ha Long Bay. The present data suggest that these sub-populations are stable and actively reproducing. Densities of *G. catbaensis* on islands in Ha Long Bay were, on average, 6 animals / 100 m, some magnitudes higher than on Cat Ba Island (0.08 – 0.17 animals / 100 m, Ngo et al. 2016b). The species is not evenly distributed over the small islands, but only occurs along limited habitat sites. A survey on a population of the closely related *G. luii* in the north of Vietnam revealed a similar low density of 0.08 specimens / 100 m (Ngo et al. 2016b). Extremely high exports and a local extirpation have been reported for this species in the past (Stuart et al. 2006).

Similar research on another enigmatic Vietnamese gecko species, *Cnemaspis psychedelica,* endemic to small islands in the south of Vietnam, revealed a density of 12–19.2 animals / 100 m and an estimated population size of 365–732 individuals (Ngo et al. 2016a). The population of the threatened gecko, *Gonatodes daudini* endemic to St. Vincent and the Grenadines, was estimated at abundances of 87–218 animals / ha in 2010 and has significantly declined to 19 animals / ha) in 2018 (with a total population of 9952 individuals) and over-harvesting being reported as the major threat to the species (Bentz et al. 2011; Shepherd et al. 2019). Overharvesting for the international trade has also been reported as a major threat to *Lygodactylus williamsi*, an electric blue gecko endemic to a small range in Tanzania. Flecks et al. (2012) estimated a density of 353 specimens / ha and a total population size of 148,684 ± 112,365 adults of this species. As such, these geckos are examples for range restricted, endemic species with small populations that appear to be especially threatened by trade. As a consequence, all three gecko species have been recently included (*C. psychedelica* and *L. williamsi* at CoP17) or proposed for inclusion (*Gonatodes daudini* for CoP18) on CITES Appendix I to regulate international trade in these species.

The recorded abundances in *G. catbaensis* also appear to be extremely low compared to other threatened and endemic geckos. The lowest abundances of *G. catbaensis* were found at touristic sites on Cat Ba Island. Conversely, abundances were relatively stable on the islands in Ha Long Bay, which comprised intact habitats and were not affected by human activities, as they were too small and inaccessible. Harvesting has not been recorded on these small islands so far. Thus, it is likely that the much lower densities of the species on Cat Ba Island are the result of anthropogenic pressures, especially collection for the pet trade.

Conclusions

As *G. catbaensis* was found to occur in very low densities and has extremely restricted habitat ranges (small islands) that are subject to stochastic weather events, the species appears to be extremely vulnerable to harvesting, which appears to be the case for the entire genus.

Endemic species are considered to be especially vulnerable to over-exploitation (Janssen and Indenbaum 2019). The present study confirmed that tiger geckos are not only locally used, but a subject of the international pet market. Compared to the low densities and small populations in the wild, the number of specimens currently found in the international trade appears to be considerably large. As such, it can be assumed that ongoing uncontrolled harvesting might further imperil *Goniurosaurus* spp. in the future. Tiger geckos are neither sufficiently protected by law nor part of conservation programmes, due to the lack of substantial knowledge on the species conservation status and probably due to the general lack of public as well as political interest in biodiversity conservation. To date, exact impacts of trade on the species cannot be identified, as data of legal trade are only recorded for species listed in the CITES Appendices in most countries. However, at the time of writing, the inclusion of all *Goniurosaurus* species from China and Vietnam in CITES Appendix II has been proposed by China, Vietnam and the EU to be decided at the Conference of the parties (CoP18) in May-June 2019, in Sri Lanka.

Recommendations for conservation

Some *Goniurosaurus* taxa are only found within protected areas in Vietnam, for example *G. catbaensis* in Cat Ba National Park and Ha Long Bay World Heritage site, *G. huuliensis* in Huu Lien Nature Reserve, *G. lichtenfelderi* in Bai Tu Long National Park and animals can only be collected with appropriate permits from local authorities. At the time of writing this manuscript, all *Goniurosaurus* species native to Vietnam have been included in Group IIB of the Governmental Decree 06/2019/ND-CP, which came into force on 10 March 2019 and prohibits the collection of and trade in respective species without permits in Vietnam (The Government of Vietnam 2019). In China, *G. hainanensis* was listed as a species of terrestrial wildlife, which are beneficial or of important economic or scientific value. Furthermore, *G. hainanensis* and *G. bawanglingensis* were listed as wildlife under special protection in Hainan Province. According to Janssen and Shepherd (2019), all *Goniurosaurus* species, endemic to Japan, are currently listed under the law for the Conservation of Endangered Species of Wild Fauna and Flora (LCES), which prohibits – amongst others – the collection and selling of respective species. Eight species of *Goniurosaurus* have been recently included in the IUCN Red List and were assessed in the threat categories VU, EN and CR. In fact, it has been specifically paid more attention regarding conservation activities for *G. catbaensis* after its inclusion in the IUCN Red List as "Endangered" in 2016 (Nguyen et al. 2016). Training programmes have been held by IUCN Vietnam since November 2018 to enhance monitoring skills of researchers in Ha Long Bay and Cat Ba National Park in order to protect wild sub-populations of *G. catbaensis*. Signboards highlighting the conservation needs of *G. catbaensis* have been provided to the scientific department of Ha Long Bay (Fig. 6), which represents a first step towards meaningful conservation of the species.

Chung tay bảo vệ loài

Thạch sùng mí cát bà ở Vịnh Hạ Long

Protection of the Cat Ba Tiger Gecko in Ha Long Bay

Tên khoa học:
Goniurosaurus catbaensis ZIEGLER, NGUYEN, SCHMITZ, STENKE, RÖSLER, 2008
Được mô tả năm 2008
Hiện chỉ ghi nhận tại đảo Cát Bà và Vịnh Hạ Long
Hãy chung tay bảo vệ loài Thạch sùng mí cát bà
Bảo tồn sinh cảnh sống:
- Hãy bảo vệ rừng tại Vịnh Hạ Long
- Hãy bỏ rác vào thùng, không tô vẽ, phá hủy hang động
Không săn bắt Thạch sùng mí và các loài động vật hoang dã

Scientific name:
Goniurosaurus catbaensis ZIEGLER, NGUYEN, SCHMITZ, STENKE, RÖSLER, 2008
A recently discovered species in 2008
Currently known only from Cat Ba Island and Ha Long Bay
Together we protect the Cat Ba Tiger Gecko
Habitat protection:
- Protect forest of Ha Long Bay
- Put the garbage into wastebasket, do not paint, destroy caves and limestone cliffs
Don't collect the Tiger Gecko and other wildlife species

Figure 6. Signboard handed over to the Ha Long Bay Management Department to point to the threats and conservation need of the Cat Ba tiger gecko in English and Vietnamese languages.

To further improve the conservation status of *Goniurosaurus* species, we recommend the following measures:

- The inclusion of *Goniurosaurus* spp. in the Appendices of CITES in order to better control and monitor trade in wild specimens. The current proposal to include all *Goniurosaurus* species from China and Vietnam in CITES Appendix II should be supported. Likewise, we acknowledge that the Japanese *Goniurosaurus* clade may warrant listing in the CITES Appendices. Therefore, a listing could be considered in the future, as also proposed by Janssen and Shepherd (2019). A listing of the Japanese species in Appendix III, as suggested by Janssen and Shepherd (2019), might be a favourable interim solution.
- As the lack of adequate information on the biology and conservation status of single species can impede conservation measures, a timely assessment of the remaining *Goniurosaurus* species in the IUCN Red List is strongly advised. Therefore, more species specific research is needed to fully understand conservation requirements.
- It is likely that, with more research, further cryptic species or new occurrences of this genus will be discovered in the future. Thus, it is strongly recommended to conceal exact locality data as well as detailed descriptions of localities for such new species or population records in order to prevent targeted collection, as has happened in the past.
- We recommend to improve or establish coordinated ex-situ breeding programmes for all species and to build up a stable captive population in order to a) serve as the backup population for restocking measures (also in the light of extreme population declines e.g. due to stochastic weather events) and b) to meet the demand for tiger geckos in the trade and thereby reduce the pressure on wild populations.

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3.2. Modeling the environmental refugia of the endangered Lichtenfelder's Tiger Gecko (*Goniurosaurus lichtenfelderi*) towards implementation of transboundary conservation

The author of the thesis and Dr. Dennis Rödder mainly contributed to the planning of the study. Data collection, analyses, preparation of graphics and the writing of the paper were mainly carried out by the author of the thesis. Prediction models were corrected by Dr. Dennis Rödder. Extend field surveys were conducted in 2020 by Huy Quoc Nguyen and Tien Quang Phan.

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RESEARCH ARTICLE

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Modeling the environmental refugia of the endangered Lichtenfelder's Tiger Gecko (*Goniurosaurus lichtenfelderi*) towards implementation of transboundary conservation

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This article is part of a Special Issue entitled *Transboundary Conservation Under Climate Change*, compiled by Mary E. Blair, Minh D. Le and Ming Xu.

Abstract

Climate change has potential effects on global biodiversity by shifting the optimal distribution of terrestrial organisms, particularly species with narrow distributions. *Goniurosaurus lichtenfelderi,* a forest-dwelling lizard, is found on both the island and mainland sites of northern Vietnam and southern China. The species is categorized as Vulnerable in the IUCN Red List and was recently listed in CITES Appendix II and the Vietnam Government's Decree 06 in 2019 due to severe anthropogenic impacts on its populations. In this study, we employ Maxent species distribution modeling with climatic and vegetation cover data to identify the potential distribution of *G. lichtenfelderi*. We also used this approach to assess future climate impacts on the potential distribution under different climate change scenarios. Our model predicts that the potential distribution of *G. lichtenfelderi* will shrink significantly under future scenarios and even vanish in the entire study area under novel environmental conditions of the BCC-CSM 1-1 – RCP 8.5 scenario by the 2070s. Overall, the current potential distribution is expected to shift towards higher latitudes within the next decades. The forecasted maps provide useful guidelines to implement conservation strategies to mitigate synergistic impacts from climate change and other negative anthropogenic activities. In the context of the potentially severe impacts, the border areas between China and Vietnam, Yen Tu Mountain Range, Bai Tu Long National Park, and their surroundings should be considered core refugia for the species, where conservation measures need to be prioritized in the future.

Highlights

- Discovering new sub-populations of *Goniurosaurus lichtenfelderi* during field surveys is a result of using the Maxent model to identify its potential distribution.
- The contemporary potential distribution of *Goniurosaurus lichtenfelderi* is predicted to shrink significantly and shift towards higher latitudes under climate change scenarios by the 2050s and 2070s.
- Projected core refugia for *Goniurosaurus lichtenfelderi* encompass the contiguous Yen Tu Mountain Range, Bai Tu Long National Park, and border areas in northern Quang Ninh Province, Vietnam and Fangchenggang – Chongzuo, China.
- Research and management collaborations between China and Vietnam's authorities in the near future will be essential for establishing a species and habitat conservation area in the unprotected border region between the two countries.

Keywords: Climate change, conservation, environmental variables, forest-dwelling lizard, Maxent, potential distribution, protected areas.

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Introduction

A high proportion of terrestrial organisms from around the world will face the risk of extinction due to severe effects of climate change (Huggett 2004, Thomas et al. 2004, Parmesan 2006, Huey et al. 2009, Nogués-Bravo et al. 2010, Sandel et al. 2011, Monastersky 2014, Pimm et al. 2014, Urban 2015). Wiens (2016) documented the local extirpation of almost half of 976 investigated species during the 20th century, especially in tropical regions. The IUCN listed more than 300 terrestrial species from Pacific Island nations as threatened by climate change (Taylor and Kumar 2016). Monastersky (2014) indicated that climate change accounts for approximately 7% of declines in animal populations and is expected to become more severe over time. Urban (2015) predicted that climate change presents an extinction risk to 9% of species in Asia and suggested amphibians (13%) and reptiles (9%) are the most vulnerable vertebrate groups. Narrowly-distributed species and habitat specialists with unique ecological niches are considered to be particularly vulnerable since they are less capable of responding to impacts of climate change compared to wide-ranging species (Huggett 2004, Sandel et al. 2011, Pimm et al. 2014, Markle and Kozak 2018).

As ectotherms, reptiles are generally susceptible to environmental changes. Some remote forest-dwelling species show a limited dispersal capability, and as such climate change is expected to affect those severely (Araújo et al. 2006, López-Alcaide and Macip-Ríos 2011, Fitzgerald et al. 2018, Powers and Jetz 2019, Vicente Liz et al. 2019). However, actual impacts of global climate change on reptiles have not been well understood because the distribution of most reptiles fall within remote and restricted areas, as shown for many species in Vietnam (Guisan and Hofer 2003, Segurado and Araújo 2004, Sterling et al. 2006, Urban 2015, Ngo et al. 2016, van Schingen et al. 2016a, Le et al. 2017).

Lichtenfelder`s Tiger Gecko, *Goniurosaurus lichtenfelderi* (MOCQUARD, 1897), is one of 24 species of the eublepharid genus *Goniurosaurus,* characterized by a high level of local endemism and narrow distribution (Orlov et al. 2008, Liang et al. 2018, Ngo et al. 2019a, Qi et al. 2020a, b, Zhu et al. 2020a, b, Uetz et al. 2021). This habitat specialist was first described from an island located in the Gulf of Tonkin in northern Vietnam (Orlov et al. 2008). Subsequently, the tiger gecko has been recorded from several provinces across the mainland of northeastern Vietnam and was very recently discovered in Chongzuo, southern China (Grismer 2000, Orlov et al. 2008, Nguyen et al. 2009, Gawor et al. 2016, Zhu et al. 2020b). Due to the restricted distribution, *G. lichtenfelderi* is potentially vulnerable to anthropogenic threats such as habitat loss and climate change, resembling other tiger geckos (Yang and Chan 2015, Ngo et al. 2016, Ngo et al. 2019b). Consequently, *G. lichtenfelderi* is assessed as Vulnerable (VU) in the IUCN Red List for Threatened Species (Nguyen 2018). In addition, as locally over-harvested for the international pet trade, the species has been included in CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix II and the Vietnam Government's Decree No. 06/2019/ND-CP (Group IIB) in 2019 (Ngo et al. 2019b). However, appropriate conservation measures have not yet been undertaken to safeguard *in situ* populations of this highly threatened species.

In this study, we predicted the potential distribution of *G. lichtenfelderi* using distribution records and environmental variables (including climate and vegetation cover data). The maximum entropy algorithm (MAXENT) was employed to predict the species' current potential distribution and evaluate alterations of suitable habitat for the species under different scenarios of climate change. We hypothesize that the contemporary suitable area of *G. lichtenfelderi* will contract under any scenario of climate change. Furthermore, we propose potential core refugia of *G. lichtenfelderi* based on projected suitable habitats, to improve the efficacy of conservation measures.

Methods

Study area

The study area (within 20-24 ^{0}N and 104-109 ^{0}E) with elevations ranging from 1 m to 2,139 m a.s.l. (Fig. 1), encompassing the entire known distributions of four captured *Goniurosaurus* species in northern Vietnam, was selected based on previous studies, direct observations, and interviews with local community members (Grismer et al. 1999, Vu et al. 2006, Orlov et al. 2008, Ziegler et al. 2008, Orlov et al. 2020, Zhu et al. 2020b). To obtain occurrence localities of the target species, we conducted field surveys in Hai Duong, Bac Giang and Quang Ninh provinces (both in the mainland and on offshore islands), northern Vietnam. Two new distribution records of *G. lichtenfelderi* from Ba Vi National Park (NP), Ha Noi Capital, northern Vietnam (Orlov et al. 2020) and Chongzuo, China (Zhu et al. 2020b) were also included (Fig. 1). Based on previous descriptions of its natural habitat, *G. lichtenfelderi* is only found along small streams situated within evergreen forests on granitic formations (Grismer 2000, Orlov et al. 2008, Ngo et al. pers. obs). Macro-environmental characteristics of the study region are presented in detail in Supplementary Table S1.

Data collection

Occurrence data of *G. lichtenfelderi* were compiled from literature and direct observations in the field from 2014 to 2020. Only data with precise location information were included in the analysis apart from one pseudo-presence from Chongzuo, China, for which exact information was not available (Zhu et al. 2020b). Using pseudo-data likely affects the accuracy of SDMs by potentially introducing a sampling bias. Rather than being randomly selected, the pseudo-presence point was intentionally placed in a forest with dense vegetation in Chongzuo spotted based on satellite imagery, in proximity to the recorded distribution

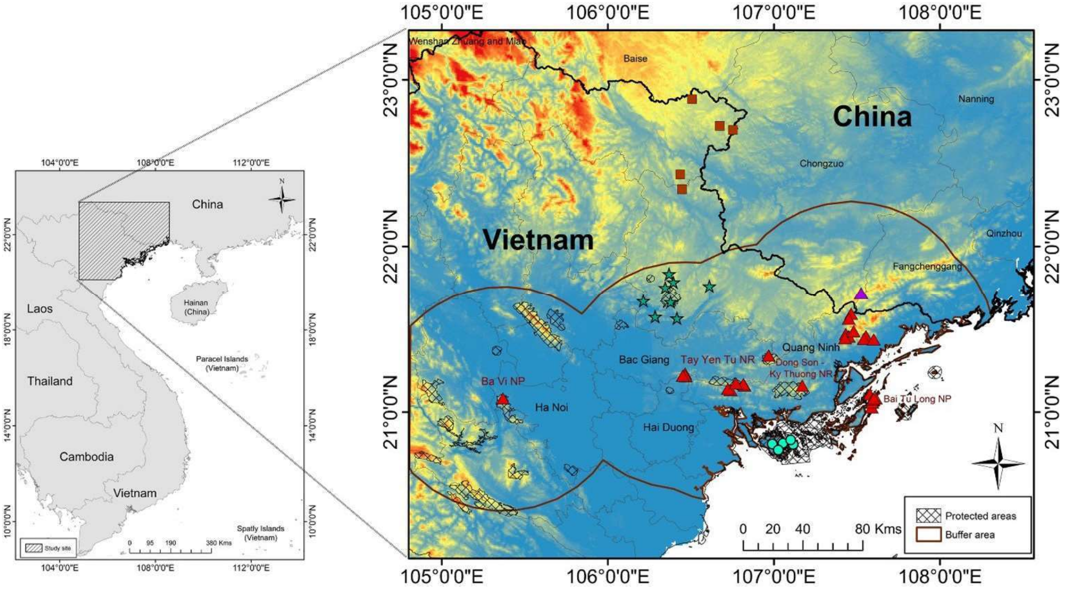

Figure 1. Map of study site in northern Vietnam and southern China, including a selected buffer area shown with a brown line representing a radius of 75 km around occurrence points of *Goniurosaurus lichtenfelderi* (red triangles – Vietnamese populations; a violet triangle – Chinese pseudo-occurrence), and other occurrences of three sister congeners (*G. catbaensis* – beryl green circles, *G. huuliensis* – teal stars and *G. luii* – brown squares). Background colors depict different elevations (delft blue to red indicating increasing elevation).

of Vietnamese populations in the border area. This pseudo-point is highly expected to be within the distribution of – or at least in close proximity to – the natural population of *G. lichtenfelderi* in China.

Coordinates of captured individuals were recorded with a GPS Garmin 64. The data will be shared upon reasonable request. A total of 190 occurrence records in the WGS84 projection of *G. lichtenfelderi* were initially selected. However, several records were removed by using the spatial filtering in packages “dismo” and “sp” in R v 3.1.2 (R Core Team 2018). Only one occurrence locality of *G. lichtenfelderi* was randomly selected within each 1 km square. Such spatial filtering can improve the quality of prediction models by decreasing geographical bias, autocorrelation effects, and uncertainty (Veloz 2009, Radosavljevic and Anderson 2014, Kiedrzyński et al. 2017). For macro-climatic data, we initially extracted 19 bioclimatic variables in the study area. The current (version 1.4) and future climatic conditions from the Coupled Model Intercomparison Project Phase 5 (CMIP5) were obtained from Worldclim (https://www.worldclim.org/) (Hijmans et al. 2005). To predict the future potential distribution of the target species, we selected future climatic data from two general circulation models (GCM) including Community Climate System Model version 4 (CCSM4) (Gent et al. 2011) and Beijing Climate Center – Climate System Model 1-1 (BCC-CSM 1-1) (Wu et al. 2014) by 2050s (average 2041 – 2060) and by 2070s (average 2061 – 2080). Two climate change scenarios of representative concentration pathways (RCPs): RCP 4.5 and RCP 8.5, representing intermediate and the most severe levels of accumulation of greenhouse gas concentrations on the future climate, were used for each model, respectively (Moss et al. 2010, Van Vuuren et al. 2011). For vegetation cover data, we extracted the Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) within the study area using Moderate Resolution Imaging Spectroradiometer (MODIS) sensor data in recent five years from 2015 to 2019 (https://earthexplorer.usgs.gov/). The mean, maximum, minimum, median, range, standard deviations (STD), and mean, maximum, minimum of warmest and coldest quarters for the enhanced vegetation index (EVI) and normalized difference vegetation index (NDVI) were generated using Quantum GIS software (QGIS Version 3.12.0, Development Team. 2020. Available at http://qgis.osgeo.org [Downloaded 25 March 2020]). These environmental variables are listed in Supplementary Table S1.

To remove the influence of multicollinearity on the modeling process, a pairwise Pearson correlation analysis of 36 environmental variables was conducted using the package “raster”. Highly correlated variables with Pearson’s correlation coefficients (r) larger than |0.7| were removed. Consequently, 28 variables were excluded and only 11 remaining variables were included in the final analyses (Supplementary Table S2). Six out of these selected variables were replaced in accordance with each future climate model, while five remaining variables of vegetation cover were used for the vegetation model. The climatic and vegetation cover variables were used separately to model the potential distributions in order to account for both broad-scale climatic factors delimiting the species potential distribution and fine-scale habitat availability within this potential distribution. This procedure allowed us to disentangle the potential impact of climate change from those of land-use changes, which are largely unknown in the near future.

Species distribution modeling

Occurrence data of *G. lichtenfelderi* and six selected climate variables under current conditions and future scenarios, and five remaining ones under current vegetation cover conditions were separately imported into the Maxent software (Phillips et al. 2006) v. 3.4.1 (https://biodiversityinformatics.amnh.org/opensource/maxent/ [accessed March 2020]) to predict suitable habitats. For a rare species with a restricted distribution, the selection of a study background area that includes very environmentally distant areas from the presences of *G. lichtenfelderi* can inflate the AUC scores (Lobo et al. 2008) (see more below). Thus, we generated a buffer background region for model building by circling an area with a radius of 75 km around occurrence points with dissolved boundaries using the R packages "dismo" and "raster". The selected buffer layer is expected to encompass all potential refugia of *G. lichtenfelderi* within the study area (Fig. 1) and improves the accuracy of discriminate use of AUC in predicted models. We did project models to areas beyond the selected buffer site within the study area to assess alternative potential refugia in the context of future climate change.

As only a small number of species presence locations were available, using the default automatic configuration may not be always appropriate and may lead to model overfitting (Shcheglovitova and Anderson 2013, Muscarella et al. 2014). In order to minimize model overfitting, we used the ENMeval package in R and employed the jackknife method to determine the optimal model parameter configuration by varying the "feature" and "regularization multiplier" parameters for both the climatic and vegetation models (e.g. Supplementary Fig. S1) (Warren and Seifert 2011, Muscarella et al. 2014, Radosavljevic and Anderson 2014). The optimal model parameter configuration was identified based on evaluation criteria including the AUC test statistic, omission rate at two different thresholds (OR-MTP, OR-10) and delta.AIC$_c$ (Warren and Seifert 2011, Shcheglovitova and Anderson 2013, Muscarella et al. 2014, Radosavljevic and Anderson 2014, Morales et al. 2017). In particular, AUC is an effective threshold-independent index to distinguish presence from absence or background (0.5 indicates that the performance of the model is no better than random, while values closer to 1.0 indicate better model performance) (Fielding and Bell 1997, Phillips et al. 2006, Peterson et al. 2011). In addition, the OR-MTP (minimum training presence omission) and OR-10 (10% training omission) rates are threshold-dependent metrics to evaluate model overfitting when their values are greater than the expectation of zero and 10%, respectively (Fielding and Bell 1997, Peterson 2011, Radosavljevic and Anderson 2014). The AIC (Akaike Information Criterion) corrected for small sample sizes reflects both model goodness-of-fit and complexity, and the model with the lowest AIC$_c$ value (i.e. delta.AIC$_c$ = 0) is considered the best model (Warren and Seifert 2011).

The minimum training presence threshold was selected in Maxent to convert continuous predicted values to binary maps of suitable and unsuitable areas (Pearson et al. 2007, Phillips et al. 2006). To train the data, we ran 100 bootstrap replications by sampling with replacement from the presence points (Phillips et al. 2006). Default values were applied for the maximum number of interactions (500), the maximum number of background points (10,000), and the prevalence of the species (0.5) (Phillips and Dudík 2008). Lastly, we selected the cloglog output format, in which values ranging from 0 to 1 indicate the probability of suitable environmental conditions for the species. In particular, values closer to 1 suggest a greater probability of species occurrence. The contribution of each variable to the current climatic and vegetation models was determined by measuring the permutation importance (Phillips and Dudik 2008).

To evaluate the accuracy of the climatic and vegetation maps, the multivariate environmental similarity surfaces (MESS) analysis was developed in recent versions of Maxent (Elith et al. 2010). MESS analysis compares the environmental similarity of variables and identifies the similarity of any given pixel to a reference set of pixels of chosen predictor variables within the whole study site. It is used to determine areas with non-analog (novel) environments where the model presents extrapolations compared to highly similar (interpolated) areas (Fitzpatrick and Hargrove 2009, Elith et al. 2010, 2011). In this study, similarity/novelty was classified into four levels: "< -10" (high extrapolation), "-10 – 0" (low extrapolation), "0 – 10" (low interpolation) and "> 10" (high interpolation). We also performed the MESS analysis to create maps for all future climate scenarios and to evaluate the alteration of novel climatic conditions from the reference conditions under the current climatic model within the whole study site.

Maxent was first run using default parameter settings to find potential new populations of *G. lichtenfelderi* using six selected climate variables and only 25 representative occurrence points collected from 2014 to 2019. The model was computed by selecting "auto features" and the regularization multiplier of "1.00". Afterwards, a short survey was conducted in Quang Ninh Province in July 2020 by interviewing local community members to confirm new populations of *G. lichtenfelderi* based on results from the initial prediction model. New coordinates were combined with previous locations to improve the model. Because low omission rates are desirable when searching for new populations (Peterson 2006, Lobo et al. 2008), we also identified optimal configurations by varying the "feature" and "regularization multiplier" parameters in the

ENMeval package to improve the predictability of 25-occurrence model.

Core distribution areas

The core refugia for *G. lichtenfelderi* were identified within highly suitable areas in terms of climate, which have values above 10% training presence cloglog threshold (high occurrence probability). This is a stricter criterion for converting to a binary map with smaller suitable habitats (Radosavljevic and Anderson 2014). We also identified buffer refugia with the least-suitable environmental conditions with values above the minimum presence cloglog threshold (medium occurrence probability) (Phillips et al. 2006, Radosavljevic and Anderson 2014). They were all combined with suitable areas in the vegetation model. To identify priority areas and assess the effectiveness of the protected areas to safeguard the species, we collected all shapefiles of nature reserves and national parks within the occurrence region of *G. lichtenfelderi* from the website of https://www.protectedplanet.net/ (accessed June 2019) (Fig. 1). The projected environmental refugia for the target species were afterwards overlaid with the protected area layer.

Results

Goniurosaurus lichtenfelderi was recorded in Yen Tu mountain range from southern Quang Ninh Province, through Bac Giang to Hai Duong provinces, and three non-contiguous areas in Ha Noi City, two islands in Quang Ninh Province, northern Vietnam and Chongzou, southern China. The species was documented in four protected areas, namely Bai Tu Long National Park (NP) and Dong Son – Ky Thuong Nature Reserve (NR) in Quang Ninh Province, Tay Yen Tu NR in Bac Giang Province, and Ba Vi NP in Ha Noi City (Fig. 1).

Using 25 occurrence records (including the pseudo-presence from China) and current climatic variables, the model using default parameter settings predicted a new potential distribution of *G. lichtenfelderi* in northeastern border areas between China and Vietnam, which was beyond its initially known range (Fig. S2A). Based on the prediction results, we conducted extensive interviews with local community members in the northern districts of Quang Ninh Province. A total of 11 new records of *G. lichtenfelderi* were documented; none of these records were located within a protected area. We produced two additional, optimized models of LHQ – 1.5 and H – 1.5 configurations using the 25 initial records, selected following evaluation metrics (Fig. S1A, Table S3, Supplementary data). Predicted maps of the optimal models highlighted suitable areas more apparently in northern Quang Ninh Province, compared to the prediction of the default model (Fig. S2).

Using a total of 36 representative occurrence records as an input, we finally selected the optimal current climatic model of H – 1.5 and the vegetation model of LQ – 1.0 following our evaluation criteria (Fig. S1B, C). Specifically, both Delta.AIC$_c$ values of the models were zero; average.test.AUCs reached high values (0.94 and 0.87, respectively); and the values of OR-MTP and OR-10 were lower than 0.04 and 0.11, respectively (Table S3). According to the results of MESS analysis, we measured high similarity (interpolated) habitat covering 10,042 km^2 (approximately 75%) in the current climate model and 7,513 km^2 (approximately 56%) in the vegetation model within the selected buffer, with very small areas of extrapolation (high novelty) in both of these models (Fig. S4A, B).

Four important climate variables (Mean diurnal temperature range (Bio2), Mean temperature of driest quarter (Bio 9), Precipitation of warmest quarter (Bio 18) and Precipitation seasonality (Bio 15)) accounted for 96% of permutation importance for the current climatic model (Fig. S3A). For the vegetation model, three variables of NDVI of STD, NDVI of Mean Coldest Quarter and NDVI of Minimum Warmest Quarter accounted for 85.9% of permutation importance (Fig. S3B).

Under the current climate conditions, the potential distribution of *G. lichtenfelderi* mainly covers the sites of occurrence and their surroundings. Moreover, we recorded potentially suitable habitat in coastal areas and other islands in Quang Ninh Province, northern Vietnam, and a few additional sites in the border area of Fangchenggang, southern China (Fig. 2A, B). In particular, climatically suitable habitat consists of a total area of 16,133 km^2, of which approximately 13,408 km^2 (83%) are within the buffered selection (Fig. 4). Under the vegetation conditions, suitable habitat only consists of a total of 7,629 km^2 (57%) within the climatically suitable area in the selected buffer (Fig. 2C, D).

With respect to future projections for the 2050s and 2070s under the scenarios of RCP 4.5 and RCP 8.5, each predicted significantly smaller suitable areas compared to that determined by the current climate model, and the range contraction reached the highest level under RCP 8.5 by the 2070s (Fig. 3, 4). In fact, the climatically suitable areas under future scenarios fluctuated considerably depending on RCPs and periods examined (Fig. 3, 4). The largest climatically suitable area under the CCSM4 – RCP 4.5 scenario by the 2050s covers 6,209 km^2 in the selected buffer (46% suitable area under the current climate conditions), while under the BCC-CSM 1-1 – RCP 8.5 scenario by the 2070s, the potential distribution of *G. lichtenfelderi* was not recorded under novel climatic conditions (0 km^2 in the selected buffer) (Fig. 3D, 4). Using a MESS analysis to compare the current climate model with the future ones, we found that high similarity (interpolated) habitats within the potential climatic distribution of *G. lichtenfelderi* were partly replaced by high novelty (extrapolated) habitats under the future scenarios (Fig. S4A, S5). Especially, under the RCP 8.5 scenarios by the 2070s, novel climatic conditions were documented in most of the study area (Fig. S5D, H). In general, the potential distribution of *G. lichtenfelderi* under future scenarios tends to shift towards higher latitudes in border areas between China and Vietnam (Figs 2, 3).

We also consider suitable areas as potential refugia for the long-term persistence of *G. lichtenfelderi* in

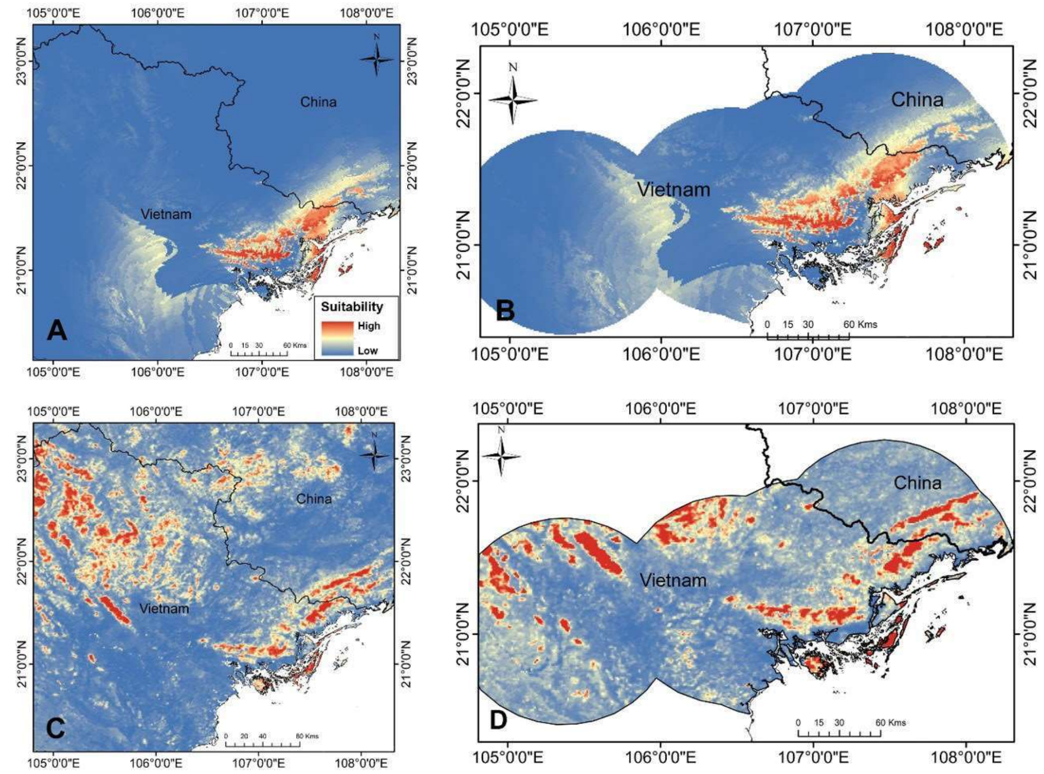

Figure 2. Current suitable distribution of *Goniurosaurus lichtenfelderi* using 36 occurrence points under current climatic conditions: A. in the study area; B. in the selected buffer; and, under vegetation cover conditions: C. in the study area; D. in the selected buffer. Red to blue colors represent high to low suitability.

the context of current environmental conditions and impacts of future climate change. In particular, the green shapes, which represent highly suitable habitats, mostly cover areas in Bai Tu Long NP, Yen Tu Mountain Range, and border areas in both China and Vietnam, and the yellow areas with lower levels of suitability cover surrounding sites (Fig. 5). A few scattered green patches are also recorded on some islands and coastal areas from Quang Ninh Province (Fig. 5).

Discussion

Modeling

To predict the potential distribution of *G. lichtenfelderi*, we used Maxent models with known and newly collected occurrence data and environmental variables (climate and vegetation). The current models of climate and vegetation performed very well based on model performance and evaluation metrics. Further, model tuning was performed to ensure model selection balance low AIC_c and omission rates (Warren and Seifert 2011, Shcheglovitova and Anderson 2013, Muscarella et al. 2014, Radosavljevic and Anderson 2014, Morales et al. 2017). Following the MESS analysis, the majority of the potential distribution of *G. lichtenfelderi* is in areas of high similarity (interpolation), while areas of high novelty (extrapolation) are beyond the potential distribution of the current models (e.g. Supplementary Fig. S4A, B). These results confirmed that the optimal models performed well, are not overfit, and are meaningful to use for future projections.

Eleven focal environmental factors (climate and vegetation) were selected to predict the potential distribution of *G. lichtenfelderi*. Being ectothermic, the selected climate variables may influence the tiger geckos' spatial distribution, physiology, behavior and reproductive biology (López-Alcaide and Macip-Ríos 2011, Ngo et al. 2019a, Vicente et al. 2019). Furthermore, all *G. lichtenfelderi* specimens were recorded along small streams and none was found outside evergreen forests (Ngo et al. pers. obs). Thus, a high level of humidity and dense vegetation coverage might associate with the natural history of *G. lichtenfelderi*. Ngo et al. (2019a) already confirmed the importance of these environmental factors for the microhabitat requirement of another congener, *G. catbaensis*. There is a significant gap in the knowledge about habitat requirements of *G. lichtenfelderi* and more studies yet need to be undertaken to understand its role in the ecosystem. Thus, the specific biological relationships and mechanisms between the selected environmental variables (macro-climate and vegetation cover) and the species remain unclear.

Finding new populations

Beyond the known distribution of *G. lichtenfelderi*, the initial and two optimal models predicted the potential habitat in a few small patches of northern

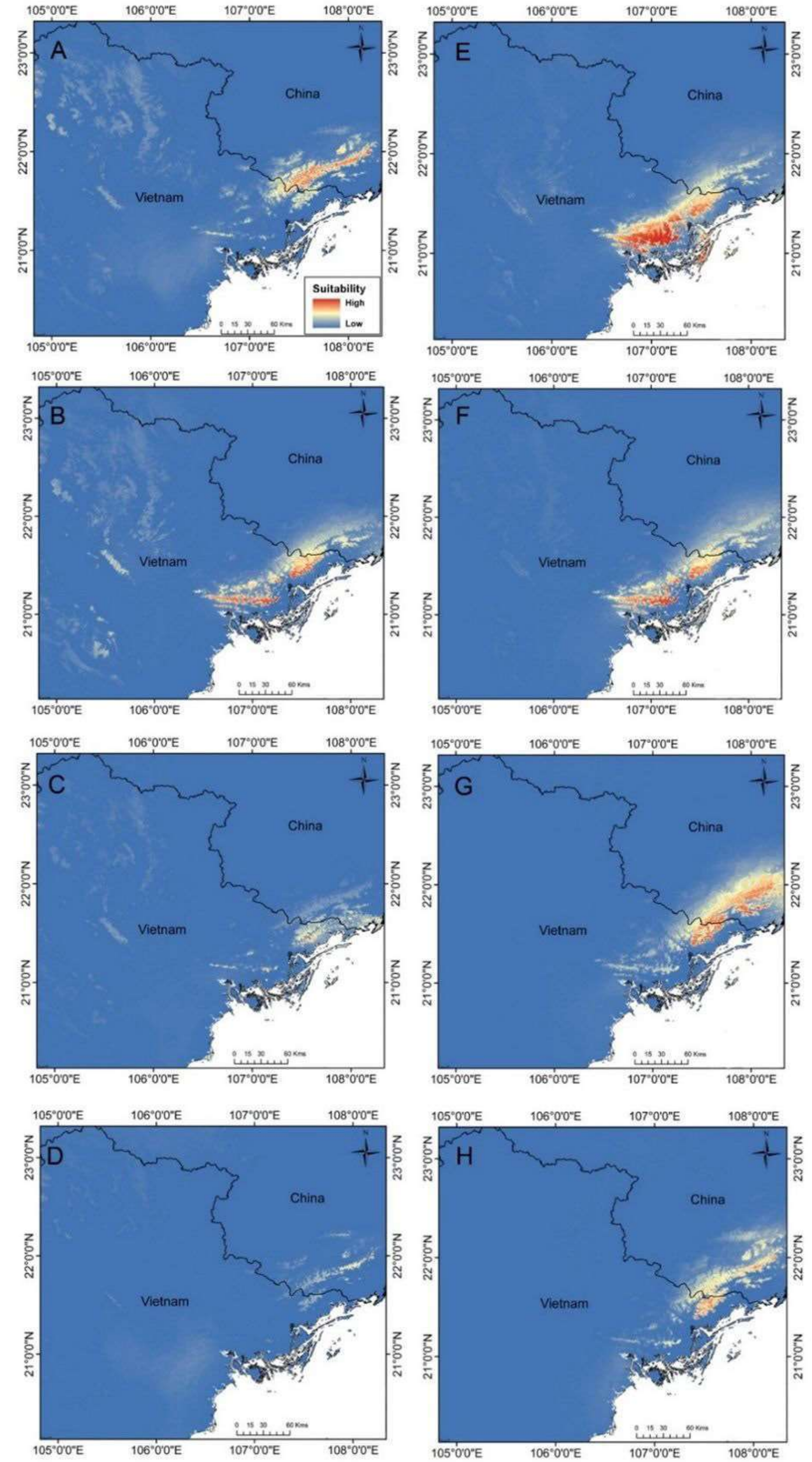

Figure 3. Projected future maps of potential distribution for *Goniurosaurus lichtenfelderi* under future climatic conditions following the circulation model BCC_CSM-1-1: A. RCP-4.5 by 2050; B. RCP-4.5 by 2070; C. RCP-8.5 by 2050; D. RCP-8.5 by 2070; and CCSM4: E. RCP-4.5 by 2050; F. RCP-4.5 by 2070; G. RCP-8.5 by 2050; H. RCP-8.5 by 2070. Red to blue colors represent high to low suitability.

