LINKING ECOLOGICAL, FORENSIC AND MOLECULAR ANALYSES WITH CONSERVATION ASSESSMENT: A CASE STUDY ON THE VIETNAMESE CROCODILE LIZARD

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Summary

The crocodile lizard *Shinisaurus crocodilurus* Ahl, 1930 - a true living fossil - is the only known living representative of the family Shinisauridae. Its current distribution range is restricted to small and isolated relict populations in South China and Northeast Vietnam. The species is strongly associated to freshwater streams within remote and intact evergreen broadleaf forests and exhibits rare reproduction strategies (e.g., ovoviviparity, multipaternity). Its unique biology and outstanding appearance makes *S. crocodilurus* an increasingly desired target species in the international pet trade, which - in concert with multiple further anthropogenic pressures - brought the species at the brink of extinction. While detrimental population declines of *S. crocodilurus* have been recorded in China, respective information on its population status, threats and ecology remained poorly known in Vietnam. The present thesis provides a combination of several ecological, systematic, molecular and forensic methods in order to identify the autecology and conservation status of *S. crocodilurus* in Vietnam and to test the utility of different methods as potential novel tools for conservation implications.

Based on annual population monitoring of *S. crocodilurus* we found, that wild populations are dramatically small, in continuous decline and we even observed several local extirpations in Vietnam within recent years. Poaching as well as ongoing habitat loss and fragmentation were identified as major causes. We further provided new and essential basic knowledge on the ecological adaptation of Vietnamese *S. crocodilurus*, emphasizing its strong ecological specialization. In order to predict the overall distribution of suitable habitats and potential further occurrences of *S. crocodilurus*, we applied species distribution models (SDMs). Following the resulting predictions, we discovered a new *S. crocodilurus* population, affirming the practical utility of such theoretical models. In this context, we also predicted an almost entire loss of suitable habitats by 2080 as consequence of global climate change. To substantiate these prognoses with actual data on temperature selection, we developed a backpack system with thermo data loggers for *S. crocodilurus*, which - being the first of its kind in lizards - proved to be applicable in the field. First data indicated the species small realized thermal niche and selection of cool temperatures, suggesting its vulnerability to climate change.

Since we identified illegal trade as critical and acute threat to *S. crocodilurus* - and mechanisms to detect the potential illegal source of traded specimens are generally lacking - we tested the applicability of isotopic markers to distinguish captive bred from wild caught specimens. This approach was herein trialed for the first time in lizards and revealed promising results, namely a clear separation of the investigated groups. Thus, this novel method might develop as a future tool in wildlife forensics.

A herein applied integrative approach revealed distinct differences in ecological adaptation, morphology and genetics between Chinese and Vietnamese crocodile lizard populations, resulting in the taxonomic separation into at least two subspecies, concordant with the split into two conservation units. This taxonomic split emphasized the immediate need of enforced *in situ* and *ex situ* conservation measures and the establishment of separated conservation breeding programs to retain the genetic integrity of each taxon. Such a conservation breeding program was initiated in the Me Linh Station for Biodiversity, Vinh Phuc, Vietnam - based on the herein conducted ecological and microhabitat analyses - in order to establish a stable reserve population with the goal of a future restocking program. In this context, we identified priority areas for conservation and provided recommendations for the establishment of new reserves and the maintenance of forest corridors, connecting existing reserves with *S. crocodilurus* populations, in order to inform authorities and policy makers. In summary, this thesis clearly demonstrated the suitability of a various spectrum of novel forensic and ecologic tools and highlights the effectiveness of a multidisciplinary strategy in species conservation, exemplary on the crocodile lizard. Furthermore, it also emphasizes the necessity of ground research, population monitoring and assessment of habitat requirements to provide a solid base for advanced techniques, to amend legislations, to protect habitats and - together with the expertise from zoological gardens - to develop and manage *ex situ* conservation programs.

Zusammenfassung

Die Krokodilschwanzechse *Shinisaurus crocodilurus* Ahl, 1930 - ein echtes lebendes Fossil - ist der einzig rezente Vertreter innerhalb der Familie der Shinisauridae. Heute kommt sie nur noch in kleinen, inselartig fragmentierten Reliktbeständen in Südchina und Nordvietnam vor. Die semiaquatische Echse zeichnet sich durch ihre hohe Angepasstheit an kleine Bergbäche in intaktem, immergrünen Tieflandfeuchtwald, sowie durch besondere Reproduktionsstrategien (Ovoviviparie und Multipaternität) aus. Ihre einzigartige Lebensweise und charismatische Erscheinung macht die Krokodilschwanzechse zu einer zunehmend begehrten Zielart im internationalen Lebendtierhandel. Wilderei, im Zusammenspiel mit weiteren anthropogenen Einflüssen, brachten die Krokodilschwanzechse in China bereits an den Rand des Aussterbens, während entsprechende Daten zum Populationsstatus, zu Bedrohungspotentialen und zur Ökologie der Art in Vietnam fehlen. Die vorliegende Arbeit exemplifiziert eine Kombination verschiedener ökologischer, systematischer und molekularer Ansätze, um den Schutz-, sowie den taxonomischen Status und die Ökologie der Krokodilschwanzechse in Vietnam zu erfassen. Weiterhin wurde die praktische Anwendbarkeit verschiedener neuer ökologischer und forensischer Methoden und ihre Eignung als potentielle Werkzeuge für den Artenschutz überprüft.

Unsere jährlichen Bestandsschätzungen ergaben, dass wildlebende Populationen in Vietnam extrem klein sind. Außerdem verzeichneten wir innerhalb der letzten Jahre einen stetigen Populationsrückgang und sogar lokale Ausrottungen. Wilderei und kontinuierlich zunehmender Lebensraumverlust und -fragmentierung identifizierten wir hierbei als Hauptursachen. Außerdem liefert die vorliegende Arbeit essentielle und neue Erkenntnisse über die ökologische Anpassung vietnamesischer Krokodilschwanzechsen und verdeutlicht ihre besondere ökologische Spezialisierung. Um die generelle Verbreitung geeigneter Lebensräume, sowie potentielle noch unbekannte Vorkommen zu prognostizieren, verwendeten wir sogenannte "Nischenmodellierung" (SDMs). Diese Vorhersagen führten zu der Entdeckung einer bislang unbekannten *S. crocodilurus* Population, wodurch außerdem die praktische Anwendbarkeit von SDMs - die zwar in der ökologischer Forschung häufig angewendet, jedoch kaum auf Praktikabilität getestet sind - bestätigt wird. Außerdem berechneten wir mittels SDMs das fast komplette Verschwinden geeigneter Lebensräume bis 2080, als Konsequenz des Klimawandels. Um diese theoretische Vorhersage mit faktischen

Daten zur Temperaturselektion von *S. crocodilurus* im natürlichen Lebensraum zu untermauern, entwickelten wir ein Rucksacksystem mit Temperaturdatenloggern. Dieses System erwies sich im Feld als äußerst tauglich und stellte außerdem die erste Studie ihrer Art mit aquatischen Echsen dar. Erste Daten indizieren eine sehr schmale Realnische bezüglich der Temperatur, nämlich die Selektion von konstant kühleren Temperaturen, wodurch - unter Berücksichtigung der SDMs - eine enorme Gefährdung der Krokodilschwanzechse durch die globale Erwärmung prognostiziert wird.

Da illegaler Handel als besonders akute Gefährdung der Krokodilschwanzechse identifiziert wurde und geeignete Mechanismen, den illegalen Ursprung gehandelter Tiere eindeutig bestimmen zu können grundsätzlich fehlen, testeten wir erstmalig die Eignung von Stabilisotopensignaturen der Haut von Echsen, um legale Nachzuchten von illegalen Wildfängen zu unterscheiden. Diese Pilotstudie erzielte erste vielversprechende Ergebnisse und indiziert das Potential dieser neuen Methode, als zukünftiges forensisches Werkzeug im Artenschutz Anwendung zu finden.

Ein hier angewandter integrativer Ansatz zeigte außerdem, dass sich vietnamesische und chinesische Krokodilschwanzechsen in ihrer ökologischen Einnischung, Morphologie und Genetik unterscheiden, was in einer taxonomischen Separation in mindestens zwei Unterarten resultierte, einhergehend mit der Differenzierung von zwei Schutzeinheiten. Die taxonomische Aufspaltung unterstreicht den unmittelbaren Bedarf erhöhter Schutzmaßnahmen - im Speziellen in Vietnam - sowie den Aufbau getrennter Erhaltungszuchtprogramme, um die genetische Integrität der beiden Taxa zu erhalten. Ein solches Erhaltungszuchtprogramm im Hinblick auf eine zukünftige Wiederauswilderung wurde - basierend auf den vorangegangenen ökologischen Untersuchungen - in der Me Linh Station für Biodiversität, Vinh Phuc Provinz, Vietnam initiiert.

Insgesamt demonstrierte diese Arbeit die Nützlichkeit von diversen neuen forensischen und ökologischen Werkzeugen und betont - am Beispiel der Krokodilschwanzechse - die Effektivität einer multidisziplinären Strategie im Artenschutz. Außerdem wurde die Wichtigkeit von Grundlagenforschung, wie zum Beispiel Populations- und Lebensraumanalysen herausgestellt, welche - zusammen mit der Expertise zoologischer Gärten - die solide Basis für moderne Methoden, Schutzprogramme und Regelungen zum Artenschutz bildet.

1. Introduction

1.1 Global biodiversity crisis: Facing earth's sixth mass extinction

"Nowadays, biodiversity is greater than ever before", with especially high values of species richness and endemic species occurring in the tropics (Collen et al. 2014; Rohde and Muller 2005; Figure 1). However, biodiversity is globally shrinking rapidly with estimated current extinction rates of species that are 100 to 1,000 times higher than natural extinction rates (Ceballos et al. 2015; Pereira et al. 2010; Urban et al. 2012). Causal for this current "biodiversity crisis" are anthropogenic pressures with habitat destruction, climate change, environmental pollution and unsustainable overexploitation of natural resources representing the most severe stressors that - in interaction - may drive numerous species into an extinction vortex (Böhm et al. 2013; Monastersky 2014; Pereira et al. 2010; Fagan and Holmes 2006). In contrast to the earth' previous mass extinctions, caused by natural catastrophes such as volcanism or meteorites, human impacts are currently assumed to cause the earth's sixth mass extinction of species (Barnosky et al. 2011; Ceballos et al. 2015).

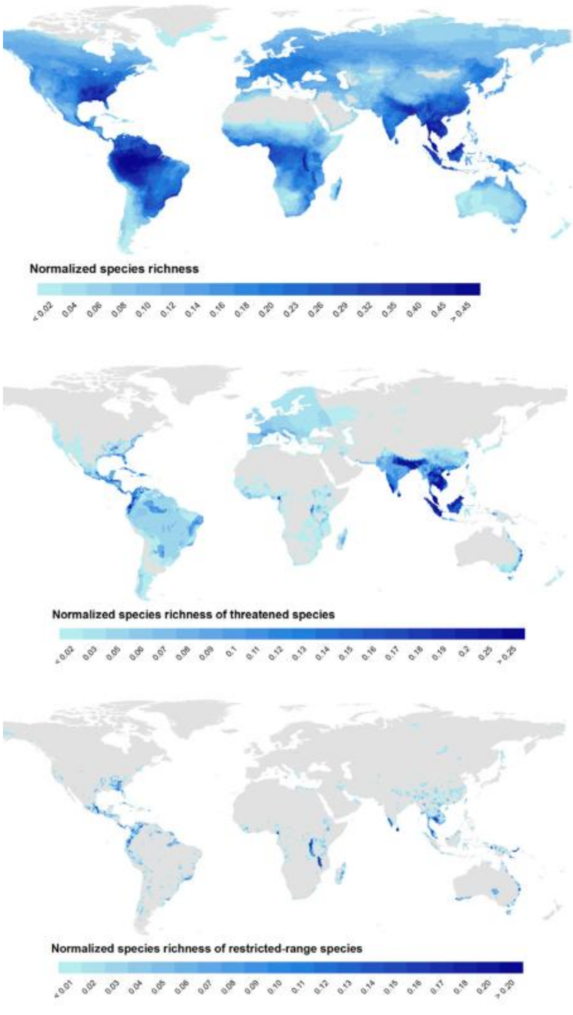

Figure 1. Global patterns of freshwater species diversity, threat and endemism. Obtained from Collen et al. 2014.

Multiple studies provided growing evidence that species associated to freshwater ecosystems, which harbor a rich diversity of species and habitats, are especially vulnerable and in decline (Collen et al. 2009; 2014; Darwall et al. 2011; Galewski et al. 2011). Even though freshwater systems cover less than 1% of the earth's surface, they provide important services and goods, including market goods such as drinking water, transportation, electricity generation, pollution disposal, and irrigation as well as non-market goods, such as biodiversity (Collen et al. 2014;

Gleick 1993; Wilson and Carpenter 1999). Due to the connectivity of freshwater systems, multiple interacting anthropogenic stresses, such as fragmentation, alteration of flow through dam construction, channelization, overexploitation, increasing levels of organic and inorganic pollution, invasive species and diseases, might cause profound negative impacts on associated species communities (Collen et al. 2014; Darwall et al. 2009; Dudgeon et al. 2006; Revenga et al. 2005, Strayer and Dudgeon 2010; Vörösmarty et al. 2010). On top to these multiple direct stressors, global climate change is expected to perceptibly affect the integrity and function of freshwater systems (Collen et al. 2014; Dudgeon et al. 2006). While the diversity and general extinction risk of species in freshwater ecosystems remain understudied, Collen et al. (2014) demonstrated that extinction risk for freshwater species is consistently higher than for their terrestrial counterparts. However, of the worldwide described species, only 4% have been substantially investigated and assessed, exacerbating to infer solid and universal valid conclusions on species' vulnerability, especially of poorly studied groups such as reptiles (Monatersky 2014).

1.1.1 Excursus 1: The fate of reptiles - Generally overlooked but highly vulnerable

Reptiles evolved more than 250 million years ago, distributed globally, adapted to almost every region and habitat type on earth, developed diverse traits and strategies and reached a peak of diversity and species richness in Asia (Uetz 2000). However, due to the lack of common interest in reptiles compared to more charismatic animals, reptiles are generally overlooked and still imperfectly studied (Böhm et al. 2013). Of the worldwide described reptile species, only about 50% have been assessed by the IUCN (International Union for the Conservation of Nature) Red List of Threatened Species yet, whereof about 20% are classified as “data deficient” (IUCN 2016). It is currently discussed in what extent recent living reptiles are being affected by the current extinction crisis (Böhm et al. 2013). Nevertheless, the number of reptile species assessed for the IUCN Red List in one of the Endangered categories is steadily increasing. To effectively and targeted protect species, comprehensive information about their distribution, systematics, ecology and threat level is essential to highlight urgent conservation cases and to inform respective authorities and politicians with appropriate information. Böhm et al. (2013) presented the first global analysis of extinction risk in reptiles, displaying that the proportion of threatened reptile species is highest in freshwater environments, tropical regions and oceanic islands and that highest data deficiencies were found in tropical areas such as Southeast Asia. Thus, they emphasized the urgent need of research attention to be focused on tropical regions, which currently experience most dramatic rates of habitat loss. Accordingly, a recent review of Winter et al. (2016) assessing 106 studies on climate change impacts on reptile and amphibian populations, identified Asia as region exhibiting the biggest knowledge gaps. They highlight the especially great need of research in less convenient areas by accepting lower sample sizes than for studies on common species.

1.1.2 Excursus 2: Tropical lizards as main victims of global climate warming?

Besides current high rates of habitat loss and deforestation, tropical ecosystems are assumed to become critically affected by global climate change. Even though climate warming in the tropics might be lower than at higher latitudes, recent studies considering organism physiology predict that climate warming will be even more severe in the tropics compared to temperate regions (Root et al. 2003; Parmesan 2007; Tewksbury et al. 2008). According to Tewksbury et al. (2008), climate warming will most severely affect species living in warm climates, in aseasonal environments, or being specialized to specific temperatures and having limited acclimation capacity. Tropical forest ectotherms are assumed to be particularly vulnerable, because they use to live in constant shade and are not adapted to high operative temperatures in open habitats. Tewksbury et al. (2008) suggest that an increase in the temperatures of lizards may cause alarming declines in their Darwinian fitness, since they usually lack adequate behavioral traits to evade rising temperatures (Figure 2). Two natural compensatory responses to climate

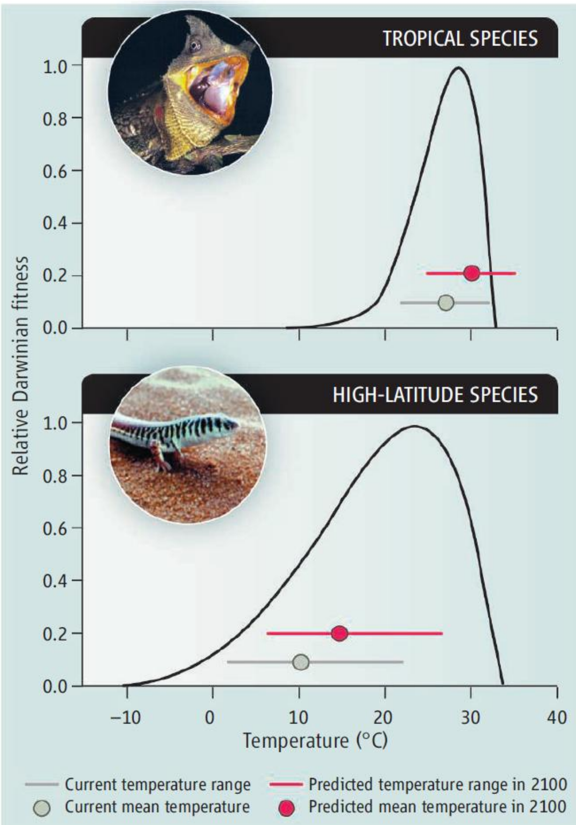

Figure 2. Tropical terrestrial ectotherms (top) are suggested to have narrower thermal tolerances than higher-latitude species (bottom) and live closer to their physiological optima. Thus tropical lizards may be highly vulnerable even to modest climate warming. Obtained from Tewksbury et al. (2008).

change are either range shifts to more favorable thermal environments or the adjustment to altered environments by behavioral or physiological plasticity, while the failure of adaption might result in extinction (Sinervo et al. 2010). With respect to range restricted tropical lizards, Sinervo et al. (2010) predicted that the limited ability to respond to climate change with range shifts or local adaptation will cause a 35-40% extinction risk of global lizard populations by 2080 with thermo-conforming and viviparous species being most vulnerable. Range shifts in species, as major consequence of climate warming, are expected to occur especially along altitudinal gradients and are assumed to induce complex changes in local species assemblages in the future (Parmesan and Yohe 2003; Root et al. 2003). Urban et al. (2012) predicted that climate change will mostly threaten species communities that have i) narrow niches such as in the tropics, ii) vary in dispersal and iii) compete strongly. They emphasized that impacts of climate change on biodiversity have been underestimated by neglecting competition and dispersal abilities. Furthermore, the ability of species to migrate to alternative environments may be also prevented by increasing fragmentation of habitats and the lack of corridors or stepping stones connecting existing habitats. Mitchell et al. (2008) reported that *Sphenodon* - a living fossil - was adapted to past climate changes, but is nowadays severely threatened by global warming due to the strong fragmentation of suitable habitats.

1.1.3 Excursus 3: The scope of the global reptile trade

According to Nijman et al. (2012), wildlife trade is the core problem of biodiversity conservation and sustainable development, including all sales and exchanges of wild specimens and resources by humans (Abensperg-Traun 2009; Broad et al. 2003). Nijman (2010) stated that - related to economic growth - also the international demand for exotic wildlife and its products has distinctly increased, which caused a likewise rise in the extent of wildlife trade, exacerbated by globalization, human population growth and increasing buyer power.

Exotic reptiles are globally traded in large volumes to supply the increasing demand for living pets, skin, food and traditional medicine (Nijman 2010; Nijman et al. 2012). In combination with additional stressors such as habitat loss and degradation (Gibbons et al. 2000), climate change (e.g., Araujo et al. 2006; Sinervo et al. 2010; Tewksbury et al. 2008) and environmental pollution (Guillette et al. 1994), unsustainable harvesting of wildlife emerged as a contributing factor to population declines or even extinction of species (Gibbons et al.

2000; Shepherd and Ibarrondo 2005; Stuart et al. 2006). Even though extinction processes that are causally linked to trade activities remain relatively undocumented (Jenkins et al. 2014), recent studies identified international harvest as second largest threat to the survival of many reptile species (Böhm et al. 2013). It is evident that species, which are range restricted, endemic or ecologically specialized, which have low fecundity, a high age at first maturity or long generation times, are especially vulnerable to over-collection (Böhm et al. 2013; Nijman et al. 2012; Webb et al. 2002). As one representative example, *Goniurosaurus luii*, a prominent Tiger Gecko, which is adapted to karst formations in Northern Vietnam and Southern China, became rapidly extinct at its type locality shortly after its original description as consequence of overharvesting (Stuart et al. 2006). Similar scenarios have been also recorded for other charismatic lizards in Vietnam (e.g., Auliya et al. 2016; Ngo et al. 2016a, 2016b). In order to classify the threat level of a species, to evaluate the impact of trade on local populations and to develop wildlife management strategies, population size estimation and monitoring provide essential data and measures, which lack for almost all reptile species (Ngo et al. 2016a; Reed et al. 2003; Traill et al. 2007). With respect to the increasing numbers of globally threatened species (IUCN 2016), the protection and sustainable conservation of species requires more attention than ever (Aulyia et al. 2016; Ceballos et al. 2015; Dirzo and Raven 2003; Pimm et al. 1995).