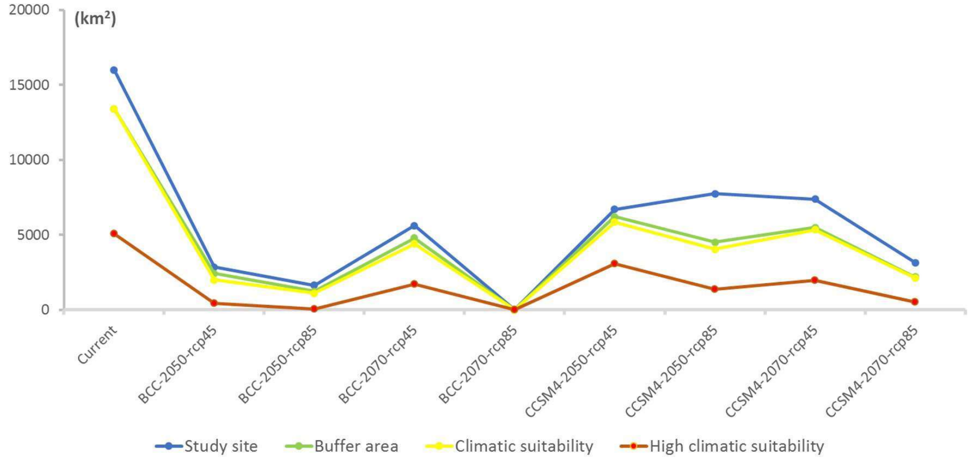

Figure 4. Predicted suitable habitat areas of *Goniurosaurus lichtenfelderi* under different conditions of current and future scenarios. (Blue line represents the climatically suitable area in the study site; green line in the selected buffer background; yellow line within the background area of climatic suitability, brown line within the background area of high climatic suitability in the buffer area).

Quang Ninh Province, Vietnam (Fig. S2). According to these findings, extensive surveys were conducted and 11 new locations of *G. lichtenfelderi* were recorded. The effectiveness of Maxent modeling to find new populations was documented in a study of another forest-dwelling lizard, *Shinisaurus crocodilurus*, in northern Vietnam (van Schingen et al. 2016a). We recorded sympatric occurrences of these two lizards by interviews with local community members in at least five locations. The discovery of new occurrence locations of *G. lichtenfelderi* from the predicted maps could provide further justification for the use of the geographically close pseudo-presence points, as we included in this study from Chongzuo, China. In our case, with so few presence points to work from, selecting a carefully vetted pseudo-presence point did not detract from model performance and likely significantly improved model results to predict the population of *G. lichtenfelderi* in China.

The updated model using 36 occurrence points predicted other potential distribution sites of *G. lichtenfelderi* in Fangchenggang, southern China, a few islands and coastal areas in Quang Ninh Province, and a few small areas in Bac Ninh and Vinh Phuc provinces, northeastern Vietnam (Fig. 2A, B). Over the last two decades, intensive surveys have never documented the species in Tam Dao National Park, Vinh Phuc Province (Nguyen et al. 2009). However, additional overlooked populations of *G. lichtenfelderi* will likely be discovered from one or more of the remaining unstudied potential areas.

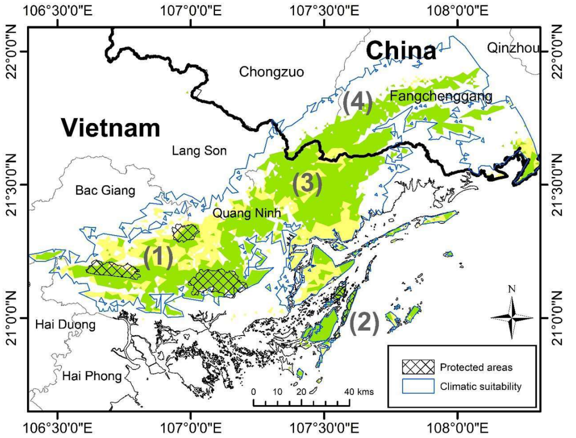

Figure 5. Proposed refugia throughout the range of *Goniurosaurus lichtenfelderi* under current models (the green areas serve as core habitats of suitable vegetation within the yellow areas of high climatic suitability and the blue lines cover all climatically suitable areas in the buffered background): (1) Refugium 1 – Yen Tu Mountain Range; (2) Refugium 2 – Bai Tu Long National Park; (3) Refugium 3 – in northernmost Quang Ninh Province; (4) Refugium 4 – in Fangchenggang and Chongzuo, southernmost China.

Potential refugia

To date, only a few studies used the Maxent approach to predict the potential distribution of lizard species in Vietnam (e.g. *Goniurosaurus catbaensis* and *Shinisaurus crocodilurus*) under contemporary and future conditions (van Schingen et al. 2016a, b, Le et al. 2017, Ngo et al. 2019a). The potential distribution of these species was predicted to be diminished due to the impact of climate change. The projected distribution range of *G. lichtenfelderi* is consistent with the trend of contraction under climate change. Novel climatic conditions will arise within the current potential distribution of *G. lichtenfelderi*, even the currently realized niche of *G. lichtenfelderi* will be totally replaced following the BCC-CSM 1-1 – RCP 8.5 model projection by the 2070s (Figs 3, 4). Overall, the potential distribution of *G. lichtenfelderi* tends to shift towards higher latitudes, following a similar trend in many terrestrial species (Walther et al. 2002, Deutsch et al. 2008, Lenoir and Svenning 2013).

Under contemporary climatic conditions, the potential distribution of *G. lichtenfelderi* mostly covers occurrence areas and their surroundings (Fig. 2A). The current map shows that the potential range of *G. lichtenfelderi* does not overlap with the occurrence extent of each remaining *Goniurosaurus* congeners in Vietnam, except for *G. catbaensis* from the Gulf of Tonkin, northern Vietnam (Fig. 1, Fig. 2A). However, the potentially sympatric patches only occur in a few small karst islands in Ha Long Bay, Quang Ninh Province and Cat Ba NP, Hai Phong City, where the target species has never been recorded, as it has only been found in granite-dwelling forests (Ziegler et al. 2008, Gawor et al. 2016, Ngo et al. 2019a). It is likely that *G. lichtenfelderi* has a distinct climate niche from other congeners of the *G. luii* group in Vietnam. A macroclimatic niche divergence may explain the evolutionary split between the granite-adapted *G. lichtenfelderi* species and the karst-dwelling *G. luii* group (Orlov et al. 2008, Ziegler et al. 2008), but these hypotheses should be further investigated in the future.

Since all *Goniurosaurus* species are likely narrowly-distributed habitat specialists and adapted to unique ecological niches, we only identified stable refugia for *G. lichtenfelderi* under the impacts of climate change, rather than identifying colonization areas (Orlov et al. 2008, Ngo et al. 2019a, b). A potential refugium, which is highly suitable in the context of current environmental conditions and can be relatively stable under climate change scenarios, can potentially increase landscape connectivity to facilitate genetic exchange between subpopulations (Littlefield et al. 2017). Thus, we suggest that key regions safeguard wild populations of *G. lichtenfelderi* include the contiguous Yen Tu Mountain Range (Refugium-1), Bai Tu Long NP (Refugium-2), northern parts of Quang Ninh Province, northern Vietnam (Refugium-3), and border areas in Fangchenggang and Chongzuo, China (Refugium-4) (Fig. 5). Within these refugia, three protected areas (Bai Tu Long NP, Tay Yen Tu NR and Dong Son – Ky Thuong NR) contain a significant proportion of the area of habitat suitability (approximately 365 km^2).

Thus, we highly recommend that these three protected areas and the green regions in Figure 5 be prioritized as core areas. Their surrounding regions bounded with yellow patches can function as buffer zones; for example, Hai Duong Province and the area between Dong Son – Ky Thuong NR and Tay Yen Tu NR can serve as corridors connecting the core localities (Fig. 5). Van Schingen et al. (2016a) also highlighted the importance of key forest areas within Yen Tu Mountain Range and the border region in Quang Ninh Province to maintain genetic exchange and the long-term persistence of another threatened lizard, *Shinisaurus crocodilurus*. We assume that other sympatric species will also be affected by the contraction of suitable habitats due to the impact of climate change and they should be safeguarded in the same manner.

We highlight the importance of the two border-region refugia in Quang Ninh Province, northern Vietnam (Refugium-3), and Fangchenggang and Chongzuo, southernmost China (Refugium-4), in relation to *in situ* conservation actions. In particular, under the potential threat of climate change, the currently suitable refugia of *G. lichtenfelderi* are likely to shrink and move towards higher latitudes in the border areas between China and Vietnam within the next decades. Moreover, the two refugia shape a contiguously suitable and unfragmented habitat for the species between the two countries (Fig. 5). Therefore, research and management collaborations between authorities of China and Vietnam are essential to protect the remaining wild populations of *G. lichtenfelderi*.

With respect to the primary region in Bai Tu Long NP, surrounding islands and coastal areas in Quang Ninh Province, increasing sea levels and catastrophic events caused by the global climate change may be a potential threat to the island-populations of *G. lichtenfelderi*. As Ngo et al. (2019a) documented in a case study of *G. catbaensis,* a sub-population seemed to be wiped out from a former occurrence site on Cat Ba Island, Hai Phong City after an extreme flood event. In fact, the target species only occurs in restricted areas along small streams on granitic rocks, thus, microhabitat degradation is expected to be exacerbated in the summer by heavy rains (Ngo et al. pers. obs).

Anthropogenic impacts and conservation

Previous observations have already shown the degradation and fragmentation of habitats in the Yen Tu Mountain Range caused by direct anthropogenic threats, namely deforestation as a consequence of coal mining, timber logging and tourism development, and waste deposition (van Schingen et al. 2014, 2015, Ngo et al. pers. obs). Habitat fragmentation likely generates potential barriers that prevent genetic exchange through dispersal between *G. lichtenfelderi* populations. These anthropogenic impacts will make the species even more vulnerable to the impacts of climate change. In fact, no *G. lichtenfelderi* individual was observed along stream sections in close proximity to the residential and agricultural regions (Ngo et al. pers. obs). We assume that the actual habitat loss could be worse than the predicted loss under climate change scenarios.

In addition, all narrowly distributed tiger geckos are extremely vulnerable to over-exploitation (Yang and Chan 2015, Ngo et al. 2019b). A large number of wild animals of *G. lichtenfelderi* were over-harvested to meet high demand from the local and international pet markets. Particularly, *G. lichtenfelderi* was the most common tiger gecko species in the international pet trade from 1999 to 2018 comprising 43.6% (7,281 individuals) of all imported individuals into the United States (US) (Ngo et al. 2019b).

Synergistic effects of habitat degradation and over-exploitation will certainly lead to an exacerbation of climate change impacts in the future. The species may even go extinct before climatic effects become more apparent. Thus, mapping optimal refugia and identifying key regions for *G. lichtenfelderi* will help to enhance the effectiveness of stringent conservation strategies to mitigate the impacts of climate change and other human disturbances. Furthermore, we propose to establish a species and habitat conservation area for the highly threatened species *G. lichtenfelderi* and *Shinisaurus crocodilurus* in unprotected areas in the border area between China and Vietnam.

Conclusion

In this study, we predicted the potential distribution of *G. lichtenfelderi* by using the maximum entropy algorithm in the Maxent software under current and future environmental scenarios across its range in northern Vietnam and southern China. This resulted in successful field surveys documenting new occurrence records for the species. Contemporary potential distributions were mainly recorded in the occurrence areas of *G. lichtenfelderi* and their surroundings. Suitable refugia for *G. lichtenfelderi* are expected to decline significantly and shift towards higher latitudes under climate change scenarios by the 2050s and 2070s.

Border areas between China and Vietnam, Yen Tu Mountain Range and granitic habitats on islands in Quang Ninh Province, including three protected areas, are considered core refugia for the implementation of conservation measures. Unprotected areas in the border region between China and Vietnam covering a high proportion of suitable habitat for both endangered species of *G. lichtenfelderi* and *S. crocodilurus* are proposed to be set aside as a species and habitat conservation area in the future.

The results of the present study are expected to form a baseline for formulating and implementing appropriate *in situ* conservation measures to safeguard wild populations of *G. lichtenfelderi* and facilitate research management collaborations between authorities from China and Vietnam to mitigate impacts of climate change and other human disturbances.

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Author Contributions

HNN, HQN, TQP and LRG conducted field surveys. HNN and DR computed the species distribution models. HNN, TQN, LRG, DR and TZ wrote the manuscript.

Supplementary Material

The following materials are available as part of the online article at https://escholarship.org/uc/fb

Figure S1. Four evaluation criteria resulting from three models made across a range of feature-class combinations and regularization multipliers.

Figure S2. Predicted suitable habitat for *Goniurosaurus lichtenfelderi* under current climate conditions using 25 occurrence points with a set of different configurations.

Figure S3. Permutation importance in the current prediction models.

Figure S4. Multi-Environment Similarity Surface (MESS) maps of novel habitat for current models.

Figure S5. Multi-Environment Similarity Surface (MESS) maps of novel habitat for the circulation models of climate change.

Table S1. Current climate and vegetation cover conditions within the study site.

Table S2. Multicollinearity test results showing Pearson correlation coefficients of eleven selected environmental variables used in species distribution modeling.

Table S3. Evaluation metrics of Maxent ENMs generated by ENMeval for the optimal climatic and vegetation models.

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3.3. Vulnerability of an endemic Tiger Gecko (*Goniurosaurus huuliensis*) to climate change: modeling environmental refugia and implications for *in-situ* conservation

The author of the thesis and Dr. Dennis Rödder mainly contributed to the planning of the study. Data collection, analyses, preparation of graphics and the writing of the paper were mainly carried out by the author of the thesis. Scripts for species distribution models were provided and corrected by Dr. Dennis Rödder. Extend field surveys were conducted by Huy Quoc Nguyen.

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Vulnerability of an endemic Tiger Gecko (*Goniurosaurus huuliensis*) to climate change: modeling environmental refugia and implications for in-situ conservation

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Abstract. Detailed information on potentially suitable habitats and forecasted alterations thereof under climate change scenarios are critical for the conservation planning of endangered taxa, in particular those with small distribution ranges. The Huu Lien Tiger Gecko, *Goniurosaurus huuliensis*, is a is a micro-endemic species in northern Vietnam. The species is listed in the IUCN Red List as Critically Endangered and in CITES Appendix II due to habitat loss and overexploitation for the international pet trade. Climate change has been globally acknowledged to impact on many species and it likely has negative influences on *G. huuliensis*. In this study, an ensemble modeling technique is employed, trained with climate and vegetation cover conditions, to identify the contemporary potential distribution of this species and assess its alterations under different climate change scenarios. Our predictions suggest that the current potential distribution of *G. huuliensis* mostly covers the known sites of occurrence and their surroundings. These areas will narrow significantly and/or shift towards higher latitudes under novel climate conditions as can be expected according to future IPCC scenarios. To safeguard in-situ populations of *G. huuliensis* in the context of the potentially severe impacts, we provide a core refugia map that identifies key regions for priority conservation measures, including Lang Son Province and small sites in Bac Giang and Thai Nguyen Provinces, northern Vietnam. We highly recommend that the Huu Lien Nature Reserve be selected as a "centre" to kick-off conservation actions for the target species.

Key words. Squamata, Eublepharidae, climate, core refugia, Ensembles of Small Models (ESMs), Huu Lien Nature Reserve, potential distribution, vegetation.

Introduction

The Huu Lien Tiger Gecko, *Goniurosaurus huuliensis* Orlov, Ryabov, Nguyen, Nguyen & Ho, 2008, which is one of five known *Goniurosaurus* species in Vietnam, has only been recorded from the type locality in the Huu Lien Nature Reserve (NR), Lang Son Province (Orlov et al. 2008, Nguyen et al. 2009, Nguyen 2011). This endemic species is found in the evergreen forest on isolated karst formations at altitudes from 300 to 370 m a.s.l. (Orlov et al. 2008). Although discovered recently, *G. huuliensis* was assessed as Critically Endangered (CR) in the IUCN Red List of Threatened Species (Nguyen 2018), and this species was included in CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix II and the Vietnam Government's Decree No. 06/2019/ND-CP (Group IIB) in 2019 (Ngo et al. 2019b).

Recent investigations provided convincing evidence for the endangered status of *G. huuliensis*. In particular, this habitat specialist has, to date, only been recorded from a very small karstic range within the Huu Lien Nature Reserve, with the estimated extent of occurrence (EOO) being 30 km² (Orlov et al. 2008, Nguyen et al. 2009, Nguyen 2018). Similar to other Tiger Geckos, the effective population status of *G. huuliensis* is expected to be extremely small (Ngo et al. 2016, 2019a, b). Furthermore, wild Tiger

Geckos, including *G. huuliensis*, have been locally overharvested for the international pet trade (Ngo et al. 2019a, b). These severe impacts may be driving this species to the brink of extinction.

Climate change is a global threat to biodiversity and ecosystems, which drives large-scale shifts in species distribution, and may lead to a decline of species abundance, and even extirpation or extinction of many terrestrial organisms (Huggett 2004, Thomas et al. 2004, Parmesan 2006, Huey et al. 2009, Monastersky 2014, Pimm et al. 2014, Urban 2015, Taylor & Kumar 2016, Markle & Kozak 2018, Weiskopf et al. 2020). Besides direct anthropogenic impacts, climate change may also affect *G. huuliensis* in a negative manner. On the one hand, as a poikilothermic lizard, basic physiological functions, such as locomotion, growth and reproduction are determined mainly by environmental conditions (Araújo et al. 2006, Fitzgerald et al. 2018, Ngo et al. 2019a, Vicente et al. 2019). On the other hand, a species within a small distribution range and a habitat specialist being dependent on specific ecological niches will be less capable of responding to novel environmental conditions as a result of climate change (Huggett 2004, Orlov et al. 2008, Sandel et al. 2011, Pimm et al. 2014, Markle & Kozak 2018). Thus, this target species is considered to be particularly susceptible to climate change.

To date, no in-situ conservation action has yet been implemented to protect wild populations of *G. huuliensis*. General conservation plans have already been proposed for all Tiger Geckos (Ngo et al. 2019b). However, in-situ conservation measures have been only conducted to safeguard another threatened congener, *Goniurosaurus catbaensis*, from northern Vietnam, after Ngo et al. (2016, 2019a) provided detailed insights into its population status and utilized microhabitat, and Le et al. (2017) used a Maxent approach to predict its potential distribution for conservation.

A considerable gap between designing general conservation plans and practical application of conservation measures can limit the efficiency level of conservation efforts (Dudley & Parish 2006). In fact, conservationists normally encounter an obstacle for priority conservation efforts when trying to identify where potentially suitable habitats and core refugia are (Baumgartner et al. 2018), even though all populations should be protected in the same manner. Recently, the development of species distribution models (SDMs), based on species' geographic coordinates and their environmental data, have been able to predict the potential distributions of species (Guisan & Zimmermann 2000, Guisan & Thuiller 2005). Several techniques to compute SDMs have been recently applied for some rare lizards in Vietnam (van Schingen et al. 2016, Le et al. 2017, Ngo et al. 2019a, Ngo et al. 2021). However, the modelling of endangered or rare species has its limitations when only a few recorded occurrences are combined with many predictor variables, which may lead to the model overfitting (Breiner et al. 2015). Recently, the ensembles of small models (ESMs) approach, a novel strategy of fitting SDMs, has been employed to overcome the limitations of using a single SDM technique and improve the qualitative outcome of predictions where only a small number of presence coordinates is available (Breiner et al. 2015, Cola et al. 2017).

The present study aims to predict the potential distribution of *G. huuliensis* based on geographic locations and environmental variables (including climate and vegetation data) under both current conditions and different future scenarios. The ESMs technique is employed to compute the models and to project the ensemble through space and time. We hypothesize that the currently suitable area of *G. huuliensis* will shrink significantly under the impacts of climate change. We also intend to identify potential core refugia of *G. huuliensis*, based on the simulated outcomes, to improve the efficacy of in-situ conservation measures.

Materials and methods

Study area

The study area (within 20–24°N and 104–109°E) with altitudes ranging from 1 to 2,139 m a.s.l. (Fig. 1) was selected as the background area, encompassing all known distribution ranges of the five *Goniurosaurus* species occurring in northern Vietnam (Grismer et al. 1999, Vu et al. 2006, Orlov et al. 2008, Ziegler et al. 2008, Nguyen et al. 2009, Nguyen 2011). *Goniurosaurus huuliensis* is known only from the karst forest at its type locality in the Huu Lien NR, Lang Son Province, northern Vietnam (Orlov et al. 2008). Further populations of *G. huuliensis* are expected to be found in surrounding areas, including all districts of Lang Son Province and adjoining provinces (e.g., Bac Giang, Cao Bang, Quang Ninh and Bac Giang Provinces), where we directly observed karst habitats resembling those known from the type locality.

Data collection

Occurrence data of *G. huuliensis* were compiled from literature, direct observations, and interviews with local people (Orlov et al. 2008). We conducted field surveys in the Huu Lien NR in April and August 2019, and from June to July 2020. An extensive initiative interviewing local people was carried out in July 2020 in all districts of Lang Son and surrounding provinces (Bac Giang, Cao Bang, Quang Ninh, Thai Nguyen) in order to possibly detect as yet unrecorded populations of *G. huuliensis.*

Coordinates of each captured individual were recorded with a GPS Garmin 64, but will be shared upon request. A total of 80 occurrence records in the WGS84 projection of *G. huuliensis* were initially collected. However, several records were removed to reduce the density of recorded locations by using spatial filtering in the packages "dismo" and "sp" in R v 3.1.2 (R Core Team 2018) due to duplicates or near-duplicates for some localities. Only one occurrence locality was randomly selected within each 1-km square. Spatial filtering can improve the quality of prediction models by decreasing geographical bias, autocorrelation effects, and uncertainty (Veloz 2009, Radosavljevic & Anderson 2014). A total of 27 representative occurrence points were finally used for modelling.

For environmental data, we selected bioclimatic and vegetation cover variables. Nineteen climatic variables for current (averages between 1960–1990, version 1.4) and future conditions from the Coupled Model Intercomparison Project Phase 5 (CMIP5) were obtained from Worldclim (https://www.worldclim.org/) (Hijmans et al. 2005). To predict the future potential distribution of the target species, we selected future climatic data from two general circulation models (GCM), including Community Climate System Model version 4 (CCSM4) (Gent et al. 2011) and Beijing Climate Center – Climate System Model 1-1 (BCC-CSM 1-1) (Wu et al. 2014) as expected for the 2050s (average 2041–2060) and the 2070s (average 2061–2080). Two climate change scenarios of representative concentration pathways (RCPs): RCP 4.5 and RCP 8.5, representing intermediate and the most severe levels of accumulation of greenhouse gas concentrations in the future climate, were used for each model, respectively (Moss et al. 2010, van Vuuren et al. 2011). For vegetation cover data, we extracted the Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI) from within the study area using Moderate Resolution Imaging Spectroradiometer (MODIS) sensor data from five years, 2015 to 2019 (https://earthexplorer.usgs.gov/). The mean, maximum, minimum, median, range, standard deviations (STD), and mean, maximum, minimum of warmest and coldest quarters for the enhanced vegetation index (EVI) and normalized difference vegetation index (NDVI) were generated using Quantum GIS (QGIS Version 3.12.0, Development Team. 2020, available at http://qgis.osgeo.org [downloaded on 25 March 2020]).

Eleven variables were eventually kept for the prediction approaches based on the selection previously made by Ngo et al. (2021) for another tiger gecko, *G. lichtenfelderi*. Particularly, six climatic variables (i.e., Bio-2: Mean Diurnal Temperature Range, Bio-3: Isothermality, Bio-9: Mean Temperature of Driest Quarter, Bio-15: Precipitation Seasonality, Bio-18: Precipitation of Warmest Quarter, Bio-19: Precipitation of Coldest Quarter) were used to train the climatic model, while five remaining variables of vegetation cover (e.g., NDVI of Mean Coldest Quarter, NDVI of Minimum Coldest Quarter, NDVI of Minimum Warmest Quarter, NDVI of STD, and EVI of Range) were used for a separate model based on the vegetation structure. The climate and vegetation cover variables were used separately to model the potential distribution, in order to account for both broad-scale climatic factors delimiting the species' potential distribution and fine-scale habitat availability within this potential distribution. Future scenarios of potential vegetation structure are not available for limiting these variables. This procedure allowed us to disentangle the potential impact of climate change from those of land-use changes, which are largely unpredictable.

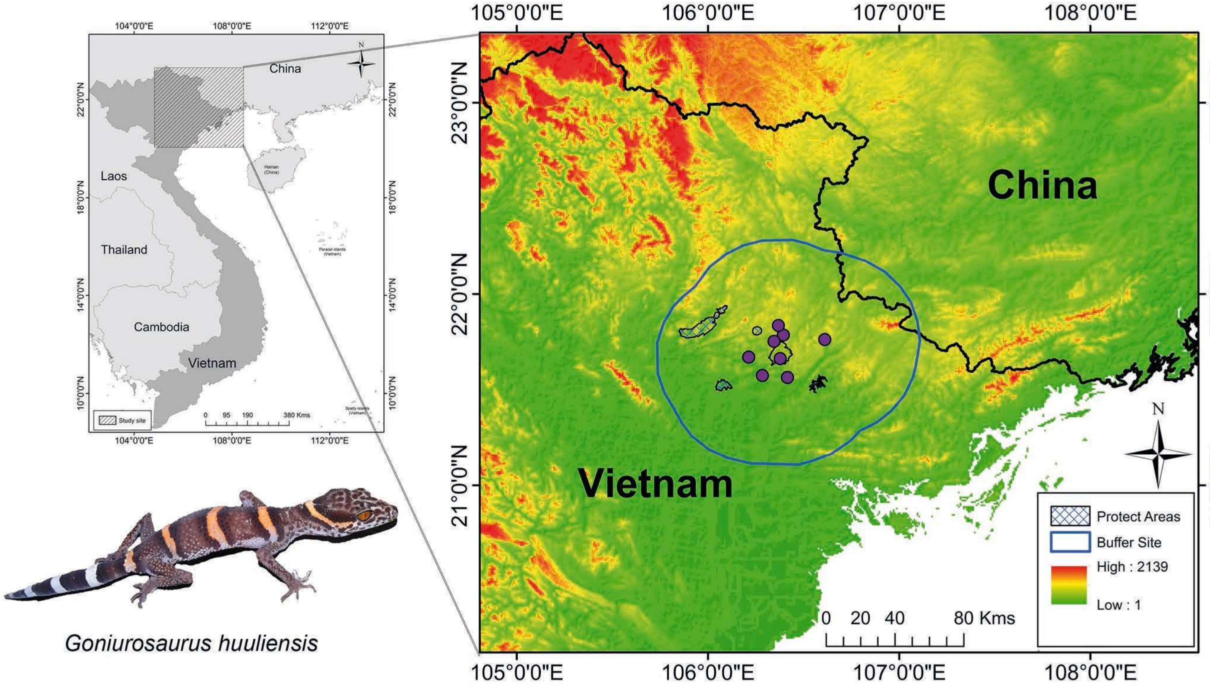

Figure 1. Map of study site in northern Vietnam and southern China, including the selected buffer representing a radius of 50 km around occurrence points (violet circles) of *Goniurosaurus huuliensis*.

Species distribution modelling

We fitted the ensembles of small models with six modelling techniques: artificial neural network (ANN), classification tree analysis (CTA), generalized linear models (GLM), generalized additive models (GAM), generalized boosting regression models (GBM), and maximum entropy modelling (Maxent). All models were calibrated with the R package "ecospat" ver. 3.1 (Breiner et al. 2015, Cola et al. 2017). To build an Ensemble of Small Models, we used subsets of the environmental predictors to create bivariate predictor combinations. In particular, six climate and five vegetation predictors resulted in 15 and 10 bivariate models (BiVa) in each of six selected modelling techniques, respectively (step-1, Supplementary Fig. S1). In step-2, we evaluated each of these bivariate models using a cross-validated model evaluation index as a Somers' D (Breiner et al. 2015). Bivariate models with a Somers' D lower than 0 (i.e., AUC < 0.5) were set to zero and not used to build ESMs. One ESM prediction was built for each modelling technique in step-3 (i.e., ANN-ESM based on bivariate ANN models, CTA-ESM on bivariate CTA models, etc.). In the final steps (Steps 4 and 5), we built a final ensemble prediction (EP-ESM) – the fitted ESM by averaging across these ESMs, again using Somers' D as a weighting factor (Supplementary Fig. S1) (Breiner et al. 2015).

All models were calibrated with presence-only data and 10,000 random background points selected within a buffer area. As the buffer we defined an area with a radius of 50 km around the occurrence points using the R packages "dismo" and "raster". The fitted ESM was also projected to areas beyond the selected buffer within the study area to show up alternative potential refugia in the context of future climate change. Fifteen-fold cross-validation, with subsets of 70% training data and 30% test data, was used to evaluate the models. We used three adjustment indices to evaluate model performance, including the AUC of the Receiver Operating Characteristic Curve to discriminate presence from absence (or background), which was then used to build a Somers' D ($D = 2 \times (AUC - 0.5)$) (Fielding & Bell 1997, Lobo et al. 2008, Elith & Graham 2009), TSS is particularly ($TSS = sensitivity + specificity - 1$) useful for the modelling of rare species and can be used to compare different modelling techniques (Allouche et al. 2006). In these indices, values closer to 1.0 indicate better model performance (Breiner et al. 2015).

To assess the predictive capabilities of our ensemble model projections, multivariate environmental similarity surface (MESS) analyses were used to quantify potential extrapolation errors (Elith et al. 2010). The respective MESS functions of the "ecospat" package were used (Cola et al. 2017). The MESS analysis compares the environmental similarity of any given grid cell within the study site to a reference set of grid cells of chosen predictor variables. It is used to identify extrapolation in areas with novel environmental conditions beyond the training range of the models, as is indicated by negative values (Elith et al. 2010, 2011). In this study, similarity/novelty was classified into four levels: "< -10" (high extrapolation), "-10–0" (low extrapolation – margin), "0–10" (low interpolation – margin) and "> 10" (high interpolation). We also performed MESS analyses to create maps for each future climate scenario and to evaluate the alteration of novel climatic conditions from the reference conditions under the current climate model.

Core refugia

The core refugia for *G. huuliensis* were identified within highly suitable areas in terms of climate, which have values above the 10% training presence threshold (high occurrence probability). This is a stricter criterion for converting the probability maps to binary maps with smaller suitable habitats. We also identified buffer refugia with the least-suitable environmental conditions with values above the minimum presence threshold (intermediate occurrence probability). They were all combined with suitable areas as identified in the model based on vegetation indices. To identify priority areas and assess the effectiveness of the protected areas for safeguarding the target species, we collected all shapefiles of nature reserves within the occurrence region of *G. huuliensis* from https://www.protectedplanet.net/ (accessed in June 2019) (Fig. 1). The identified environmental refugia for the target species were afterwards layered with the protected area.

Results

As a result of our extensive interviews, we documented the occurrence of *G. huuliensis* in four districts of Lang Son Province (including the Huu Lien NR) and another district in Thai Nguyen Province, northern Vietnam. According to local people, all animals were found in the evergreen forest on karst formations. They were observed moving or resting on rocky cliffs or hiding in crevices.

Regarding the model evaluation, Maxent.Phillips-ESM and ANN-ESM were the best models, showing the highest mean values of adjustment indices with low variation, while GLM-ESM exhibited the lowest value of these indices with high variation (Supplementary Figs S2–S3). Compared to these, the fitted ensemble models (EP-ESMs) of climate and vegetation improved the accuracy of prediction as indicated with higher average values of adjustment indices. In particular, TSS, AUC and Somers'D in the fitted EP-ESMs performed well with average values of 0.80, 0.92 and 0.83 in the climate model, respectively (Supplementary Fig. S2). These values were relatively lower in the vegetation model (0.71, 0.88 and 0.75, respectively) (Supplementary Fig. S3). All selected climate and vegetation variables significantly influenced the prediction and their contribution rates were approximately equal (Supplementary Tables S1–S2).

Under current climatic conditions, the potential distribution of *G. huuliensis* covers mainly the sites of occurrence and their surroundings within the buffer area. We recorded a few additional potential sites beyond the buffer area in Vinh Phuc Province, northern Vietnam, and in border areas between China and Vietnam (Cao Bang Province) (Fig. 2A). The suitable climate range covers a total area of 63,773 km^2, of which approximately 42.5% (27,124 km^2) represent highly suitable habitat (Fig. 2B). However, within the buffer area, the suitable habitat only covers 9,577 km^2 (about 15% of the total suitable area). With regard to the vegetation model, suitable habitats of *G. huuliensis* are relatively scattered within the study site, but they are also gathered mainly around the occurrence sites. In particular, in the buffer area, the suitable area of vegetation covers 6,874 km^2 within the intermediate suitable site of climate, accounting for 71.8% (Figs 2A–2B–4). In terms of the MESS analysis, the buffer area is filled by a large area of interpolated habitat, covering 9,620 km^2 (approximately 65.0%) in the current climate model and 8,250 km^2 (approximately 56%) in the vegetation model, whereas we only recorded a very small area of extrapolation in both these models (Figs 2C–2D).

With respect to future predictions, in general, the climatically suitable area of each future scenario fluctuated depending on RCPs (e.g., 4.5 and 8.5) and periods (by the 2050s and 2070s). Under future climate scenarios, each predicted a significantly smaller area of the potential distribution compared to the contemporary one and implied a shift towards higher latitudes in the future (Figs 2–3–4). However, we recorded a novel case of the CCSM4 –

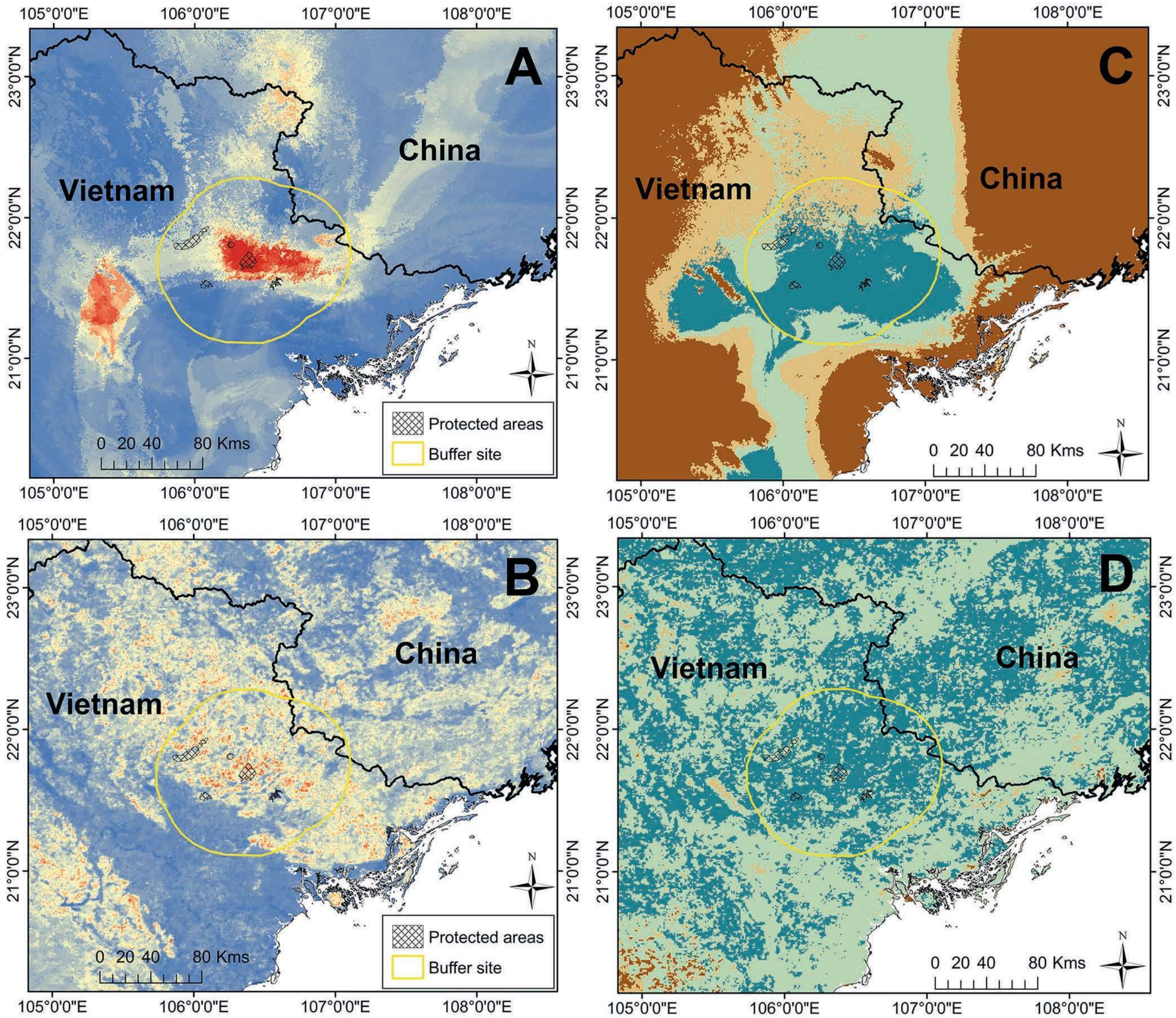

Figure 2. Projected potential distribution for *Goniurosaurus huuliensis* following (A) the climate model; (B) the vegetation model (blue to red colours indicate higher suitability). Multi-Environment Similarity Surface (MESS) map of novel habitat following (C) the climate model; (D) the vegetation model (teal colour represents high interpolation habitat, aqua colour – low interpolation, coral colour – low extrapolation, brown colour – high extrapolation).

RCP 4.5 scenario by the 2050s, covering 10,311 km^2 of the suitable area within the buffer. Afterwards, this suitable area contracted considerably under the same conditions by the 2070s (Fig. 4). The range contraction is expected to be largest under the RCP8.5 scenario by the 2070s. In particular, the CCSM4 circulation model suggests that the potential distribution may cover only 3,507 km^2 (representing 24% suitable area of the current model) (Figs 3D–4), while in the BCC-CSM 1-1 model, no highly suitable habitat for *G. huuliensis* was documented (0 km^2) and the intermediate suitable habitat within the buffer was only 2,555 km^2 (Figs 3D–4). Given the MESS projections, interpolated habitats within the current climate-based potential distribution of *G. huuliensis* will be gradually replaced by high novelty (extrapolated) habitats in the future (Supplementary Figs S4–S5). It is noteworthy that novel climatic conditions were recorded in most of the entire buffer area under the RCP 8.5 scenarios by the 2070s (Supplementary Figs S4A4–S4B4).

Discussion

Prediction models

Comparing the adjustment indices of TSS, AUC and Somers' D, we confirmed that the ensemble SDM significantly

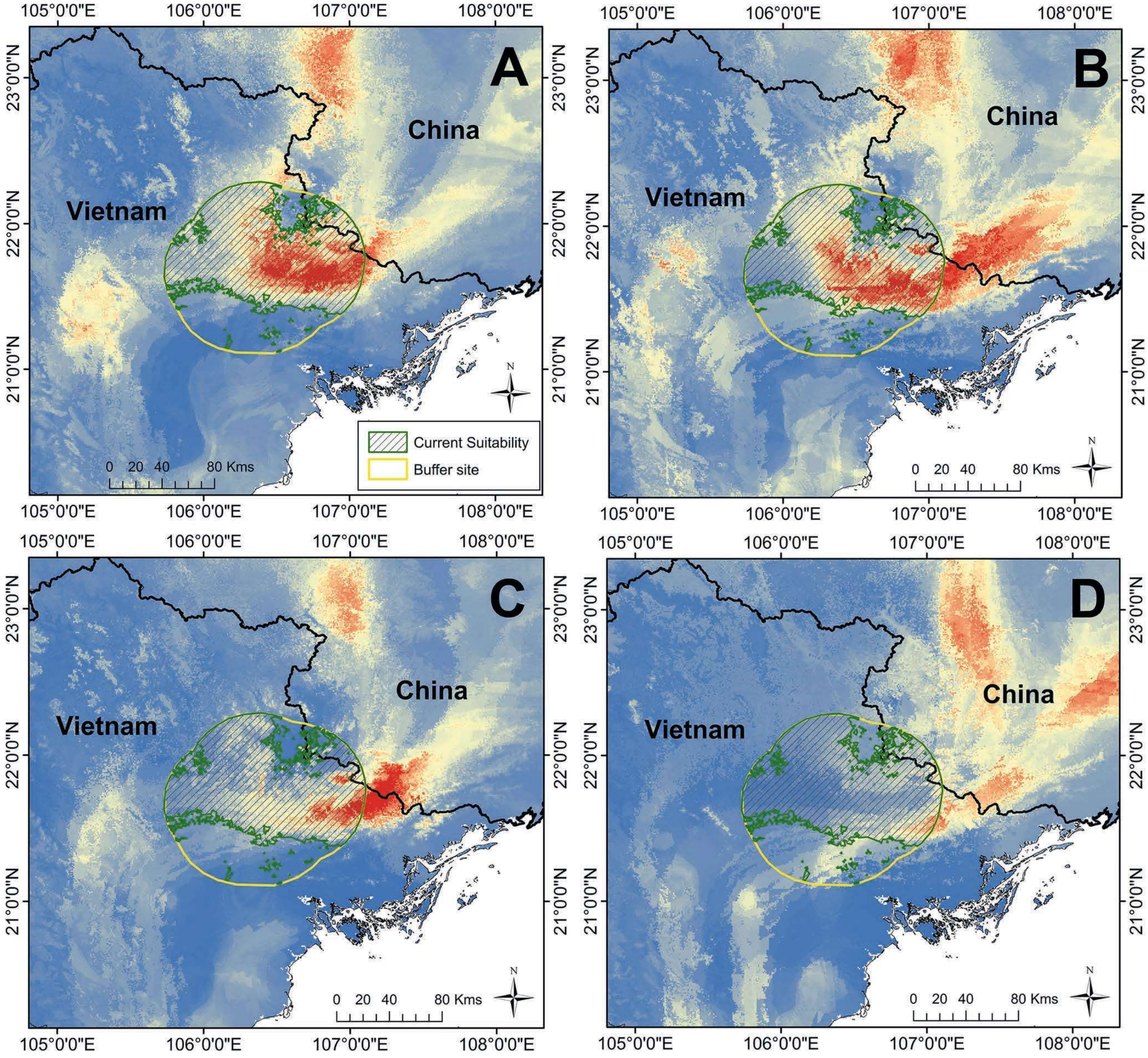

Figure 3. Projected potential distribution for *Goniurosaurus huuliensis* under different future climate scenarios (blue to red colours indicate higher suitability). The average map of two circulation models of BCC_CSM-1-1 and CCSM4 in the scenario of (A), RCP-4.5 by the 2050s; (B) RCP-4.5 by the 2070s; (C) RCP-8.5 by the 2050s; (D) RCP-8.5 by the 2070s.

improved the accuracy of model prediction for rare species. In particular, the average values of these indices in the fitted ensemble models (EP-ESMs) of climate and vegetation were generally higher than for a single modelling technique (Supplementary Figs S2–S3).

All selected climate and vegetation variables influenced the predictions, and their contributions were approximately equal in each ensemble model (Supplementary Tables S1–S2). However, the specific biological relationships between the selected environmental variables (macroclimate and vegetation cover) and the species remain unclear. Studies on two Tiger Gecko congeners, *G. catbaensis* and *G. lichtenfelderi*, recently described their microhabitat use and identified vegetation (canopy coverage) and ambient parameters (temperature, humidity, weather condition) as the most important characteristics (Ngo et al. 2019a, Ngo et al. in press). Therefore, it is undeniable that the selected environmental variables, which might play important roles in the natural history of *G. huuliensis*, constrain the current distribution range and even have effects on evolutionary processes (see also Pyron & Burbrink 2009, Rödder & Engler 2011, Zhang et al. 2014, Rato et al. 2015, Heidari 2019, Nogueira et al. 2019, Sheu et al. 2020).

In this study, we found in the current MESS map that novel climatic conditions will impact on most sites of occurrence of the other three Vietnamese Tiger Geckos (namely *G. catbaensis, G. lichtenfelderi*, and *G. luii*), and that the potential distribution of *G. huuliensis* does not or only slightly overlaps their occurrence sites (Orlov et al. 2008, Ziegler et al. 2008, Nguyen et al. 2009, Nguyen 2011, Ngo et al. 2019a, Ngo et al. 2021). The climate-based potential distribution of the granite-adapted *G. lichtenfelderi*, as simulated by the Maxent approach, showed that it does not overlap with the extent of occurrence of any karst-dwelling Tiger Gecko in Vietnam, apart from *G. catbaensis* (Ngo et al. 2021). We assume that macroclimatic niche divergence may have a central role in explaining the diversification in *Goniurosaurus* (Grinnell 1917, Rödder & Engler 2011, Fisher-Reid et al. 2012).

Implications for conservation

Under the future potential impacts of climate change, the potential distribution of *G. huuliensis* tends to narrow and shift towards higher latitudes in the border area between China and Vietnam. Similar changes were also previously predicted by using the Maxent approach for *G. catbaensis* and *G. lichtenfelderi* (Le et al. 2017, Ngo et al. 2021). Since all *Goniurosaurus* species are locally-distributed specialists adapted to unique ecological niches (Nguyen et al. 2009, Nguyen 2011, Ngo et al. 2016, 2019a, 2021, Ngo et al. in press), we only identified currently suitable areas within the selected buffer that can serve as refugia for sustainable in-situ conservation rather than areas suitable for colonization. Based on the forecasted climate and vegetation maps, we have generated a core-refugia map that identifies key regions for priority conservation. The green patches in the map (Fig. 5) represent highly suitable habitats with regard to both climate and vegetation, mostly in areas in Lang Son Province and at small sites in Bac Giang and Thai Nguyen Provinces, northern Vietnam. The yellow areas indicating

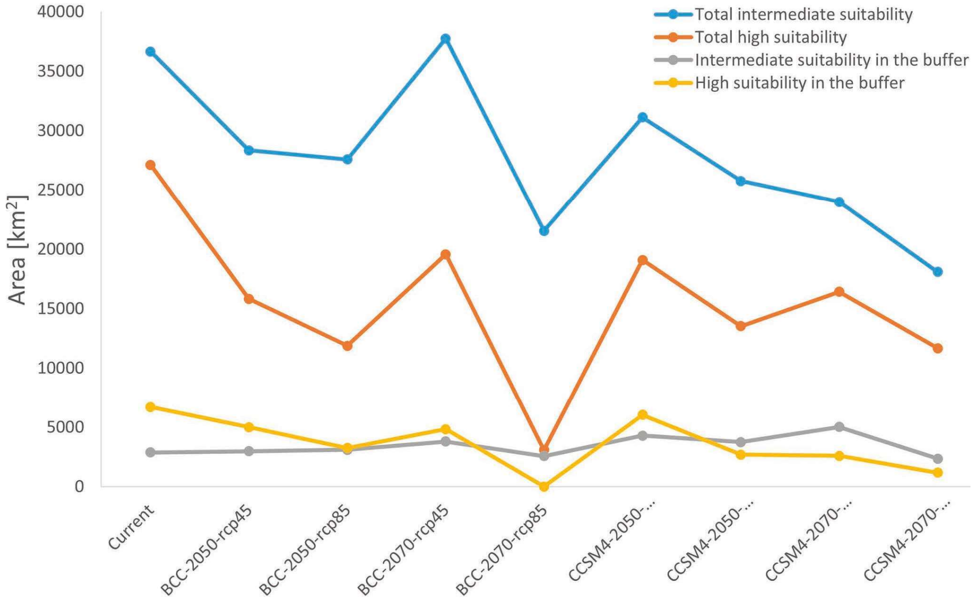

Figure 4. Projected habitable areas of suitable categories for *Goniurosaurus huuliensis* under different climate conditions of current and future scenarios (blue line represents the total areas of intermediate suitability within the study site; orange line the total areas of high suitability within the study site; grey line the areas of intermediate suitability in the buffer; yellow line the areas of high suitability in the buffer).

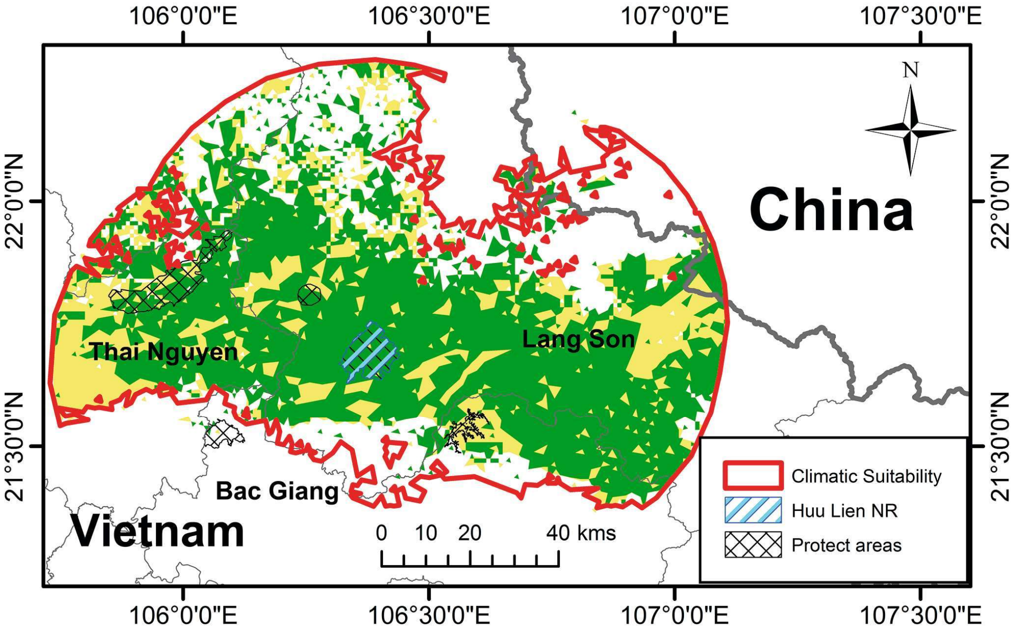

Figure 5. Proposed refugia throughout the range of *Goniurosaurus huuliensis* under the current model (green areas indicate core habitats of suitable vegetation within yellow areas of high climatic suitability and red lines cover all climatically suitable areas in the buffer area).

the intermediate level of climate suitability should be considered buffers of the core refugia (Fig. 5).

There are three nature reserves (namely Bac Me, Huu Lien and Than Sa – Phuong Hoang) within the selected green patches (Fig. 5). However, the target species has been found only in the Huu Lien NR. A large area of evergreen forest in the Huu Lien NR is rigorously protected by local people and rangers, and wild populations of *G. huuliensis* may potentially be found in previously overlooked but suitable areas there (Ngo et al. pers. obs). Thus, the Huu Lien NR plays a very important role and should be considered a "centre" for the initiation of in-situ conservation programs for *G. huuliensis*.

However, our interviews with local rangers showed that dealers are usually contacted by local hunters from the Huu Lien NR with offers of desired quantities and prices for each wild specimen. Captured animals were then handed over to the dealers in places outside the nature reserve to evade stringent inspections by local rangers. As a consequence of these illegal actions, large numbers of wild animals were collected, leading to a potentially significant decline of the wild population of *G. huuliensis* (Ngo et al. 2019a, b). Furthermore, we observed other anthropogenic impacts on the karst habitat of *G. huuliensis*, such as expanding road construction and illegal timber logging.

Thus, we recommend the establishment of a species and habitat conservation area for the threatened species *G. huuliensis*. The core-protection area should be selected from within the green patches in the core-refugia map with the "Huu Lien NR-Center" (Fig. 5). However, this suggestion may not be the best option to protect the species effectively. In fact, our selection of core refugia is only based on the outcome of our climatic and vegetation models. To enhance the practical applicability of in-situ conservation measures, conservationists should be provided with detailed biological information on the target species, such as population status, habitat requirements, and threats as well. At the moment, to mitigate the anthropogenic impacts on wild populations and their habitat, we strongly propose that monitoring of the illegal trade and protecting natural forests be intensified, as well as community education on the value of biodiversity be enhanced.

Two other lizards, *Gekko canhi* and *Scincella apraefrontalis*, were recently discovered in the Huu Lien NR (Nguyen et al. 2010, Rösler et al. 2010, Ngo et al. pers. obs). We assume that these endemic species, being sympatric with *G. huuliensis*, are likewise being negatively affected in the context of climate change. Therefore, these lizard species will also benefit from improved conservation measures in the region.

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Supplementary data

The following data are available online:

Supplementary Figure S1. Schematic of the Ensemble Small Models (ESM) for each selected modelling technique.

Supplementary Figure S2. Performance of seven Ensemble of Small Models of climate according to adjustment indices of TSS, AUC and Somers'D.

Supplementary Figure S3. Performance of seven Ensemble of Small Models of vegetation according to adjustment indices of TSS, AUC and Somers'D

Supplementary Figure S4. Multi-Environment Similarity Surface (MESS) map of the novel habitat following future circulation models.

Supplementary Figure S5. Predicted areas of novel habitats as per MESS analyses under different conditions of current and future scenarios.

Supplementary Table S1. Relative contributions (percentages) of climatic variables for ESMs.

Supplementary Table S2. Relative contributions (percentages) of vegetation variables for ESMs.

IV. DISCUSSION

4.1. Systematics, evolution and zoogeography.

The systematics of the *Goniurosaurus* genus remains challenging due to ongoing discoveries of cryptic species and no single genetic marker applied for all species at once. In Section 1.1, our phylogenetic analysis showed that all recorded *Goniurosaurus* populations from Vietnam were found to be monophyletic with low intra-specific genetic divergences. Together with morphological comparisons, the study approved the **first hypothesis** that all Vietnamese populations were assigned to one of the four known species: *G. catbaensis, G. huuliensis, G. lichtenfelderi* or *G. luii*. No individual of *G. araneus* from Vietnam was collected in this study. It is possible that the species has never occurred in Vietnam, though extensive surveys have been conducted over the last two decades in the type locality (Grismer et al. 1999; Ngo et al. 2016b).

The taxonomic reviews of *Goniurosaurus* agreed well with previous revisions and clarified some suspicions. In accordance with Liang et al. (2018) and Qi et al. (2020a, b), for example, *G. bawanglingensis* and *G. zhoui* are embedded within the *G. lichtenfelderi* group in both analyses of genetics and morphology, while they were previously assigned as sister taxa in the *G. luii* group (Grismer et al. 2002; Zhou et al. 2018). The similarity in some morphological characters compared to *G. luii* by Ngo et al. (2016b) doubted the previous recognition of a new species (namely *G. kadoorieorum* Zang & Chan 2015). With respect to *G. kadoorieorum, G. luii* recovered to be polyphyletic (Zhou et al. 2020a; Sections 1.1 – 1.2). Our molecular study confirmed that *G. murphyi*, described by Orlov & Darevsky (1999), is a junior synonym of *G. lichtenfelderi* (Grismer 2000). All mainland populations of *Goniurosaurus* found in granitic forests are conspecific with *G. lichtenfelderi* from islands in Bai Tu Long National Park, Quang Ninh Province, northern Vietnam (type locality). However, more cryptic species may be found in the Japanese group and potential discoveries of new species in southern China (Chapter 1) may take issue with the current understanding of *Goniurosaurus* systematics.

Given ancestral range estimations in Section 1.3 and Liang et al. (2018), the *Goniurosaurus* ancestor was predicted to originate from somewhere in the contiguous continental Eastern Asia, including the Ryukyu Archipelago and Hainan Island before the Eocene (Honda et al. 2014). The radiation of insular *Goniurosaurus* groups (namely *G. kuroiwae* and *G. lichtenfelderi*) was therefore considered relative to past tectonic events (e.g. the collision between India and Eurasia continents, clockwise rotation of the Philippine Sea plate) (Seno et al. 1993; Ota 1998; Tend & Lin 2004; Sterling et al. 2006; Liang et al. 2018). Once the ocean served as a geographic barrier that prevented the dispersal ability and gene flow among populations. The evolution pattern of vicariance thereby forced the cladogenesis in the two insular groups (~late Mioence), as well as in two insular tiger gecko species in Vietnam (*G. catbaensis* and *G. lichtenfelderi*) during glacial periods in the late Pliocene (Sterling et al. 2006; Heaney 2007; Ziegler et al. 2008; Herzschuh et al. 2016; Liang et al. 2018; Section 1.3).

Based on the phylogenetic analysis, we employed abiotic factors (e.g. habitat preference, altitudinal gradient, climate niche) to explain the role that they might play in the diversification of *Goniurosaurus*. For the habitat preference, a stochastic character mapping analysis in Section 1.2 approved the **second hypothesis**. In particular, karst ecosystems served as the most probable ancestral habitat for *Goniurosaurus* as well as for three out of the four monophyletic groups (*G. kuroiwae*, *G. luii*, and *G. yingdeensis* groups), while the *lichtenfelderi* group originated from non-karst habitat. Morphological flexibility to adapt to specialized habitats was verified. Some distinguishable characteristics of longer head and snout-vent lengths, larger eyes, longer trunk and limbs were recorded in the karst-adapted *Goniurosaurus* groups (Section 1.2), and occur in other karst-dwelling *Cyrtodactylus* species as well (Nielsen and Oliver 2017; Grismer et al. 2020; Kaatz et al. 2021), indicating that there is a convergent adaption that shapes karstic lifestyle within and between the gekkotan families.

The species richness varies along the elevation gradient (Sterling et al. 2006; McCain 2010; McCain and Grytnes 2010). Based on this background, the coded elevation levels were also mapped at each cladogenetic node of the full *Goniurosaurus* tree. With the exception of the most common ancestor of *Goniurosaurus* lineages (namely *G. kwanghua, G. hainanensis, G. lichtenfelderi*) at the "high" elevation, the "low" level was predicted as the most probable ancestral state at the crown tree and all group nodes (Section 1.3). Two-way altitudinal transitions, but following discordant directions, might also force the speciation within the *G. lichtenfelderi* group. In particular, low-to-high transitions along the elevation gradient upward highland refugia on Hainan Island might occur in the interglacial mid-Pliocene, and thereby tiger geckos avoided severely warmer climate conditions. Whilst the youngest cladogenesis between *G. hainanensis* and *G. lichtenfelderi* might be relative to the altitudinal partitioning as well, but vice versa *G. lichtenfelderi* colonized toward low sites in Vietnam during several glacial periods (~2 mya; Section 1.3).

We further employed ecological niche models to elucidate the role of the Grinnellian niche (e.g. climate conditions) with respect to the diversification of *Goniurosaurus*. In Section 1.3, we reconstructed profiles of predicted niche occupancy (PNO) of *Goniurosaurus* species to illustrate their niche evolution history and afterward estimated phylogenetic signals in the climatic conditions among sister taxa. As a result, phylogenetic signals were noted, but relatively low. Given maximum likelihood calculations, the main reason for the low signal is that *G. lichtenfelderi* adapted to contrasting climate conditions to those of relatives on Hainan Island. Furthermore, a wide range of climate gradients within the *G. luii* group and the heterogeneity recorded among its lineages (e.g. *G. catbaensis, G. kwangsiensis* and *G. liboensis*) caused the low signals in general, following the divergence pattern. On the other hand, all lineages of the monophyletic *G. yingdeensis* group experienced similar conditions in all climates, which supported the phylogenetic signal following the conservatism pattern (Section 1.3). Accordingly, the **third hypothesis** claiming the diversification of *Goniurosaurus* under intermixed pressures of climatic niche conservatism and divergence patterns, was confirmed.

Given the distribution of Vietnamese tiger gecko species in allopatry, *G. lichtenfelderi* is only adapted to granite-stream habitats, whereas the three remaining species are exclusively found on karst formations (Section 1.2; Sections 2.1 – 2.3). The morphological and genetic similarities between two geographically distant populations of *G. lichtenfelderi* in island and mainland sites were explained due to no significant differences between their ecological niches (Section 2.2). *Goniurosaurus catbaensis* is only documented on isolated islands, following both the vicariance and climatic divergence patterns, compared to two remaining karst relatives (Sections 1.1 – 1.2). Rivers are also regarded as a potential barrier favoring the reproductive isolation, and consequently, speciation, such as the Zuojiang River between *G. luii* and *G. araneus* within Nonggang Nature Reserve in China, coupled with the Ky Cung River between *G. huuliensis* and *G. luii* in Vietnam. These rivers likely established an eastern boundary for *G. luii* (Chen et al. 2014; Section 2.3).

4.2. Autecology and population status

In general, a range-restricted species is characterized with habitat specialization and unstable population status (e.g. small population size). As such, its populations are more vulnerable to extinction risks than wide-range relatives (Schlaepfer et al. 2005; Böhm et al. 2013; IUCN 2021). Based on this background, research in Chapter 2 provided the first knowledge of micro-habitat characteristics and assessed the population status of Vietnamese tiger geckos. In consequence, the **fourth** and **fifth hypotheses** were approved, respectively.

In Sections 2.1 – 2.3, micro-habitat descriptions confirmed previous recognitions that three tiger geckos (e.g. *G. catbaensis, G. huuliensis* and *G. luii*), are indeed limestone karst-dwelling lizards in evergreen forests (Grismer et al. 1999; Orlov et al. 2008; Nguyen 2011). Their habitats are relatively similar, typically characterized with a high vegetation coverage, low height to the ground, high humidity, stable ambient temperature and dry rocky substrates. Crevices of open karst caves as well as single rocks were identified as their shelters. The habitat preference of *G. lichtenfelderi* is significantly different. The species is adapted to shore areas along small stream sections, comprising rocky shelters on the soil floor, low height to ground, and covered with high micro-vegetation coverage in evergreen forests on granitic mountains (Section 2.2).

In Section 2.1, new sub-populations of *G. catbaensis* were recorded outside of the type locality on adjacent islands from Ha Long Bay, Quang Ninh Province. The population size on the four islands was estimated to be relatively stable, consisting of about 124 and 129 individuals (Section 3.1). However, the population size of *G. catbaensis* on Cat Ba Island (type locality) was estimated to be exceedingly small (16 to 24 individuals; Ngo et al. 2016b). According to a report of Gewiss (2020) belonging to our *Goniurosaurus* project, a mean population size of approximately 195 individuals and an effective one of 164 matures of *G. lichtenfelderi* can be assumed, covering four populations in both island and mainland sites. These values only reflected the surveyed sites and might not capture the entire range of *Goniurosaurus* taxa. However, their total population size is assumed to be relatively small. To overcome this limitation, the global population of *G. huuliensis* was estimated with a potential size of 1,447 individuals within integrated suitable habitats. The maximum size can achieve up to 2,855 ind. within the karst habitats in the whole extent of occurrence – EOO (Section 2.4). The maximum value seems to be much larger than the actual population size due to the inclusion of suboptimal habitats, but is still relatively lower than the required minimum viable population to ensure the long-term persistence of the species (Reed et al. 2003; Traill et al. 2007).

With respect to densities of comparable relatives, *G. lichtenfelderi* was observed with a low density of 0.31 – 0.76 ind./ 100 m (Gewiss 2020), which is much higher than an estimation by Ngo et al. (2016b) for *G. catbaensis* on Cat Ba Island, Hai Phong City (0.08 – 0.17 ind./ 100 m) and for *G. luii* in Ha Lang District, Cao Bang Province (0.08 ind./ 100 m). Whilst the highest density was also documented in *G. catbaensis* (Section 3.1), but on other islands in Ha Long Bay, Quang Ninh Province (6 ind./ 100 m). Furthermore, *G. huuliensis* was documented to occur in a small density of only 0.64 ind./ 100 m (Section 2.4). The mean density of tiger geckos is considerably lower compared to other global threatened reptiles, such as *Cnemaspis psychedelica* (12 – 19.2 ind./ 100m), or *Physignathus cocincinus* (1.98 – 2.64 ind./ 100m) (Ngo et al. 2016b; Nguyen et al. 2018a).

4.3. Threats and implications for conservation

4.3.1. Anthropogenic threats.

As considered one of the most susceptible reptile groups due to the high level of endemism, increasing human pressures can drive the fate of *Goniurosaurus* into the global extinction vortex (Böhm et al. 2013; Ngo et al. 2016b; Cox et al. 2022). The differences among intra-specific population sizes and densities of *Goniurosaurus* species could be a result of integrative human impacts, depending on their level of pressure (Gewiss 2020; Section 2.4; Section 3.1). In particular, the lowest population sizes and densities were documented in the most disturbed populations (such as *G. catbaensis* on Cat Ba Island, *G. luii* in Cao Bang Province, *G. lichtenfelderi* in Yen Tu Mountain), whereas the highest values were recorded in intact areas (such as *G. catbaensis* on four islands in the Ha Long Bay, *G. lichtenfelderi* in Bai Tu Long NP and Hai Duong Province). Gewiss et al. (2020) also recorded that population densities and sizes of the water dragon, *Physignathus cocincinus,* in disturbed sites in northern Vietnam were considerably lower in undisturbed areas. Therefore, under human impacts, *Goniurosaurus* species are prone to extinction under human impacts. In this context, the **fifth hypothesis** concerning human impacts on *Goniurosaurus* populations was further confirmed.

- Overexploitation of tiger geckos for international trade.

Recorded in pet markets since the 1990s, *Goniurosaurus* species are particularly threatened by extinction through over-exploitation. Consequently, *G. luii* might be extirpated from the type locality at Pingxaing, China, and extensive surveys over the last two decades have no longer found Vietnamese populations of *G. araneus* (Grismer et al. 1999; Yang & Chan 2015; Ngo et al. 2016b; Section 1.1). In Section 3.1, an attempt was carried out to outline domestic and international trade activities in *Goniurosaurus* species. All five native *Goniurosaurus* species in Vietnam (with the exception of *G. araneus* from China) have been frequently offered in local pet shops and on internet platforms as well. However, most of them have been exported to Indonesia and Thailand and from there to final destinations of the United States (U.S.) and the European Union (EU). A large number of *Goniurosaurus* individuals have been indeed imported into the U.S., noted for online trade in the EU, and available in European reptile fairs as well. These observations, during random physical as well as internet market surveys, only reflect snapshots of trade in tiger geckos.

Stuart et al. (2006) proposed that captive breeding can reduce the high demand for wild-caught animals. In fact, a large number of tiger geckos for sale were labeled as captive-bred in the EU and the U.S. (Section 3.1). However, wild-caught animals of *Goniurosaurus* species were still imported in large quantities. High commercial revenues were identified as the main motivator that provoked the wild exploitation of all Vietnamese tiger geckos (Section 3.1). Due to the extremely high global demand for tiger geckos, captive-bred animals otherwise might be not available in quantities to meet the demand yet. Furthermore, wild-caught individuals likely have a high mortality rate during transport and stockpiling in poor conditions (Section 3.1). Under severe impacts of overexploitation for trade, all rare tiger gecko species quickly become threatened by extinction, which highly favored the **sixth hypothesis**.

- Potential threats of climate change to *Goniurosaurus*.

Climate change has been globally acknowledged to impact many tropical species (Ohlemüller et al., 2008; Bellard et al. 2014; Newbold 2018; Winter et al. 2016; Trew & Maclean 2021). However, it was unknown whether it may have detrimental influences on populations of *Goniurosaurus*. As a result of climate change, the increase in the frequency and intensity of natural catastrophes in tropical regions, such as drought, storms, floods and sea level rising are imperiling global biodiversity, especially in Vietnam (US Forest Service 2011; Taylor & Kumar 2016). From the type locality in Viet Hai Commune, on Cat Ba Island an extensive flood in 2015 might have eliminated a large proportion of the wildlife community, including *G. catbaensis* species (Section 2.1), though the site, as Ngo et al. (2016b) recommended before, should be an area of conservation priorities. Therefore, found on isolated islands, *Goniurosaurus* populations at the lowland are predicted to lose their distribution in the future due to the sea level rising (Taylor & Kumar 2016).

In the SDM sections, climate and vegetation cover data were trained to identify the potential distribution of each species and assess its alterations under future climate change scenarios. In particular, predictions for *G. catbaensis* (Section 2.1), *G. lichtenfelderi* (Section 3.2) and *G. huuliensis* (Section 3.3) showed that their potential distribution mostly cover the known sites of occurrence and surroundings. Under future scenarios of climate change, the potential distribution of *G. lichtenfelderi* and *G. huuliensis* were projected to shift towards higher latitudes, shrink in the next decades and even vanish in their entire distribution. In this context, the **seventh hypothesis** of climate change was approved.

As described in Section 1.3, species that evolved in niche conservatism pattern are generally more susceptible to climate change and vice versa (Wiens & Graham. 2005; Hadly et al. 2009; Wiens et al. 2010; Peterson 2011; Pyron et al. 2014; Ahmadi et al. 2021). In this study, sister taxa in the *G. yingdeensis* group, on Hainan Island and some in the *G. luii* group were identified to evolve within a narrow climate gradient (Section 1.3). However, this does not mean that the remaining *Goniurosaurus* species are unharmed by climate change. In particular, *G. lichtenfelderi* thriving in the niche divergence and *G. huuliensis* in the conservatism, are both potentially impacted by climate change (Sections 3.2 – 3.3, respectively).

As habitat specialists, tiger geckos are unable to migrate long distances to reach potential refugia where more favorable climate conditions may mitigate pressures of climate change (Sections 2.1 – 2.3, Sections 3.2 – 3.3). Whilst the increase of forest degradation and conversion are imperiling the last survival chance of tiger geckos under climate change due to the loss of suitable habitats and the lack of micro-corridors. Served as the exclusive preferred habitat and refugia under climate change, the existence of 80 % of the tiger gecko species depends entirely on the integrity of karstic ecosystems (Section 1.2). Resembling what is occurring in Southeast Asia as Clements et al. (2006) documented, karst formations as the habitat of *G. catbaensis, G. huuliensis* and *G. luii* have been dramatically quarried to extract materials for the cement production, and expand road constructions and other infrastructures associated with urbanization and tourism. Furthermore, large forest areas were strongly fragmented due to timber logging, and those areas were gradually replaced by industrial crops or grassy hills. Being sympatric with another threatened lizard (namely *Shinisaurus crocodilurus*), habitats of *G. lichtenfelderi* across the Yen Tu Mountain range have been severely impacted by anthropogenic activities, such as coal mining, water pollution, timber logging and construction works for the tourism, leading to habitat degradation and loss (van Schingen et al., 2014; 2015; Gewiss 2020; Section 2.2). These additional impacts can make tiger geckos even more vulnerable to climate change.

4.3.2. Conservation relevance.

- Preventing over-exploitation of tiger geckos

From Chapter 1 to Chapter 3, understandings of systematics, evolution, ecology, population status and human threats (e.g. overexploitation for trade, habitat loss and climate change) emphasized that tiger gecko species are vulnerable to extinction. Therefore, conservation measures are urgently required and should no longer be delayed. With respect to the unsustainable trade, disclosing exact localities in new species and population descriptions may increase the human accessibility to wild populations (Yang & Chan 2015; Lindenmayer & Scheele. 2017). Thus, it is strongly recommended to conceal exact locality data (e.g. coordinates) in order to prevent targeted collection in *Goniurosaurus* species (Section 3.1).

Coupled with captive breeding efforts proposed by Stuart et al. (2006), the problems relative to overexploitation of wild-caught tiger geckos can be tackled. However, the lack of regulations was insufficient to stop poaching and prevent the exploitation of wild animals.

Furthermore, the scale of the international demand for tiger geckos, as well as trade levels and patterns were uncontrollable. Based on the background, all species – namely *Goniurosaurus* spp. – from China and Vietnam were listed in CITES Appendix II and Vietnamese species were also nationally protected under the Governmental Decree 06/2019/ND–CP (Group IIB). With the current legislation, not only new species, namely *G. chengzheng, G. gezhi*, *G. gollum*, *G. kwanghua* and *G. varius* from China, but also potentially newly discovered species are automatically listed in CITES Appendix II (Qi et al. 2020a, 2020b; Zhu et al. 2020a, 2020b, 2021; Section 3.1). Recently, the listing of all six endemic *Goniurosaurus* species from Japan in CITES – Appendix III has come into force. Detailed descriptions of the morphology among Vietnamese tiger gecko species together with an identification key of all species provided in Section 1.1 can assist relevant authorities (e.g. local rangers, CITES authorities, officers) to identify *Goniurosaurus* taxa and enforce regulations more effectively.

- Conservation: *in-situ* and *ex-situ* measures

Listed in the IUCN Red List as "Endangered" based on sufficient information on population status, ecology and threats, *G. catbaensis* has received more attention in conservation (Nguyen et al. 2016). In the workshop proceeding – "Values and solutions to conserve biodiversity in Ha Long Bay and Cat ba Archipelago" held in Ha Long City in 2017, Nguyen et al. (2018b) highlighted the unique biological value of reptiles in Cat Ba National Park and proposed *G. catbaensis* as a high-profile flagship species of the Ha Long World Heritage and Cat Ba Biosphere Reserve. Training programs have been held by IUCN Vietnam since November 2018 to enhance monitoring skills of researchers and local rangers in Ha Long Bay and Cat Ba National Park. Some signboards and a thousand leaflets highlighting the conservation needs of *G. catbaensis* we provided to local scientific departments and tourists (Section 3.1).

Of 24 described tiger gecko species, 16 have been considered threatened (listed as VU, EN and CR) following the "Criterion–B1" of restricted range (IUCN 2021). Apart from the threatened *G. catbaensis*, none of conservation measures have been yet implemented to safeguard the rest of tiger gecko species due to lacking understanding of population status, species ecology and threats. Under the challenge of increasing human impacts, the fate of globally threatened *Goniurosaurus* species cannot wait for conservation trials. Updated in this study, the biological knowledge indeed is the basis to propose recommendations and solutions to implement conservation activities truly effective in the future.

- Following the climatic niche conservatism, tiger gecko species are highly vulnerable to human pressures, especially to climate change. In terms of conservation planning, high priorities of protection and conservation attention to limit the effects of climate change should be implemented to prevent extinction of these species (Section1.1).
- At least forests associated with granite streams of *G. lichtenfelderi* and karst formations of remaining Vietnamese tiger geckos need higher protection levels to enable their long-term persistence. Besides stringent regulations to stop the illegal trade, it is necessary to improve

the capacity building for local rangers and forest protection departments as well as community education to enhance the effectiveness of forest protection and the awareness of local communities about the biodiversity value (Sections 2.1 – 2.3).

- Since *Goniurosaurus* species are narrow habitat specialists, stable refugia for tiger gecko species should be identified, rather than identifying colonization areas under climate change. In Chapter 3, key refugia of *Goniurosaurus* species were suggested, including forests in Yen Tu Mountain Range, Bai Tu Long NP and border areas between China and Vietnam for *G. lichtenfelderi* (Section 3.2) and most forests in Lang Son Province for *G. huuliensis* (Section 3.3). Within these refugia, protected areas (e.g. Bai Tu Long NP, Tay Yen Tu NR and Dong Son – Ky Thuong NR, and Huu Lien NR), which contain a significant proportion of suitable habitat, should be considered "centers" for the future *in-situ* conservation programs of *G. lichtenfelderi* and *G. huuliensis,* respectively. However, approximately 85% of the total population, for example, of *G. huuliensis* may exist beyond the Huu Lien NR boundaries (Section 2.4). Without law enforcement and forest ranger patrolling in there, a plan to establish a species and habitat conservation area is an adequate conservation solution to safeguard all *in-situ* populations. As such priorities of conservation policies to protect natural ecosystems and their high-profile flagship species (e.g. tiger geckos) can be re-assessed rather than priorities for the economic development.
- Due to the similarity in microhabitat use, a solution of one-size-fits-all type in conservation measures was proposed in Section 2.3 to apply between the two karstic species (namely, *G. huuliensis* and *G. luii*), and can also expand to other karst-dwelling *Goniurosaurus* species. Likewise, the concordance of macro- and micro-niches between two distant populations in island and mainland sites of *G. lichtenfelderi*, in Section 2.4, favors the possibility of applying the same measures for all.
- Following the special issue: "Transboundary conservation under climate change", in Section 3.2 for *G. lichtenfelderi*, the importance of border regions is prioritized for *in-situ* conservation actions. Therefore, research and management collaborations between authorities of China and Vietnam are essential to protect the remaining populations of *G. lichtenfelderi*. We further recommend supervision along border regions between Vietnam and neighboring countries (such as China, Indonesia, Thailand) that should be improved and become more stringent to prevent the illegal trade in tiger geckos.

Establishing coordinated *ex-situ* breeding programs for all tiger geckos is highly recommended to build a stable captive population. In fact, an *ex-situ* conservation breeding program for all Vietnamese tiger gecko species has been established at the Me Linh Station for Biodiversity since 2018 by our team based on a long-term cooperation between the Institute of Ecology and Biological Resources (IEBR), Vietnam and Cologne Zoo, Germany, with the main purpose to establish an assurance population for each species. First breeding successes were recently recorded for *G. catbaensis* and *G. lichtenfelderi* at the station (Pham et al. 2019; 2021). Recently, Cologne Zoo set up a conservation breeding program for all Vietnamese tiger geckos as well, which resulted in good and continuing breeding success (Ziegler and Rauhaus 2022). The plan is to establish a conservation breeding network, already including several European zoos, and to expand the network into the USA (Ziegler and Rauhaus 2022). With both domestic and foreign breeding programs assurance colonies are developed which could facilitate future repatriation/ restocking once being required to recover disturbed wild populations. Together such a strategy complies with the „One Plan Approach“ proposed by the IUCN SSC Conservation Breeding Specialist Group (CBSG), which aims to develop integrative strategies for the protection of threatened animal species, viz. to promote the cooperative interaction of *in-situ* and *ex-situ* measures and expert group, thus implying the development of management strategies and conservation actions by all responsible parties for all populations of a species, whether inside or outside their natural range (e.g., Ziegler and Rauhaus 2022).

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Appendix 1.

Additional Publications and Manuscripts.

1.2. Phylogeny and evolution of habitat preference in *Goniurosaurus* (Squamata: Eublepharidae) and their correlation with karst and granite-stream-adapted ecomorphologies in species groups from Vietnam

Grismer L.L., **Ngo N.H.**, Qi S., Wang Y.Y., Le D.M. and Ziegler T. (2021). Phylogeny and evolution of habitat preference in *Goniurosaurus* (Squamata: Eublepharidae) and their correlation with karst and granite-stream-adapted ecomorphologies in species groups from Vietnam. Vertebrate Zoology 71: 335–352.

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Phylogeny and evolution of habitat preference in *Goniurosaurus* (Squamata: Eublepharidae) and their correlation with karst and granite-stream-adapted ecomorphologies in species groups from Vietnam

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Abstract

Maximum likelihood (ML) and Bayesian inference (BI) analyses using two mitochondrial (16S and cyt *b*) and two nuclear (CMOS and RAG1) genes and 103 specimens recovered the first phylogenies of all 23 extant species of *Goniurosaurus*. The analyses strongly supported the recognition of four monophyletic species groups with identical inter-specific relationships within the *kuroiwae, lichtenfelderi,* and *yingdeensis* groups but discordant topologies at some nodes within the *luii* group. Both analyses recovered a polyphyletic *G. luii* with respect to *G. kadoorieorum*, and owing to the lack of diagnostic characters in the latter, it is considered a junior synonym of *G. luii*. A stochastic character mapping analysis of karst versus non-karst habitat preference suggested that karstic landscapes may have played a major role in the evolution and diversification of *Goniurosaurus.* A karst habitat preference is marginally supported as the most probable ancestral condition for *Goniurosaurus* as well as for the *kuroiwae*, *luii,* and *yingdeensis* groups. However, a non-karst habitat preference is marginally supported as the most probable ancestral condition for the *lichtenfelderi* group. Multivariate and univariate ecomorphological analyses of the karst-adapted *G. catbaensis, G. huuliensis,* and *G. luii* of the *luii* group and the granite-stream-adapted *G. lichtenfelderi* of the *lichtenfelderi* group demonstrated that their markedly statistically different body shapes may be an adaptive response that contributes to habitat partitioning in areas of northern Vietnam where they are nearly sympatric.