Lenzen et al. (2012) identified Europe as one of the major global consumers in the live reptile trade. Thus Europe needs to take responsibility for the conservation of species in theirs countries of origin (Gruttke 2004).

Commercial captive breeding of reptiles might theoretically reduce the pressure on wild populations, however concrete evidence exist for numerous cases of exports of wild-caught individuals labeled as “captive-bred” (Lyons and Natusch 2011; Nijman et al. 2012). Mechanisms to identify such fraudulent claims of mislabeling in order to ban and fine illegal trade pathways are broadly lacking. As a first step, the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Secretariat commissioned a case study on methodologies to differentiate between wild and captive-bred CITES-listed snakes in the trade (CITES 2013). Nevertheless, knowledge on trade pathways and impacts on target species is broadly lacking and enforced activities such as the development of control mechanisms to regiment the international trade in reptiles are urgently required to approach the global wildlife crime.

1.2 Vietnam: A hotspot of biodiversity, but center of illegal wildlife trade

Vietnam is situated within one of the earth's biodiversity hotspots, classified by the presence of especially high numbers of endemic and endangered species (Myers et al. 2000), ranked as the 16th most biodiversity rich country in the world, comprising 110 key biodiversity areas (Mittermeier et. al. 2004) and stands out for its high level of endemism (Queiroz et al. 2013). Furthermore, the country hosts two World Natural Heritage Sites, five Ramsar wetlands, eight United Nations Education, Scientific and Cultural Organization (UNESCO) Biosphere Reserves and two Association of Southeast Asian Nations (ASEAN) Heritage Parks (Queiroz et al. 2013). Vietnam is of global importance for its impressive naturally occurring biodiversity and is further recognized as one of the world's richest countries in agro-biodiversity. Due to the high degree of landscape-level variation among ecoregions, Vietnam is characterized by a diverse topography, climate, soils and geology with 14 ecoregions recognized by WWF (see Figure 3).

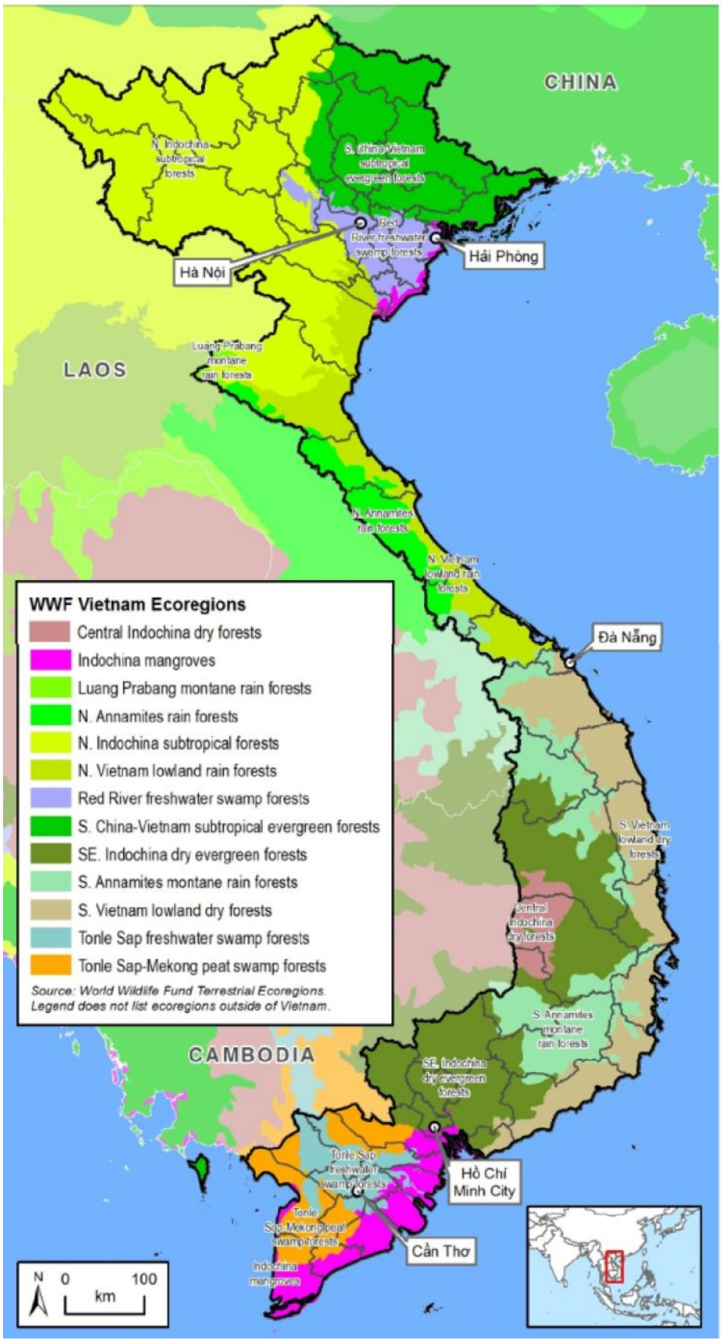

Figure 3. Map of Vietnams ecoregions by WWF.

However, Vietnam's biodiversity is still underestimated (Queiroz et al. 2013). Especially in terms of reptile diversity, Vietnam is recognized as one of the earth' most well known countries, with a total of 465 recorded species to date (Uetz 2000; www.reptile-database.org, assessed 29 September 2016) and many new reptile species steadily being discovered (e.g., Nguyen et al. 2013; Ziegler and Nguyen 2010). Among new discoveries are still charismatic and colorful species such as the Psychedelic Rock Gecko (*Cnemaspis psychedelica*) or the Cat Ba Tiger Gecko (*Goniurosaurus catbaensis*), which are both island endemics of Vietnam (Grismer et al. 2010; Ziegler et al. 2008).

Vietnam is ranked as a global center of biodiversity yet, but remaining biodiversity and tropical forests seriously decline due to various anthropogenic pressures (CEPF 2012). Related principal threats to biodiversity are habitat loss due to forest clearance for agriculture, grazing land, coal mining, fire wood collecting, illegal timber logging, illegal trade in wildlife, pollution, weak protected area management and infrastructure development without proper impact avoidance or mitigation measures (Queiroz et al. 2013).

The current high deforestation rate (Sodhi et al. 2010) resulted in a tremendous degradation of lowland subtropical evergreen broadleaf forest in Northeast Vietnam, which harbors a unique biodiversity, comprising many rare and endemic species (Sterling et al. 2006; Le and Ziegler 2003; Sourcebook 2004). This particular forest type is considered as especially vulnerable and fast disappearing in Southeast Asia (Meijaard and Sheil, 2008) and has already been substantially cleared in northern Vietnam (Tordoff et al. 2000), with some fragmented remains extending from Bac Giang Province to the Chinese border in Quang Ninh Province and partly covered by three nature reserves (NRs) namely Yen Tu, Tay Yen Tu and Dong Son-Ky Thuong NRs (Sourcebook 2004). Furthermore, Vietnam's freshwater biodiversity is considered to be under severe threat (Carew-Reid et al. 2010). Besides, Vietnam belongs to the earth's five countries, which are most vulnerable to climate change with increasing frequency and severity of typhoons, changing seasonal rainfall distribution, altered flow regimes, sea level rise or alternating temperature regimes as consequences (US Forest Service 2011).

In addition, Vietnam is recognized as one of the major exporters of living exotic reptiles and amphibians, as well as wildlife products to supply the international market (Aulyia et al. 2016; Mott 2006). Based on the alarming high level of illegal wildlife trade, Vietnam was ranked first in the WWF Wildlife Crime Scorecard (Queiroz et al. 2013). Simultaneously, appropriate national legislations and measures to efficiently control the harvesting and trade in wildlife are lacking and the enforcement of existing legislation is weak (Queiroz et al. 2013). Concrete evidence suggests that illicit trade represents the greatest hazard to Vietnams' wildlife even in reserves, with most of the wildlife products being illegally exploited for commercial purposes (Ngoc et al. 2008). Illegal cross-border wildlife trafficking-run by organized gangs, who are commonly linked to human and arm trafficking - is a serious problem in Vietnam, due to the lack of proper custom enforcements and high profits, generated by the trade in animals and plants (WCS 2012). According to observations, 76% of vehicles crossing the border from Quang Ninh Province, Vietnam to China used illegal crossing points in order to avoid inspections (WCS 2012). Furthermore, Vietnamese ports have comparably low seizure rates, resulting in increased illicit cross-border trafficking via Vietnam to China. Thus, poor law enforcement in combination with high profits, boosts the overexploitation of endangered species in Vietnam (CEPF 2012).

Furthermore, comprehensive knowledge on ecological traits, population sizes and distribution ranges is lacking for almost all species, which is crucial for species conservation measures as well as to predict the ability of single species to cope with alternating environmental conditions such as global climate change. Thus, basic research on biology, population status and threats is urgently required for most species in the country.

1.3 *Shinisaurus crocodilurus*

1.3.1 Systematics, distribution and natural history

The crocodile lizard *Shinisaurus crocodilurus* Ahl, 1930 is the only living representative within the monotypic family Shinisauridae, which represents a morphological conserved and independent taxonomic lineage. Oldest known fossil relatives are *Dalinghosaurus longidigitus* from the early Cretaceous (Evans and Wang 2005), *Bahndwivici ammoskius* from the Eocene (Conrad 2006) and *Merkurosaurus ornatus* from the lower Miocene (Klembara 2008), which form the independent and monophyletic phylum Shinisauria, based on their high morphological similarities with recent *Shinisaurus* (Conrad 2008; Conrad et al. 2011). Due to the high conservatism and presence of numerous primitive characters (e.g., the presence of a lacrimal, supratemporal, tabular, pineal eye, and palatal teeth and a complete supratemporal fenestra, supratemporal arch and a complete post orbital bar), *S. crocodilurus* is recognized as a true living fossil (Hu et al. 1984; Zhang 1991; Zhao et al. 1999). The recent discovery of a further shinisaurid skin fossil (USNM PAL 540708, Figure 4) from North America demonstrated that shinisaurs remained unchanged in the distribution of scales and patterns of scale size during the Cenozoic (Conrad et al. 2014, see Figure 4). In addition, the especially high anatomic and osteologic similarities between *Bahndwivici ammoskius* and

Shinisaurus crocodilurus revealed an extremely high overall conservatism within shinisaurs even over the past 50 million years (Conrad et al. 2014).