Keywords

Asia, stochastic character mapping, systematics, synonymy, tiger geckos

Introduction

Eublepharid geckos of the genus *Goniurosaurus* Barbour, 1908 comprise 23 saxicolous specialists (Uetz et al. 2021) that extend from the Ryukyu Archipelago in Japan, southward through East Asia to northern Vietnam. *Goniurosaurus* is a well-defined monophyletic group (Grismer 1988) comprised of four monophyletic species groups: the *kuroiwae* group containing six species endemic to the Ryukyu Archipelago, Japan; the *lichtenfelderi* group with five species from insular and mainland China and northern Vietnam; the *luii* group with eight species from northern Vietnam, some of its offshore islands, and southern China; and the *yingdeensis* group consisting of four species from southern China (Kurita et al. 2008; Nguyen et al. 2009; Nguyen 2011; Wang et al. 2013; Honda and Ota 2017; Liang et al. 2018; Qi et al. 2020a, 2020b; Zhu et al. 2020a, 2020b). Apart from these species, *Goniurosaurus sinensis* Zhou, Peng, Huo and Yuan, 2019 is likely a junior synonym of another species from Hainan Island, China and not included herein (Qi et al. in progress). Phylogenetic relationships within *Goniurosaurus* have never been strongly supported nor consistent among different studies (e.g. Wang et al. 2013; Liang et al. 2018; Qi et al. 2020a, 2020b; Zhu et al. 2020a, 2020b). This protracted state of discordance results, in part, from researchers focusing on different species groups as opposed to the entire genus, as well as using different genes or different combinations of genes with varying combinations of ingroup and outgroup species—all variables that bear significantly on tree construction (Wiens 1998; Zwickl et al. 2002; Heath et al. 2008; Wiens and Morrill 2011; Wainwright and Price 2016). The most commonly used genetic markers have been the mitochondrial genes 12S and 16S rRNA and cytochrome *b* (cyt *b*). Liang et al. (2018) were the first to address the challenges of properly aligning rRNA (Pyron et al. 2013) and constructed a well-supported mito-nuclear data set using 16S, cyt *b*, and the nuclear genes oocyte maturation factor MOS (CMOS), and recombination activating 1 (RAG1). Zhu et al. (2020b) also used this mito-nuclear combination, but examined only relationships within the *lichtenfelderi* group.

In an effort to continue building a more global understanding of the phylogenetic relationships within *Goniurosaurus,* we expanded the mito-nuclear data set of Liang et al. (2018) to include 103 individuals as opposed to 31 and 23 as opposed to 17 species, which for the first time, includes all extant species of the genus (Table 1). We used this phylogeny in a stochastic character state mapping (SCM) analysis (Revell 2012) of habitat preference to explore the role karstic landscapes may have played in the evolution and diversification of *Goniurosaurus* and if

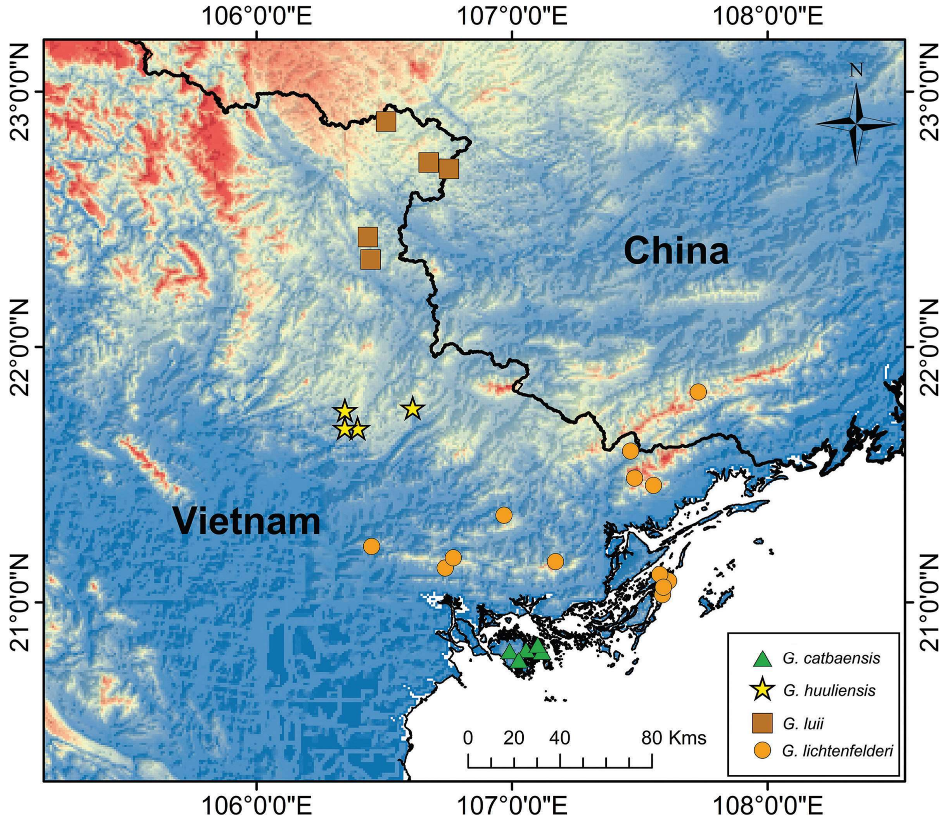

Figure 1. Distribution of the four recorded species of *Goniurosaurus* in Vietnam and China used in the ecomorphological analysis.

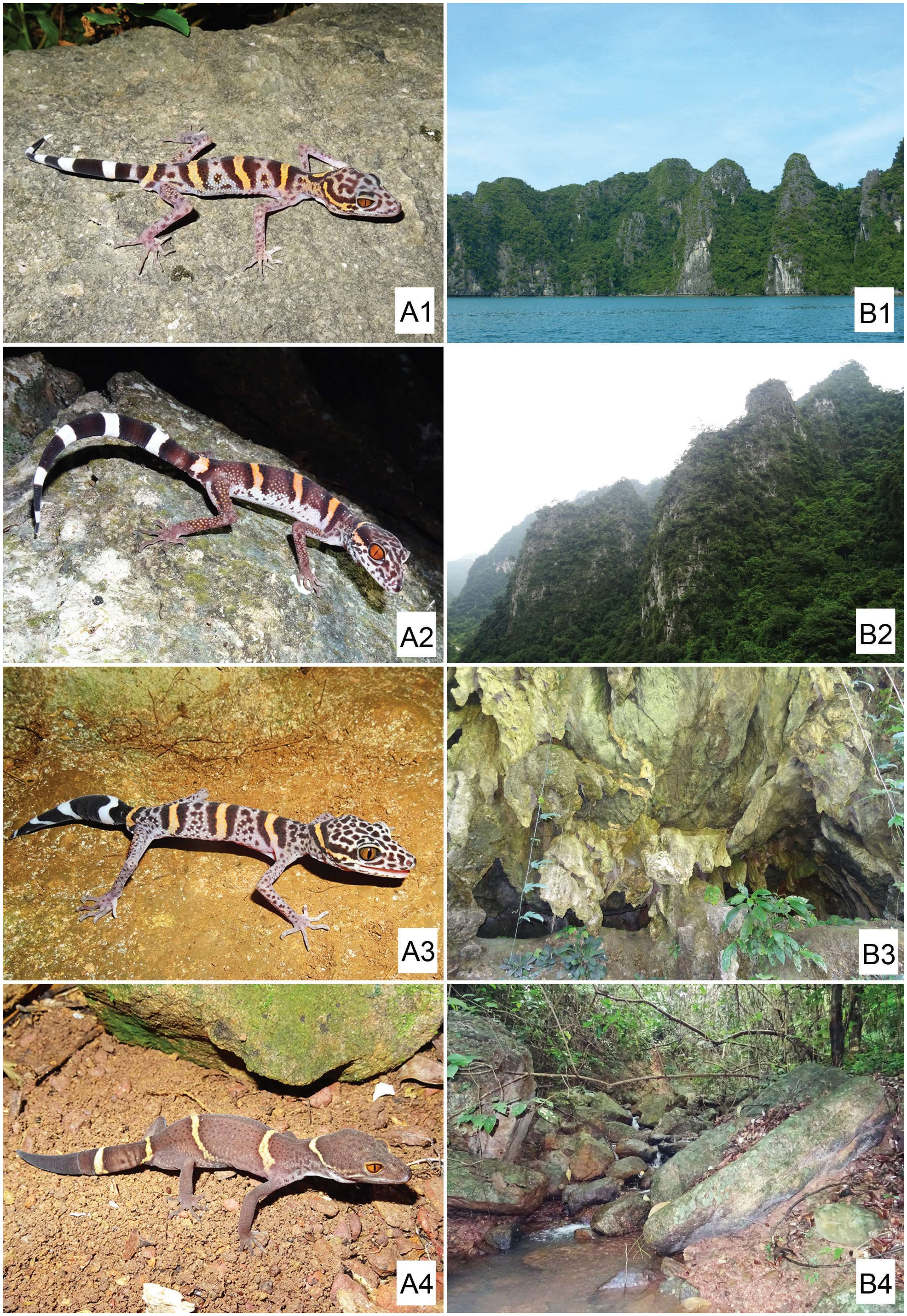

Figure 2. A1–A3. Photos of karst-adapted species (*Goniurosaurus catbaensis*, *G. huuliensis*, and *G. luii*, respectively), A4. Granite-stream-adapted species (*G. lichtenfelderi*), B1 – B3. Karst habitats of the *luii* group, B4. Granite-stream habitat of the *lichtenfelderi* group. Photos by Hai Ngoc Ngo.

Table 1. Species and GenBank accession numbers of the sequenced specimens used herein.

Species/Specimen	**16s**	**cytb**	**CMOS**	**RAG1**
Goniurosaurus araneus	AB308460			
G. araneus ECNU-V0008	MT533259			
G. araneus JFBM15830			HQ426537	HQ426286
G. bawanglingensis BL-RBZ-021	MH247190	MH247201	MH247212	MH247223
G. bawanglingensis BL-RBZ-022	MH247191	MH247202	MH247213	MH247224
G. bawanglingensis BL-RBZ-023	MH247192	MH247203	MH247214	MH247225
G. bawanglingensis BL-RBZ-024	MH247193	MH247204	MH247215	MH247226
G. bawanglingensis SYS r002162	MT995758	MT995773		
G. catbaensis G33	MW741550	MW650944		
G. catbaensis G34	MW741551	MW650945		
G. catbaensis G35		MW650946		
G. catbaensis MHNG 2699.49	EU499389			
G. gezhi ECNU-V0038	MT533260			
G. gezhi ECNU-V0040	MT533261			
G. gezhi ECNU-V0042	MT533262			
G. gezhi ECNU-V0046	MT533263			
G. gezhi ECNU-V0047	MT533264			
G. gollum SYS r002420	MT995784	MT995787	MW727559	MW727594
G. gollum SYS r002421	MT995785	MT995788	MW727560	MW727595
G. gollum SYS r002422	MT995786	MT995789	MW727561	MW727596
G. hainanensis BL-RBZ-041	MH247194	MH247205	MH247216	MH247227
G. hainanensis BL-RBZ-042	MH247195	MH247206	MH247217	MH247228
G. hainanensis SYS r000349	KC765080			
G. hainanensis JK1	AB308458			
G. huuliensis Gohu	AB853453	AB853479		
G. huuliensis G21		MW650936		
G. huuliensis G23		MW650937		
G. huuliensis G24		MW650938		
G. kadoorieorum ECNU-V0058	MT533258			
G. kadoorieorum ECNU-V0060	MT533265			
G. kadoorieorum ECNU-V0061	MT533266			
G. kuroiwae Goku1 Northern Okinawa	AB853448	AB853473		
G. kuroiwae Goku2 Southern Okinawa	AB853445			
G. kuroiwae Goor1 Southern Okinawa	AB853446	AB853467		
G. kwanghua ECNU-V0003	MK782788	MK782782	MK782776	MK782770
G. kwanghua ECNU-V0004	MK782789	MK782783	MK782777	MK782771
G. kwanghua ECNU-V0005	MK782790	MK782784	MK782778	MK782772
G. kwangsiensis ECNU-V0009	MK782786	MK782780	MK782774	MK782768
G. liboensis SYS r000217	KC900230			
G. lichtenfelderi ECNU-V0007	MK782785	MK782779	MK782773	MK782767
G. lichtenfelderi IEBR 3692	JF799756			
G. luii ECNU-V0012	MK782787	MK782781	MK782775	MK782769
G. luii Golu2			EF081254	
G. luii Golu3	AB853452	AB853478		
G. luii SYSr 000255	KC765083			
G. luii SYSr 000256	KC765084			
G. luii ZFMK 87057	EU499391			
G. luii TG00795				HQ426287
G. orientalis Goku3	AB853446			
G. orientalis Goor2	AB853443	AB853461		
G. orientalis Goor3		AB853462		
G. sengokui Gose1	AB853444	AB853463		
G. sengokui Gose2		AB853464		
G. sengokui KUZ 62087			HQ876393	
G. splendens Gosp1	AB853451	AB853477		
G. splendens Gosp2	AB853449			
G. splendens Gosp3	AB853450			

Species/Specimen	16s	cytb	CMOS	RAG1
G. toyamai Goto1	AB853447	AB853468		
G. toyamai Goto2		AB853469		
G. varius SYS r002331	MT995754	MT995769	MW727556	MW727590
G. varius SYS r002332	MT995755	MT995770		
G. varius SYS r002333	MT995753	MT995768		
G. varius SYS r002362	MT995756	MT995771	MW727557	MW727592
G. varius SYS r002363	MT995757	MT995772	MW727558	MW727593
G. varius SYS r002485	MW721828	MW727532	MW727562	MW727597
G. varius SYS r002486	MW721829	MW727533	MW727563	MW727598
G. yamashinae Goya1	AB853442	AB853460		
G. yamashinae Goya2	AB853441	AB853459		
G. yamashinae Goya3		AB853458		
G. yingdeensis Field number DYA01	MW721830	MW727534	MW727574	MW727605
G. yingdeensis Field number DYA02	MW721831	MW727535	MW727575	MW727606
G. yingdeensis Field number HS01	MW721832	MW727536	MW727576	MW727607
G. yingdeensis Field number HS02	MW721833	MW727537	MW727577	MW727608
G. yingdeensis Field number LT01	MW721834	MW727538	MW727580	MW727611
G. yingdeensis Field number LT02	MW721835	MW727539	MW727581	MW727612
G. yingdeensis SYS r000549	KC765082			
G. yingdeensis SYS r000550	KC900231			
G. yingdeensis SYS r001271	MT995759	MT995774	MW727547	
G. yingdeensis SYS r001272	MT995760	MT995775	MW727548	
G. yingdeensis SYS r001493	MT995761	MT995776	MW727551	
G. yingdeensis SYS r0002115	MT995762	MT995777		
G. zhelongi Field number HW01	MW721838	MW727540	MW727578	MW727609
G. zhelongi Field number HW02	MW721839	MW727541	MW727579	MW727610
G. zhelongi Field number MDA01	MW721836	MW727542	MW727582	MW727613
G. zhelongi Field number MDA02	MW721837	MW727543	MW727583	MW727614
G. zhelongi Field number TZ01	MW721840	MW727544	MW727584	MW727615
G. zhelongi Field number TZ02	MW721841	MW727545	MW727585	MW727616
G. zhelongi SYS r000816	KJ423105	MT995778	MW727570	
G. zhelongi SYS r001491	MT995763	MT995779	MW727549	
G. zhelongi SYS r001492	MT995764	MT995780	MW727550	
G. zhelongi SYS r002108	MT995765	MT995781		
G. zhoui BL-RBZ-001	MH247196	MH247207	MH247218	MH247229
G. zhoui BL-RBZ-004	MH247197	MH247208	MH247219	MH247230
G. zhoui BL-RBZ-006	MH247198	MH247209	MH247220	MH247231
G. zhoui BL-RBZ-007	MH247199	MH247210	MH247221	MH247232
G. zhoui BL-RBZ-008	MH247200			
G. zhoui SYS r002213	MT995766	MT995782	MW727553	
G. zhoui SYS r002214	MT995767	MT995783	MW727554	
Eublepharis macularius	AB853454	AB853480		

habitat preference coevolved with ecomorphology in near sympatric species of the *luii* and *lichtenfelderi* groups in Vietnam (Ngo et al. 2021; Figs. 1, 2).

Materials and methods

Genetic data and phylogenetic analyses

Genomic DNA was extracted from muscle tissue samples, using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Primers used for 16S were r16S-5L (5'- GGTMMYGCCTGCCCAGTG -3') and 16Sbr-H (5'- CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991), for cyt *b* the primers were L14731 (5'- TGGTCTGAAAAACCATTGTTG-3') (Honda et al. 2014) and H15149m (5'- GCMCCTCAGAAKGATATTTGYCCTCA-3') (Chambers and MacAvoy 1999), for CMOS the primers were FU-F (5'- TTTGGTTCKGTCTACAAGGCTAC -3') and FU-R (5'- AGGGAACATCCAAAGTCTCCAAT -3') (Gamble et al., 2008) , and for RAG1 the primers were R13 (5'- TCTGAATGGAAATTCAAGCTGTT -3') and R18 (5'- GATGCTGCCTCGGTCGGCCACCTTT -3') (Groth and Barrowclough 1999). The PCR procedure was performed with an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min (Liang et al. 2018). PCR products were purified with spin columns and then sequenced with forward primers using BigDye Terminator Cycle Sequencing Kit as per the guidelines on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co., Ltd.

We constructed Maximum Likelihood (ML), Bayesian Inference (BI), and Bayesian Evolutionary Analysis by Sampling Trees (BEAST) phylogenetic trees using a concatenated data set composed of 3070 base pairs (bp) of the mitochondrial genes, 16S (633 bp) and cyt *b* (1075 bp), and the nuclear genes, CMOS (472 bp) and RAG1 (890), from 103 specimens of 23 species of *Goniurosaurus* with varying degrees of sequence coverage across the samples (Table 1). Concatenation followed the comparison of separate gene trees to confirm there were no major discordances. One species, *Eublepharis macularius*, served as an outgroup (Grismer 1988; Jonniaux and Kumazawa 2008) to root the trees. Sequence data and GenBank accession numbers are listed in Table 1.

A Maximum likelihood (ML) analysis partitioned by gene was implemented using the IQ-TREE webserver (Nguyen et al. 2015; Trifinopoulos et al. 2016) preceded by the selection of substitution models using TIM2+F+I+G4 for 16S and cyt *b* and HKY+F for CMOS and RAG1. To avoid over parameterization, protein coding genes were not partitioned by codon. One-thousand bootstrap pseudoreplicates via the ultrafast bootstrap (UFB: Hoang et al. 2018) approximation algorithm were employed, and nodes having UFB values of 95 and above were considered strongly supported (Minh et al. 2013). We considered nodes with values of 90–94 as well-supported. A Bayesian inference (BI) analysis was carried out in MrBayes 3.2.3. (Ronquist et al. 2012) on XSEDE using the CIPRES Science Gateway (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010) employing default priors and models of evolution that most closely approximated those selected by the BIC and used in the ML analysis. Two independent Markov chain Monte Carlo (MCMC) analyses for each data set were performed—each with four chains, three hot and one cold. The MCMC simulations ran for 100 million generations. Trees were sampled every 10,000 generations, and the first 10% of the trees from each run from each data set were discarded as burn-in. The parameter files from both runs were checked in Tracer v1.6 (Rambaut et al. 2014) to ensure that convergence and stationarity of all parameters had effective sample sizes (ESS) well-above above 200. Post-burn-in sampled trees from each respective run were combined and 50% majority-rule consensus trees were constructed. Nodes with Bayesian posterior probabilities (BPP) of 0.95 and above were considered highly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). We considered nodes with values of 0.90–0.94 as well-supported.

An input file was constructed in Bayesian Evolutionary Analysis Utility (BEAUti) v. 2.4.6 using a relaxed lognormal clock with unlinked site models, linked trees and clock models, and a Yule prior and run in BEAST on CIPRES (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010). bModelTest was used to numerically integrate over the uncertainty of substitution models of each gene while simultaneously estimating phylogeny using Markov chain Monte Carlo (MCMC). MCMC chains were run for 100,000,000 generations and logged every 10,000 generations. The BEAST log file was visualized in Tracer v. 1.6.0 (Rambaut et al. 2014) to ensure effective sample sizes (ESS) were well-above 200 for all parameters. A Maximum clade credibility tree using mean heights at the nodes was generated using TreeAnnotator v.1.8.0 (Rambaut and Drummond 2014) with a burn-in of 1000 trees (10%). Nodes with BPPs of 0.95 and above were considered strongly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). We considered nodes with values of 0.90–0.94 as well-supported.

Ancestral state reconstruction

The BEAST tree was converted to newick format and pruned using the *drop.tip*() command (Paradis and Schliep 2018) in the R package ape [v.3.4.3] to include only the earliest diverged individual of each species. Habitat preference (karst or non-karst; see below) was mapped onto the tree using SCM implemented in the R package Phytools (Revell 2012) in order to derive probability estimates of the ancestral states at each node. A transition rate matrix was identified that best fit the data by comparing the corrected Akaike Information Criterion (AICc) values in the R package ape (Paradis and Schilep 2018). Three transition rate models were considered: a 2-parameter model having different rates for every transition type (the ARD model); a single-parameter model with equal forward and reverse rates between states (the symmetrical rates SYM model); and a single rate parameter model that assumes equal rates among all transitions (ER). Lastly, an MCMC approach was used to sample the most probable 1000 trait histories from the posterior using the *make.simmap*() command and then summarized them using the *summary*() command.

The coding of habitat preference for each species was determined from the literature and field observations of the authors (Table 2). A species' habitat preference was coded as "karst" if it had a strong association with karstic habitats. Many such species may range into forested areas or even other rock types (granite or volcanic) if nearby but their densities are much greater in karstic areas. A species was coded as "non-karst" if detected only in forested areas or forested areas with other rock types (e.g. granite). These species never show any strong preference for karstic microhabitats even if such habitats exist within their range.

Morphological data and analyses

An ecomorphological analysis was conducted using four of the five recorded species from Vietnam (Grismer et al. 1999; Vu et al. 2006; Orlov et al. 2008; Ziegler et al. 2008; Nguyen et al. 2009; Nguyen 2011; Orlov et al.

Table 2. Habitat preferences of the species of *Goniurosaurus*.

Species	**1° habitat**	**2° habitat**	**Source**
***kuroiwae* group**			
G. splendens	karst	forest	Nakamura and Ueno (1963), H. Ota pers. comm., L. Grismer pers. obs.
G. toyamai	forest		H. Ota pers. comm.
G. kuroiwae North	forest		H. Ota pers. comm., L. Grismer pers. obs.,
G. kuroiwae South	karst	forest	Nakamura and Ueno (1963), H. Ota pers. comm., L. Grismer pers. obs.
G. yamashinae	karst	forest	H. Ota pers. comm., L. Grismer pers. obs.,
G. sengokui	karst	forest	Werner et al. (2004), H. Ota pers. comm.
G. orientalis	karst		H. Ota pers. comm.
***yingdeensis* group**			
G. gollum	karst		Qi et al. (2020a)
G. yingdeensis	karst	granite	Wang et al. (2010), S. Qi pers. obs.
G. zhelongi	karst	granite	S. Qi, pers. obs., Wang et al. (2014)
G. varius	karst		Qi et al. (2020b)
***lichtenfelderi* group**			
G. bawanglingensis	granite	karst	Grismer et al. (2002), Orlov et al. (2008)
G. zhoui	karst	granite	Zhou et al. (2018), S. Qi pers. obs.
G. kwanghua	karst	granite	Zhu et al. (2020)
G. lichtenfelderi	granite		Orlov et al. (2008)
G. hainanensis	granite	volcanic	S. Qi pers. obs., L. Grismer pers. obs.
***luii* group**			
G. catbaensis	karst		Ziegler et al. (2008), Ngo et al. (2019a)
G. gezhi	karst		Zhu et al. (2020)
G. araneus	karst		Grismer et al. (1999)
G. kadoorieorum	karst		Yang and Chan (2015)
G. huuliensis	karst		Orlov et al. (2008)
G. luii	karst		Grismer et al. (1999), Vu et al. (2006)
G. liboensis	karst		Wang et al. (2013)
G. kwangsiensis	karst		Yang and Chan (2015)

2020) for which there existed a substantially large morphometric data set (Ngo et al. 2021): the karst-adapted *G. catbaensis* Ziegler, Nguyen, Schmitz, Stenke, and Rösler, 2008, *G. huuliensis* Orlov, Ryabov, Nguyen, Nguyen, and Ho, 2008, and *G. luii* Grismer, Viets, and Boyle, 1999 of the *luii* group and the granite stream-adapted *G. lichtenfelderi* (Mocquard, 1897) of the *lichtenfelderi* group (Figs. 1, 2). A total of 486 live individuals and 54 museum specimens of four species were examined for morphological data, comprising 194 individuals of *G. catbaensis* (21 juveniles, 93 females, and 80 males), 80 individuals of *G. huuliensis* (two juveniles, 46 females, and 32 males), and 88 individuals of *G. luii* (11 juveniles, 43 females, and 34 males) of the *luii* species group and 178 individuals of *G. lichtenfelderi* (14 juveniles, 72 females, and 92 males) of the *lichtenfelderi* group.

Measurements were taken with dial calipers to the nearest 0.1 mm on the right side of each individual. Abbreviations are as follows: snout-vent length (SVL), from tip of snout to vent; axilla to groin length (AG), from posterior edge of forelimb insertion to anterior edge of hind limb insertion; maximum body width (BW), greatest width of torso, taken at level of midbody; maximum body height (BH), from dorsal surface of body to belly; internarial distance (ID), distance between nares; head length (HL), from the tip of snout to posterior edge of occiput; maximum head width (HW); cheek height (CH), from posterior edge of labial to top of head at parietal region; interorbital distance (IO), distance between posteriormost points of eyes; diameter of auditory meatus (AD); snout to eye distance (SL), measured from tip of snout to anteriormost point of eye; diameter of eye (ED), greatest diameter of eye; eye to ear distance (EE), from posterior margin of eye to posterior margin of ear; forelimb length (FLL), from axilla to the tip of the fourth finger; hind limb length (HLL), from groin to the tip of the fourth toe. To remove potential effects of allometry, size was adjusted using the following equation: $X_{adj}=\log(X)-\beta[\log(SVL)-\log(SVL_{mean})]$, where X_{adj}=adjusted value; X=measured value; β=unstandardized regression coefficient for each population; and SVL_{mean}=overall average SVL of all populations (Thorpe 1975, 1983; Turan 1999; Lleonart et al. 2000)—accessable in the R package *GroupStruct* (available at https://github.com/chankinonn/GroupStruct). The morphometrics of each species were adjusted separately and then concatenated so as not to conflate intra- with interspecific variation (Reists 1986). All data were then scaled to their standard deviation to insure they were analyzed on the basis of correlation and not covariance and were log-transformed to insure they were normally distributed.

An analysis of variance (ANOVA) was performed on a data set coded for species to search for the presence of statistically significant mean differences ($p < 0.05$)

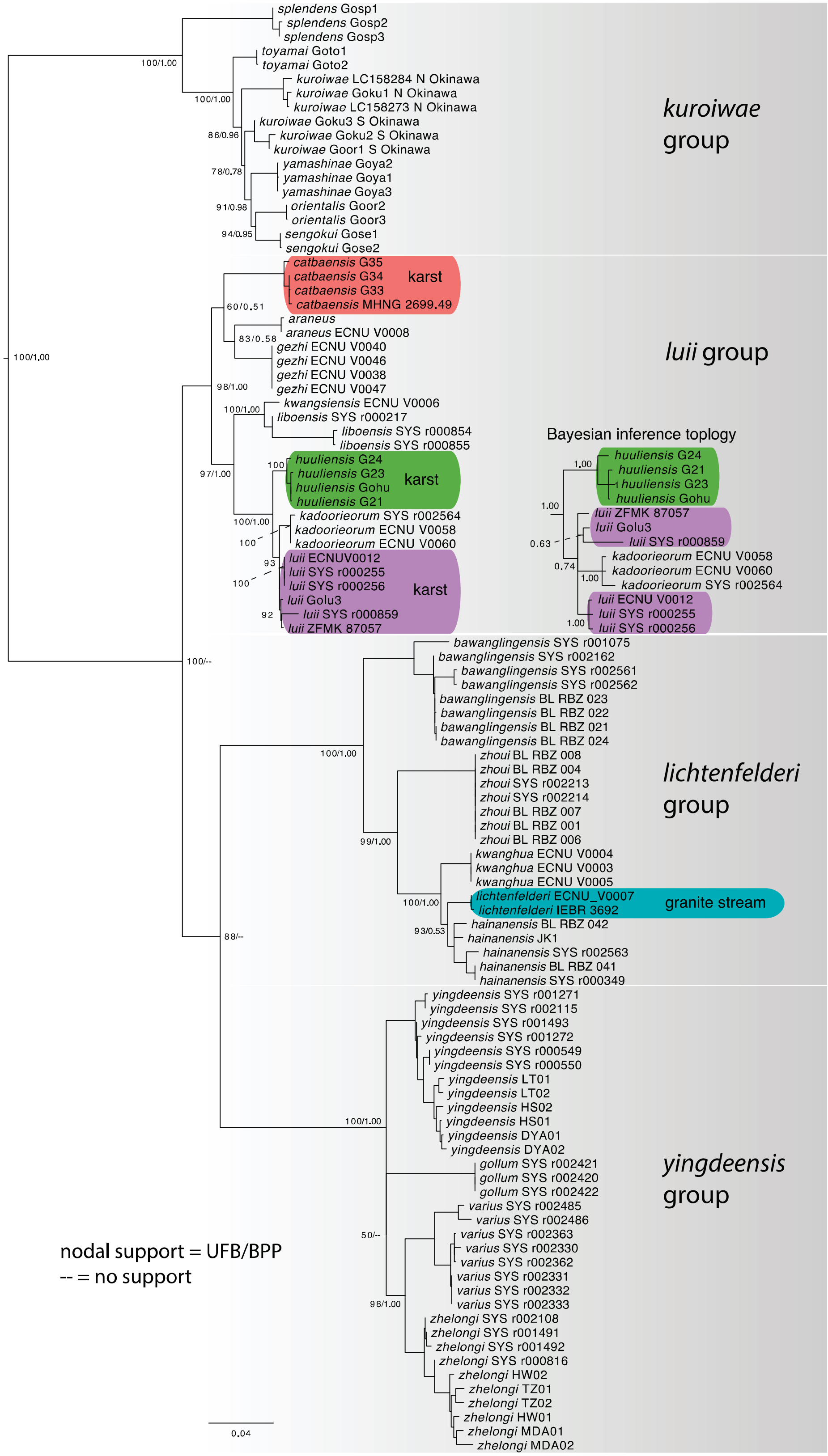

Figure 3. Mito-nuclear maximum likelihood topology with ultrafast bootstrap values (UFB) and Bayesian posterior probabilities (BPP) at the nodes. All species except *Goniurosaurus luii* had strong nodal support (100/1.00) for their monophyly. The inset in the *luii* species group is a section of the BI analysis showing the non-monophyly of *G. luii* with respect to *G. kadoorieorum.* Colored species are those used in the ecomorphological analyses.

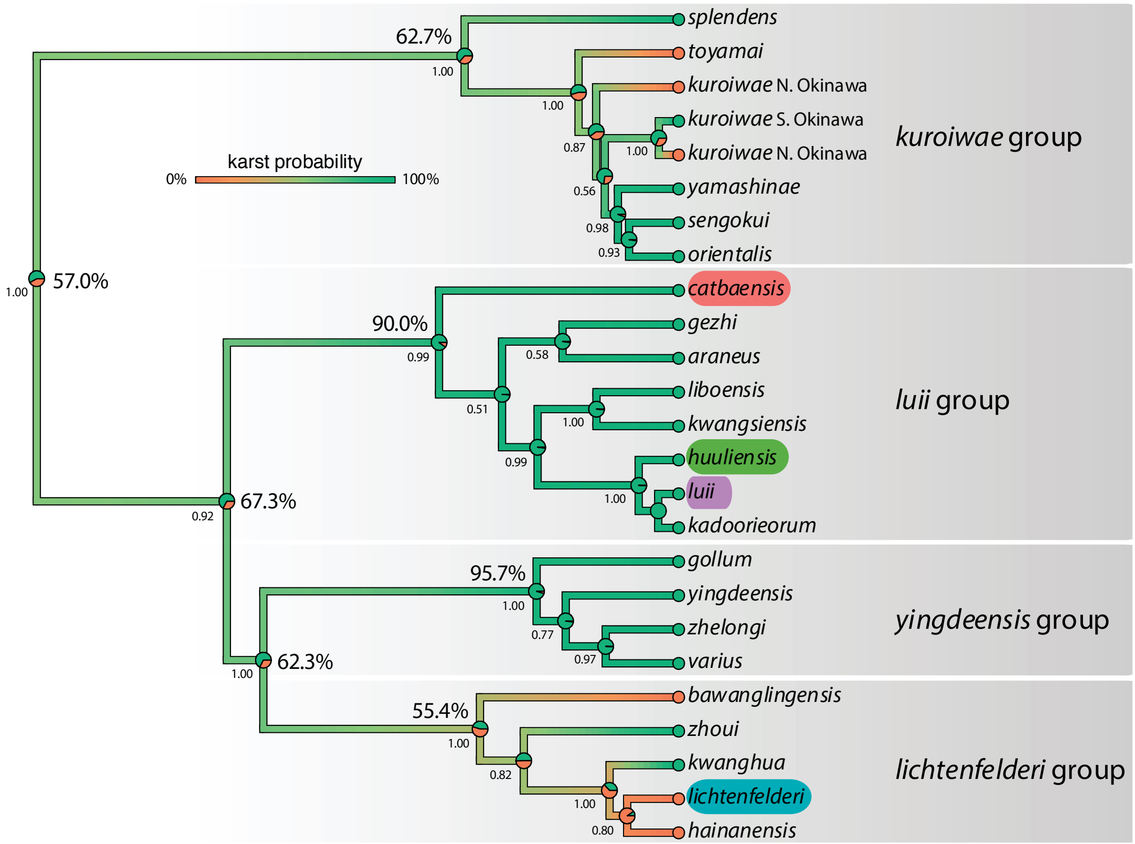

Figure 4. Stochastic character map of karst and non-karst habitat preferences on a BEAST topology. Percentages at the nodes are the probability of the ancestral habitat preference. Numbers at the nodes are BPP values. Colored species are those used in the eco-morphological analyses.

among characters across the selected subset of species in the *luii* and *lichtenfelderi* groups. Character means bearing statistical differences among species were subjected to a TukeyHSD test to ascertain which species pairs differed significantly from each other for those particular characters. A Student *t*-test was also performed on a second data set coded for only habitat preference (karst versus non-karst) to search for the presence of statistically significant mean differences ($p < 0.05$) among the same subsets of species coded for habitat. Violin plots with inserted boxplots were generated in order to visualize the range, frequency, mean, 50% quartile, and degree of differences between the dependent variables for both data sets bearing statistically different mean values.

The morphospatial clustering of the two separate data sets (species and habitat preference) were visualized using principal component analysis (PCA) along the ordination of the first two principal components (PC) using the Adegenet package in R (Jombart et al. 2010) and implemented by the *prcomp*() command. The data were log-transformed prior to analysis in order to normalize their distribution so as to ensure characters with very large or very low values could not over-leverage the results owing to intervariable nonlinearity. All statistical analyses were performed using R.3.1.2 (R Core Team 2018).

Results

Phylogenetic relationships

The ML, BI, and BEAST analyses recovered strong nodal support (UFB 98–100/BPP 1.00) for the monophyly of all four species groups with the *kuroiwae* group being the strongly supported (100/1.00) sister group to the remaining three groups (Fig. 3). The ML analysis weakly recovered (88) the *lichtenfelderi* and *yingdeensis* groups as sister lineages but the BEAST analysis recovered this relationship with strong support (1.00; Fig. 4). The BI analysis recovered the *luii* and *yingdeensis* groups as sister lineages, although the support is so low (0.51), the three groups effectively form a polytomy. The ML and BI analyses recovered the identical inter-specific relationships within the species groups but discordant relationships with the BEAST analysis regarding the *luii* group. The ML and BI analyses recovered a poorly supported (*G. catbaensis* (*G. araneus, G. gezhi*)) clade but the BEAST analysis recovered *G. catbaensis* as the strongly supported (0.99) sister species to the remainder of the *luii* group species (Figs. 3, 4, respectively). All analyses recovered a polyphyletic *Goniurosaurus luii* with respect to *G. kadoorieorum* (not shown in the pruned tree of Fig. 4).

Table 3. Difference, lower and upper ranges, and adjusted *p* values of statistically significant mean differences between species pairs for each character based on ANOVA and subsequent TukeyHSD analyses.

axilla-groin (AG)	**difference**	**lower range**	**upper range**	***p* adjusted**
huuliensis-catbaensis	0.095633573	0.072292064	0.118975082	3.37E-10
lichtenfelderi-catbaensis	–0.075967576	–0.094200659	–0.057734493	3.37E-10
luii-catbaensis	–0.029676257	–0.05225407	–0.007098445	0.004206065
lichtenfelderi-huuliensis	–0.171601149	–0.195246969	–0.14795533	3.37E-10
luii-huuliensis	–0.12530983	–0.152447204	–0.098172456	3.37E-10
luii-lichtenfelderi	0.046291319	0.023399042	0.069183596	1.60E-06
body width (BW)				
huuliensis-catbaensis	0.106179184	0.066412755	0.145945613	4.37E-10
lichtenfelderi-huuliensis	–0.083314667	–0.123599543	–0.043029791	8.67E-07
luii-huuliensis	–0.121548632	–0.167781995	–0.075315269	5.34E-10
body height (BH)				
huuliensis-catbaensis	0.094637915	0.046801122	0.142474708	2.84E-06
lichtenfelderi-huuliensis	–0.07317021	–0.121630666	–0.024709755	0.000647886
luii-huuliensis	–0.125013692	–0.180629845	–0.069397539	7.11E-08
luii-lichtenfelderi	–0.051843482	–0.098759605	–0.004927359	0.02359844
internarial distance (ID)				
lichtenfelderi-catbaensis	–0.082396274	–0.105742691	–0.059049857	3.37E-10
luii-catbaensis	–0.076638786	–0.105548379	–0.047729192	4.74E-10
lichtenfelderi-huuliensis	–0.096677642	–0.126954757	–0.066400527	3.37E-10
luii-huuliensis	–0.090920154	–0.125668004	–0.056172303	5.78E-10
head length (HL)				
huuliensis-catbaensis	0.075818967	0.058801575	0.092836359	3.37E-10
lichtenfelderi-catbaensis	–0.162875997	–0.176169033	–0.149582961	3.37E-10
luii-catbaensis	–0.029098886	–0.045559496	–0.012638276	3.82E-05
lichtenfelderi-huuliensis	–0.238694964	–0.255934217	–0.221455712	3.37E-10
luii-huuliensis	–0.104917853	–0.124702663	–0.085133043	3.37E-10
luii-lichtenfelderi	0.133777111	0.117087238	0.150466985	3.37E-10
head width (HW)				
huuliensis-catbaensis	0.036775074	0.019138869	0.05441128	6.89E-07
lichtenfelderi-catbaensis	–0.158637831	–0.172414249	–0.144861413	3.37E-10
luii-catbaensis	–0.065002064	–0.082061241	–0.047942887	3.37E-10
lichtenfelderi-huuliensis	–0.195412905	–0.213279039	–0.177546772	3.37E-10
luii-huuliensis	–0.101777138	–0.122281395	–0.081272881	3.37E-10
luii-lichtenfelderi	0.093635767	0.07633899	0.110932545	3.37E-10
head height (HH)				
huuliensis-catbaensis	0.108413032	0.073172094	0.143653969	3.37E-10
lichtenfelderi-catbaensis	–0.032237965	–0.059766217	–0.004709713	0.014142658
lichtenfelderi-huuliensis	–0.140650997	–0.176351381	–0.104950613	3.37E-10
luii-huuliensis	–0.14002168	–0.180993602	–0.099049757	3.37E-10
cheek height (CH)				
huuliensis-catbaensis	0.069595379	0.028246161	0.110944597	0.000101199
lichtenfelderi-catbaensis	–0.14807373	–0.180373429	–0.11577403	3.37E-10
luii-catbaensis	–0.057345812	–0.09734215	–0.017349473	0.001381599
lichtenfelderi-huuliensis	–0.217669109	–0.259557409	–0.175780808	3.37E-10
luii-huuliensis	–0.126941191	–0.175014741	–0.078867641	5.00E-10
luii-lichtenfelderi	0.090727918	0.050174509	0.131281326	8.27E-08
interorbital distance (ID)				
huuliensis-catbaensis	0.05692565	0.034698839	0.079152461	9.29E-10
lichtenfelderi-catbaensis	–0.211451872	–0.228814215	–0.194089528	3.37E-10
luii-catbaensis	–0.03636425	–0.057863835	–0.014864664	9.22E-05
lichtenfelderi-huuliensis	–0.268377522	–0.29089411	–0.245860933	3.37E-10
luii-huuliensis	–0.093289899	–0.1191313	–0.067448499	3.37E-10
luii-lichtenfelderi	0.175087622	0.15328859	0.196886655	3.37E-10
snout length (SL)				
huuliensis-catbaensis	0.108547374	0.083797596	0.133297152	3.37E-10

lichtenfelderi-catbaensis	–0.20706889	–0.226402034	–0.187735746	3.37E-10
lichtenfelderi-huuliensis	–0.315616264	–0.340688712	–0.290543816	3.37E-10
luii-huuliensis	–0.125435452	–0.154210112	–0.096660792	3.37E-10
luii-lichtenfelderi	0.190180812	0.16590737	0.214454254	3.37E-10
ear diameter (ED)				
huuliensis-catbaensis	–0.137477526	–0.199198043	–0.075757008	9.51E-08
lichtenfelderi-catbaensis	–0.338691314	–0.386903935	–0.290478693	3.37E-10
luii-catbaensis	–0.365692271	–0.425393393	–0.305991149	3.37E-10
lichtenfelderi-huuliensis	–0.201213788	–0.263738975	–0.138688602	3.37E-10
luii-huuliensis	–0.228214745	–0.299972435	–0.156457055	3.37E-10
eye to ear distance (EE)				
huuliensis-catbaensis	0.085091637	0.059731157	0.110452117	3.37E-10
lichtenfelderi-huuliensis	–0.06878479	–0.094475902	–0.043093678	4.25E-10
luii-huuliensis	–0.101777804	–0.131262479	–0.072293128	3.37E-10
luii-lichtenfelderi	–0.032993014	–0.057865404	–0.008120623	0.00377334
eye diameter (ED)				
huuliensis-catbaensis	0.085091637	0.059731157	0.110452117	3.37E-10
lichtenfelderi-huuliensis	–0.06878479	–0.094475902	–0.043093678	4.25E-10
luii-huuliensis	–0.101777804	–0.131262479	–0.072293128	3.37E-10
luii-lichtenfelderi	–0.032993014	–0.057865404	–0.008120623	0.00377334
forelimb length (FLL)				
huuliensis-catbaensis	–0.137477526	–0.199198043	–0.075757008	9.51E-08
lichtenfelderi-catbaensis	–0.338691314	–0.386903935	–0.290478693	3.37E-10
luii-catbaensis	–0.365692271	–0.425393393	–0.305991149	3.37E-10
lichtenfelderi-huuliensis	–0.201213788	–0.263738975	–0.138688602	3.37E-10
luii-huuliensis	–0.228214745	–0.299972435	–0.156457055	3.37E-10
hindlimb length (HLL)				
huuliensis-catbaensis	–0.137477526	–0.199198043	–0.075757008	9.51E-08

The mito-nuclear data set of Liang et al. (2018) differed from all the above analyses in that their ML and BI analyses (79/0.99) placed the *yingdeensis* group as the sister group to a sister lineage comprised of the *luii* group and *lichtenfelderi* group (87/1.00).

Ancestral state reconstruction

The AICc scores for the three transition rate models of the SCM analysis were ARD = 34.547134 and SYM and ER = 32.099451. The SCM analysis using either the SYM or ER model suggests that a karst habitat preference is the most probable ancestral condition for *Goniurosaurus* (57.0% probability), the *kuroiwae* group (62.7%), the *luii* group (90.0%), and the *yingdeensis* group (95.7%; Fig. 4). The probable ancestral condition for the *lichtenfelderi* group is non-karst (55.4%). The karst habitat preference of *G. kwanghua* and *G. zhoui* of the *lichtenfelderi* group is considered to have evolved independently given that the ancestral condition of the *lichtenfelderi* group and that of the most recent common ancestor of the sister species *G. lichtenfelderi* and *G. hainanensis* was not karst-adapted (Fig. 4).

Ecomorphology

In both the species and habitat preference PCA analyses, PC1 accounted for 49.1% of the variation in the data set and loaded most heavily for limb length (FLL and HLL), snout length (SL), eye diameter (ED), interorbital distance (IO), head width (HW), and head length (HL). PC2 accounted for an additional 13.3% of the variation and loaded most heavily for body width (BW) and body height (BH) (Figs. 5, 6; Table 4).

The PCA analysis of the karst-adapted *Goniurosaurus catbaensis, G. huuliensis,* and *G. luii* of the *luii* group demonstrates that their body shapes greatly overlap in morphospace despite there being several slight, but statistically significant mean differences among them (Fig. 5; Table 3). Additionally, none of the plots of the karst-adapted species overlap with that of the granite stream-adapted species *G. lichtenfelderi* along the ordination of PC1.

The PCA analysis using habitat preference as the dependent variable among the four species, showed that the karst-adapted and granite-stream-adapted species plot separately as before along taxonomic lines and that collectively, the former have significantly longer axilla-groin lengths (AG); longer, wider, and thicker heads (HL, HW, and CH); longer snouts (SL); longer limbs (FLL and HLL); wider interorbital distances (IO); larger eyes (ED) and larger ear openings (AD) (Fig. 6). Many of these characters—longer head and snout, larger eyes, longer trunk, longer limbs—occur in many other distantly related karst-adapted species of *Cyrtodactylus* (Grismer et al. 2016a, 2020b; Kaatz et al. 2021; Nielsen and Oliver 2017), indicating that these are convergent adaptions to a karstic life style within and between the gekkotan families.

Table 4. Summary statistics and principal component analysis scores for the mensural characters for *Goniurosaurus catbaensis, G. huuliensis, G. luii,* and *G. lichtenfelderi*. Abbreviations are listed in the Materials and methods.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**	**PC14**
Standard deviation	2.62151	1.36659	1.03962	0.92238	0.82762	0.77172	0.71922	0.62534	0.54279	0.51042	0.44460	0.42745	0.41145	0.18664
Proportion of variance	0.49088	0.1334	0.0772	0.06077	0.04893	0.04254	0.03695	0.02793	0.02104	0.01861	0.01412	0.01305	0.01209	0.00249
Cumulative proportion	0.49088	0.62428	0.70148	0.76225	0.81118	0.85371	0.89066	0.9186	0.93964	0.95825	0.97237	0.98542	0.99751	1
Eigenvalue	6.87232	1.86758	1.08081	0.85079	0.68496	0.59554	0.51728	0.39105	0.29462	0.26053	0.19767	0.18272	0.16929	0.03483
AG	–0.24490	–0.08424	0.09672	0.10058	–0.56138	0.67337	–0.35690	0.01987	–0.04902	0.07718	–0.08231	0.03497	–0.02384	0.01371
BW	–0.04928	–0.61441	–0.07471	0.28744	–0.08051	–0.01832	0.20859	–0.63240	–0.03995	–0.19837	0.15593	–0.11090	0.06221	0.01347
BH	–0.04819	–0.61109	–0.04352	0.29637	0.08259	–0.06845	0.04189	0.71074	0.07724	0.07219	–0.03719	–0.04417	–0.03405	0.02105
ND	–0.18533	–0.11312	0.64606	0.12685	0.26270	–0.29150	–0.55437	–0.15934	–0.06520	0.16090	–0.04518	0.00367	0.01346	–0.01680
HL	–0.34765	0.02673	–0.05102	–0.02271	0.00465	–0.10546	–0.03065	0.01183	0.02638	–0.32873	0.17358	0.23473	–0.81798	0.00137
HW	–0.33156	–0.06751	–0.07095	–0.08475	0.05312	–0.03718	0.30763	–0.17678	0.01022	0.48788	–0.67604	–0.04173	–0.21464	0.01521
CH	–0.22962	–0.07645	–0.23672	–0.12992	0.73588	0.52586	–0.13009	–0.06367	0.04671	0.02514	0.139	0.0252	0.08193	–0.0003
IO2	–0.34276	0.00113	–0.05114	0.03079	–0.01618	–0.12015	0.10256	0.06796	–0.38288	–0.23806	–0.14615	0.67256	0.40575	–0.08672
SE	–0.32091	0.01885	–0.14557	–0.08578	–0.10147	–0.18206	–0.18393	–0.02718	0.77823	–0.28404	–0.17039	–0.03379	0.27124	–0.00567
ED	–0.32467	0.19657	0.01794	0.11714	0.06087	–0.00844	0.0395	0.12818	–0.36952	–0.45307	–0.20533	–0.65522	0.08037	–0.01989
EE	–0.08674	–0.33966	0.34954	–0.81367	–0.09736	0.04251	0.1902	0.08589	–0.05072	–0.11731	0.09913	–0.09141	0.05499	0.02214
AD	–0.19445	0.23327	0.54515	0.30447	0.06568	0.23322	0.5704	0.05955	0.28325	0.01759	0.2077	0.05512	0.05956	0.0013
FLL	–0.35278	0.08503	–0.16262	–0.01768	–0.10677	–0.18636	–0.00643	0.02061	–0.09402	0.28874	0.3664	–0.07982	0.12433	0.73937
HLL	–0.34892	0.04532	–0.17931	–0.04073	–0.1368	–0.17019	0.00654	0.02352	–0.04871	0.36988	0.42336	–0.16275	0.07515	–0.666

Discussion

Geckos in general are particularly well-adapted to karstic landscapes (see Luu et al. 2016; Grismer et al. 2014, 2020a, 2021 and references therein; Google Scholar search using key words "karst" and "Gekkonidae") and *Goniurosaurus* is no exception, being that 19 of its 23 species (83%) occupy karstic habitats (Grismer et al. 1994, 1999; Orlov et al. 2008; Ziegler et al. 2008; Yang and Chan 2015; Honda and Ota 2017; Zhou et al. 2018; Ngo et al. 2019a; Qi et al. 2020a, 2020b; Zhu et al. 2020a, 2020b). It is clear that karstic landscapes have played a significant role in the evolution and diversification of *Goniurosaurus* being that it is the probable ancestral habitat preference for the genus and three of the four species groups. Even the ancestor of the non-karst adapted ancestor of the *lichtenfelderi* group was karst-adapted (Fig. 4). Furthermore, within the species groups, the limited data herein would suggest that the karst-adapted species are specialized, range-restricted endemics (Grismer et al. 1994, 1999; Orlov et al. 2008; Ziegler et al. 2008; Yang and Chan 2015; Honda and Ota 2017; Zhou et al. 2018; Ngo et al. 2019a; Qi et al. 2020a, 2020b; Zhu et al. 2020a, 2020b). With the exception of *G. lichtenfelderi,* all the non-karst-adapted species are restricted to islands in the Ryukyu Archipelago (*kuroiwae* group) or Hainan Island (*lichtenfelderi* group). It may be that the absence of competition and/or predators in these insular habitats widened the fundamental niches of their ancestors and allowed some species to become more generalized in their habitat preference, which should be tested using new techniques combining phylogenetic history, character evolution, and ecological reconstruction programs.

Systematics of the *luii* group

The ML and BI analyses of Liang et al. (2018) and the BEAST analysis herein (Fig. 4) recovered *Goniurosaurus catbaensis* as the strongly supported sister species to the remainder of the *luii* group. Whereas the ML and BI analysis herein, recovered *G. catbaensis* as the very poorly supported (60/0.51) sister species of the *G. araneus* plus *G. gezhi* clade (Fig. 3). Given that three of the five analyses strongly supported the former relationship and two analyses poorly supported the latter, we prefer the placement of *G. catbaensis* as the sister species to the remainder of the *luii* group (Fig. 4). Given the very low nodal support of the latter, it essentially renders that portion of the tree a polytomy and as such, does not effectively contradict the strongly supported sister species position of *G. catbaensis* in the other trees.

Goniurosaurus kadoorieorum of the *luii* group (represented by only 16S) is nested within *G. luii* in

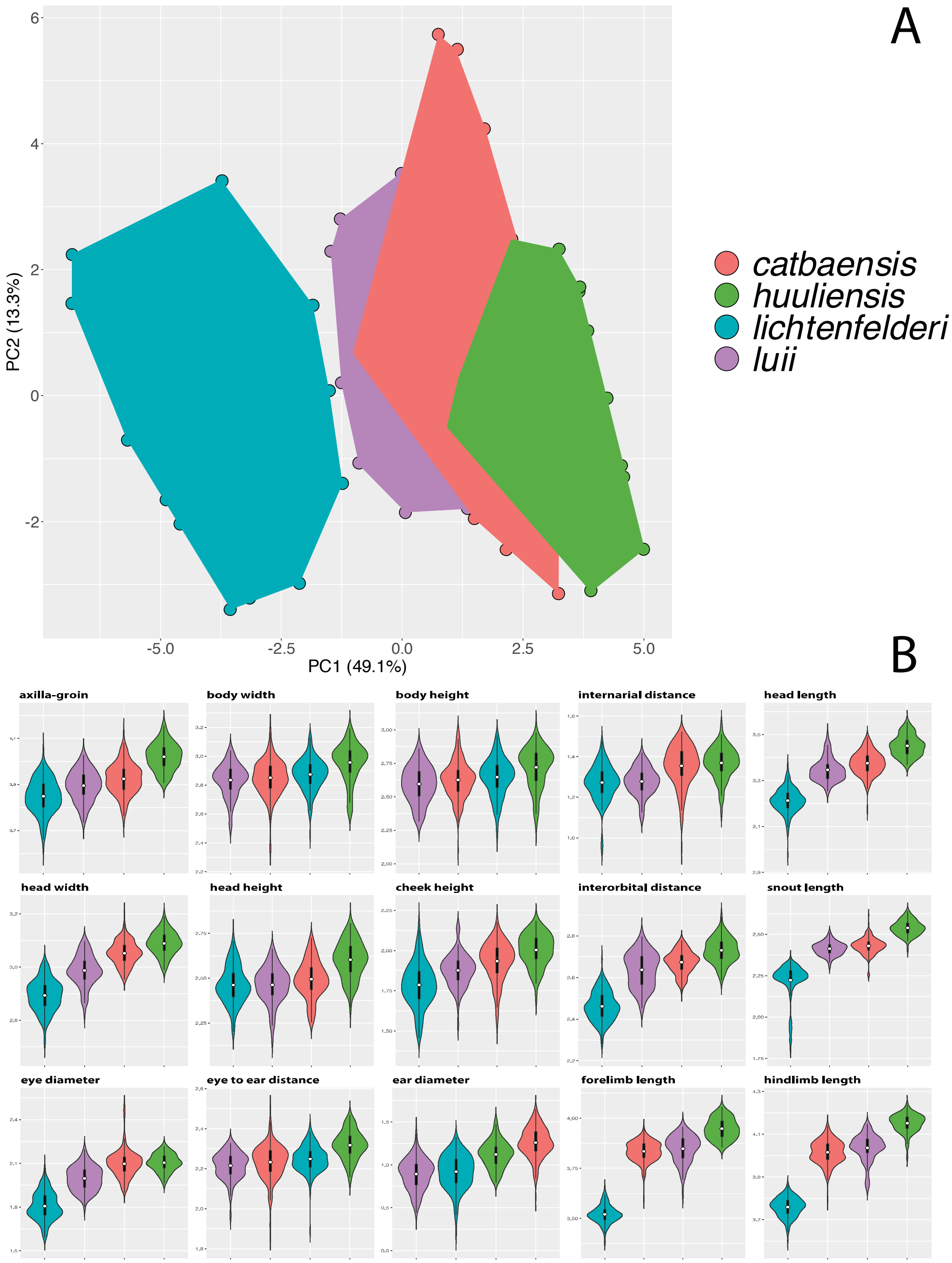

Figure 5. A. Principal component analysis of the karst-adapted species *G. catbaensis, G. huuliensis,* and *G. luii* of the *luii* group and the granite-stream-adapted *G. lichtenfelderi* of the *lichtenfelderi* group. B. Violin plots overlain with box plots showing the range, frequency, mean (white dot), and 50% quartile (black rectangle) of the size-adjusted morphometric characters.

both the ML and BI analyses, rendering *G. luii* polyphyletic (Fig. 3). The same relationship was recovered in the 16S phylogeny of Zhu et al. (2020a). This, and the lack of diagnostic characters separating *G. kadoorieorum* from *G. luii* (Yang and Chan 2015; Ngo et al. 2016), indicates the two species should be considered conspecific and as such, *G. kadoorieorum* Yang and Chan, 2015 is relegated here to a junior synonym of *G. luii* Grismer, Viets, and

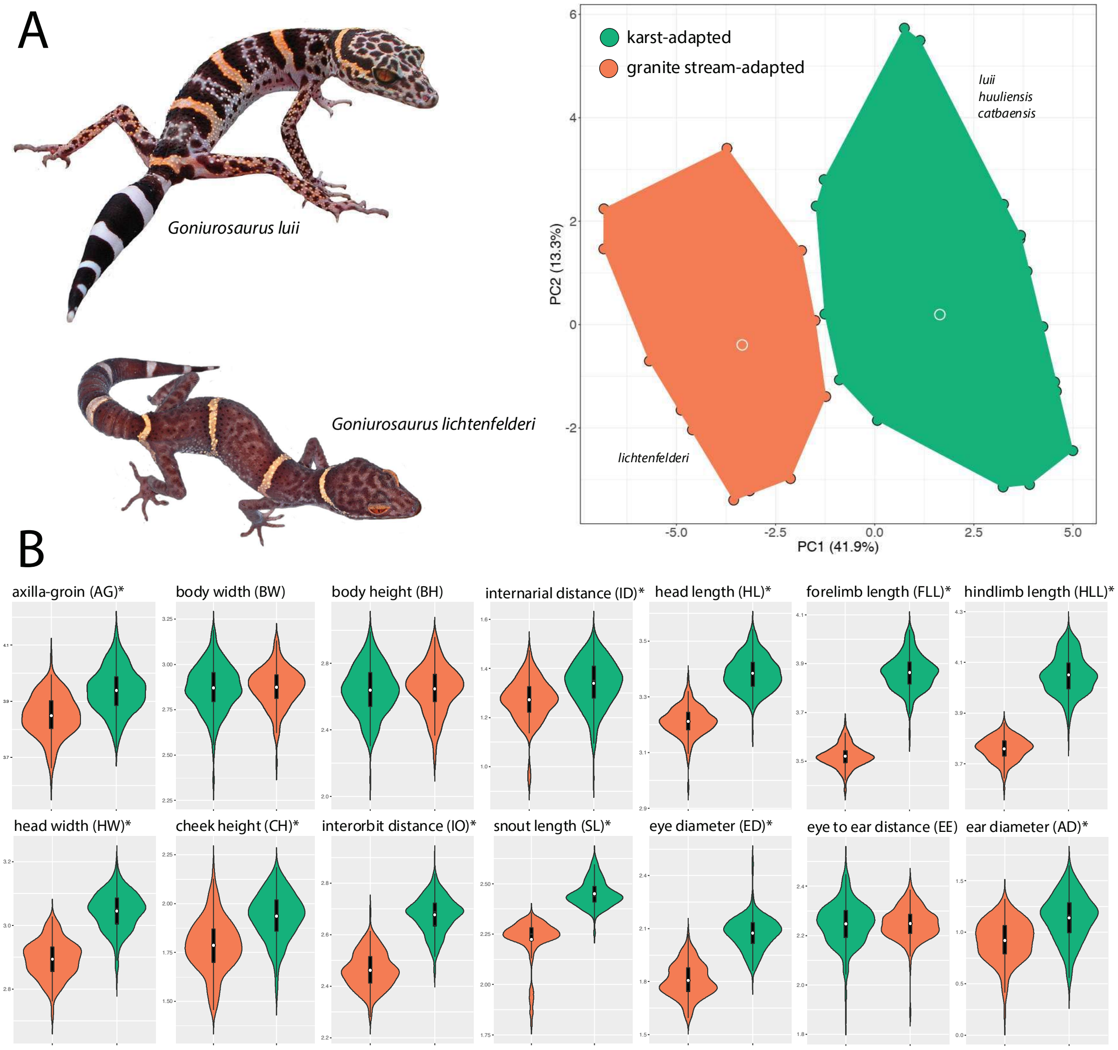

Figure 6. A. Principal component analysis using karst habitat preference and granite stream habitat preference as the dependent variables for *G. catbaensis, G. huuliensis,* and *G. luii* of the *luii* group and *G. lichtenfelderi* of the *lichtenfelderi* group. B. Violin plots overlain with box plots showing the range, frequency, mean (white dot), and 50% quartile (black rectangle) of the size-adjusted morphometric characters. Asterisks denote characters bearing statistically significant mean differences between the karst and granite-stream-adapted species based on student *t*-tests. Upper photo from Vuu et al. (2008). Lower photo from Hai Ngoc Ngo.

Boyle, 1999. In all analyses, *G. huuliensis* is consistently recovered as the sister species to *G. luii sensu lato* and its species status is not questioned (Figs. 3, 4).

Conservation

Wide-ranging more inclusive studies pertaining to ecosystems management are becoming commonplace in light of climate change and widespread habitat destruction. Such studies reconcile data from a broad range of disciplines in order to address issues that may bear on ecosystems management. Foundational to many of these studies is a basic understanding of species ecology and habitat preference—correlated here with ecomorphology (Cabral et al. 2009; Harfoot et al. 2014). Baseline information on habitat and microhabitat requirements of any species are paramount to understanding how they interact with, and navigate through, their environment (e.g. Grant and Grant 2008; Greene 2005; Losos 2010) and as such, the contextualization of ecosystem management may ultimately turn on these simple points (Meiri 2018; Sinervo et al. 2010).

Integrating the phylogenetic patterns of biodiversity and the morphological adaptations of habitat preference that, in part, underpin species radiations, can fundamentally contribute to conservation management programs (Grismer et al. 2020a, 2021; Erwin 1991; Vane-Wright et al. 1991; Williams et al. 1991; Vázquez and Gittleman 1998; Moritz et al. 2000; Forest et al. 2007; Sgro et al. 2010; Harvey et al. 2011; Rolland et al. 2012; Winter et al. 2012; Shaffer et al. 2015; Beaumont and Wang 2019; Fay et al. 2019; Holderegger et al. 2019)—especially in the karstic regions of northern Vietnam where anthropogenic impact is degrading the habitat and reducing the density of localized populations of *Goniurosaurus* (Ngo et al. 2019b). Northern Vietnam and many of its offshore islands in the Gulf of Tonkin, harbor large areas of fragmented karstic habitats scattered across their landscapes (Cerrano et al. 2006; Do 2001, 2014; Luo et al. 2016; Ngo et al. 2019a) that are inhabited by an exceptionally large number of endemic plants and animals (Do 2001; Sterling et al. 2006; Clements et al. 2008; Luo et al. 2016; von Oheimb et al. 2017). The obligate restriction of many species to fragmented karstic environments—such as all species of the *luii* and *yingdeensis* groups—functionally transforms these environments into habitat islands (Clements et al. 2006, 2008; von Oheimb et al. 2017), which in some cases, bear an unprecedented degree of range-restricted endemism (e.g. Sgro et al 2012; Harvey et al. 2011; Grismer et al. 2018a, 2021).

Unfortunately, *Goniurosaurus* species are particularly attractive (Fig. 2) and over-harvested for the illegal pet trade (Stuart et al. 2006; Yang and Chan 2015; Ngo et al. 2019b). This is an additional threat to these range-restricted endemics from imperiled karstic environments (Grismer et al. 1997; Orlov et al. 2008; Ziegler et al. 2008; Nakamura et al. 2014; Yang and Chan 2015; Honda and Ota 2017; Zhou et al. 2018; Ngo et al. 2019a; Qi et al. 2020a,b; Zhu et al. 2020a,b). In fact, in areas of China and Vietnam, many populations have suffered huge declines in numbers, or even extirpation at some localities, due to the illegal commercial pet trade (Stuart et al. 2006; Yang and Chan 2015; Ngo et al. 2019b, 2021). We hope that this study will bring more clarity to the plight of this genus and continue to serve ongoing conservation and management programs.

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1.3. Extraordinary species richness of *Goniurosaurus* genus in allopatry: Understanding niche evolution and the need of conservation measures

Ngo N.H., Grismer L., Nguyen Q.T., Le D.M., Qi S., Rödder D. and Ziegler T. (Manuscript) Extraordinary species richness of *Goniurosaurus* genus in allopatry: Understanding niche evolution and the need of conservation measures.

Extraordinary species richness of *Goniurosaurus* genus in allopatry: Understanding niche evolution and the need of conservation measures.

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Abstract

Global biodiversity crisis is identified with a dominant cause of extremely high rates of extinction, especially for reptiles due to anthropogenic impacts. In addition to typical criteria in the IUCN Red List to evaluate the status of threatened species, the rate of niche evolution can help to estimate the level of ecological flexibility and adaptive capacity of species to environmental shifts. Highly restricted-range endemism in disjunct karst ecosystems Tiger geckos of the genus *Goniurosaurus* offer a unique opportunity to investigate mechanisms of species radiation and allopatric evolution. Dated phylegentic reconstruction revealed that the first cladogenesis of *Goniurosaurus* ancestor took place during the Eocene (~ 45.3 mya) and continued radiate across East Asia until the Pliocene. Using ecological niche modellings (ENM) approaches, we reconstructed the history of niche occupancy and calculated the rate of climatic niche evolution by estimations of the phylogenetic signal during cladogenesis within the genus *Goniurosaurus* from China and Vietnam. Our results provide evidence of both phylogenetic niche conservatism and divergence among Chinese and Vietnamese *Goniurosaurus* species. Coupled with estimations of ancestral ranges and habitats, we thereby suggest related hypotheses that shaped the extraordinary richness of *Goniurosaurus* and their allopatric distribution. Following the pattern of niche conservatism, range-restricted *Goniurosaurus* species, which are identified to be likely more susceptible to environmental alteration, should be received higher priorities of conservation attention and protection.

Keywords. Cladogenesis, conservatism, divergence, environmental shifts, phylogenetic signal, radiation, Tiger geckos.

Introduction

The acceleration of the sixth mass extinction of wildlife is the most serious threat to the persistence of wild populations and will lead to a global biodiversity crisis with extremely high rates of extinction, especially for reptiles (Gibbons et al. 2000; Böhm et al. 2013; Monastersky

2014; Marshall et al. 2020). Causes for this crisis and an increasing number of threatened species are severe anthropogenic impacts, including unsustainable over-exploitation, habitat fragmentation and loss, invasion of non-native species, pollution and climate change (Böhm et al. 2013; Ceballos et al. 2015; Marshall et al. 2020; Chapple et al. 2021). Despite these threats of extinction, a few thousand reptiles have been discovered in the last decade through extensive field works and integrative analyses using morphology and genetics in order to construct phylogeny based on taxonomies. It is noteworthy that the majority of these newly discovered species are range-restricted endemics and many have been listed as threatened in the IUCN Red List under criteria B and D2 on the basis of restricted geographical range (Böhm et al. 2013; IUCN 2019; Chapple et al. 2021; Uetz et al. 2021). For range-restricted endemics, small population sizes are potentially prone to anthropogenic impacts or stochastic events in this era of an uncertain future and could become extinct in a short period of time (IUCN 2019). In terms of genetic diversity, range-restricted endemics usually have a low level of diversity due to their small population size (Frankham 1996; Hartl & Clark 1997) and occur in unique habitats or microhabitats and thus, are incapable of responding to rapid ecological shifts potentially threatening them under the model of global change—especially climate change. On the other hand, widespread species ranging throughout a variety of habitats on the latitudinal diversity gradient, are more likely to have the necessary genetic diversity to adapt to non-analog ecological conditions (Ferrière et al. 2004; Giennap et al. 2008; Allendorf et al. 2012; Lavergne et al. 2013; Lai et al. 2019; Ahmadi et al. 2021). Using a large phylogenetic data set from five vertebrate groups, including reptiles, Willoughby et al. (2015) suggested that the IUCN criteria of population size and species range extent are however not concordant to the level of genetic diversity. In order to estimate the rate of adaptive capacity to ecological shifts, given the relationship between geographic extent and genetic diversity, the typical criteria in the IUCN Red List are insufficient and in need of additional categories.

It has long been acknowledged that ecological conditions are the major factors that determine a species' distribution and range limits as well as being a driver of speciation (Darwin 1859; MacArthur 1984; Orr and Smith 1998; Schluter 2009; Glor & Warren 2010). In particular, a species range is limited by its physiological range of tolerance to ecological factors—defining the fundamental niche, in which a species can sustain a stable population size (Grinnel 1917; Kearney & Porter 2014). These coupled with biotic interactions and dispersal barriers, delimit and constrain the realized niche within the full range of the fundamental niche, thus reducing the occupied geographic range within its potential distribution (Hutchinson 1957; Soberón 2007). Thus, if environmental changes occur too rapidly and overcome physiological tolerances, populations likely fail to adapt to the new conditions, which may lead to their extirpation (Futuyma 2010; Cahill et al. 2013). Otherwise, under normal circumstances, ecological divergence can be one of the main drivers of speciation and is the evolutionary response in populations with high levels of ecological flexibility and adaptive capacity (Thuiller et al. 2005; Schluter 2009; Lavergne et al. 2013; Smith et al. 2019; Ahmadi et al. 2021). In terms of niche conservatism, species that have retained their ancestral ecological characteristics and have evolved slowly in homogenous environments over their evolutionary time (Harvey & Pagel 1991; Wiens & Graham. 2005; Hadly et al. 2009; Peterson 2011; Pyron et al. 2014), are more susceptible to the negative influences of rapid environmental change (Wiens et al. 2010; Lavergne et al. 2013; Ahmadi et al. 2021). Therefore, understanding the niche evolution is particularly important in properly evaluating the status of threatened species and thereby proposing science-based measures of conservation (Richardson et al. 2010; Wiens et al. 2010; Moritz et al. 2013). Recent efforts have integrated ecological niche modelling (ENMs) with phylogenetic data to identify the mechanism of niche evolution, enabling researchers to test hypotheses of diversification processes and the non-randomness of species distribution among closely related species (Evans et al. 2009; Hof et al. 2010; Salamin et al. 2010; Broennimann et al. 2012; Lavergne et al. 2013; Rato et al. 2015; Ahmadi et al. 2021).

The high level of restricted-range endemism in Tiger geckos of the genus *Goniurosaurus*, which comprises four species groups restricted to disjunct karst ecosystems scattered among granitic mountains, offers a unique opportunity to investigate mechanisms of species radiation and evolution (Honda et al. 2014; Liang et al. 2018; Ngo et al. 2021; Grismer et al. 2021). Recently, systematic issues concerning the genus *Goniurosaurus* were resolved and updated with recent discoveries based on integrative approaches including morphology and phylogenetics (Liang et al. 2018; Grismer et al. 2021; Ngo et al. 2021). In particular, 24 tiger geckos are assigned to one of four monophyletic species groups: the *kuroiwae* group with six exclusively insular species from the Ryukyu Archipelago, Japan; the *lichtenfelderi* group with five species and the *luii* group with nine species distributed throughout insular and mainland sites in China and Vietnam; and the *yingdeensis* group with four continental species in southern China (Fig. 1) (Nguyen et al. 2009; Nguyen 2011; Wang et al. 2013; Honda & Ota 2017; Liang et al. 2018; Qi et al. 2020a, 2020b; Zhu et al. 2020a, 2020b; Zhu et al. 2021). It is noteworthy that most Tiger geckos have been only recorded from a single locality or island, and all are allopatric (Ngo et al. 2021). Oceanic archipelagos and disjunct karst systems serve as geographic barriers that prevent gene flow and dispersal (Heaney 2007; Sterling et al. 2006). Allopatry coupled with a multitude of ecological niches including variable climatic conditions offered by a complex karstic topography across different latitudes they occupy, likely account for such a high level of restricted-range endemism in *Goniurosaurus* (Clements et al. 2006; Sterling et al. 2006). Liang et al. (2018) related the evolutionary history of *Goniurosaurus* in part with information of divergence times among its lineages. That research underlined the central role of the continental island of Hainan as a facilitator of the species diversification within the *lichtenfelderi* group. Using an ancestral habitat reconstruction analysis at phylogenetic levels, Grismer et al. (2021) shed light on the causal relationship between habitat types and evolution, in which karstic ecosystems were highly supported as the most probable ancestral condition for *Goniurosaurus* as well as for three out of the four groups (e.g. *kuroiwae*, *luii*, and *yingdeensis* groups). The *lichtenfelderi* group, however, was recovered to have evolved from granitic habitat.

Section 1.3.

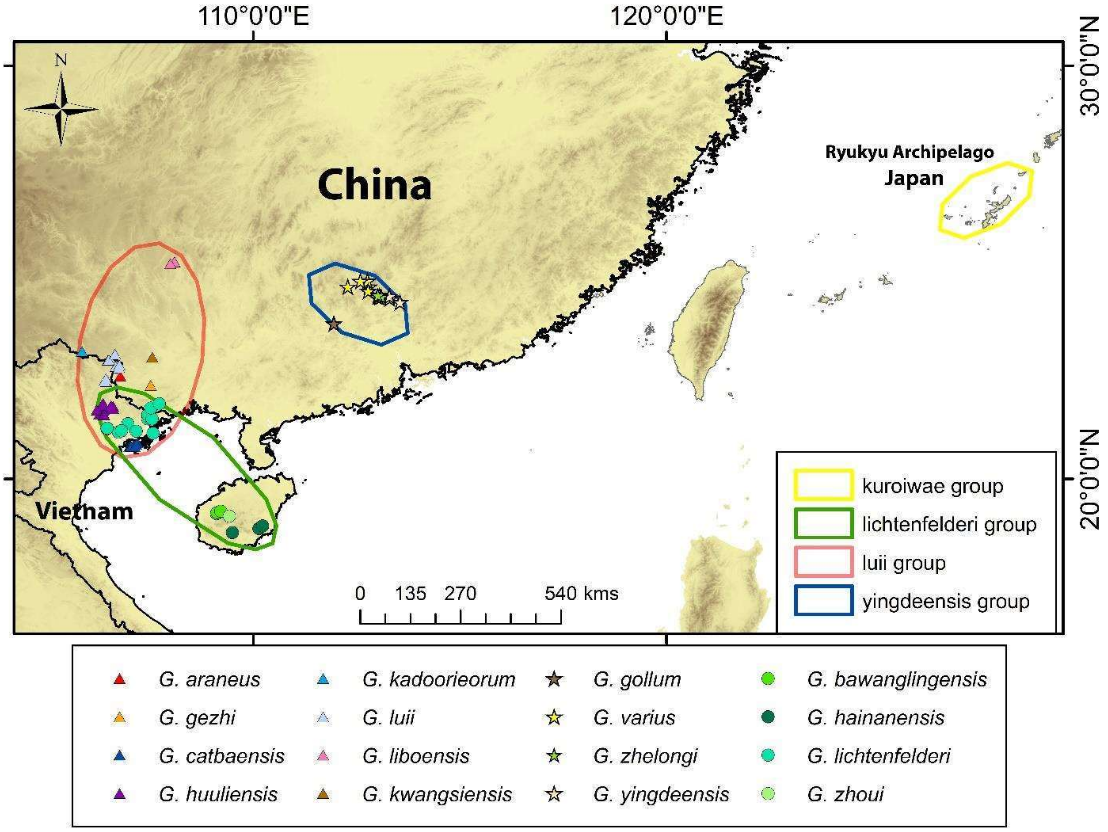

Figure 1. Geographic ranges of four *Goniurosaurus* groups and representative locations of 16 sister taxa in China and Vietnam.

Goniurosaurus is considered one of the most threatened genera under ongoing severe human impacts (e.g. unsustainable over-exploitation for the pet trade and habitat loss) (Ngo et al. 2019). Furthermore, as highly endemic ectotherms with limited dispersal capacity, potential factors—including small population size, stochastic events and climate change—can also push the *Goniurosaurus* species to the brink of extinction (Ngo et al. 2016; 2019; Ngo et al. 2021; 2022). To safeguard *Goniurosaurus* populations, 18 of all Tiger gecko species have been listed as near threatened (NT), vulnerable (VU), endangered (EN) and critically endangered (CR) in the IUCN Red List. Based on convincing evidence of over-harvesting, legal regulations of all Tiger gecko species from CITES listings have recently come into force (Ngo et al. 2019; 2021). In spite of a wealth of studies on morphology, phylogeny, and assessments of demography and ecology (Ngo et al. 2016; 2019; 2021; 2022; Liang et al. 2018; Grismer et al. 2021), a sufficient understanding of this group's radiation and allopatric distribution in relation to ecological processes over evolutionary time remains to be further investigated in order to establish science-based conservation measures.

In this study, we use available molecular data published by Grismer et al. (2021) and Zhu et al. (2021), to reconstruct a dated phylogenetic tree including all *Goniurosaurus* species. The phylogenetic analysis from the dated tree is combined with approaches of ENM using macro-climatic data to identify the rate of climatic niche evolution among *Goniurosaurus* sister taxa from China and Vietnam. Based on these results, we mainly intend to answer the question whether incipient cladogeneses among *Goniurosaurus* groups and afterward speciations were driven by niche conservatism or niche divergence. The past tectonic events and vicariance within the karstic ecosystems, including isolated islands, are assumed to have causal interactions with those mechanisms of niche evolution. By which, we aim to elucidate hypotheses on speciation and allopatric distribution of *Goniurosaurus* species. In terms of conservation planning, we further intend to identify *Goniurosaurus* species following the pattern of niche conservatism, which allows us to propose that those species should be received higher priorities of conservation attention and protection, as being more vulnerable to environmental changes.

Materials and Methods

Genetic data and estimation of divergence times

Genomic DNA was extracted from muscle tissue samples, using a DNA extraction kit from Tiangen Biotech (Beijing) Co., Ltd. Primers used for 16S were r16S-5L (5’-GGTMMYGCCTGCCCAGTG -3’) and 16sbr-H (5’- CCGGTCTGAACTCAGATCACGT-3’) (Palumbi et al. 1991), for cytb the primers were L14731 (5’- TGGTCTGAAAAACCATTGTTG-3’) (Honda et al. 2014) and H15149m (5’- GCMCCTCAGAAKGATATTTGYCCTCA-3’) (Chambers and MacAvoy 1999), for CMOS the primers were FU-F (5’- TTTGGTTCKGTCTACAAGGCTAC -3’) and FU-R (5’-AGGGAACATCCAAAGTCTCCAAT -3’) (Gamble et al., 2008) , and for RAG1 the primers were R13 (5’- TCTGAATGGAAATTCAAGCTGTT -3’) and R18 (5’- GATGCTGCCTCGGTCGGCCACCTTT -3’) (Groth and Barrowclough 1999). The PCR procedure was performed with an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min (Liang et al. 2018). PCR products were purified with spin columns and then sequenced with forward primers using BigDye Terminator Cycle

Sequencing Kit as per the guidelines on an ABI Prism 3730 automated DNA sequencer by Shanghai Majorbio Bio-pharm Technology Co., Ltd.

We constructed Maximum Likelihood (ML), Bayesian Inference (BI), and Bayesian Evolutionary Analysis by Sampling Trees (BEAST) phylogenetic trees using a concatenated data set composed of 3070 base pairs (bp) of the mitochondrial genes, 16s (633 bp) and cytb (1075 bp), and the nuclear genes, CMOS (472 bp) and RAG1 (890), from 103 specimens of 24 species of *Goniurosaurus* with varying degrees of sequence coverage across the samples (see Grismer et al. 2021:Table 1). Concatenation followed the comparison of separate gene trees to confirm there were no major discordances. One species, *Eublepharis macularius*, served as an outgroup (Grismer, 1988; Jonniaux and Kumazawa, 2008) to root the trees. See Grismer et al. (2021:Table 1) for all sequence data and GenBank accession numbers.