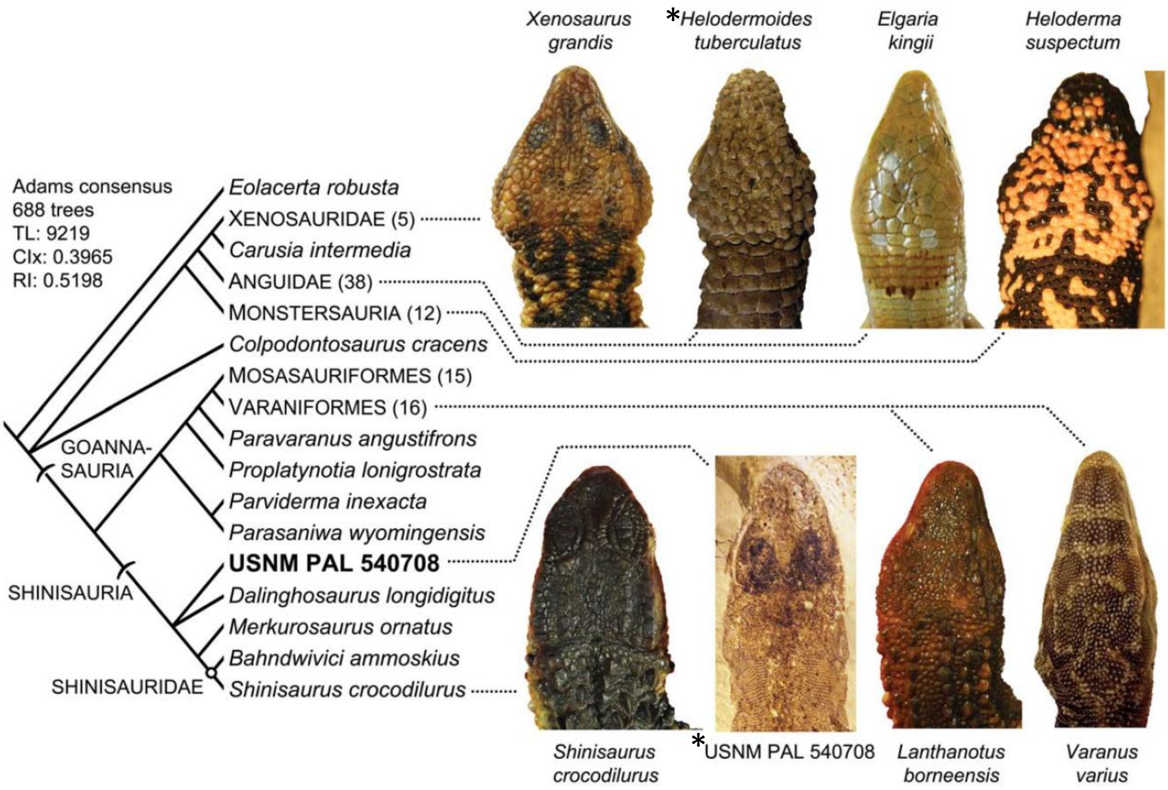

Figure 4. Combined-evidence analysis based on the data compiled by Conrad et al. 2014, illustrating the scale-type evolution in Anguimorpha. Asterisks indicate extinct taxa. USNM PAL 540708 is a recent discovered skin fossil of a shinisaur. "Domed/thickened osteoderms like those of *Helodermoides tuberculatus* and *Heloderma suspectum* may be plesiomorphic for Anguimorpha, because they are present in *Parviderma inexacta* and *Proplatynotia longirostrata*. Tubercular osteoderms are present in xenosaurids. Nonglyptosaurine anguids possess enlarged cranial plates. Keeled osteoderms are known for *Dalinghosaurus longidigitus*, *Merkurosaurus ornatus*, *Bahndwivici ammoskius*, *Shinisaurus crocodilurus*, and some varaniforms (e.g., *Necrosaurus*). Few *Varanus* are known to possess osteoderms." Modified after Conrad et al. (2014).

Systematically, *S. crocodilurus* is an anguimorph lizard, forming an independent lineage within the clade Platynota. Regarding extant taxa, *S. crocodilurus* was previously placed within the family Xenosauridae, (McDowell and Bogert 1954; Zhao *et al.* 1999) and longtime considered to be the extant sister taxon of the clade *Xenosaurus* (Estes et al. 1988; Gao and Norell 1989; Evans and Wang 2005). However, more comprehensive investigations of the cranial anatomy and muscular system indicated that *Shinisaurus* is a much more primitive species, exhibiting numerous differences in comparison to *Xenosaurus* and assured its independent and primal position within the Anguidae (Hu et al. 1984; Rieppel 1980; Wu und Huang 1986; Townsend et al. 2004). Several studies on squamate phylogeny, investigating either morphologic or genetic characters, revealed slightly different phylogenetic relationships among related genera throughout the last years (e.g., Conrad 2006; Conrad et al. 2008; Townsend et al. 2004). Most recent integrative studies, combining morphologic and molecular traits in extant and extinct species assumed, that *Lanthanotus borneensis* and *Varanus* are the closest living relatives to *S. crocodilurus*, also sharing the presence of keeled osteoderms (Conrad et al. 2011, 2014; Figure 5).

Figure 5. Basal relationships of Anguimorpha based on a combined data set of morphology and molecular data, and including extant and fossil taxa. Solid branches indicate relationships supported in the strict and Adams consensuses; outlined branches indicate those relationships recovered only in the Adams consensus. Taxon names printed in lighter (grey) type are those not scored for the characters used in this analysis. Obtained from Conrad et al. 2011.

Fossil evidence suggests a former broad distribution range of shinisaurid representatives with several records from North America, Europe and Asia (Conrad 2006; Conrad et al. 2014; Klembara 2008). However, the distribution range of *S. crocodilurus* is nowadays restricted to small and isolated relict populations in southern China and northern Vietnam. The species had been first collected in 1928 and was described in 1930 as new species, genus and family from Guangxi Autonomous Region in China (Ahl 1930). Afterwards it has been also recorded from neighboring Guangdong and Hunan provinces and only in 2003 *S. crocodilurus* was for the first time discovered in North Vietnam (viz. Quang Ninh Province) by Le and Ziegler (2003).

The species epithet derived from its phenotypic resemblance to crocodiles, such as the presence of dorsal osteoderms, which are forming two characteristic rows on the tail surface and are dispersed throughout the entire dorsal body surface. Concerning coloration, crocodile lizards exhibit diverse patterns, ranging from cream or yellow to light red, vivid red, vivid blue or grey. Juveniles generally have a triangular yellowish colored dorsal snout surface during the first month.

Crocodile lizards are associated to aquatic freshwater habitats, namely vegetated streams within intact and remote evergreen broadleaf forests (Ning et al. 2006; Wu et al. 2007). Only rarely found in lizards, *S. crocodilurus* is lecitotroph viviparous (ovoviviparous) giving birth to living juveniles within the water. Huang et al. (2015) recently found that the primary mating mode of the species is being polyandrous and polygynous, showing multipaternity in offspring of the same clutch. In natural habitats *S. crocodilurus* reaches maturity relatively late with about three to four years (Zollweg and Kühne 2013). Animals give birth once a year to usually two to 12 offspring (Zhang 2006; Zollweg and Kühne 2013).

1.3.2 At the brink of extinction

Although crocodile lizards succeeded in surviving on earth for a huge period of time, they are nowadays at the brink of extinction in China, due to a combination of diverse human stressors (Huang et al. 2008). Due to economic reasons habitats are destroyed, degraded and fragmented by forest clearance for agricultural use, logging for the sale of wood, for mining or trees are substituted by more profitable plants such as tea shrubs (Huang et al. 2015). Indirect negative consequences are the decrease of water holding capacity of soils, the drying up or pollution of freshwater systems by pesticides or other toxic substances, released by mining activities. The building of small scale dam construction further changes the natural courses of streams (Huang et al. 2008). In the Chinese culture, the use of crocodile lizards as traditional medicine can be traced back for several hundred years (CITES 2016). Due to its low activity, crocodile lizards were traditionally believed to cure insomnia (Herpin and Zondervan 2006; Hoffmann 2006; Nguyen et al. 2014). Furthermore the species is locally consumed as food source for home requirements (Herpin and Zondervan 2006;

Huang et al. 2008). In addition, the combination of its convenient size, primeval morphological traits and various color pattern made the species a desired pet among international hobbyists and thus also of high commercial value (e.g., Huang et al. 2008; Lau et al. 1997; CITES 2016).

In China, wild populations suffered dramatic declines of locally up to 90% between 1978 and 2004 to only 950 individuals, while five subpopulations were even reported to have completely vanished (Huang et al. 2008). Recent research indicates that *S. crocodilurus* is meanwhile facing extinction at most remaining sites, except of the monitored and protected population in Daguishan Nature Reserve (NR) in China (Zollweg 2015). Increasing international demand is still fueling unsustainable over-collection of the species and the pressure on wild populations. Respective knowledge on the conservation and population status, ecology and impacts of threats to *S. crocodilurus* in Vietnam is lacking until recently.

Its unique combination of numerous unique life history traits, special adaptions and restricted and fragmented distribution range in concert with its monotypic taxonomic status, long evolutionary history and status as living fossil, makes the species highly suitable to exemplary study evolutionary mechanisms and ecological adaptation and suits to assess impacts of climate change. Due to its restricted distribution, local adaptation and sedentarism, the species further suits for a long-term monitoring of populations with a high chance of capturing the whole population. This premise provides an ideal scenario to monitor and evaluate impacts of latest anthropogenic stressors such as habitat destruction and over-collection on an evolutionary ancient species. Its high conservation concern, charismatic and prominent appearance and increasing international interest qualify the species as a flagship species being of high value to protect Vietnams last remaining parts of evergreen lowland forests with its unique fauna and flora.

1.4 Objectives

The present thesis depicts a case study, assessing the impacts of direct and indirect anthropogenic stressors - exemplified on the Vietnamese crocodile lizard - and presents different approaches to facilitate the implementation of concrete conservation measures. The first part of this thesis displays basic research results on ecological requirements and adaptation, population sizes and trends, as well as on threats to *S. crocodilurus* in Vietnam. This study especially focused on the evaluation of impacts of climate change and international trade, which are currently assumed to distinctly imperil numerous reptile species. Thereupon, a major goal of this thesis was to establish and evaluate the applicability of novel tools to enforce species conservation, which remains globally challenging.

Using locality data and environmental parameters, species distribution models (SDMs) were applied in order to identify overall habitat suitability and to predict potential further occurrences of *S. crocodilurus*. Since such theoretical models are broadly applied, but only scarcely proven in the field, the present work assessed the suitability of SDMs to forecast occurrences of unknown populations by the subsequent conduction of excursions at predicted sites. Besides, future scenarios of habitat suitability for *S. crocodilurus* were predicted in order to assess the impact of global climate change on the species. In this context the species thermal niche within natural microhabitats was assessed in addition to these theoretical prognoses. Therefore, a backpack system with thermo data loggers was herein developed and tested for *S. crocodilurus,* representing the first of its kind study in aquatic lizards.