A Maximum likelihood (ML) analysis partitioned by gene was implemented using the IQ-TREE webserver (Nguyen et al. 2015; Trifinopoulos et al. 2016) preceded by the selection of substitution models using TIM2+F+I+G4 for 16s and cytb and HKY+F for CMOS and RAG1. To avoid over parameterization, protein coding genes were not partitioned by codon. One-thousand bootstrap pseudo-replicates via the ultrafast bootstrap (UFB: Hoang et al. 2018) approximation algorithm were employed, and nodes having UFB values of 95 and above were considered strongly supported (Minh et al. 2013). We considered nodes with values of 90–94 as well-supported. A Bayesian inference (BI) analysis was carried out in MrBayes 3.2.3. (Ronquist et al. 2012) on XSEDE using the CIPRES Science Gateway (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010) employing default priors and models of evolution that most closely approximated those selected by the BIC and used in the ML analysis. Two independent Markov chain Monte Carlo (MCMC) analyses for each data set were performed—each with four chains, three hot and one cold. The MCMC simulations ran for 100 million generations. Trees were sampled every 10,000 generations, and the first 10% of the trees from each run from each data set were discarded as burn-in. The parameter files from both runs were checked in Tracer v1.6 (Rambaut et al. 2014) to ensure that convergence and stationarity of all parameters had effective sample sizes (ESS) well-above above 200. Post-burn-in sampled trees from each respective run were combined and 50% majority-rule consensus trees were constructed. Nodes with Bayesian posterior probabilities (BPP) of 0.95

and above were considered highly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). We considered nodes with values of 0.90–0.94 as well-supported.

An input file was constructed in Bayesian Evolutionary Analysis Utility (BEAUti) v. 2.4.6 using a relaxed lognormal clock with unlinked site models, linked trees and clock models, and a Yule prior and run in BEAST on CIPRES (Cyberinfrastructure for Phylogenetic Research; Miller et al. 2010). bModelTest was used to numerically integrate over the uncertainty of substitution models of each gene while simultaneously estimating phylogeny using Markov chain Monte Carlo (MCMC). MCMC chains were run for 100,000,000 generations and logged every 10,000 generations. The BEAST log file was visualized in Tracer v. 1.6.0 (Rambaut et al. 2014) to ensure effective sample sizes (ESS) were well-above 200 for all parameters. A Maximum clade credibility tree using mean heights at the nodes was generated using TreeAnnotator v.1.8.0 (Rambaut and Drummond 2014) with a burn-in of 1000 trees (10%). Nodes with BPPs of 0.95 and above were considered strongly supported (Huelsenbeck et al. 2001; Wilcox et al. 2002). We considered nodes with values of 0.90–0.94 as well-supported.

Ancestral range estimation

The resulting BEAST tree was used to estimate the ancestral range at each node using the R package BioGeoBEARS (Matzke 2013, 2014). We followed the dating scheme of Jonniaux and Kumazawa (2008) in order to compare it with the BEAST and treePL (Smith and O'Mera 2021) analyses of divergence times of Liang et al. (2018). BioGeoBEARS allows for both probabilistic inferences of ancestral geographic ranges and statistical comparisons of range expansion from different models in a likelihood framework employing the Akaike Information Criterion (AIC) to allow the data to choose the best fitting model. Available models in BioGeoBEARS include a likelihood version of the parsimony-based Dispersal Vicariance Analysis DIVA ("DIVALIKE") (Ronquist 1997), the likelihood-based Dispersal-Extinction Cladogenesis (DEC) model of the LAGRANGE program (Ree and Smith 2008), and the Bayesian-based BayArea ("BAYAREALIKE") (Landis et al. 2013). Additionally, each model incorporates founder-effect speciation (+J) which is purported to be particularly important when reconstructing biogeographic scenarios of insular lineages (Matzke 2014). However, the DEC and the DEC +J models must be viewed with caution as it has been demonstrated that they are not time-dependent, thus precluding anagenesis in the speciation process and deferring all speciation to nearly instantaneous cladogenic events at the nodes (Ree & Sanmartín 2018). For a geography input file, we classified *Goniurosaurus* species into four areas following the division of Liang et al. (2018), including (1) China: in northern Guangdong Province occupied with four species of *yingdeensis* group (Qi et al. 2020); (2) Hainan Island (China) with four species of *lichtenfelderi* group (except for *G. lichtenfelderi*); (3) Ryukyu Archipelago (Japan) with six species of *kuroiwae* group; and (4) Vietnam: from Red River fault in northern Vietnam, northward to southern Guizhou Province, China, with nine species of *luii* group and *G. lichtenfelderi*. These areas were not randomly selected given the distinct geography of four monophyletic species groups of *Goniurosaurus* with an exception of *G. lichtenfelderi* within the range of *luii* group in Vietnam (Ngo et al. 2021; Grismer et al. 2021). Each species occurs in only a single region and as such was allowed to assign only a single selected area in the analysis.

Ancestral elevation reconstruction

In order to estimate the elevation at each cladogenetic node in the tree, we employed a stochastic character mapping (SCM) analysis implemented in R software using the package phytools based on the BEAST tree of *Goniurosaurus*. Elevation levels (namely low, medium and high) were mapped onto the tree using SCM implemented in the R package phytools (Revell, 2012) in order to derive probability estimates of the ancestral states at each node. The coding of elevation level for each species was determined from the literature and field observations of the authors (Fig. S1). A transition rate matrix was identified for best fit the data by comparing the corrected Akaike Information Criterion (AICc) in the R package ape (Paradis and Schilep, 2018). Three transition rate models were considered: a 2-parameter model having different rates for every transition type (the ARD model); a single-parameter model with equal forward and reverse rates between states (the symmetrical rates SYM model); and a single rate parameter model that assumes equal rates among all transitions (ER). Lastly, an MCMC approach was used to sample the most probable 1000 trait histories from the posterior using the *make.simmap*() command and then summarized them using the *summary*() command.

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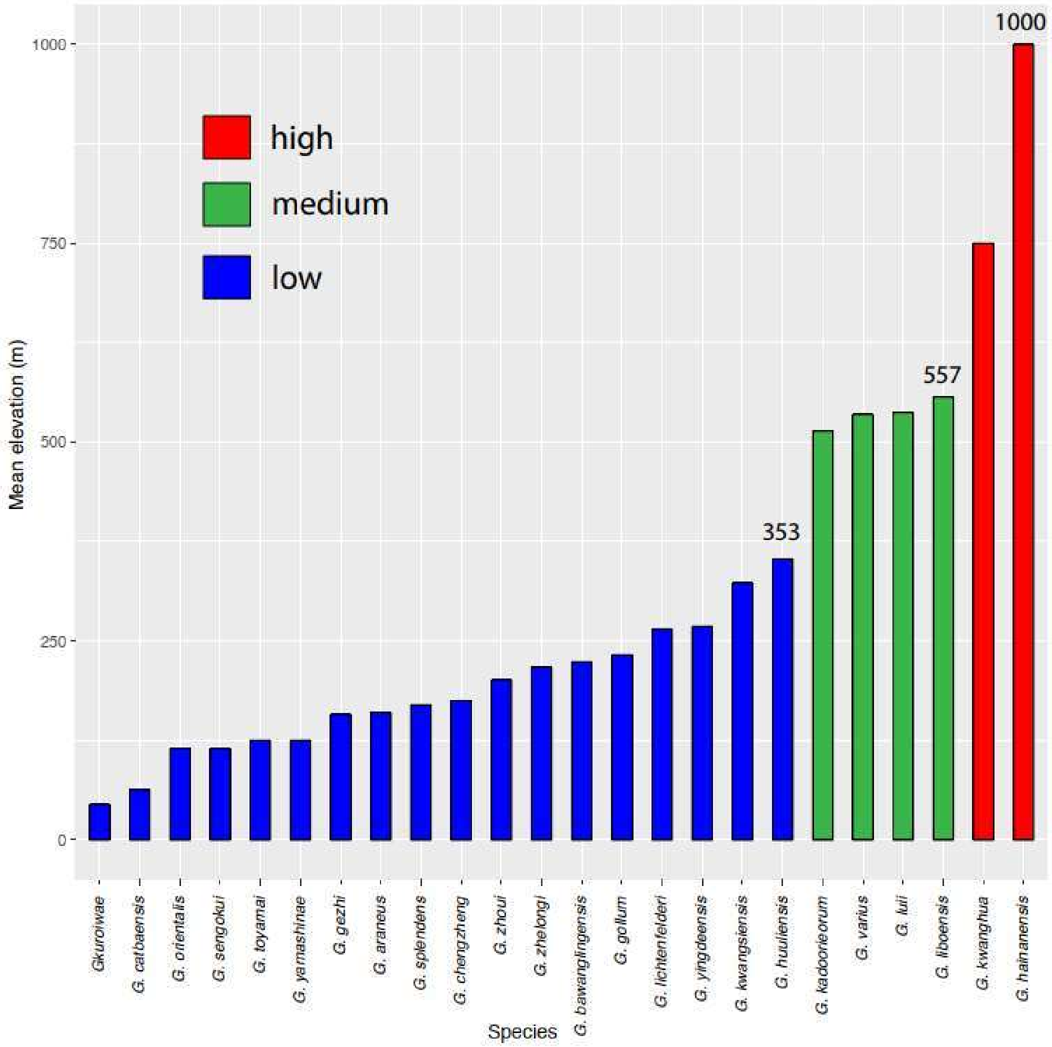

Figure S1. The mean elevation was recorded in all *Goniurosaurus* species.

Occurrence records and environmental data

A total of 223 occurrence records of 16 *Goniurosaurus* taxa in China and Vietnam were collected from our field surveys in the two last decades by ourselves and others (Fig. 1). In order to improve the quality of prediction models due to geographic bias and autocorrelation effects as results of duplicates, we randomly selected only one record within each 1 km square by using spatial filtering functions from the package“spThin” in R v 3.1.2 (R Core Team 2018; Aiello-Lammens et al. 2015). Consequently, 160 representative records of three *Goniurosaurus* groups (including *lichtenfelderi* – 44 records, *luii* – 79 records and *yingdeensis* – 38 records, see details in Table S1) were used for following analyses and models.

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In terms of environmental data, nineteen climatic variables for current conditions with a spatial resolution of 30 arc-second (averages between 1970 – 2000) were obtained from Worldclim (http://www.worldclim.org/, version 2.0) (Fick & Hijmans 2017). To mitigate the influence of multicollinearity of predictors on the models, we used a pairwise Pearson correlation analysis to eliminate highly correlated climatic variables with coefficient values (r) larger than |0.7| (Dormann et al. 2013. Our final data set comprised seven climatic variables including Mean Diurnal Range (BIO2), Isothermality (BIO3), Max Temperature of Warmest Month (BIO5), Mean Temperature of Wettest Quarter (BIO8), Precipitation of Wettest Month (BIO13), of Driest Month (BIO14) and Warmest Quarter (BIO18), which were selected for subsequent analyses.

Climatic niche comparisons

To compare the macro-climatic niche among *Goniurosaurus* groups and sister taxa of each group, all pairwise tests were computed by using the package "ecospat" in R software (Di Cola et al. 2017). The niche equivalency and two-way similarity tests, presented in detail by Warren et al. (2008) and Broennimann et al. (2012), were calculated between *Goniurosaurus* group pairs in this study. The degree of overlap in climatic-niche space was further evaluated using Schoener's D, which varies from 0 (no overlap) to 1 (complete overlap) (Schoener, 1970; Kohn and Riggs, 1982; Warren et al. 2008). However, the niche overlap indices among species-pairs cannot be reliably estimated due to the limited availability of records with less than ten in most Tiger geckos (Rödder et al. 2011). Following a jackknifing approach proposed by Rödder et al. (2011) to overcome the limitation, all available *Goniurosaurus* records of each monophyletic group were pooled into a dataset and then repeated "leave-one-species-out" to calculate Schoener's D values. We subsequently used the value of 1-D as the relative degree of overlap between the omitted one and all remaining species.

Each macro-climatic niche was described in a orthogonal space combining the first two principal components in the PCA-env approach (Broennimann et al. 2012). The overlap level of given macro-climatic niche spaces was evaluated by direct observations on the same environmental background. To define the available macro-climatic space, we constructed a minimum convex polygon (MCP) covering a total of selected *Goniurosaurus* records using the package "adehabitatHR" in R software (Calenge 2006).

Species distribution modellings In terms of species distribution modelling (SDM), the potential distribution of eachTiger gecko was predicted by using an ensemble of small models (ESMs) combining six modelling techniques: artificial neural network (ANN), classification tree analysis (CTA), generalized linear models (GLM), generalized additive models (GAM), generalized boosting regression models (GBM), and maximum entropy modelling (Maxent). All models were calibrated using the "biomod2" package (Breiner et al. 2015; Thuiller et al. 2016; Cola et al. 2017). A subset of environmental factors which contains principal components (PCs) as the result of a principal component analysis (PCA) with the seven selected climatic variables using the function "rasterPCA" of "RStoolbox" package in R software, was employed in the ESMs.

Although the potential distribution of each species was projected within the entire background, all models were only trained with presence-only data and 10,000 random points selected within the MCParea. For *Goniurosaurus* species recorded with one or two occurrences (Table S1), we selected four or three more pseudo-presences, respectively. With at least five occurrences, the prediction model can be technically trained with climatic variables. We created a subset of 30 random locations for each of those species within a radius of 5 km around using "dismo" and "raster" packages in R software. The hierarchical cluster using "cluster" and "factoextra" in R software filtered those selected pseudo-presences with the most similar values of climatic PCs and elevation (downloaded from the worldclim with a spatial resolution of 30 arc-second) to occurrences' values. Subsets of 70% training data and 30% test data were used to evaluate species models. We employed two perfomance indices of AUC and TSS, and their values closer to 1.0 indicate better model performance (Breiner et al. 2015).

History of niche occupancy

Following an approach proposed by Evans et al. (2010), the evolutionary history of climatic niche occupancy of *Goniurosaurus* species in China and Vietnam was reconstructed throughout predicted niche occupancy (PNO) profiles in the "phyloclim" package. The suitable probability derived from ESMs of each *Goniurosaurus* species was re-scaled to the sum of 1, and corresponding PC scores binned into 1,000 grid cells in order to obtain the PNO profile for each PC. From these PNO profiles, we resampled 1,000 times using the generalized least squares method with an assumption of Brownian motion evolution to estimate the climatic spectrum on the BEAST dated tree of *Goniurosaurus* species in China and Vietnam (Rato et al. 2015; Ahmadzadel et al. 2016; Ahmadi et al. 2021).

Ancestral climatic state estimation

Phylogenetic signals were examined to measure the level of similarity in lineage traits of climatic PCs in correlation with phylogenetic relatedness. Each of climatic PCs was tested for the phylogenetic signal based on values of Blomberg's K (Blomberg et al. 2003) and Moran's I (Münkemüller et al. 2012) using the "phylosig" function of the package "phytools" in R software. A phylogenetic signal is identifed when $p < 0.05$, meaning that Blomberg's K and Moran's I are significantly different from zero. In particular, values of K range from zero to infinity, where $K < 1$ indicates weak phylogenetic signal, and $K > 1$ strong signal, implying that sister taxa are, respectively, more or less divergent in environmental traits. In terms of Moran's I, the value reaches to +1 indicating a perfect cluster of similar traits, whereas I of -1 indicates the highest autocorrelation of dissimilar traits.

We illustrated the ancestral state of climatic PCs to assess the pattern of climatic evolution of Tiger geckos by using the "contMap" function of the "phytools" package in R software (Revell et al. 2012; 2013). For climatic input data, we averaged the climatic spectrum of each climatic PC from the PNO profiles for each Tiger gecko. The best model to describe trait evolution was ranked based on the AICs tests for four commonly proposed models: Brownian motion (BM), Ornstein–Uhlenbeck (OU), Early Burst (EB) and Lambda, using the "fitContinuous" function of "geiger" package (Pennell et al. 2014). We computed the maximum likelihood (ML) to predict the climatic state under the selected evolution model at the internal nodes and states along edges of each branch were interpolated based on the equation from Felsenstein (1985) with a color gradient.

Result

Phylogenetic relationships and divergence dating

The time-calibrated BEAST analysis recovered a phylogeny with well-supported nodes (BPP ≥ 90) throughout the tree (Fig. 2). The divergence dates are similar to those calculated by Liang et al. (2018) and fall well within the range of their calculated highest posterior density (HPD). This is especially true for the timing of the origin and divergence within each species group.

The phylogeny indicates that *Goniurosaurus* diverged from its sister lineage during the Eocene at approximately ~45.3 mya and continued to radiate across East Asia up until the Pliocene. Diversification within four monophyletic species groups occurred in their respective geographic regions and began in the mid-Miocene between ~13.4–7.7 mya (million years ago) and continued to at least the mid-Pliocene ~2 mya (Fig. 2).

In the *kuroiwae* clade, the first cladogenesis event occurred ~13.3 Mya with an emergence of *G. splendens*, and the most recent divergence was estimated at ~3.8 Mya between *G. orientalis* and *G. sengokui*. Although the *yingdeensis* group diverged by the early Oligocene, the first divergence within its lineages was very late at ~ 7.7 Mya. A new species of *G. varius* in this group diverged most recently at ~3.7 Mya. In the *lichtenfederi* group, *G. bawanglingensis* first split from its sister lineaged ~11.3 Mya. A pair of *G. hainaensis* and *G. lichtenfelderi* later diverged ~1.9 Mya. Regarding the *luii* group, the first divergence was estimated at ~10.3 Mya and the lastest ~2.6 Mya between *G. huuliensis* and *G. luii*. Recent studies confirmed that *G. kadoorieorum*, described by Yang & Chan (2015), is a synonym of *G. luii* (Grismer et al. 2021; Ngo et al. 2021) and their evolutionary time thus was considered as 0 Mya.

Ancestral reconstruction of geographical ranges and elevation

The BioGeoBEARS model comparisons indicate that the DEC+J model has the best fit to the data and most likely to infer the correct ancestral range at each node being that it had the lowest AIC-wt score (Table S2). Despite the noted criticisms of the +J parameter (Ree & Sanmartín 2018), it is noteworthy that all the trees generated in all the analyses generally recovered the same ancestral range for each node, thus converging on the same biogeographical scenario. The analyses indicate that the respective ancestral regions for each group are the same regions in which each group radiated—the exception being *G. lichtenfelderi* of the *lichtenfelderi* group of the Hainan Region being found in the Vietnam Region. Liang et al. (2018) considered this to be a recent dispersal event. The geographical origin of *Goniurosaurus* is less clear. The analysis indicates that all the deep nodes outside those of the species groups, recovered an essentially equal probability that *Goniurosaurus* originated anywhere across the combined areas. Given the young age and relatively recent geographic isolation of the Ryukyu Archipelago and Hainan Island from late Mioence (Kimura

2000; Sterling et al. 2006; Honda et al. 2014; Liang et al. 2018), it is safe to posit the *Goniurosaurus* evolved in continental East Asia. With additional fieldwork and the potential discovery of new species north of Guangdong Province, the region of origin could be further refined.

Table S2. Model testing for the BioGeoBEARS analysis with and without found-event speciation (+J). Models tested: dispersal-extinction-cladogenesis (DEC); Bayesian analysis of biogeography when the number of areas is large (BayArea); and dispersal-vicariance (DIVA). d = rate of dispersal; e = rate of extinction.

LnL	numparams	d	E	j	AICc	AICc_wt
DEC	-13.43754938	2	0.253901095	8.60E-09	0	31.42055331
DEC+J	-11.01490786	3	1.00E-12	1.00E-12	0.027306884	29.17267286
DIVALIKE	-15.8110847	2	0.375558282	0.940700302	0	36.16762394
DIVALIKE+J	-11.0447176	3	1.00E-12	0.413357214	0.024990954	29.23229234
BAYAREALIKE	-21.27821842	2	0.607985713	1.89701353	0	47.10189138
BAYAREALIKE+J	-12.39951255	3	1.00E-07	1.00E-07	0.035891293	31.94188224

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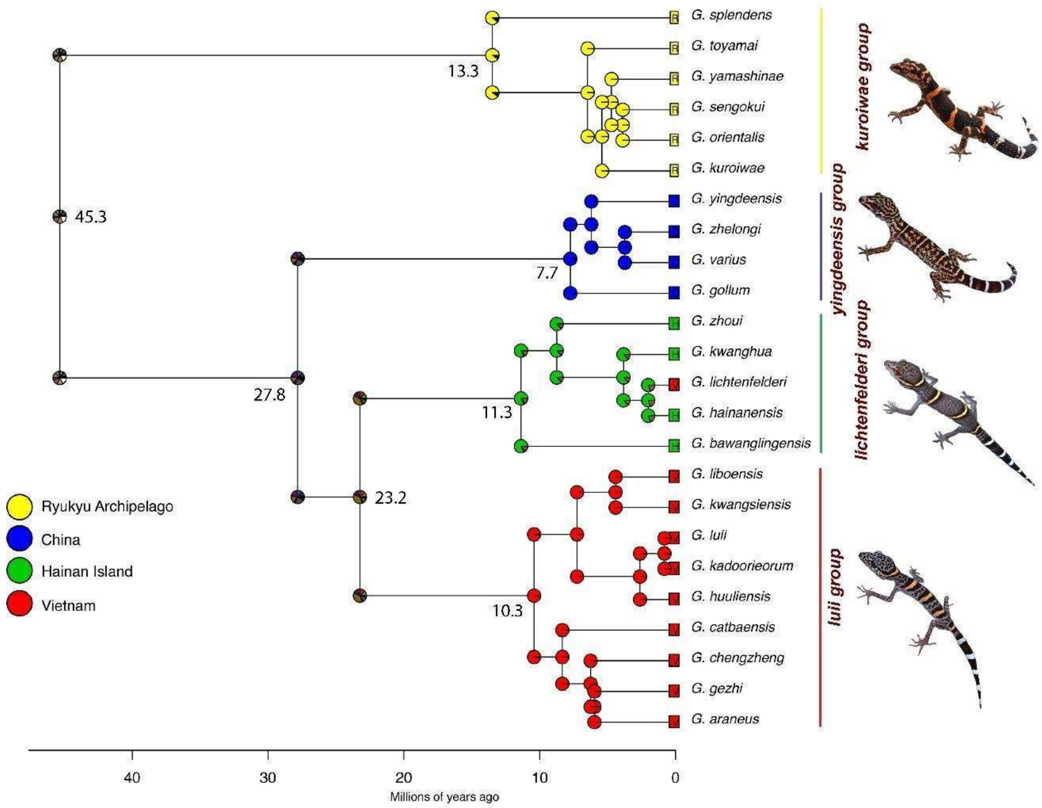

Figure 2. The ancestral geographical range of *Goniurosaurus* species reconstructed under BIOGEOBEARS using DEC+J model on the dated BEAST tree. Single capital letters and colors indicate the current biogeographic unit of each species. Pie charts with colors in the corner of the cladogram indicate the estimated probability of each area inherited from the ancestor after the cladogenetic process.

Regarding the habitat trait elevation, all *Goniurosaurus* species have been recorded in areas ranging from 40 m to 1000 m elv (Fig. S1). The AIC scores for the three transision models were ARD = 47.0085, SYM = 43.60873, and ER = 40.07807. The SCM using an ER model predicted that low elevations are the most probable ancestral state for *Goniurosaurus* and the low level was recorded for all four species groups as well. However, the most common ancestor of three sister lineages, namely *G. kwanghua, G. hainanensis, G. lichtenfelderi* of the *lichtenfelderi* group, independently evolved at the high elevation, although *G. lichtenfelderi* only is found at low elevations (Fig. 3).

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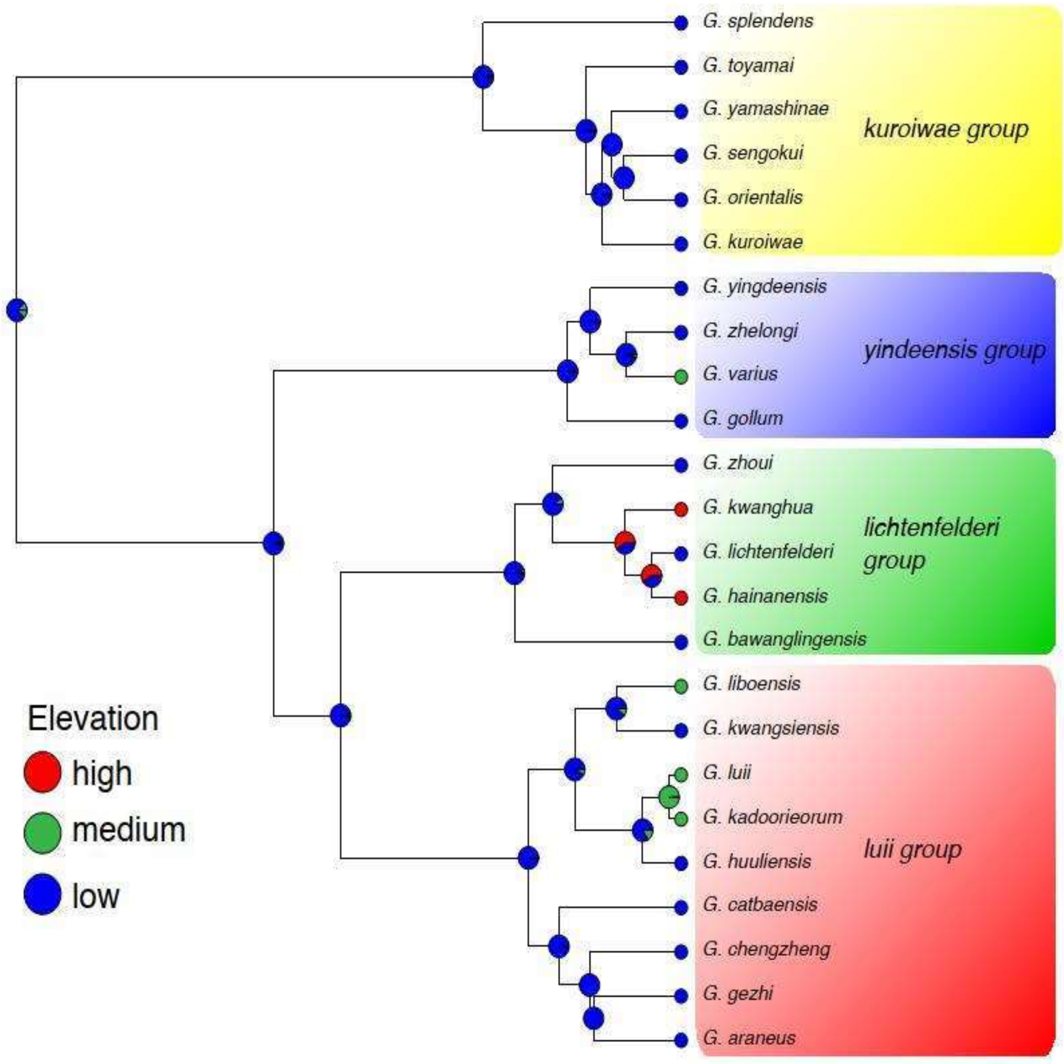

Figure 3. Stochastic character map of elevation of *Goniurosaurus* species on the BEAST tree. Color circles at the cladogenetic nodes are the probability of the ancestral elevation occupation.

Niche overlap comparison

Climatic niche space of three *Goniurosaurus* groups in China and Vietnam were illustrated on a two-dimensional space of the first axis (PC1) and the second axis (PC2) accounting for 38.64 % and 26.94 % of the overall variation in the PCA-env analysis, respectively (Fig. 4). The analysis revealed no overlaps among their climatic space, especially the niche space of *yingdeensis* group which is entirely separated from the remaining groups (Fig. 4). The climatic variables BIO2 (Mean Diurnal Range) and BIO14 (Precipitation of Driest Month) dominantly influenced the climatic space of *yingdeensis* group, whereas *lichtenfelderi* group's space strongly correlated Bio13 (Precipitation of Wettest Month) and Bio18 (Precipitation of Warmest Quarter), and *luii* group to BIO3 (Isothermality), BIO5 (Max Temperature of Warmest Month) and BIO8 (Mean Temperature of Wettest Quarter) (Fig. 4). . Niche overlap between group-pairs in terms of Schoener's D only ranges from nearly 0 (*yingdeensis* and two remaining groups) to very limited overlap with D = 0.15 (*lichtenfelderi* and *luii* groups). Furthermore, the climatic spaces occupied by the three groups were neither significantly similar ($p > 0.05$) nor equivalent ($p = 1.00$) in all group-pair comparisons (Table S3; Fig. S2).

Table S3. Macro-climatic niche comparisons among *Goniurosaurus* groups, including niche overlaps in terms of Schoener's index (D) and measurements of niche similarity and niche equivalency.

Goniurosaurus groups		Niche Overlap (D)	Niche similarity		Niche equivalency
a	b		a → b	b → a	
lichtenfelderi	*luii*	0.15	0.396	0.386	1.00
	yingdeensis	<0.001	1.00	1.00	1.00
luii	*yingdeensis*	0.0013	0.802	0.792	1.00

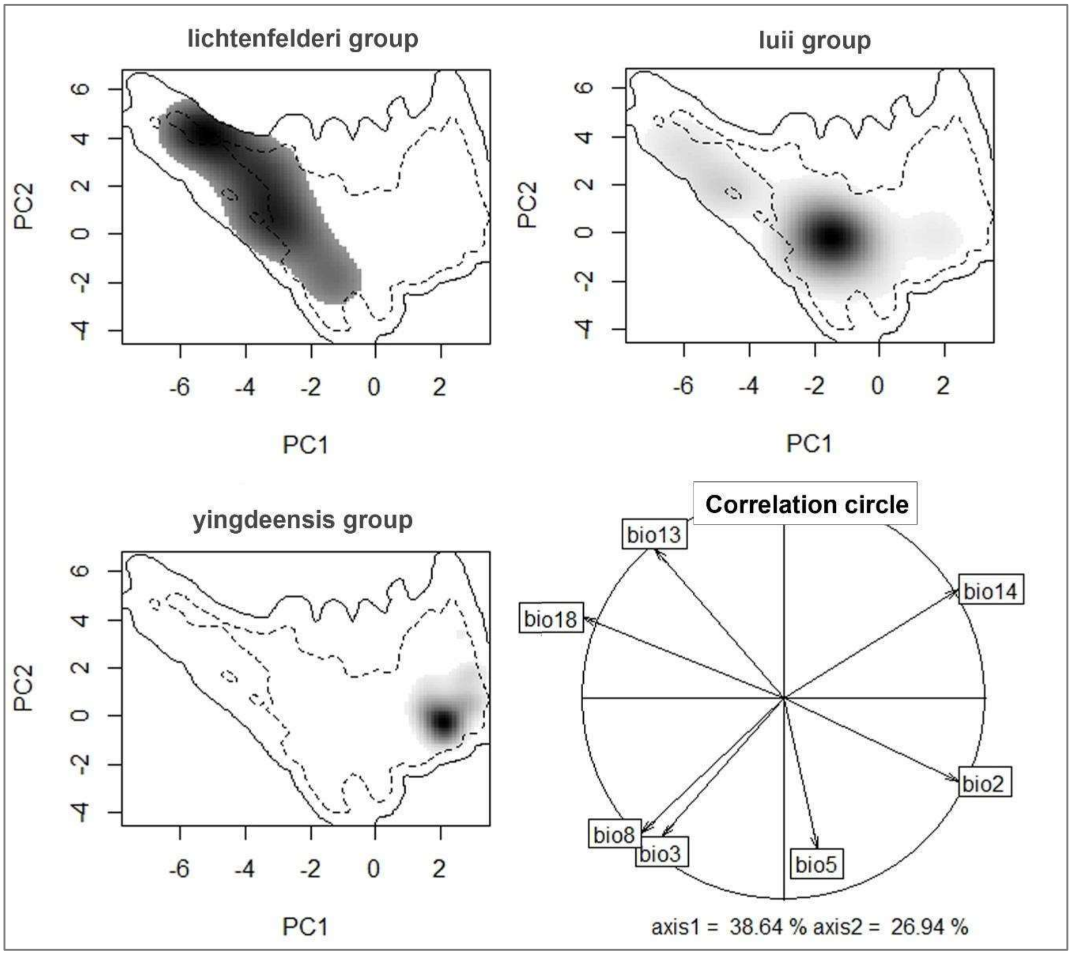

Figure 4. Climatic niche space occupied by *Goniurosaurus* groups according to the PCA-env including *lichtenfelderi* group (granite-stream adapted group) – *luii* group and *yingdeensis* group (karst adapted group), and contribution of climatic variables presented along the first two axes (PC1 and PC2). The solid (100%) and dashed contour (50%) lines illustrate the available macro-climate space.

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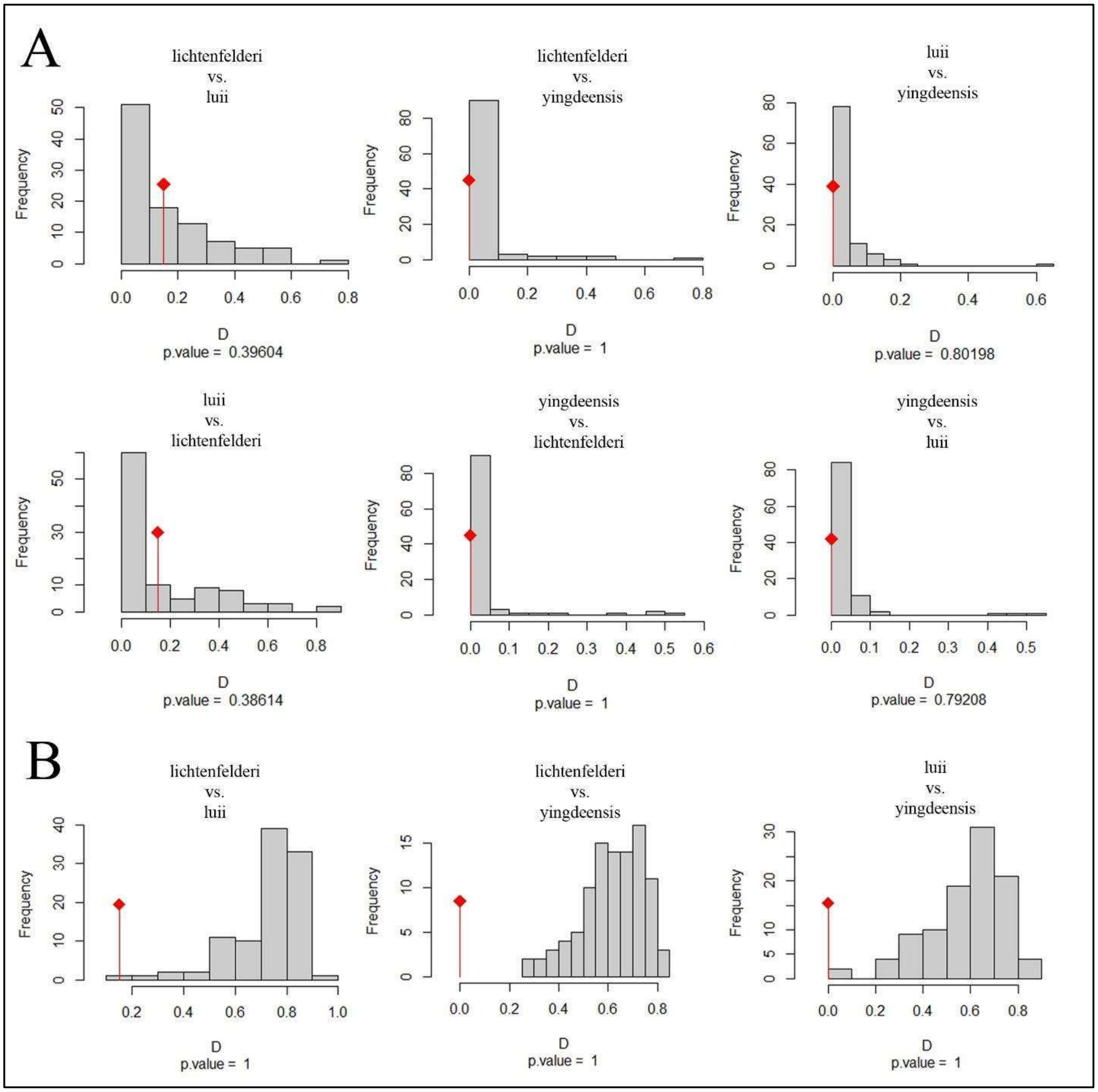

Figure S2. Histograms showed the observed niche overlap D between given *Goniurosaurus* group-pairs with diamond bars, and simulated niche overlaps (grey bars) on tests of A. Niche similarity in two directions; and B. Niche equivalence, were calculated from 100 iterations.

To evaluate the level of niche overlap among species, we used a Jackknifing approach using all pooled species records of each group and omitting the records of each species for one test. The very low value of Schoener's D, which means the high value of 1-D, was only recorded in some omitted species from their group, including *G. lichtenfelderi* (1-D = 0.998) of the *lichtenfelderi* group, *G. catbaensis* (0.844), *G. liboensis* (0.418) of the *luii* group, and *G. varius* (0.61) of the *yingdeensis* group (Table 1). Our results from the PCA-env analysis suggest that the niche space of their group shrunk significantly when their records were omitted (Fig. S3).

Table 1: Niche Overlap of Schoener's Index (D) of 16 *Goniurosaurus* species of three groups in China and Vietnam, each calculation excludes one species of each group; and 1-D indicates the relative degree of overlap between the omitted one and all remaining species of each group.

Entire species of each group but excluding species	**D**	**1 – D**
***lichtenfelderi* group**		
G. bawanglingensis	0.894	0.106
G. hainanensis	0.959	0.041
G. lichtenfelderi	0.002	0.998
G. zhoui	0.975	0.025
***luii* group**		
G. araneus	0.861	0.139
G. catbaensis	0.156	0.844
G. gezhi	0.94	0.06
G. huuliensis	0.809	0.191
G. kadoorieorum	0.930	0.07
G. kwangsiensis	0.925	0.075
G. liboensis	0.582	0.418
G. luii	0.851	0.149
***yingdeensis* group**		
G. Gollum	0.93	0.07
G. varius	0.39	0.61
G. yingdeensis	0.85	0.15
G. zhelongi	0.65	0.35

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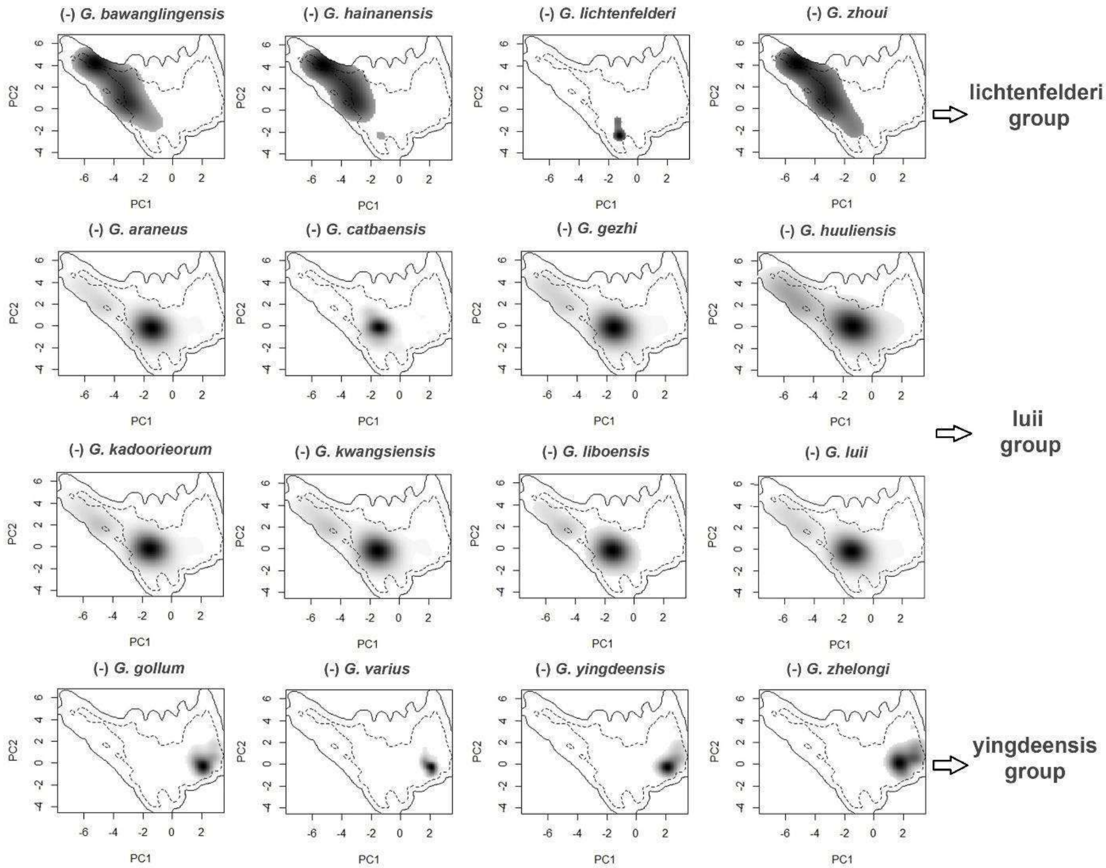

Figure S3. Climatic niche spaces of remaining species in the PCA-env after excluding records of one species from pooled coordinate data of each *Goniurosaurus* group along the first two axes (PC1 and PC2). The solid (100%) and dashed contour (50%) lines illustrate the available macro-climate space.(-) indicates that the *Goniurosaurus* species was omitted.