Since the EU, but especially Germany represents a major importer of exotic living animals and Vietnam is recognized as one of the major wildlife exporter, the present thesis assessed the relevance of *S. crocodilurus* in the international trade and resulting impacts on wild populations. Even though numerous exotic species are subject of international trade, knowledge on concrete trade impacts and pathways is generally lacking, but is crucial for legislation enforcement, to control the trade and to facilitate sustainable wildlife management. In this context, this thesis highlighted the responsibility of the EU for - and aimed to elucidate ways to improve the conservation of - endangered wildlife within its range countries exemplary on the crocodile lizard. In that regard, the applicability of isotopic markers to distinguish between legally captive bred and illegally wild caught *S. crocodilurus* in the trade, was herein tested for the first time in lizards in order to develop a potential forensic tool to detect wildlife crime.

Since *S. crocodilurus* populations from China and Vietnam are geographically separated, an integrative approach - comparing ecological niche adaptations, genetic and morphological traits among populations - was herein applied to identify the taxonomic status of respective populations.

In summary, the primary objectives of this dissertation were:

i) The evaluation of the conservation status and impacts of anthropogenic stressors on the crocodile lizard in Vietnam, including: a) an estimation of its wild population size and population trends; b) the assessment of its ecological niche including thermal niche; c) the prediction of future impacts of global climate change on *S. crocodilurus* and evaluation of the species' ability to cope with.

ii) To elucidate the role of trade in *S. crocodilurus* on wild populations and the establishment of a forensic tool to discriminate between captive bred and wild caught specimens.

iii) To assess the taxonomic status of extant *S. crocodilurus* populations in Vietnam and China.

The following hypothesis were postulated:

1) Wild *S. crocodilurus* populations in Vietnam are extremely small, declining and prone to extinction due to multiple anthropogenic stressors.

2) SDMs suit to forecast the presence of unknown *S. crocodilurus* populations.

3) *S. crocodilurus* is a habitat specialist, adapted to cool temperatures. Climate change will negatively affect the species.

4) Unsustainable international trade in *S. crocodilurus* distinctly imperils wild populations in Vietnam.

5) Isotopic markers suit as novel forensic tool to identify the source of *S. crocodilurus* specimens due to dietary differences in "captive" and "wild" individuals.

6) Extant *S. crocodilurus* populations represent more than one taxon.

2. Results

2.1 Autecology and conservation status of *S. crocodilurus* in Vietnam

Chapter 1:Current status of the Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam with implications for conservation measures

Chapter 2: First Ecological Assessment of the Endangered Crocodile Lizard, *Shinisaurus crocodilurus*, Ahl, 1930 in Vietnam: Microhabitat characterization and habitat selection

Chapter 3: Potential distribution and effectiveness of the protected area network for the crocodile lizard *Shinisaurus crocodilurus* in Vietnam

Chapter 4: Discovery of a new crocodile lizard population in Vietnam: population trends, future prognoses and identification of key habitats for conservation

Chapter 5: Will climate change affect the Vietnamese crocodile Lizard? Seasonal variation in microclimate and activity pattern of *Shinisaurus crocodilurus vietnamensis*

2.2 Impacts of trade and implications for conservation

Chapter 6: Trade in live reptiles and its impact on reptile diversity: the European pet market as a case study

Chapter 7: Last chance to see? A Review of the Threats to and Use of the Crocodile Lizard

Chapter 8: Can isotope markers differentiate between wild and captive reptile populations? A case study based on crocodile lizards (*Shinisaurus crocodilurus*) from Vietnam

2.3 Linking *in situ* with *ex situ* conservation

Chapter 9: Is there more than one Crocodile Lizard? An integrative approach reveals Vietnamese and Chinese *Shinisaurus crocodilurus* represent separate conservation and taxonomic units

Chapter 10: New insights into the biology and husbandry of Crocodile lizards including the conception of new facilities for *Shinisaurus crocodilurus vietnamensis* in Vietnam and Germany

2.1 Autecology and conservation status of *S. crocodilurus* in Vietnam

Chapter 1: Current status of the Crocodile Lizard Shinisaurus crocodilurus Ahl, 1930 in Vietnam with implications for conservation measures

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Current status of the Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam with implications for conservation measures

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Current status of the Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam with implications for conservation measures. - The Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 is a monotypic species, with a distribution range restricted to small and isolated areas in southern China and northern Vietnam. Habitat destruction and illegal poaching are the main causes of alarming population declines and even extinction of some wild populations in China. While the Chinese population was estimated to comprise only 950 individuals in 2004, the existing status of the Vietnamese population remains unknown, since its discovery in 2002. Our work provides the first estimation of the population size of *S. crocodilurus* in Vietnam, which is essential baseline data for future conservation strategies. Our field research revealed a dramatically small population size of less than 100 mature individuals. This value falls substantially below published threshold sizes of several thousand individuals, required for the long-term persistence of a species. Our research highlights the urgent need to improve the conservation activities for this species in its natural habitats and suggests means for a translocation program to restore (minimum viable sizes of) the wild populations in northern Vietnam.

Keywords: Population size - PIT tags - MVP - Conservation planning - Restoration - Southeast Asia - Yen Tu Mountain.

INTRODUCTION

The Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 (Fig. 1) is the only living representative of the monotypic family Shinisauridae and recognized as a true "living fossil" (Hu *et al.*, 1984; Huang *et al.*, 2008; Le & Ziegler, 2003; Zhao *et al.*, 1999). The species has specific habitat requirements such as undisturbed rocky streams within the evergreen rainforests with a known geographic range restricted to few small and isolated areas in northern Vietnam and southern China (Huang *et al.*, 2008; Le &

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FIG. 1
Shinisaurus crocodilurus in its natural habitat in Tay Yen Tu NR, Vietnam. Photo: M. van Schingen.

Ziegler, 2003; van Schingen *et al*., 2014). The Crocodile Lizard is threatened by extinction, with illegal poaching and habitat loss being recognized as the major threats to this species in China. Its resemblance to a crocodile makes it a desired target species on the international pet market and its reduced activity and low metabolism makes the species an easy prey of illegal poachers (Huang *et al*., 2008; Le & Ziegler, 2003; Wang *et al*., 2009). As a result *S. crocodilurus* is experiencing alarming population declines and even extirpation at some localities in China (Huang *et al*., 2008). In 1990 the species was finally listed by the Committee on the International Trade in Endangered Species (CITES) on appendix II in an attempt to diminish the trade with the species and to minimize further population declines (Huang *et al*., 2008). However, a study conducted in 2004 on the Chinese population concluded that only 950 individuals remained in the wild and revealed dramatic local declines of up to 90% in 25 years (Huang *et al*., 2008). Today the populations of *S. crocodilurus* in China have likely declined even more, while the existing status of the Vietnamese subpopulation remains unknown since its discovery in 2002 (Le & Ziegler, 2003).

In Vietnam the species was reported from three different localities, all in areas with some degree of protection: Tay Yen Tu Nature Reserve (NR) in Bac Giang Province, and Yen Tu NR and Dong Son - Ky Thuong NR in Quang Ninh Province (Le & Ziegler, 2003; Hecht *et al*., 2014; van Schingen *et al*., 2014), being at least 10 km apart from each other. All three sites are part of the last remaining contiguous lowland rainforest of Northeast Vietnam, which harbours a unique fauna not being found elsewhere in the country and which is zoogeographically related to southern China (Nguyen, 2011).

Appropriate estimations of the population size provide essential information for the classification of the threat level of a species and are crucial for wildlife management and management of the long-term survival of populations and species (Reed *et al.*, 2003; Traill *et al.*, 2007). Several studies support the notion that the size of the "minimum viable population" (MVP) is in reality much higher than the threshold sizes proposed by the IUCN and lie in the dimension of several thousand individuals (e.g., Reed *et al.*, 2007; Traill *et al.*, 2010).

This study includes a preliminary evaluation of the existing status of *S. crocodilurus* in Vietnam and provides information and evidence for the necessity of immediate conservation measures to protect this species in its natural habitat. Subsequently we provide recommendations for future conservation strategies of *S. crocodilurus*.

METHODS

Study sites: The surveyed sites were selected based on the previous discovery of three subpopulations of *S. crocodilurus* in Yen Tu NR, Uong Bi District, Quang Ninh Province (21°06' - 21°11'N, 106°37' - 106°43'E) in 2002; in Tay Yen Tu NR, Son Dong District, Bac Giang Province (21°09' - 21°23'N, 106°38' - 107°02'E) in 2010 and the recent discovery in Dong Son - Ky Thuong NR in Hoanh Bo District, Quang Ninh Province (21°05' - 21°12'N, 106°56' - 107°13'E) in 2013 (Le & Ziegler, 2003; Hecht *et al.*, 2014; van Schingen *et al.*, 2014), see Fig. 2. Tay Yen Tu and Yen Tu NRs are contiguous forest areas with Mount Yen Tu forming the highest peak (1068 m a.s.l.) and are linked in the East to the Dong Son - Ky Thuong NR by a forest corridor. The vegetation is dominated by evergreen broadleaf forest and intermixed with bamboo forest within the Dong Son - Ky Thuong NR. The study sites are part of the last remaining evergreen forest in Northeast Vietnam, which has been substantially cleared off from the eastern side of the Red River.

Field survey: Field surveys were conducted in June and July 2013, during the non-hibernation season of the Crocodile Lizard. Due to its strong association with lentic habitats and a diurnal life-mode, the riverine vegetation of selected rocky streams was sampled upstream during repeated night excursions between 6:45 and 10:30 pm, when animals were expected to rest on perches above the water. Captured animals were tagged and released on the exact same place on the following day between 12:00 am and 7:00 pm.

A total of 14 different stream transects were sampled, ranging from 515 to 3500 m in length. In the western side of the Yen Tu mountain range located within the Tay Yen Tu NR, six streams between elevations of 350-500 m a.s.l. were surveyed. On the eastern side of the Yen Tu range, four stream transects within the Yen Tu NR at elevations between 700-850 m a.s.l. and four streams in Dong Son - Ky Thuong NR at elevation between 200-350 m a.s.l. were surveyed. Coordinates and elevations of each captured individual were recorded with a GPS.