History of climatic evolution

The first four PCs from the PCA analysis of the seven selected climatic variables accounting for 89.1 % of the overall climatic variation (thereof PC1: 39.3 %, PC2: 23.9 %, PC3: 18.1 % and PC4: 7.8 %), were employed together with geographic records for the SDMs of each species. The performance indices TSS and AUC in the fitted EP-ESM of each Tiger geckos performed well with average values being greater than 0.8 (Fig. S4). Based on results of climatic niche models for the potential distribution (Fig. S5), we reconstructed on the BEAST dated tree the ancestral niche occupancy profiles of Chinese – Vietnamese *Goniurosaurus* groups, which presented only the niche evolution pattern of divergence among given *Goniurosaurus* groups

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and intermixed with the conservatism pattern among sister species (Fig. 5).

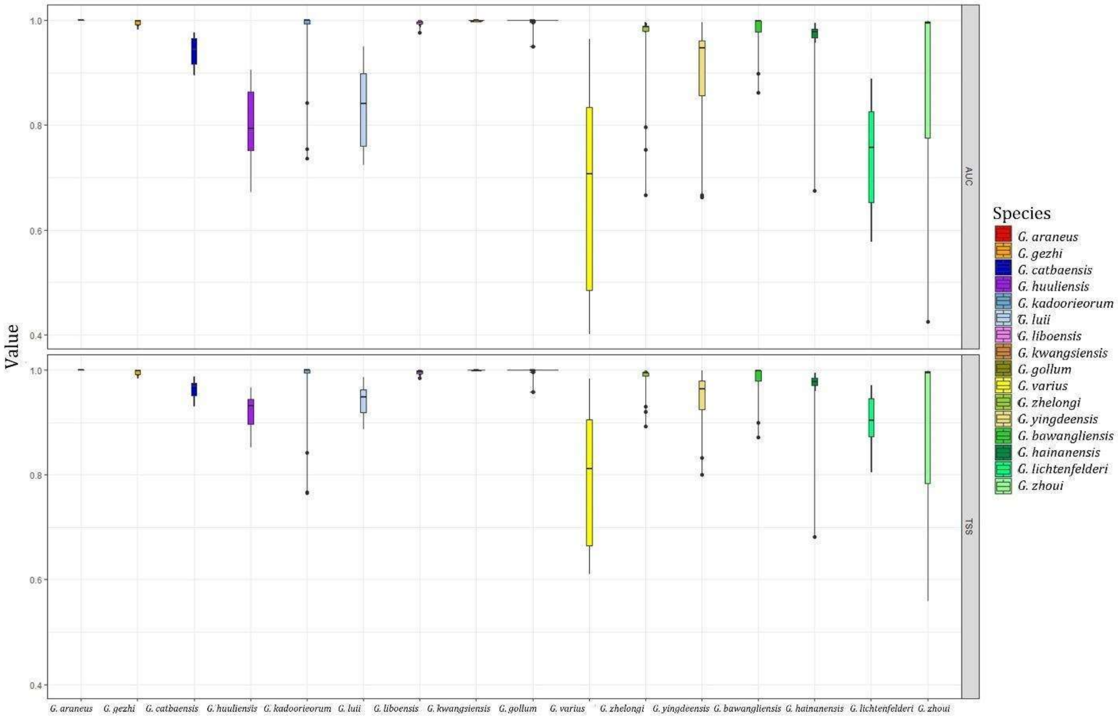

Figure S4. Performance of ensemble models of 16 *Goniurosaurus* species in China and Vietnam according to adjustment indices of AUC and TSS. Higher values indicate better-performing models.

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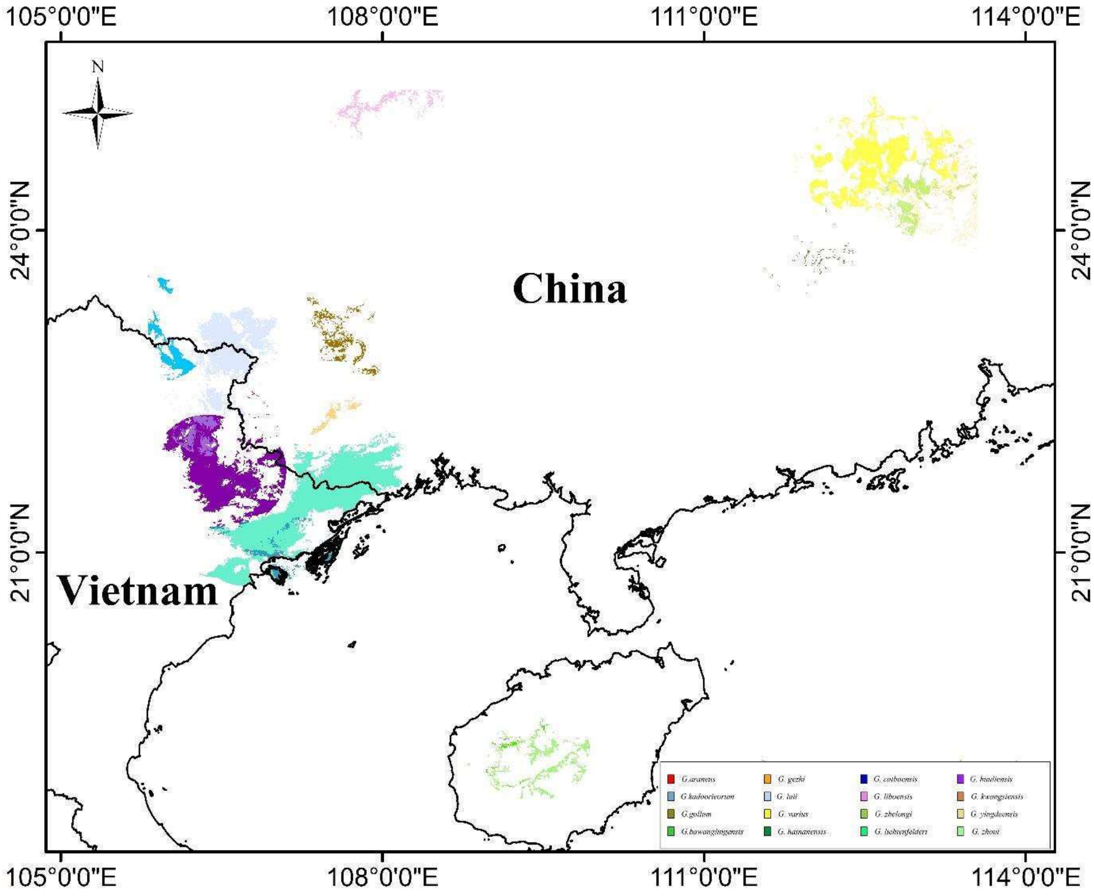

Figure S5. Potential distributions of 16 *Goniurosaurus* species in China and Vietnam were predicted by ensembling models using first four climatic PCs variables.

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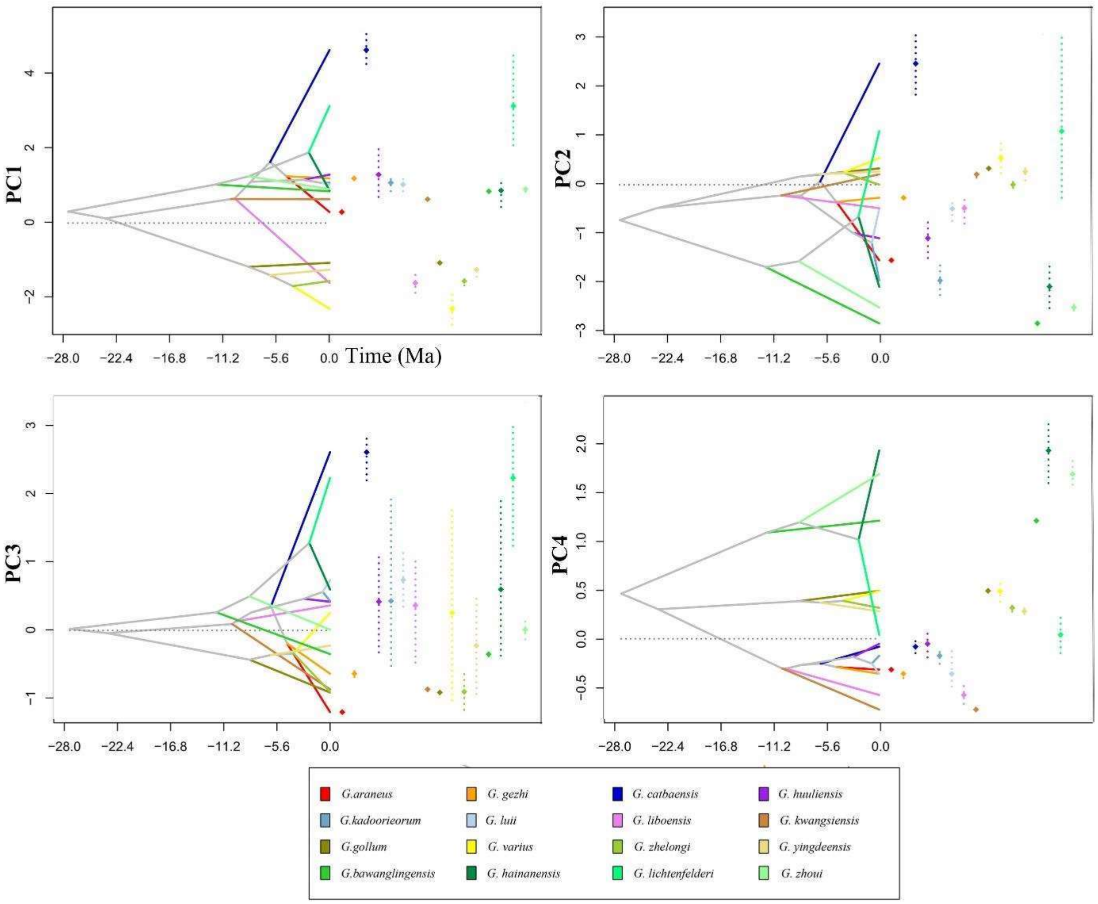

Figure 5. Inferred history of the evolution of climatic tolerances in first four climatic PC variables among 16 *Goniurosaurus* species of three groups (*lichtenfelderi*, *luii* and *yingdeensis*) in China and Vietnam. Internal nodes represent the mean of climatic tolerances as inferred for the most recent common ancestor of the extant taxa defined by that node. The 80% central density of climatic tolerance for each extant taxon is indicated by vertical dashed lines and points that mark the respective mean values.

We only detected a phylogenetic signal in PC1 and PC4 (P-values ≤ 0.05) and no signal in PC2 and PC3 (P-values > 0.05). The signals were relatively low according to Blomberg's K values, being < 1 and Moran's I values only reached 0.16 (Table S4). The AICc test suggested the Brownian motion (BM) model as most likely scenario (Table S4). Consequently, the ancestral state estimation indicated a mix of both climatic patterns, which were similar to the analysis of ancestral niche occupancy (Fig. 5; 6). In particular, the phylogenetic divergence was documented among three given groups in PC4 and PC2, and between *yingdeensis* group and both remaining groups in PC1 and PC3. Within each group, we only documented some species evolved following the climatic divergent pattern, such as *G. lichtenfelderi* of the *lichtenfelderi* group in four PC variables; *G. catbaensis* in the first three PCs; *G. kwangsiensis* in PC2 and PC3, and *G. liboensis* in PC1 (Fig. 5; 6).

Table S4. Results of the relative rate of phylogenetic signal in climatic PCs based on values of Blomberg's K and Moran's I, using the phylosig function of phytools package; and assessing the best evolutionary models of climatic variables in the ancestral state estimation based on AICc statistics, using the fitContinuous function of geiger package. AICc: Akaike Information Criterion for small sample-sized data.

		PC1	**PC2**	**PC3**	**PC4**	**AICc-total**
Blomberg's K	K	0.56	0.25	0.34	0.47	
	P-value	**0.01**	0.47	0.24	**0.05**	
Moran's I	I	0.13	-0.07	-0.02	0.16	
	P-value	**0.03**	0.52	0.25	**0.026**	
BM	-InL	**-29.96**	**-27.56**	**-23.19**	**-14.73**	**209.5**
	AICc	**67.9**	**63.12**	**54.38**	**37.47**	
	AICc_Delta	**0**	**0**	**0**	**0**	
OU	-InL	-29.31	-27.56	-22.77	-14.73	218.9
	AICc	70.26	66.76	57.17	41.11	
	AICc_Delta	2.33	3.64	2.80	3.64	
EB	-InL	-29.31	-27.56	-22.77	-13.35	215.9
	AICc	70.26	66.63	57.17	38.32	
	AICc_Delta	2.33	3.51	2.80	0.85	
Lambda	-InL	-29.96	-27.56	-23.19	-14.74	221.0
	AICc	71.56	66.76	58.01	41.11	
	AICc_Delta	3.64	3.64	3.64	3.63	
WN	-InL	-31.38	-27.56	-23.19	-18.16	219.2
	AICc	70.75	63.12	54.38	44.31	
	AICc_Delta	3.64	0	0	6.84	

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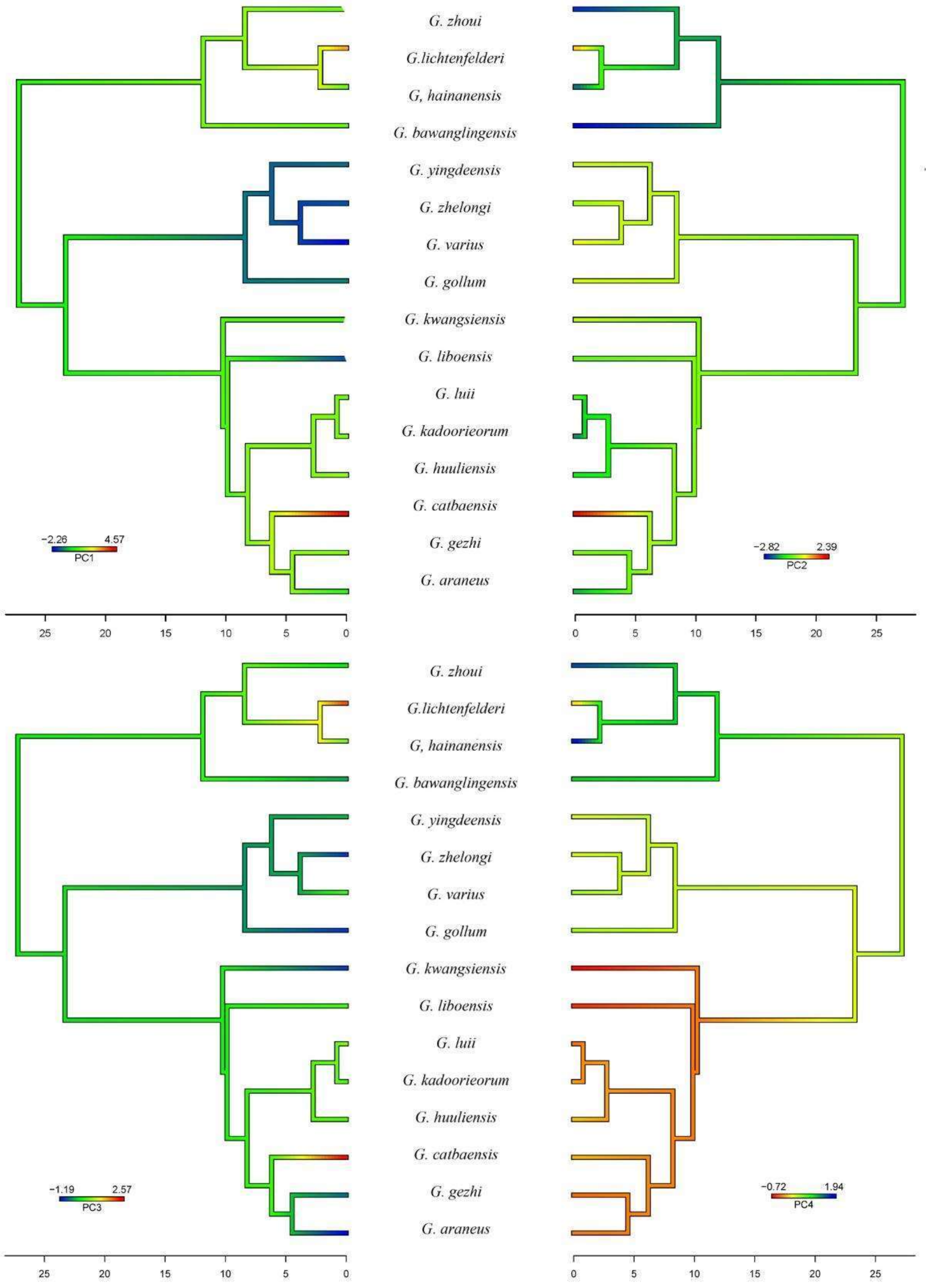

Figure 6. Ancestral state estimation of four climatic PCs variables for 16 given *Goniurosaurus* species in China and Vietnam, reconstructed based on the Brownian motion (BM) model, using the contMap function in the phytools R package

Discussion.

Following a single pattern of allopatric evolution, the cladogenesis in all Tiger geckos was assumed to be related to past orogeny processes that facilitated the geographic distinction (e.g. disjunctive limestone habitats, islands) and consequently led to the reproductive isolation (Honda et al. 2014; Liang et al. 2018; Ngo et al. 2021). However, the simple rough factor of vicariance might only play an indirect role and cannot explain the mechanism of radiation among restricted-range sister taxa of *Goniurosaurus*. Coupled with reconstructive models of biogeographic history and ecological niches during cladogenesis, we better understand evolutionary mechanisms that shaped the extraordinary richness of *Goniurosaurus* and their allopatric distribution.

Given ancestral range estimations in this study and Liang et al, (2018), rather than connecting to a particular location predicted at the crown cladogenesis node, *Goniurosaurus* ancestor originated somewhere in the contiguous continental Eastern Asia, including Ryukyu Archipelago and Hainan Island before the Eocene (Honda et al. 2014). On the other hand, the analyses revealed that the current regions in which each monophynetic *Goniurosaurus* group radiated are respectively their ancestral regions. The radition of insular *Goniurosaurus* groups is therefore considered in relation to past tectonic events. In particular, the current geographic isolation of the Ryukyu Archipelago from the eastern asian continent could be the result of young Philippine Sea plate's movement, which subducted under the Eurasian plate, rotated itself clockwise and dragged the Ryukyu northeastward in the late Paleocene (Seno et al. 1993; Ota 1998; Tend and Lin 2004). Consequently, the first radiation of *Goniurosaurus* took place with a cladogenesis of the *kuroiwae* group by the early Eocene (~45 Mya). The analyses of ancestral niche occupancy and state indicate the pattern of niche divergence in all PC climatic variables forced the subsequent emergence of *yindeensis* group by the middle Oligocene (Fig. 4; 5). Although the *kuroiwae* group was not yet employed in those analyses, due to inhabiting the highest latitudes (Fig. 1) with characteristics of the sub-tropical climate, we assumed the group thus evolved under the pattern of climatic divergence as well (Honda et al, 2014; Aiba et al. 2020). On the other hand, the uplift of Tibetan Plateau related to a tectonic event of India's collision with Eurasia, has strongly influenced the area, including northern Vietnam and southeastern China, with the dominant weather of tropical monsoon climate (Sterling et al. 2006; Miao et al. 2013). The subsequent intension of monsoon climate since the Oligocene could force the climatic niche divergence and consequently evolution between two remaining groups of *lichtenfelderi* and *luii* at that time. In this study, we documented the very low overlap between their niche space in the PCA-env (Fig. 4) and the discordance in their climatic niche spectrum (in PC2 and PC4) reconstructed by the PNO approach (Fig. 5). Furthermore, all *Goniurosaurus* groups were strongly supported with the karstic habitat as their ancestral condition, apart from the *lichtenfelderi* group with the ancestral habitat of granitic formation (Grismer et al. 2021). Given the ancestral range, the ancestor of *lichtenfelderi* group distributed at the Hainan Island, whereas the *luii* group was highly related to the contiguous mainland site in China and Vietnam (Fig. 6) (Liang et al. 2018). The collision between India and Eurasia continents together with the clockwise rotation of the young Philippine Sea plate probably forced the Hainan Island to the currently isolated position, which supported the evolution pattern of vicariance for the emergence of *lichtenfelderi* group (Seno et al. 1993; Sterling et al. 2006; Liang et al. 2018).

With the central role as Liang et al. (2018) identified, Hainan Island strongly supported as the most probable ancestral range of the *lichtenfelderi* group and its heterogeneous environments among populations potentially forced the speciation. In particular, the phylogenetic split between *G. bawanglingensis* and the most recent common ancestor of *G. zhoui* and *G. kwanghua* was highly correlated to the difference of preferred habitats. *G. bawanglingensis* only recorded in the granitic habitat, while *G. zhoui* and *G. kwanghua* are karstic-dwelling Tiger geckos (Grismer et al. 2021). Increasing volcanism by the late Miocene period might shape the mixture of karst and granite formations on Hainan Island (Ho et al. 2000; Liang et al. 2018). The emergence of other species within the *lichtenfelderi* group might generally associate with climatic cycles in the Pliocene period (Herzschuh et al. 2016). In particular, a lowland to highland transition likely occurred in some populations of *Goniurosaurus* on Hainan Island to avoid severely warmer climate conditions in the interglacial period. Our reconstruction of ancestral elevation revealed that *G. bawanglingensis* and *G. zhoui* inhabited the low elevations, following the ancestor of *lichtenfelderi* group, whereas the most recent common ancestor of remaining *lichtenfelderi* lineages preferred the high elevation (Fig. 7). In fact, the species richness varies along the elevation gradient (Sterling et al. 2006; McCain 2010; McCain and Grytnes, 2010). Thus, we suggest that the altitudinal partitioning has led to the reproductive isolation and consequently speciation between karstic species (e.g. *G. zhoui* and *G. kwanghua*) and granitic species (e.g.

G. hainanensis and *G. lichtenfelderi*). The youngest cladogenesis in *lichtenfelderi* group between *G. hainanensis* and *G. lichtenfelderi* is probably due to the combined action of vicariance and adaptation to contrasting environments. We assumed that granitic Hainan populations as the most recent common ancestor of *G. hainanensis* and *G. lichtenfelderi* radiated to the low elevation and then colonized to mainland sites and some isolated islands in Vietnam during several glacial periods in the late Pliocene due to the sea level decline (Herzschuh et al. 2016; Liang et al. 2018). Repeated falls (> 50 m) of the sea level connected numerous islands and the mainland through the periodic emergence of temporary land bridges, which allowed colonization and re-colonization among isolated populations (Li et al. 2010; Liang et al. 2018). The granite-dwelling populations in Vietnam were gradually isolated by increasing the sea level, and eventually evolved into *G. lichtenfelderi* due to limited gene flow which has been increasingly supported by the non-physic barrier of various climatic niches. We documented the pattern of niche divergence in four climatic PCs between *G. lichtenfelderi* and Hainan populations (Fig. 5; 6). The low phylogenetic signal in *Goniurosaurus* was strongly influenced due to the heterogeneity of occupied climate conditions in the *luii* group, in which the high rate of speciation was estimated from the late Miocene to early Pliocene (Fig 5; 6). In particular, three out of given eight species followed the evolutionary pattern of climatic niche divergence in the luii group, including *G. catbaensis* documented in first three PCs, *G. kwangsiensis* in PC2 and PC3, and *G. liboensis* in PC1 (Fig. 5; 6). On the other hand, the remaining species in the *luii* group and all species in the *yingdeensis* group adapted to similar gradients of climatic spaces, representing the high degree of niche conservatism in all climatic PCs. The slow rate of climatic niche evolution in the past probably explains why the first speciation in the *yingdeensis* group took place later, compared to the remaining groups, although its ancestor split much earlier (Fig. 2). Following the convergent climatic pattern, the allopatric speciation in the *luii* and *yindeensis* groups could be explained due to the disjunct distribution offered by the complex karstic ecosystems with multiple micro-ecological niches (Clements et al. 2006), and gradually limited gene flow among *Goniurosaurus* populations. Furthermore, recent studies highlighted the role of large rivers such as the Red River and canyons as potential barriers among *Goniurosaurus* sister taxa (Chen et al. 2014; Qi et al. 2020; Ngo et al. 2021; Zhu et al. 2021).

Morphological and molecular data together with ecological approaches have been commonly employed to clarify taxonomic uncertainties (Rissler et al. 2007; Leache et al. 2009; Zhang et al. 2014). The high overlap of occupied climatic gradients strongly supports the genetic and morphological similarities between *G. kadoorieorum* and *G. luii* (Ngo et al. 2021; Grismer et al. 2021). Given their niche resemblance, we also propose that *G. kadoorieorum* should be classified as a junior synonym of *G. luii*.

Conservation

Because of high number of endemic species, *Goniurosaurus* has been considered one of the most susceptible lizard groups under severe human impacts within the period of the sixth mass extinction of wildlife (Ngo et al. 2019; 2021; 2022; Grismer et al. 2021). Following the pattern of niche conservatism, some sister taxa in the *yingdeensis* group, on Hainan Island and in the *luii* group (e.g. *G. luii* – *G. huuliensis* and *G. gezhi* – *G. araneus*) have evolved within a less diverse ecological gradient. Thus, their adaptive capacities will be likely less flexible to environmental changes, which serves as a warning signal for their high level of vulnerability. Together with general assessments of small population size, restricted and disjunctive distribution (Ngo et al. 2016; 2019; 2021; 2022), these species—with slower evolutionary rates in their climatic niches—are more vulnerable to anthropogenic impacts of demographic decline, habitat fragmentation, and especially climate change. Therefore, higher priorities concerning conservation attention and protection should be immediately implemented to prevent these species from reaching the brink of extinction. One-size-fits-all types of conservation measures might be applicable among sister taxa following the pattern of niche conservatism. However, our recommendations of priority conservation for these species, do not mean that the remaining *Goniurosaurus* species which evolved according to the divergence niche pattern, are unharmed under human impacts. Typically, species that have features of rapid evolutionary rates in the past and broader ecological tolerances, thus quickly adapting toward environmental changes are less vulnerable (Thuiller et al. 2005; Schluter 2009; Lavergne et al. 2013; Smith et al. 2019; Ahmadi et al. 2021). However, species distribution modellings (SDMs) recently predicted potential distributions of *G. catbaensis, G. lichtenfelderi* (following the niche divergence pattern) and *G. huuliensis* (following the niche conservatism) and evaluated impacts of climate change. Consequently, their wild populations were all predicted to be negatively impacted under all scenarios of climate change, and their refugia even completely vanished under a scenario of the most intense effect in the near future (Le et al. 2017; Ngo et al. 2021; 2022). The threat of extinction is even coming earlier when other severe human impacts have been documented as well, including over-exploitation for the pet trade, habitat loss, and pollution (Ngo et al. 2019; 2021). Although approaches to identify the pattern of niche evolution of *Goniurosaurus* are necessary for conservation priorities, the species' endangered level should be further assessed based on comprehensive biology knowledge of population status, ecology, genetics, and impacts of human actions. The combination of biological knowledge is expected to significantly improve the efficacy of conservation plans and actions to safeguard species of *Goniurosaurus* in the future.

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Section 1.3.

Table S1. Sixteen *Goniurosaurus* species of three groups in China and Vietnam and their detailed information of geographic locations and habitat types (Question mark indicates unknown distribution).

Species	**Habitat**	**Site**	**Country**	**Coordinates**	**Source**
lichtenfelderi	Granite	Island – Mainland	Vietnam – China	44	
G. lichtenfelderi	Granite	Island – Mainland	Vietnam – China	35	Orlov et al. (2008); Ngo et al. (2022)
G. hainanensis	Granite	Island	China	4	S. Qi. pers. obs.; L. Grismer. pers. obs.
G. bawanglingensis	Granite	Island	China	4	Grismer et al. (2002)
G. zhoui	Karst	Island	China	1	Zhou et al. (2018); S. Qi. pers. obs.
luii	Karst	Island – Mainland	China – Vietnam	79	
G. luii	Karst	Mainland	Vietnam – China	27	Grismer et al. (1999); Vu et al. (2006)
G. catbaensis	Karst	Island	Vietnam	16	Ziegler et al. (2008); Ngo et al. (2019a)
G. huuliensis	Karst	Mainland	Vietnam	29	Orlov et al. (2008); Ngo et al. (2021)
G. araneus	Karst	Mainland	China (Vietnam?)	1	Grismer et al. (1999); Chen et al. (2014)
G. gezhi	Karst	Mainland	China	1	Zhu et al. (2020)
G. liboensis	Karst	Mainland	China	2	Wang et al. (2013)
G. kwangsiensis	Karst	Mainland	China	1	Yang and Chan (2015)
G. kadoorieorum	Karst	Mainland	China	1	Yang and Chan (2015)
yingdeensis	Karst	Mainland	China	38	
G. yingdeensis	Karst	Mainland	China	11	Wang et al. (2010); S. Qi. pers. obs.
G. zhelongi	Karst	Mainland	China	16	Wang et al. (2014); S. Qi. pers. obs.
G. gollum	Karst	Mainland	China	2	Qi et al. (2020a)
G. varius	Karst	Mainland	China	9	Qi et al. (2020b)
Total – 16 species	**Granite – Karst**	**Island – Mainland**	**China – Vietnam**	**160**	

2.3. Ecological niche overlap of two allopatric karst-adapted tiger geckos (*Goniurosaurus*) from northern Vietnam: Microhabitat use and implications for conservation

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Ecological niche overlap of two allopatric karst-adapted tiger geckos (*Goniurosaurus*) from northern Vietnam: Microhabitat use and implications for conservation

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ABSTRACT

Anthropogenic pressures have caused a substantial decline of global biodiversity and have been further reported to strongly affect the ecological performance of species in their habitat, especially reptiles. Understanding the ecology of species and how species respond to habitat alterations is basic knowledge to develop conservation programs and address biodiversity loss. All five species of tiger geckos (*Goniurosaurus*) in Vietnam are known to be threatened by extinction due to anthropogenic impacts such as habitat degradation and harvesting for the international pet trade. However, conservation actions have only been initiated for the better-studied species. This study provides detailed basic data on microhabitat use of two allopatric tiger gecko species which are sister taxa (*Goniurosaurus huuliensis* and *G. luii*). In total, 145 geckos (including 59 records of *G. huuliensis* and 86 records of *G. luii*) were observed during field surveys. All *Goniurosaurus* individuals were mostly recorded in the forest on karst formations, covered with evergreen broad-leaved woody trees, intermixed with ferns, shrubs and vines. Microhabitats of the two species were relatively similar in other traits, such as high vegetation coverage, high humidity, stable ambient temperature and dry-rock substrates. A multiple factor analysis supported that the ecological niche spaces of the two species highly overlap, even though their distribution ranges are geographically separated. We further found no intra-specific niche segregation in both species. The present data provide baseline knowledge for both *in-situ* and *ex-situ* conservation measures to protect species in the genus *Goniurosaurus*.

KEYWORDS

Core refugia, *Goniurosaurus huuliensis, Goniurosaurus luii*, human impacts, karst-dwelling, niche conservatism.

Introduction

A species’ distribution range is limited by multiple constraints of species’ physiological tolerances, biotic interactions and dispersal barriers. Within the distribution range, the realized niche space of a species contains the necessary resources to maintain a stable population density and allow reproduction (Grinnel 1917; Schoener 1974; Toft 1985; Van Buskirk 2005; Bowles et al. 2006; Indemaur et al. 2010; Menin et al. 2011). A non-equilibrium of ecosystems due to past and ongoing human impacts may strongly affect ecological performances of species, especially of ectotherms, and may consequently lead to a substantial decline in the species' abundance or even to species loss (Huey 1991; Sunday et al. 2014). Monastersky (2014) indicated that habitat loss/degradation and climate change have been regarded as the most severe threats to the global biodiversity and are assumed to become worse over time. Understanding the ecology of species and how species respond to natural alterations under human impacts are highly necessary to propose adequate conservation measures in order to protect species and ecosystems more effectively (Lichstein et al. 2002; Amo et al. 2007; Pelegrin et al. 2009).

Karst and granite formations from the Ryukyu Archipelago, Japan southwards through southern China (including the Hainan Archipelago) to northern Vietnam (including the Gulf of Tonkin) provide various natural habitats for a total of 24 *Goniurosaurus* (i.e. "Tiger gecko") species (Grismer et al. 1999; Orlov et al. 2008; Ziegler et al. 2008; Nguyen et al. 2009; Yang and Chan 2015; Honda and Ota 2017; Zhou et al. 2018; Qi et al. 2020a, b; Zhu et al. 2020a, b; Grismer et al. 2021; Ngo et al. 2021b; Uetz et al. 2022). Owing to restricted geographic ranges and small population sizes, all species of *Goniurosaurus* are considered to be susceptible and vulnerable to anthropogenic impacts, such as habitat degradation and climate change (Yang and Chan 2015; Ngo et al. 2016, 2019b, 2021a, 2022). Besides, several tiger gecko species have been over-exploited to supply the international pet trade (Ngo et al. 2019b), which has already led to local extirpations in a few areas in China and Vietnam (Grismer et al. 1999; Stuart et al. 2006; Yang and Chan 2015; Ngo et al. 2016; Ngo et al. 2019b). Consequently, all species of *Goniurosaurus* have been recently listed in CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) to regulate the international trade (Ngo et al. 2019b; CITES Notification No. 2020/068), whereof 17 species have been evaluated to be threatened (listed as VU, EN and CR) in the IUCN Red List (IUCN 2021).

In Vietnam, four tiger gecko species, namely *G. araneus, G. catbaensis, G. huuliensis* and *G. luii*, have been found exclusively in evergreen forests on karstic formations, and only *G. lichtenfelderi* from granitic-stream habitats (Nguyen et al. 2009; Nguyen 2011; Grismer et al. 2021; Ngo et al. 2021b). Due to their small population sizes and high rates of exploitation for the pet trade, all Vietnamese tiger geckos are further nationally protected by the Governmental Decree 06/2019/ND–CP (Management of endangered, precious and rare species of forest fauna and flora in Vietnam) Group IIB, thus, exploitation and trade in wild animals of these species are not allowed without permits proved by relevant national authorities (Ngo et al. 2019b). Based on studies of the population status and the species ecology, *in-situ* conservation measures have been implemented to protect wild populations of *G. catbaensis* in Ha Long Bay and Cat Ba National Park in northern Vietnam (see Ngo et al. 2016, 2019a). Using species distribution models (SDMs), Ngo et al. (2021a) predicted the potential distribution and identified the core refugia for *G. lichtenfelderi*. Ngo et al. (2022) used these SDMs results, coupled with detailed descriptions of microhabitat use, as a scientific foundation to kick-off conservation activities for this species. In the case of *G. araneus,* despite extensive surveys over the last two decades, its distribution in Vietnam has been questionable (Grismer et al. 1999; Ngo et al. 2021b). For the two remaining species, *G. luii* and *G. huuliensis*, their ecology and habitat use have been poorly studied so far.

The Huulien Tiger gecko, *G. huuliensis*, was considered endemic to its type locality in Huu Lien Nature Reserve (NR), Lang Son Province, northern Vietnam (Figs. 1, 2C) (Orlov et al. 2008, Nguyen et al. 2009, Nguyen 2011). Recently, new populations of *G. huuliensis* were discovered outside of Huu Lien NR, viz. Than Sa - Phuong Hoang Nature Reserve in Thai Nguyen Province (Fig.1; Ngo et al. 2021b). The Lui Tiger gecko, *G. luii*, was first discovered in southern China and afterward found in northern Vietnam (Figs. 1, 2D) (Grismer et al. 1999; Vu et al. 2006). Based on morphological and phylogenetic analyses, the two tiger gecko species are considered as sister taxa in the *G. luii* group, consisting of seven more relatives (Orlov et al. 2008; Ngo et al. 2021b; Grismer et al. 2021).

Due to the small area of occupancy, wild populations of the two species are assumed to be relatively small and highly susceptible to anthropogenic impacts (Ngo et al. 2016; 2019b; 2021a). A preliminary population size estimation for *G. luii* indicated that its wild populations are indeed rather small with only a few individuals per locality (Ngo et al. 2016). For *G. huuliensis,* it was predicted that the potential distribution will be diminished or even completely vanished by the 2070s due to climate change (Ngo et al. 2021a). Recently, *G. huuliensis* – Critically Endangered (CR) and *G. luii* – Vulnerable (VU) were assessed in the IUCN Red List (Nguyen 2018; Nguyen et al. 2021).

The present study aimed to provide insights to the microhabitat characteristics of the two less studied tiger gecko species and assessed intraspecific and interspecific differences in their habitat use. Despite their geographical distribution being contiguous, the two tiger gecko species occur in allopatry (Ngo et al. 2021b). We herein assessed whether there is niche partitioning between *G. huuliensis* and *G. luii* pointing to a nonphysical barrier. This ecological knowledge will provide a basis for *in-situ* and *ex-situ* conservation measures of tiger geckos and their habitats.

Materials and methods

Study sites

Study sites were selected based on previous observations, literature and interviews with local people and rangers in northern Vietnam. *Goniurosaurus huuliensis* is known from its type locality in Huu Lien NR, Lang Son Province, northern Vietnam at elevations from 300 up to 370 m a.s.l. (Figs. 1, 2A) (Orlov et al. 2008). The landscape of Huu Lien Nature Reserve is predominately covered by evergreen forests on karst formations. In particular, 9,734 ha or 91% of the nature reserve is limestone karst, of which 9,082 ha (93% of the total) is forested (Anon 1990). In Vietnam, *G. luii* has been found from Ha Lang and Trung Khanh districts of Cao Bang Province along the border between China and Vietnam (Grismer et al. 1999, Vu et al. 2006), and was afterward recorded in Trang Dinh District of Lang Son Province (Ngo et al. 2021b), in close proximity to its type locality in China (Figs. 1, 2B).

Field surveys

Field surveys were conducted to collect data on microhabitat parameters for *G. luii* in June 2014, May, July and September 2019, and for *G. huuliensis* in April and August 2019 as well as in June 2020. We carried out a few daytime excursions to interview local people for potential unknown sites of occurrence and to assess the presence of human impacts in the forest. A total of 17 survey transects, comprising five transects in the area of occupancy of *G. huuliensis* and 12 in the area of occupancy of *G. luii,* were set up along forest paths or patrolling trails of forest rangers and were selected based on evidence for the presence of the species in the area. Crop hills in close proximity to the survey transects were also checked for the occurrence of tiger geckos. Night excursions were conducted between 20:00 and 05:00 of the next day, since the target species are nocturnal and spend their daytime hidden in rocky crevices (Grismer et al. 1999; Werner et al. 2005; Vu et al. 2006; Orlov et al. 2008; Nguyen 2011). During the few daytime surveys, no animals were recorded. Animals were captured by hand and subsequently released at their collecting site after taking measurements of snout-vent length, body temperature and sexual identification.

Microhabitat characterization

Regarding microclimatic parameters, the air temperature [^{0}C] and relative air humidity [%] at each location of captured animals were measured with a digital thermometer (TFA Dostmann/Wertheim Kat. Nr.30.5015). We used an infrared thermometer (Measupro IRT20) to measure temperatures [^{0}C] at the substrate surface and at the body surface of animals (on the belly). We further documented the weather conditions of each surveyed day (raining or not).

The following microhabitat characters were further recorded: substrate type (classified as rock (bare rock), rock – moss (rock covered with moss), rock – vines – leaves (rock covered with vines and leaves), wood, soil, rock on ground), substrate condition (dry or wet), substrate angle [^{0}] (between the substrate surface axis and the horizontal axis, ranged from 0^{0} – 90^{0}), height [m] (vertical distance from the ground to the animal), exposure (outside or inside cave/crevice) and canopy [%] (percentage of vegetation coverage above each animal – estimated by direct observation). The activity status of each animal was recorded as resting, feeding or moving. In addition, the time of observation was recorded. Coordinates of captured individuals, together with elevation values, were recorded with a GPS Garmin 64. The accurate data of coordinates will be only shared upon request by the authors.

To identify intra-specific differences in the microhabitat use, sex of each captured individual was determined based on the presence (in males) or absence (in females) of large swollen hemipenal bulges. Each individual was assigned to an age class by measuring the body length (Snout-vent length: SVL < 85 mm = juveniles, SVL ≥ 85 mm = adults; see Ngo et al. 2021b).

Statistical analyses

Statistical analyses were performed using R.3.1.2 (R Core Team 2018). A Shapiro–Wilk test was used to check the assumption of normality. A Kruskal–Wallis test, combined with a Mann–Whitney pairwise test, was performed to determine differences of microhabitat parameters among age and sex classes. A Wilcoxon test was used to identify the difference in habitat use between *G. huuliensis* and *G. luii*. Additionally, we used a Chi-square test to examine intra- and inter-specific differences of categorical variables. A Linear Regression was computed to test for the linear relationship between body and air/ substrate temperatures. For these tests, we applied a significance level of P-value = 0.05.

We performed a Multiple Factor Analysis (MFA) by using the collected ecological dataset, comprising two qualitative groups – “Species”, “Habitat” (e.g. activity status, occupied position, substrate type, substrate condition) and four quantitative groups – “Canopy”, “Climate” (e.g. humidity and air temperature), “Elevation” and “Micro-position” (e.g. occupied height). This ordination test was performed by using the packages “factoextra” (Kassambara and Mundt 2020) and “FactoMinerR” (Le et al. 2008) in the software R. The approach indeed was applied to identify active groups and variables that account most for the variation within the dataset. Similar coded colors in the MFA scatter plot surrounded with convex hulls were presented in order to visualize the ecological niche space of *G. huuliensis* and *G. luii*. Their ecological spaces were presented within two different spatial coordinates of dimension axes (Dim1 and Dim2; Dim3 and Dim4 – showing the highest eigenvalues). To evaluate the overlap, the first four Dim-values of each tiger gecko individual were extracted to identify the difference between the two species using the Wilcoxon test.

Results

Microhabitat use

The microhabitat characterization for the two species was based on an ecological dataset of 145 individuals of *Goniurosaurus* observed during field surveys. Thereof 59 individuals (1 juvenile, 34 females, 20 males, 4 unsexed adults) were identified as *G. huuliensis* and 86 individuals (11 juveniles, 41 females, 30 males, 4 unsexed adults) were assigned as *G. luii*. *Goniurosaurus huuliensis* was found at various elevations from 176 up to 500 m a.s.l. (384 ± 9.6 m), whereas *G. luii* was documented at higher elevations from 338 up to 719 m a.s.l. (465 ± 8.9 m) (Fig. 3H, Table 1; Wilcoxon test, W = 740, P-value < 0.001). Individuals of the two species were mainly recorded in the forest on limestone formations, covered with evergreen broad-leaved woody trees, intermixed with ferns, shrubs and vines (Figs. 2A, B). Only three individuals of *G. luii* were found on single rocks in two crop hills in Cao Bang Province. Usually, the two species were not evenly distributed along the transects, rather found more clumped at a few sites, often around large cave formations.

The mean ambient air temperature in habitats of *G. huuliensis* was 26.7 ± 0.2 ^{0}C (22.7 – 28.9 ^{0}C, n = 58), which was slightly higher than that in habitats of *G. luii* (25.6 ± 0.2 ^{0}C, 21.9 – 28.6 ^{0}C, n = 71) (Fig. 3A, Table 1; Wilcoxon test, W = 2984, P-value < 0.001). This difference was also documented for substrate and body temperatures, with significantly higher values for *G. huuliensis* (Figs. 3B, C, Table 1, Wilcoxon tests, P-values < 0.05). The body temperature was positively correlated with the ambient air temperature as well as the substrate temperature (Linear regression, $r_s > 0$, P-values < 0.001). The relative humidity was similar in habitats of both species and ranged between 58 – 100 % (Fig. 3D, Table 1; Wilcoxon test, W = 2205, P-value = 0.7 > 0.05).

With regard to the substrate type, the majority of individuals of both species were found on bare rock (at least 72.9 %), while *G. luii* was never found on rocks on the ground (Fig. 4A). Both species were rarely observed on other substrates such as wood or the forest floor (Figs. 4A1, 4A2). Individuals of *G. luii* were generally found sitting on steeper angles (mean 68.3^0, n = 24) than *G. huuliensis* (mean 55.3^0, n = 34) (Fig. 3E, Table 1; Wilcoxon test, W = 451, P-value < 0.05). The majority (at least 75%) of animals in both species were found outside of, but in the close vicinity to, caves and rocky crevices as well as on dry substrates (Figs. 4B1, B2, D1, D2; Chi-square tests, P-values > 0.05). The canopy coverage was relatively less dense in microhabitats of *G. huuliensis* (65.18 ± 4.0 %; 5 – 100 %, n = 59) compared to *G. luii* (83.73 ± 2.9 %; 0 – 100, n = 71) (Fig. 3G, Table 1; Wilcoxon test, W = 1347, P-value < 0.001). We found *G. huuliensis* at an average height to the ground of 0.7 ± 0.2 m (0 – 2.5 m, n = 59), which was relatively lower than in *G. luii* (0.9 ± 0.1 m, 0 – 3.0 m, n = 86) (Fig. 3F, Table 1; Wilcoxon test, W = 1440, P-value < 0.001). During night surveys, the majority of animals were found resting (*G. huuliensis*: 67.8 % – 40 individuals; *G. luii*: 85.9 % – 61 individuals), whereas a few individuals of both species were found actively foraging and feeding (Chi-square test, $Chi^2 = 4.89$, d.f. = 2, P-value > 0.05). An individual of *G. luii* was observed feeding on a large cave cricket. Regarding intraspecific comparisons, we did not find any significant differences in the microhabitat use between sexes or age classes (Chi^2-tests and Kruskal-Wallis' tests, P-values > 0.05).

No individual of both species was encountered during the daytime surveys. At night, both species were observed active from approximately 20:00 h to 03:00 h, with the exception of one individual of *G. luii*, which was observed between 3:00 h – 5:00 h (Fig. 5). The recorded time of highest activity in both species was between 21:00 h – 01:00 h (n = 103; Fig. 5).

The MFA analysis identified ecological sets of habitat, climate, canopy and elevation as active groups (Fig. 6A) and the first four important dimensions (Dim 1 – 4) accounted for 50.3 % of the variation in the data set (Fig. 6C). In particular, dimension 1 accounted for

17.1 % of the variation, and was loaded most heavily by canopy coverage, elevation and two climatic variables (air temperature and humidity). Three remaining dimensions accounted for slightly lower percentages of observed variation and were loaded primarily by elevation (Dim 2), Canopy (Dim3) and Humidity (Dim4) (Fig. 6B). The MFA analysis further showed that the ecological space of *G. huuliensis* highly overlaps with that of *G. luii* according to dimension axes of Dim 1, Dim 3 and Dim 4 (Fig. 6C; Wilcoxon tests, P-values > 0.05). However, dimension 2 (Dim 2) is significantly different between the two tiger gecko species (Fig. 6C-1; Wilcoxon test, W = 594, P-value < 0.001).

Habitat degradation

Limestone mountains as the habitat of the two tiger gecko species have been destroyed and quarried to extract materials for cement production, and to expand road constructions and infrastructure associated with urbanization (Fig. 7A). Furthermore, a large area of the protected forests were found strongly fragmented due to timber logging activities, and those areas were gradually replaced by industrial crops or became grassy hills (Fig. 7B).

Discussion

Microhabitat characterization

The present microhabitat characterization confirmed previous observations, namely that *G. huuliensis* and *G. luii*, are indeed limestone karst-dwelling lizards in evergreen forests (Grismer et al. 1999; Orlov et al. 2008; Nguyen 2011; Grismer et al. 2021; Ngo et al. 2021b). Only three individuals of *G. luii* were found on single rocks in crop hills, in close proximity to the forest, which might be only temporary sites, visited during foraging. In general, the microhabitats of the two species were highly similar, characterized by the high vegetation coverage, high humidity, stable ambient temperature and the presence of dry rocks. Rock crevices of open caves, as well as single karstic rocks, were identified as shelters for *G. huuliensis* and *G. luii* to escape from predators and to hide during the day. These characteristics are relatively similar to those of another karst-dwelling sister species, namely *G. catbaensis*, which occurs on isolated islands in northern Vietnam (Ngo et al. 2019a). However, the MFA ordination test of niche spaces for *G. huuliensis* and *G. luii* revealed that their slight discordance is mainly related to an elevation segregation (Fig. 6C).

Not surprisingly, the body temperature of each species was positively correlated with the environmental temperatures, confirming that physiological functions are interlinked with environmental conditions as for most ectothermic species (van Schingen et al. 2015; Ngo et al. 2018, 2019a; Vicente et al. 2019). Single tests documented interspecific differences in air and substrate temperatures between microhabitats of the two species. These differences might be explained by naturally varying weather conditions among the relatively short survey periods. Therefore, we recommend long-term monitoring to assess potential effects of environmental conditions on the adaptation of the two tiger geckos. We further noted a wide range in humidity (58 – 100%) in habitats of both tiger gecko species, whereof the lowest values (humidity < 65%, n = 29) were mostly recorded before 0:00 h (about 86 %). Probably, the different daytimes and weather events, such as rains cause this high range in humidity.

Intraspecific competition was frequently reported for high-density populations which share resources, such as mating and nest-sites, especially when the optimal space is limited (Irschick et al. 2005; van Schingen et al. 2015; Ngo et al. 2016). Niche partitioning can reduce the pressure of intra-competition and has been documented in other Vietnamese lizards regarding the height of the resting position from the ground that differed between age classes (e.g. *G. catbaensis, Cnemaspis psychedelica, Shinisaurus crocodilurus*) (van Schingen et al. 2015; Ngo et al. 2018, 2019a). In the present study, we did not record any significant difference in the microhabitat characteristics among age or sex classes in both species. This could be due to the low observed population densities, which might mitigate the frequency of encounters (e.g. male – male) and thus limit intraspecific competition.

Allopatric speciation

Previous studies and our observations have never documented *G. luii* and *G. huuliensis* at the same locality (Vu et al. 2006; Orlov et al. 2008; Ngo et al. 2016), thus, they are considered to be allopatric in northern Vietnam. In particular, *G. luii* is currently known from areas stretching across the North of Lang Son Province, northwards of Cao Bang provinces, northern Vietnam to southern China. *Goniurosaurus huuliensis* has been recorded in similar karstic formations, but only in isolated areas from the South of Lang Son Province (Fig. 1) (Grismer et al. 1999; Orlov et al. 2008; Nguyen 2011; Ngo et al. 2021b). Ecological conditions are considered the major factors of non-physical barriers that determine range limits, as well as being a driver of speciation (MacArthur 1984; Orr and Smith 1998; Schluter 2009; Glor and Warren 2011). Disjunct karst formations along the elevation gradient can facilitate isolated microhabitats in close proximity with one another, which can promote allopatric speciation as a result of the long-term reproductive isolation and genetic drift (Körner 2004; Clements et al. 2006; Hoorn et al. 2018). Although the species occurrences are geographically separated and differ in elevation (176 up to 719 m a.s.l), we found that their micro-niche space highly overlapped and that microhabitat characteristics are similar between the two species, which indicates a high level of niche conservatism (Wiens and Graham 2005; Wiens et al. 2010; Pyron et al. 2014). That would be in line with the morphological and genetic similarities between the two phylogenetic sister species (Grismer et al. 2021; Ngo et al. 2021b). Recent studies have highlighted the role of large rivers and canyons as potential barriers among *Goniurosaurus* sister taxa in continental sites from China and Vietnam (Chen et al. 2014; Qi et al. 2020a; Ngo et al. 2021b; Zhu et al. 2020a). The cladogenesis, viz. the allopatric speciation of *G. huuliensis* and *G. luii* could be explained by the emergence of a local river (Ky Cung River) in Lang Son Province (Fig. 1), which might have limited interbreeding populations. Notably, distribution ranges of *G. luii* and *G. araneus* in China are separated by the Zuojiang River (Chen et al. 2014), which is coupled with Ky Cung River and has likely established the continuous eastern distribution boundary of *G. luii* (Fig. 1).

Conservation

The present microhabitat characterization of *G. huuliensis* and *G. luii* is intended to assist conservationists in collaboration with local rangers and other relevant authorities to carry out conservation actions more effectively. Typically, karst formations in undisturbed forests provide key habitats for these two species of *Goniurosaurus*. However, as we documented by interviews with local communities and direct observations in the field, several of these unique habitats have been dramatically degraded or destroyed by human disturbances. The less dense coverage of vegetation recorded in wild populations of *G. huuliensis* from Huu Lien NR may be due to timber logging activities. We recommend a higher protection of the species' core refugia by local rangers to ensure the long-term persistence of range-restricted populations and the integrity of their natural habitats. Recently, species distribution models have been applied in order to predict the potential distribution of *G. huuliensis* and identify the core refugia (Ngo et al. 2021a). Based on these models coupled with ecological findings, a proposed plan to establish a protected area for conservation of *G. huuliensis* (Ngo et al. 2021a) promises success in the future. Due to the similarity in microhabitat use, one-size-fits-all type of conservation measures can be applied between the two species, and can even expand for other karst-dwelling *Goniurosaurus* species.

The basic understanding of microhabitat use of tiger geckos contributes to improve husbandry conditions and thereby also enhances the potential success for future reintroductions. In particular, the slight differences in microhabitat characteristics (e.g., lower air and substrate temperatures, steeper angle of substrates, higher perch sites and more dense coverage observed for *G. luii* compared to those of *G. huuliensis*) should be considered for *ex-situ* measures. Recently, *ex-situ* conservation breeding programs for all Vietnamese tiger gecko species have been established at the Me Linh Station for Biodiversity, Vietnam and Cologne Zoo, Germany, which resulted in first and continuing breeding success (Pham et al. 2019, 2021; Ziegler and Rauhaus 2022). The establishment of a conservation breeding network is planned, which already includes several European zoos, and is expanding into the USA (Ziegler and Rauhaus 2022). Both in-country and foreign breeding programs assure that reserve populations can be developed, which could facilitate future repatriation/ restocking if it is required to recover disturbed wild populations.

This study emphasized the importance of studying the natural history of species in order to inform conservation activities. While more species are continuously being described, data on their ecology and threat status are lacking in many cases. Further reptile species have been recently discovered in the study sites during the last decade, for example, *Gekko canhi* (Rösler et al. 2010), *Oligodon nagao* (David et al. 2012), *Gekko adleri* (Nguyen et al. 2013b), *Hemiphyllodactylus zugi* (Nguyen et al. 2013a) and *Achalinus juliani* (Ziegler et al. 2019). Especially considering the ongoing anthropogenic impacts, such as over-exploitation (Ngo et al. 2019), climate change (Ngo et al. 2021) and habitat degradation, further ecological studies on other species are needed to better understand the different roles and interactions of species in their habitats to better protect remaining ecosystems as a whole.

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Bernardes, who assisted during field surveys in 2014. Field surveys were partially funded by Cologne Zoo, the Rufford Foundation (Project: 30597-1). Cologne Zoo is the partner of the World Association of Zoos and Aquariums (WAZA): Conservation Project 07011, 07012 (Herpetodiversity Research, Amphibian and Reptilian Breeding and Rescue Stations). This research was supported by the Vietnam Academy of Science and Technology (Project code: NVCC 09.02/22-23). Research of Hai Ngoc Ngo in Germany is funded by the German Academic Exchange Service (DAAD).

Disclosure statement

No potential conflict of interest was reported by the authors.

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Ecological niche overlap of two allopatric karst-adapted tiger geckos (*Goniurosaurus*) from northern Vietnam: Microhabitat use and implications for conservation

Hai Ngoc Ngo[a,f,g], Huy Quoc Nguyen[b], Phan Quang Tien[c], Hieu Minh Tran[c], Truong Quang Nguyen[c,d], Mona van Schingen-Khan[e], Thomas Ziegler[f,g]

Tables

Table 1. Environmental parameters characterizing the microhabitat of *Goniurosaurus huuliensis* and *Goniurosaurus luii*.

Parameters	***Goniurosaurus huuliensis*** Min – Max (Mean ± SE)	***Goniurosaurus luii*** Min – Max (Mean ± SE)
Elevation [m]	176 – 500 (384 ± 9.6, n = 59)	338 – 719 (465 ± 8.9, n = 71)
Humidity [%]	58 – 100 (75.2 ± 1.5, n = 58)	59 – 85 (74.0 ± 0.9, n = 71)
Air Temp [^{0}C]	22.7 – 28.9 (26.7 ± 0.2, n = 58)	21.9 – 28.6 (25.6 ± 0.2, n = 71)
Substrate Temp [^{0}C]	20.4 – 26.8 (24.0 ± 0.3, n = 32)	17.7 – 28.2 (23.0 ± 0.2, n = 64)
Body Temp [^{0}C]	20.2 – 27.1 (24.2 ± 0.3, n = 32)	17.5 – 28.5 (23.3 ± 0.2, n = 64)
Canopy coverage [%]	5 – 100 (65.18 ± 4.0, n = 59)	0 – 100 (83.73 ± 2.9, n = 71)
Height [m]	0 – 2.5 (0.7 ± 0.2, n = 59)	0 – 3.0 (0.9 ± 0.1, n = 86)
Position angle [^{0}]	0 – 90 (55.3 ± 5.4, n = 34)	20 – 90 (68.3 ± 3.9, n = 24)

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Figures

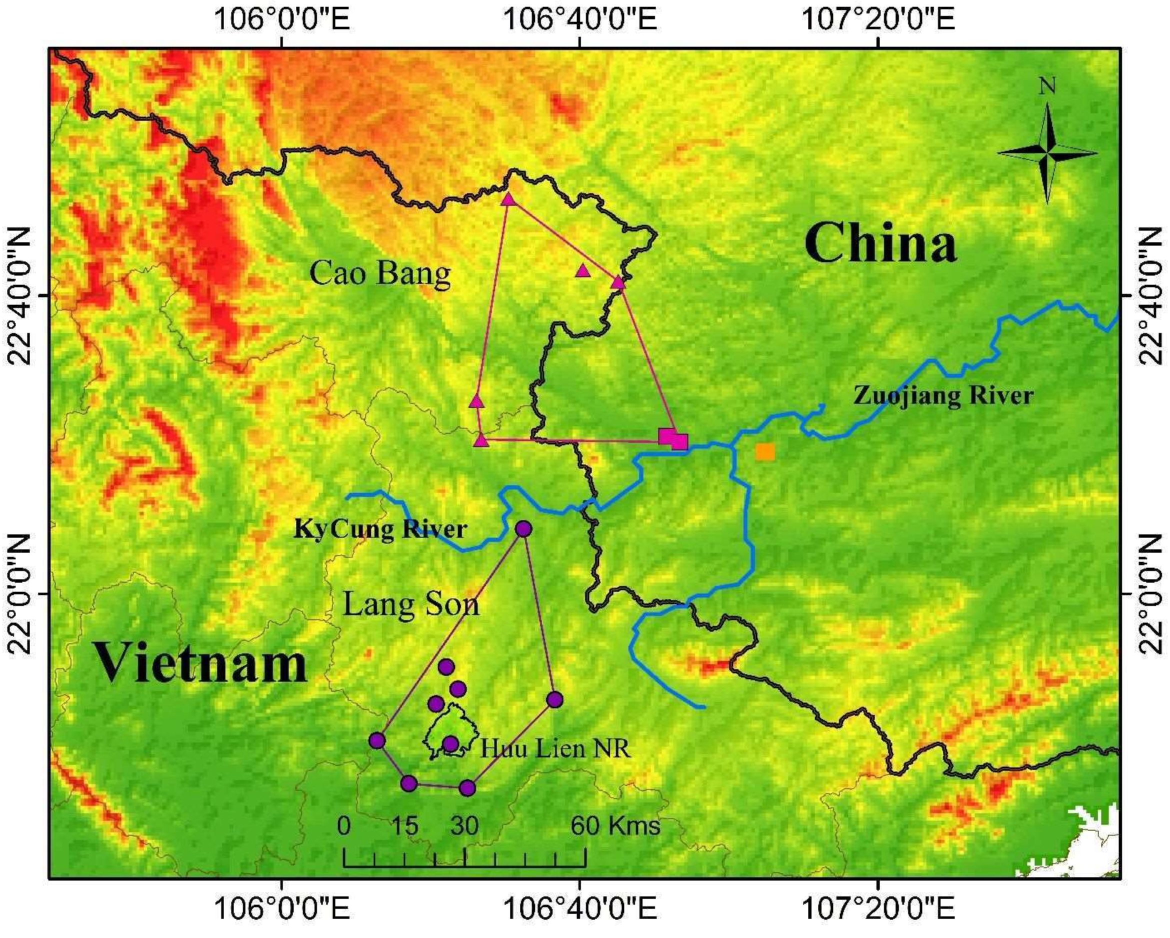

Figure 1. Geographic distribution of *Goniurosaurus huuliensis* (violet circles covered by a violet line) and *Goniurosaurus luii* (pink triangles – northern Vietnam; pink squares – southern China – covered by a pink line). An orange square represents the distribution of another tiger gecko species, namely *Goniurosaurus araneus* in China.

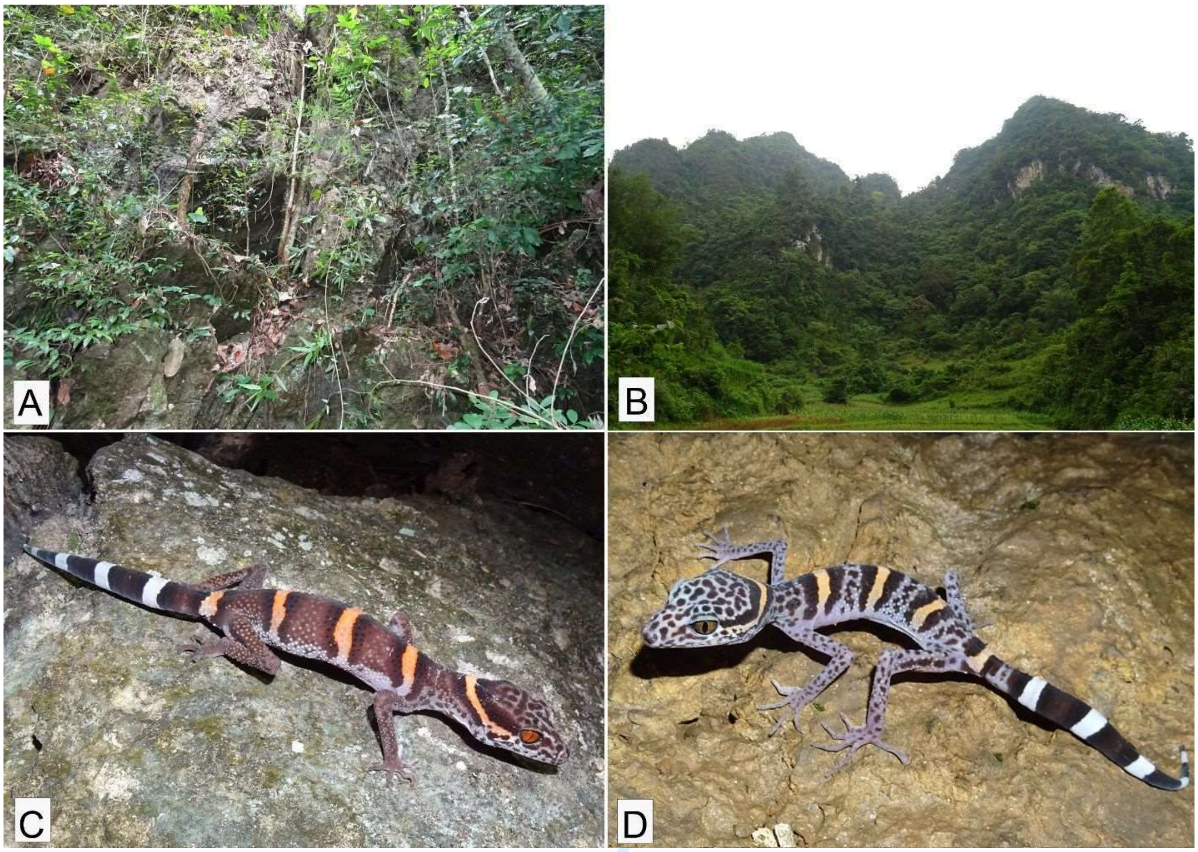

Figure 2. A. Karst mountain inhabited by *Goniurosaurus huuliensis*; B. Microhabitat of *Goniurosaurus luii*; C. *G. huuliensis*, and D. *G. luii* on rock substrate (Photographed by Hai N. Ngo).

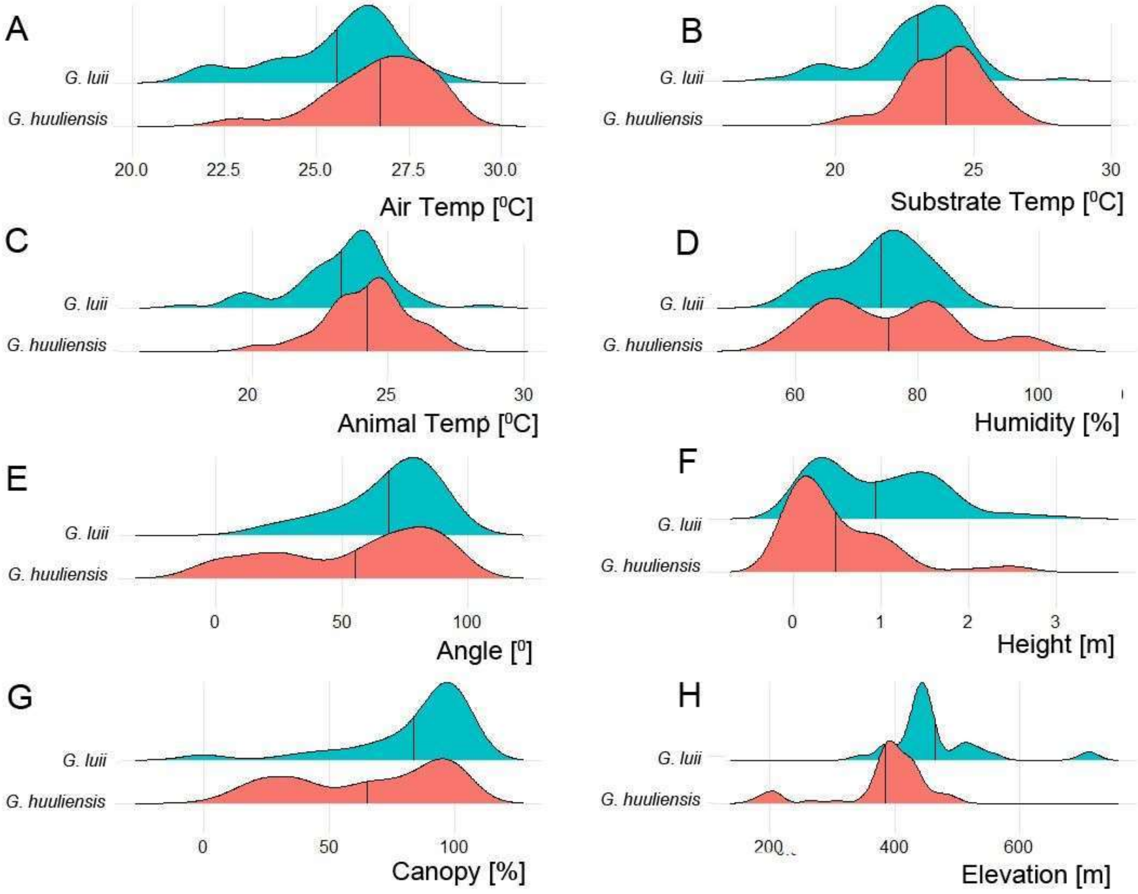

Figure 3. Microhabitat characters of *Goniurosaurus huuliensis* and *Goniurosaurus luii.* A. Air temperature; B. Substrate surface temperature; C. Animal temperature; D. Relative air humidity; E. Substrate angle; F. Occupied height; G. Canopy coverage; H. Elevation.

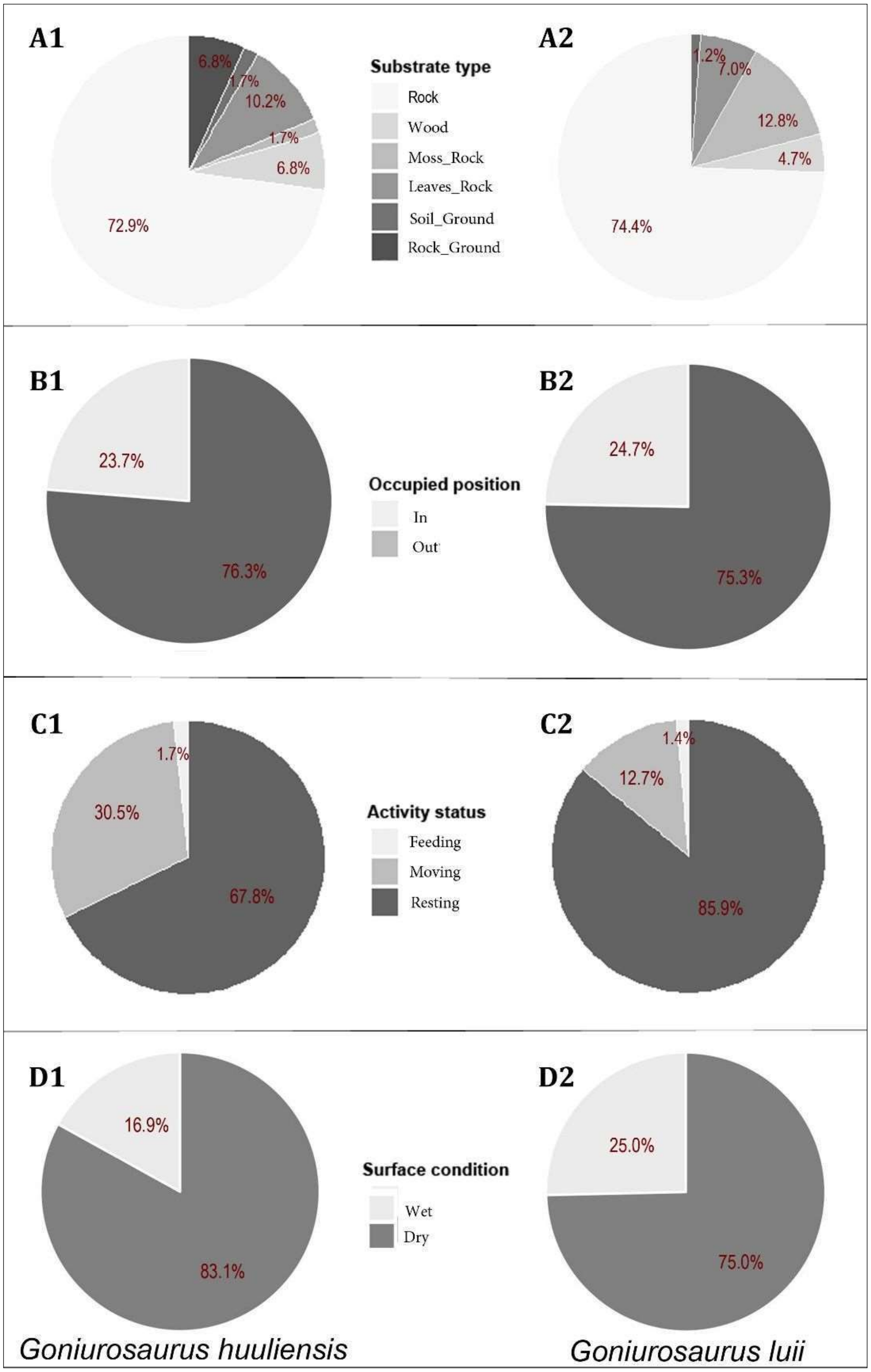

Figure 4. Microhabitat parameters and activity of *Goniurosaurus huuliensis* (1) and *Goniurosaurus luii* (2) A. Substrate type; B. Position to cave/ crevice; C. Activity status; D. Substrate moisture.

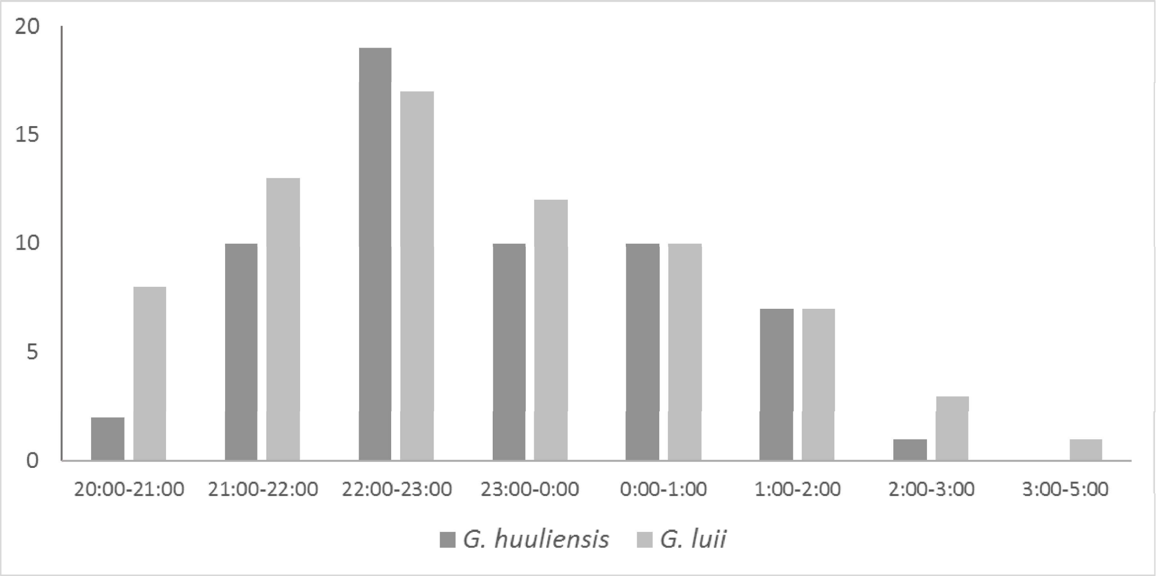

Figure 5. The number of observed individuals of *Goniurosaurus huuliensis* and *Goniurosaurus luii* at different time intervals.

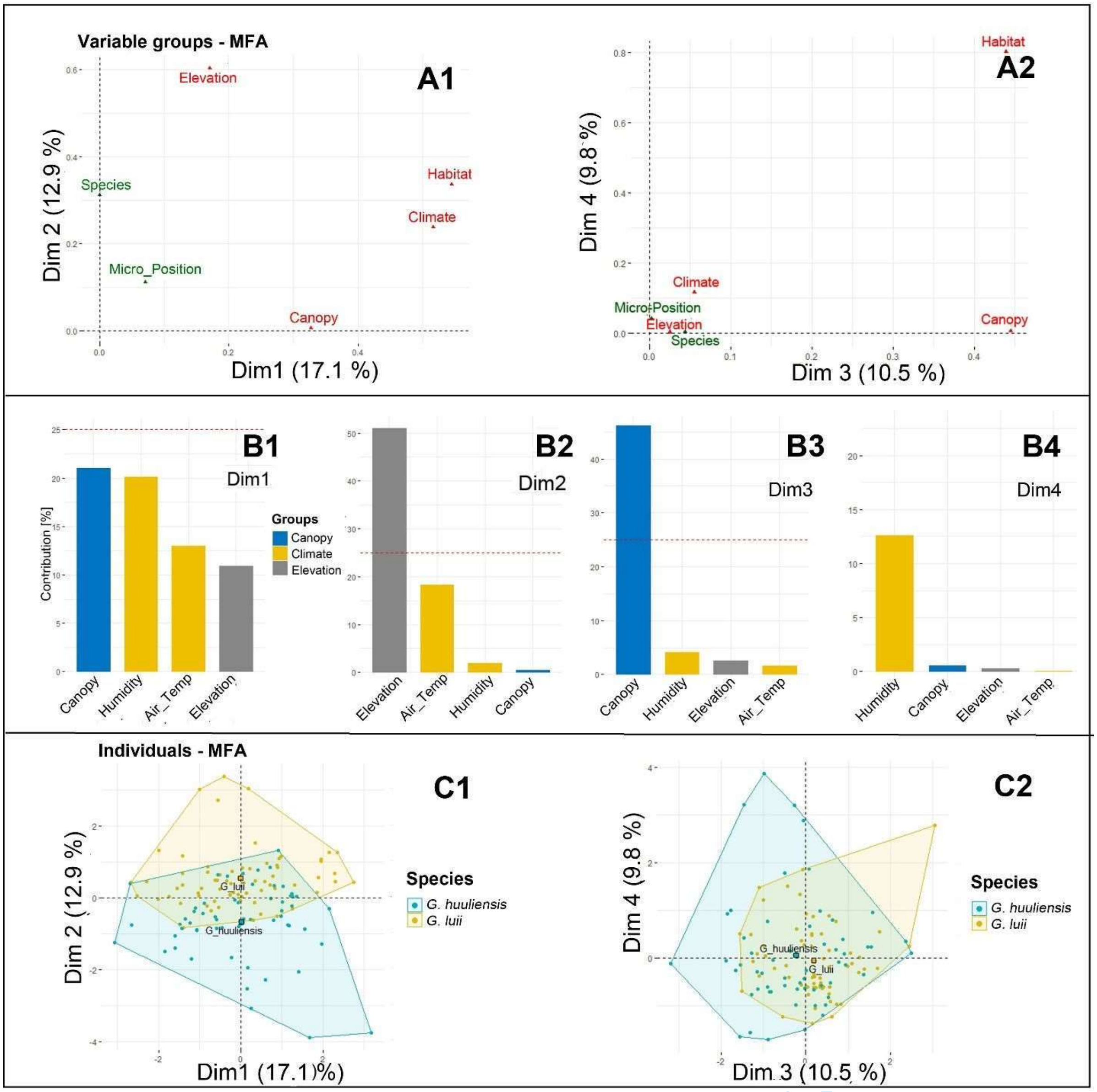

Figure 6. A. Scatterplots of all variable groups on coordinates of (1) Dim1 – Dim2, (2) Dim3 – Dim4 axes in the Multiple factor analysis (MFA); B. The first four important variables in the contribution of (1) Dim1, (2) Dim2, (3) Dim3, (4) Dim4; C. Scatter diagrams illustrating ecological-spaces of *Goniurosaurus huuliensis* and *Goniurosaurus luii* on coordinates of (1) Dim1 – Dim2, (2) Dim3 – Dim4 axes.

Figure 7. Anthropogenic impacts in natural habitats of *Goniurosaurus huuliensis* and *Goniurosaurus luii* A. Quarrying for cement productions; B. Timber logging (Photographed by Hai N. Ngo).

Appendix 2.

Other Publications during the Thesis research.

1. Ngo N.H., Nguyen Q.T., Nguyen V.T., van Schingen M. and Ziegler T. (2018) Microhabitat selection and communal nesting in the insular Psychedelic Rock Gecko, *Cnemaspis psychedelica,* in Southern Vietnam with updated information on trade. Nature Conservation 31: 1– 16.

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