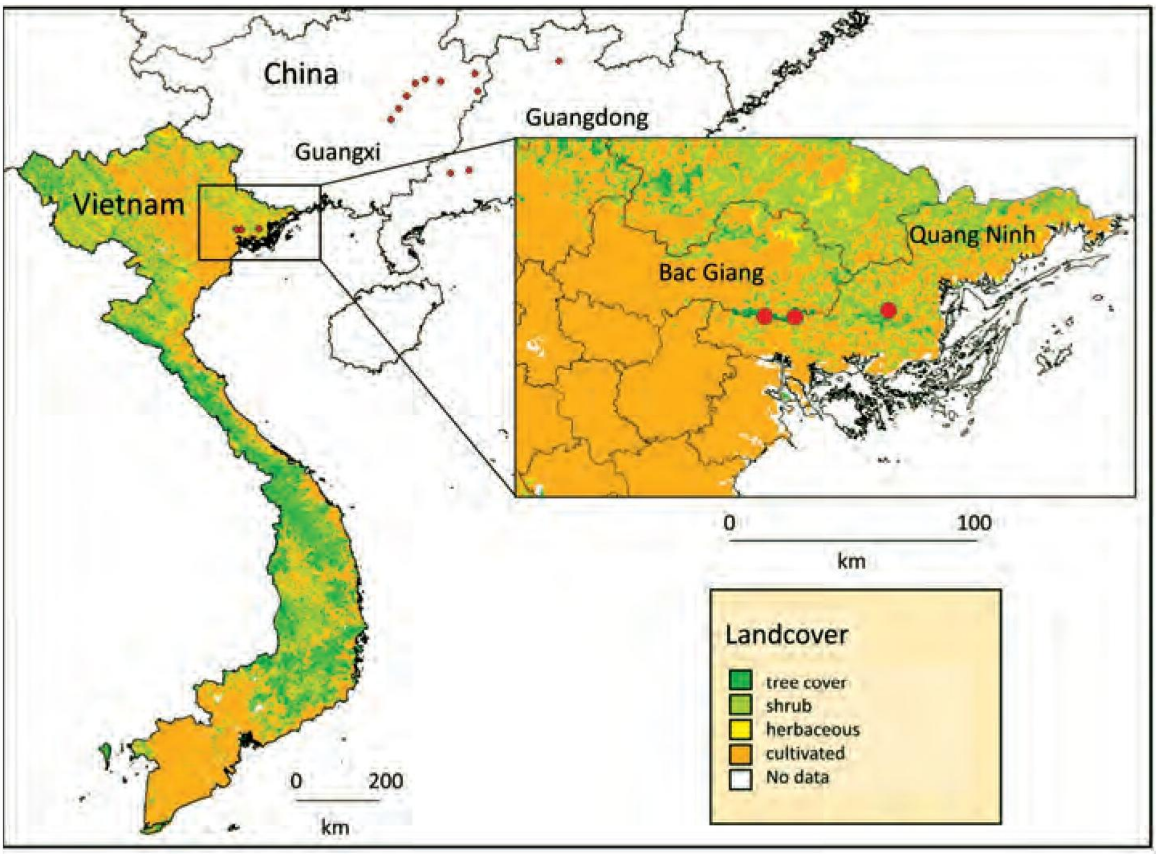

FIG. 2
Map of the current distribution of *Shinisaurus crocodilurus*. Red dots represent occurrence records.

Based on snout vent-length (SVL), individuals were classified into three different age groups, viz. juvenile (SVL < 100 mm), subadult (100 mm ≤ SVL < 140 mm) and adult (SVL ≥ 140 mm). Injuries were recorded, with special attention to the caudal region that was used as a measure of multivariate stressors.

Tagging: For the long-term monitoring of population dynamics of the species, individuals were tagged with passive integrated transponder (PIT) tags. PIT tags are commonly applied both in studies of vertebrates and invertebrates (Smyth & Nebel, 2013). Its use has established as a safe and reliable method, with low mortality rates and virtually no implications on moving speed, growth rate and health of the animal (Keck, 1994; Smyth & Nebel, 2013). It is also recommended by CITES to identify captive-bred animals, and to monitor illegal harvests as well as the international trade of species at risk (Gibbons & Andrews, 2004).

A unique PIT tag (ISO FDX-B, 9 x 1.4 mm) was inserted under the skin on the left body side behind the shoulder of each captured individual. The puncture was closed with petrolatum. The functioning of all microchips had been tested earlier with a reader (Breeder Reader LC, Planet ID GmbH). Tagged individuals did not show any signs of injury resulting from the injection and were released within 24 hours of capture. Recaptures were identified and released immediately.

Calculation: The population size was estimated by applying a capture-recapture method modified for *S. crocodilurus* by Huang *et al*. (2008). Accordingly, we calculated an "invisibility rate" which was adopted for *S. crocodilurus* to compensate for animals present but not seen during the surveys. This method was selected to obtain comparable data to the estimates on the Chinese population. The calculation of the "invisibility rate" was based on three consecutive time surveys in intervals of 1-12 days within the Tay Yen Tu NR: $N = \sum [n (1 + i)]$, where N is the total population size, n is the number of observed individuals along a stream transect and i is the "invisible rate" index: $i = [\sum(b_n - a_n)] / \sum a_n$, where a_n is the number of observed individuals in the transect n during the first survey and b_n is the total number of observed individuals in transect n. The transect n equals the surveyed stream.

Statistical analyses: Statistical analysis was performed with the program PAST (Hammer *et al.*, 2001). A Chi^2-Test was applied to test for differences among age classes and the occurrence of injuries between different localities. Significant difference was declared for $p < 0.05$ ($p < 0.05$ = *, $p < 0.01$ = ** and $p < 0.001$ = ***).

RESULTS

Population size: During the field research *S. crocodilurus* was found in seven different streams of three nature reserves. A total of 62 individuals were captured and 32 recapture events took place during the survey. Based on a calculated invisibility rate index of 1.35 the total population in Vietnam was estimated to comprise about 98 individuals, from which only 59 were considered to be mature (Tab. 1). The highest density of *S. crocodilurus* was found in Tay Yen Tu NR (28 individuals per km transect stream), while densities were lowest in Dong Son - Ky Thuong NR, ranging from 1 to 6 individuals per km transect stream (Tab. 2).

Population structure: The number of individuals capable of reproduction is crucial for the survival of the population and thus serves as measure to evaluate the endangerment of species (IUCN, 2013). *S. crocodilurus* reaches maturity at about three years (Yu *et al.*, 2009). The age is highly related to the animals size, whereby the snout-vent length proved to be the most appropriate measure corresponding to body size in lizards, as tails are prone to be injured (Meiri, 2010). A frequency histogram of the snout-vent length of captured *S. crocodilurus* revealed two maxima at 85 and 150 mm (Fig. 3A). This pattern shows that the wild population investigated in our study consisted of relatively high numbers of juveniles and young adults, but only of few subadults and big adults. A conspicuous reproduction success was observed in the Tay Yen Tu NR, related to the high proportions of juveniles, which represented 57.5% of this subpopulation (Fig. 4A). A significantly smaller success was reported in Yen Tu (8.3%) and in Dong Son - Ky Thuong (9.1%) NRs ($Chi^2 = 19.31$, $df = 4$, $p = 0.0007$; Fig. 4A). Taking into account the whole Vietnamese population, the number of adults and juveniles was represented with high percentages of about 47.6% and 39.7%, respectively, while subadults only contribute with about 12.7% (Fig. 4A).

Besides spatial differences in the population structure, high temporal fluctuations were also observed. In comparison to a previous field survey conducted by our team in June and July 2010 in Tay Yen Tu NR, the recent survey revealed: a more than tenfold increase in the number of juveniles; while the frequency of observed adults had almost doubled ($Chi^2 = 8.591$, $df = 2$, $p = 0.0136$; Fig. 4B).

TAB. 1. Estimated wild population size of *Shinisaurus crocodilurus* in Vietnam.

Nature Reserve	Tay Yen Tu	Yen Tu	Dong Son - Ky Thuong
$Subtotal_{mature}$ (all)	20 (51)	17 (21)	22 (26)
$Total_{mature}$ (all)		59 (98)	

TAB. 2. Abundances of observed *Shinisaurus crocodilurus* in Vietnam: *Ad* = adults, *Sub* = subadults, *Juv* = juveniles.

Nature Reserve	Transect [m]	Ad_{obs}	Sub_{obs}	Juv_{obs}	$Total_{obs}$	$Density_{obs}$ [Ind_{obs}/ km]
Tay Yen Tu						
1	842	3	2	2	7	8.3
2	1200	11	1	21	33	27.5
Yen Tu						
1	514	8	0	0	8	15.6
2	1600	2	0	1	3	1.9
Dong Son - Ky Thuong						
1	650	2	1	1	4	6.2
2	3500	1	3	0	4	1.1
3	830	2	0	0	2	2.4

A comparison of tail conditions among the three nature reserves showed that 25, 58 and 70 % of the observed individuals from Tay Yen Tu NR, Yen Tu NR and Dong Son - Ky Thuong NR, had regenerated tails, respectively (Chi^2 = 8.036, df = 2, p = 0.018; Fig. 3B). The habitats within Dong Son - Ky Thuong NR were the closest to local villages, had the lowest elevations (200-350 m a.s.l.) and thus were more easy to access, in comparison to the other locations. Furthermore in this area the streams were broader, less vegetated and did not comprise as many waterfalls, backwater pools and shelters like in the other two reserves. The highest visual encounters of sympatric occuring reptiles such as the Waterdragon *Physignathus cocincinus* were also found within the habitat of *S. crocodilurus* in Dong Son - Ky Thuong NR.

CONSERVATION STATUS

Threats: The major threats to the population of *S. crocodilurus* in Vietnam are habitat loss and habitat alterations caused by intensive coal mining and illegal timber logging (Ziegler *et al*., 2008; pers. obs.). Coal-mining leads not only to fragmentation but also results in the contamination of the forest floor and forest streams, threatening the water-associated organisms (Fig. 6A). In Tay Yen Tu NR, the species' habitat was seriously disturbed. The forest has been opened throughout the nature reserve in order to build roads and facilitate coal-mining (Fig. 6B). The mining area has been steadily expanding and meanwhile almost touched a stream habitat of *S. crocodilurus*. During one night survey in 2013, a huge hydraulic excavator was observed working in a distance of less than 50 m to a habitat stream of *S. crocodilurus*. In addition, huge parts of the forest have been cleared by slash and burn agriculture or have been harvested for the paper industry (Fig. 5). Habitat destruction was also hazardous in the Dong Son - Ky Thuong NR, caused in main parts by the activities of Hoanh Bo Forest Enterprise

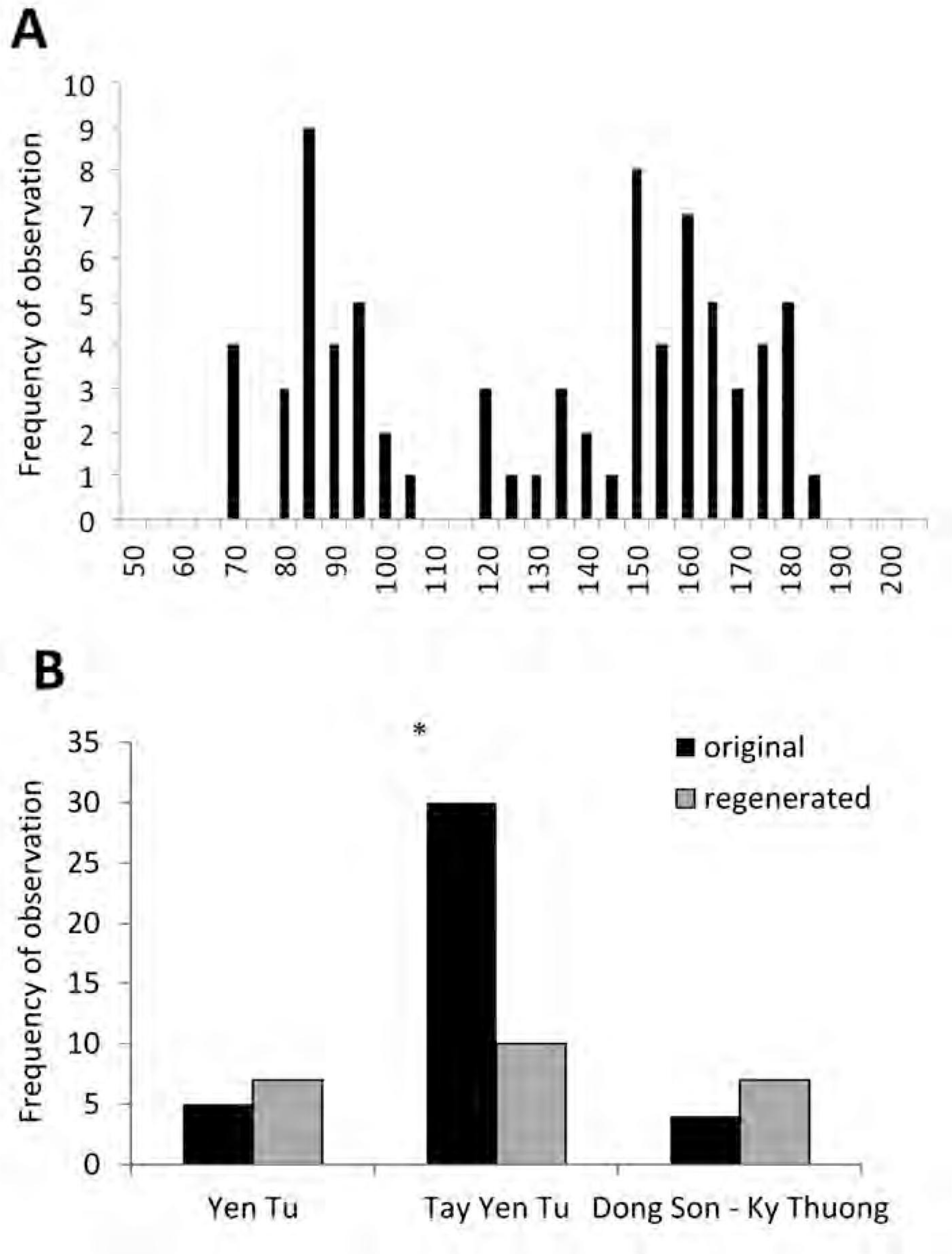

FIG. 3

opulation structure of *Shinisaurus crocodilurus* in Vietnam. (A) Frequency histogram of snout-ent length of all encountered animals; (B) Frequency of individuals with original or regenerated ils for each nature reserve, $p < 0.05$.

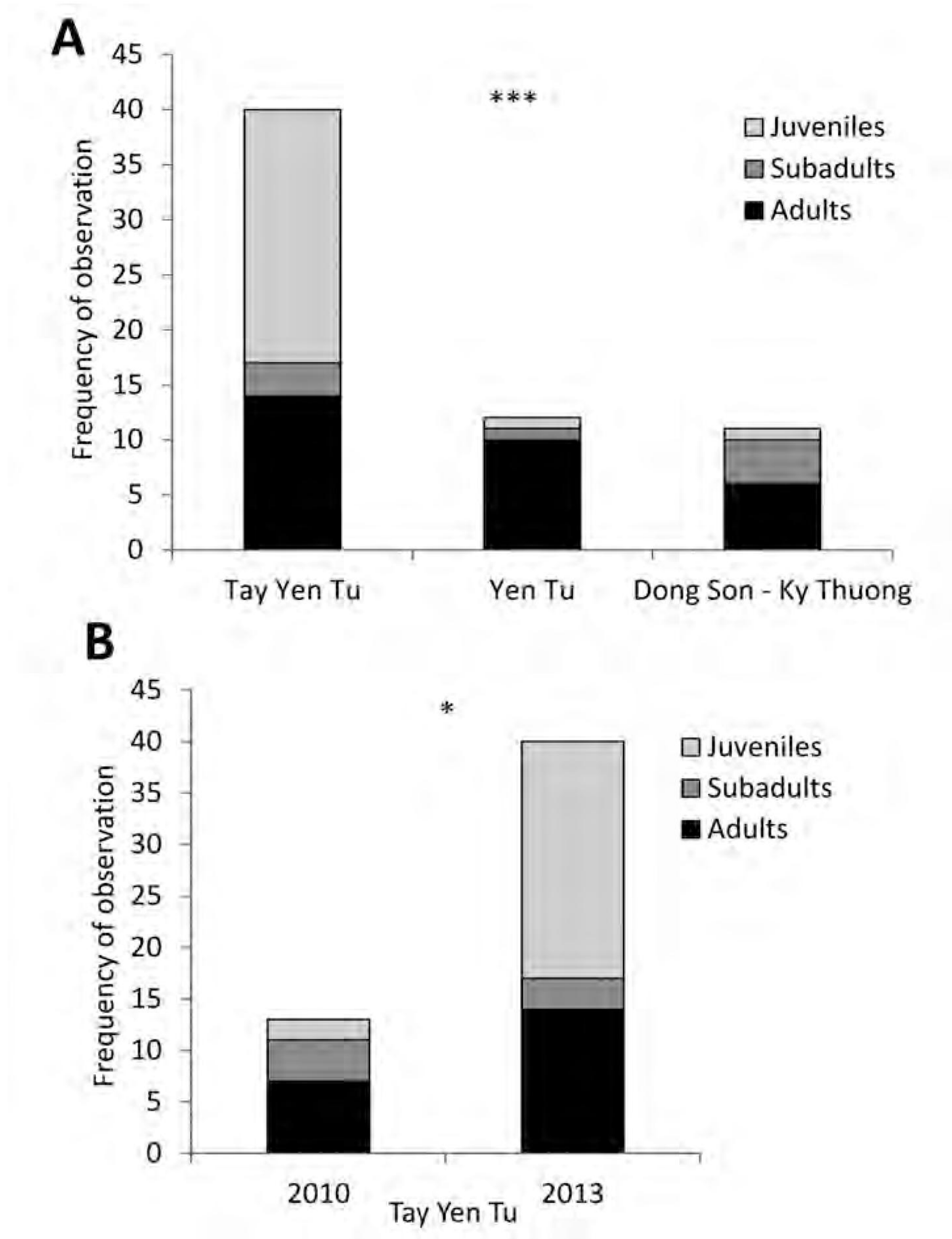

FIG. 4

Distribution of different age classes of *S. crocodilurus* in Vietnam. (A) Frequency of observed juveniles, subadults and adults from three nature reserves, $p < 0.001$; (B) Frequency shift (of juveniles, subadults and adults) in Tay Yen Tu NR between 2010 and 2013, $p < 0.05$.

FIG. 5
Slash and burn practices in Tay Yen Tu NR, Vietnam. (A) example of forest fire; (B) burned area cleared for agricultural purposes or exploration by the paper industry. Photos: M. Bernardes & M. van Schingen.

(see also Birdlife International, 2004). The construction of logging roads throughout the Dong Son - Ky Thuong NR has facilitated illegal logging and increases the accessibility of almost all areas within the nature reserve (Tordoff *et al.*, 2000). We could prove the observation of Tordoff *et al.* (2000) that hunting posed a severe threat to the biodiversity in Dong Son - Ky Thuong, as our interviews with local people showed that they indeed randomly collect amphibians and reptiles within the nature reserve as food source, and that Crocodile Lizards were collected for traditional medicine (Fig. 6C). Le & Ziegler (2003) already reported that illegal poaching for the pet trade threatens the Vietnamese population. Sold as "baby crocodile" for 100.000 to 200.000 Vietnam Dong (about 7-15 US Dollars), the Crocodile Lizard is a big seller especially among tourists. This observation agreed with our findings that Vietnamese specimens apparently also ended up in the international pet trade, as they are being offered already for sale in the internet (e.g., Doelle *in lit.*, 2013; *pers. obs.*). The high demand for the species, especially in European countries immensely increases the hunting pressure on the wild populations.

DISCUSSION

The persistence of populations in the wild depends on the size of viable subpopulations and the exchange and speed of recolonization from nearby habitats, with particularly small and range-restricted populations being highly prone to extinction in various animal species (Hanski, 1991; Reed *et al.*, 2003; Traill *et al.*, 2007). In terms of the ongoing alarming global loss of biodiversity, guidelines to link extinction risk to population size have high priority in conservation biology (Lawton & May, 1995; Reed *et al.*, 2003; Shaffer *et al.*, 2002). The concept of a 'minimum viable population' (MVP; Shaffer, 1981) has been frequently applied in terms of species recovery and conservation management programs, with relevance to the IUCN Red List's criteria concerning small and range-restricted populations (e.g., Clark *et al.*, 2002; Reed *et al.*, 2003; Traill *et al.*, 2007, 2010). The MVP is defined as the smallest threshold size, which is required for a population or a species to have a predetermined probability of persistence for a given length of time (Reed *et al.*, 2003; Shaffer, 1981). Experiments on isolated subpopulations revealed a local extinction of subpopulations with $n < 50$ and persistence with $n > 50$ individuals (Berger, 1990). With respect to reptiles and amphibians Traill *et al.* (2007) summarized MVPs ranging from 3611 to 6779 individuals and stated that MVPs generally lie in the range of several thousand individuals. Reed *et al.* (2003) concluded that a population size of at least 7000 adults in any vertebrate is required to cope with evolutionary and demographic constraints in the long-term. The population size of *S. crocodilurus* in Vietnam was preliminary estimated to comprise about 59 mature individuals and thus being dramatically smaller than the Chinese population with 950 estimated individuals in 2004 (Huang *et al.*, 2008). The high incidence of juveniles, most concise within the Tay Yen Tu NR implicates that the reproduction capability of the population is not entirely constrained by certain stressors, but rather secondary hazards as habitat degradation and poaching are assumed to limit the population persistence in the long-term, comparable to the Chinese population (Huang *et al.*, 2008).

FIG. 6

Main threats to *S. crocodilurus* in Vietnam. (A) Coal-mining exploration close to the species' habitat; (B) Opening of the forest with roads to facilitate coal-mining throughout the nature reserves; (C) Preserved *S. crocodilurus* in alcohol, used for traditional medicine in Quang Ninh Province. Photos: M. Bernardes & M. van Schingen.

The order of injuries in specimens differed among the three sites and was highest within the Dong Son - Ky Thuong NR. An unfavourable habitat structure, the occurrence of predators or competitors and human impacts might be potential reasons for higher rates of violated specimens in this reserve.

Furthermore, our study revealed that *S. crocodilurus* is strongly sedentary, as no migration between habitat streams in striking distance was proved within three years. In long-term view the restricted migration ability might reduce the gene flow and thus endanger the continuance of the species. The extremely small subpopulation sizes of about 20 mature individuals within each nature reserve make the species prone to fall into an extinction vortex (Gilpin & Soulé, 1986). Strong fluctuations within populations make them especially prone to extinction, even though populations generally underlie some level of fluctuations (e.g., Bjǿrnstad & Grenfell, 2002; Ranta *et al.*, 2006). In this context our study revealed that the subpopulation from Tay Yen Tu NR had more than doubled from 2010-2013, including a more than 11-fold increase in the proportion of juveniles. This high incidence of juveniles was observed nowhere else in Vietnam. However, the duration of survival appeared strongly restricted as only one of 13 individuals, marked in 2010 was recaptured in 2013. Since *S. crocodilurus* reaches sexual maturity only after three years, the survival during this period is crucial for the maintenance of its populations (Zhang, 2006; Yu *et al.*, 2009).

CONSERVATION MEASURES

Habitat protection: Based on the observation of various threats to the habitat of *S. crocodilurus* in Vietnam (e.g., continuously expanding coal-mining area in the direction of the habitat streams, habitat fragmentation from roads made for coal exploration and logging companies, forest clearance and natural forest fires), we strongly recommend a protection status elevation of the nature reserves in close collaboration with the authorities of the reserves. As many Crocodile Lizard populations are distributed outside or within the buffer regions of the NRs (van Schingen *et al.*, 2014), an extension of the protected area network should be further considered. Apart from the protection of the macrohabitat, we recommend that at least the habitat streams need higher protection to enable the long-term persistence of the species. An agreement with the operators of local coal-mining companies is necessary to protect the minimum area required for the survival of the population, which would be feasible as the species is strongly sedentary and restricted to few specific streams (Ning *et al.*, 2006; van Schingen *et al.*, 2014). Roads, which are increasingly created throughout Tay Yen Tu and Dong Son - Ky Thuong NRs to facilitate coal-mining and timber logging (Tordoff *et al.*, 2000; *pers. obs.*), should be directed around the habitat streams.

Wildlife trade control: To control the trade, an enhancement of the conservation status of *S. crocodilurus* by the assessment of the species for the IUCN Red List is recommended just as an upgrade of the CITES appendix. We also propose to include *S. crocodilurus* in the list of protected species in Vietnam. Illegal collections for the pet trade should be controlled by forest ranger stations through patrols at touristic sites like Tay Yen Tu and Yen Tu NRs. As *S. crocodilurus* is a habitat specialist (Ning *et al.*, 2006; van Schingen *et al.*, 2014; Wu *et al.*, 2007), only occurring along specific streams, this measure would be feasible and effective. A public awareness campaign (e.g., brochure, poster, signboard) should be conducted for local communities inside protected areas and within their buffer zones.

Population restoration: First molecular analysis of the extant subpopulations revealed no significant genetic difference (Ziegler *et al.*, 2008). However, a broader genetic analysis to evaluate the closer taxonomic relationships of the extant subpopulations is recommended, as discrepancies would have a strong impact on the risk of extinction of subpopulations or even different taxa and would require a drastic enhancement of the conservation status of *S. crocodilurus* in Vietnam. However, the estimated total population size (China: 950 + Vietnam: 59) already falls below reported threshold sizes in the magnitude of several thousand individuals, which is required for the persistence of a species over a longer period (Traill *et al.*, 2010). Based on our findings a translocation program of the species to restore the wild population, particularly in Vietnam, is urgently recommended. Translocation, defined as movement of living organisms from one area to another (IUCN), forms an important tool in wildlife conservation (Germano & Bishop, 2008). Repatriations of animals into their natural habitats were frequently combined with captive-breeding programs at zoological parks (Scott & Carpenter, 1987). A restoration program of a subpopulation of *S. crocodilurus* in China (Luokeng NR, Guangdong Province) was already initiated in 2004 (Zhang, 2006). In addition, Vietnamese specimens originating from Yen Tu NR were already successfully bred in captivity at the Me Linh Biodiversity Station in Vinh Phuc Province, which was established in cooperation of the Institute of Ecology and Biological Resources, Hanoi and the Cologne Zoo, Germany (e.g., Ziegler *et al.*, 2013). Those individuals would be suitable for restocking the wild population. A reintroduction should proceed after IUCN standards and based on studies on the species' specific requirements. In addition, all captive bred specimens should be marked with PIT tags in order to monitor the development of introduced specimens and long-term population dynamics after the release to evaluate the restoration success. Moreover, tagging of wild individuals during the present study already provides a base for future long-term investigations of *S. crocodilurus* in Vietnam.

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NOTE ADDED IN PROOF

While the present paper was in press, the inclusion of the species into the IUCN Red List and the list of protected species of Vietnam took place.

NGUYEN, T. Q., HAMILTON, P. & ZIEGLER, T. 2014. *Shinisaurus crocodilurus*. *In*: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. <www,iucnredlist.org>. Downloaded on 12 June 2014.

Chapter 2: First Ecological Assessment of the Endangered Crocodile Lizard, Shinisaurus crocodilurus, Ahl, 1930 in Vietnam: Microhabitat characterization and habitat selection

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FIRST ECOLOGICAL ASSESSMENT OF THE ENDANGERED CROCODILE LIZARD, *SHINISAURUS CROCODILURUS*, AHL, 1930 IN VIETNAM: MICROHABITAT CHARACTERIZATION AND HABITAT SELECTION

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***Abstract.*—The monotypic Crocodile Lizard (*Shinisaurus crocodilurus*) is a habitat specialist, adapted to headwaters of remote mountain streams within the tropical rainforests of southern China and northern Vietnam. Due to the anthropogenic pressures namely poaching for international pet trade and local consumption as well as habitat destruction, this living fossil is now at the brink of extinction. While research on natural history had already been conducted on Crocodile Lizards in China and relevant management programs have been established there, comparable knowledge is lacking for Crocodile Lizards in Vietnam. We provide the first comprehensive habitat characterization for Crocodile Lizards in Vietnam, which is essential for species conservation and the protection of remaining natural habitats. Our results showed that perch characterization was different between age classes, and between populations in China and Vietnam. Furthermore, our study found that Crocodile Lizards had specific needs of stream physiology and water quality. We found that few inhabited streams were affected by coal-mining activities in Vietnam, suggesting the importance of immediate measures to ensure habitat conservation of Crocodile Lizards.**

Key Words.—Shinisauridae; microhabitat characterization; niche segregation; Southeast Asia; species conservation; sustainable management

INTRODUCTION

The Crocodile Lizard (*Shinisaurus crocodilurus*, Ahl 1930) represents an ancient but prominent anguid lizard clade, which was recently described as a new species, genus and family (Zhao et al. 1999; Huang et al. 2008). Furthermore, Crocodile Lizards are habitat specialists, which prefer small, remote streams along mountain ridges within undisturbed tropical rainforest (Ning et al. 2006; Huang et al. 2008; Zollweg, 2011a; van Schingen et al. 2014a). The critical taxonomic position, long evolutionary history as well as specific life-history traits and high sensitivity to environmental conditions make this species particularly important for understanding the evolution and ecology of lizards.

Currently, the distribution range of the Crocodile Lizard is restricted to fragmented sites in southern China and northern Vietnam, where suitable habitats are small, isolated, and steadily shrinking (Le and Ziegler 2003; Huang et al. 2008; van Schingen et al. 2014a). Li et al. (2012) even predicted the loss of all original habitats in China during 2081–2100 as consequence of climate change. One report revealed that Crocodile Lizards in China are suffering a tremendous decline with a rate of about 85%, and the present population size might be fewer than 1,000 individuals in China (Huang et al. 2008). A similar study in 2013 revealed that the effective population size of Crocodile Lizards might be fewer than 100 individuals in Vietnam (van Schingen et al. 2014b), which is dramatically below the threshold of viable populations (Shaffer et al. 2002; Reed et al. 2003; Traill et al. 2007; Traill et al. 2010). Poaching for the international pet trade is currently regarded as the most severe threat causing population declines and even the extinction of several wild subpopulations (Huang et al. 2008; van Schingen et al. 2015). Consequently, the protection needs of Crocodile Lizards have received increasingly attention from all around the world. The species is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and was recently classified in the IUCN Red List of Threatened species as Endangered (Nguyen et al. 2014). However, wild populations are still in peril due to extensive collection and the lack of adequate habitat protection (van Schingen et al. 2015). Immediate conservation measures such as the restocking of wild population at protected sites are needed to ensure the persistence of the species (van Schingen et al.

2014b). Information about habitat requirements and ecology of the species is essential for planning successful conservation programs. First studies on natural history of Crocodile Lizards in China were already conducted and led to the establishment of breeding facilities and the first trials in releasing animals into the wild (Long et al. 2007; Zollweg 2011b, 2012). By contrast, the natural history and habitat requirements of Crocodile Lizards from Vietnam still remain poorly studied since its discovery in 2002 (Le and Ziegler 2003).

Our study aims to provide the first habitat characterization of Crocodile Lizards in Vietnam to get an insight into its ecological requirements, thereby promoting the protection-related breeding and sustainable management plans for this species. We assumed that we would detect differences in habitat use between juveniles and adults because of observed distinct behaviors (Zollweg and Kühne 2013; Mona van Schingen, pers. obs.), which has been also recorded for other lizards (Snyder et al. 2010). Additionally, we compared our findings with previous studies on Crocodile Lizards in China to identify a potential ecological difference between extant subpopulations, which is crucial to understand the evolutionary history, taxonomic status, and threat potential of the species and respective populations. Populations of Crocodile Lizards in China and Vietnam are separated by at least 500 km (Le and Ziegler 2003) and are exposed to slightly different climatic conditions (annual moderate temperatures in northern Vietnam vs. cold winters and hot summers in southern China; Zollweg and Kühne 2013). Thus, we assumed differences in microhabitat characteristics and habitat selection between subpopulations from China and Vietnam as divergences generally evolve rapidly in allopatric lizard populations (e.g., Herrel et al. 2008).

MATERIALS AND METHODS

Study site.—We conducted fieldwork in June and July 2013 to make the data comparable to preliminary microhabitat studies done in the summer on Crocodile Lizards in China (Ning et al. 2006). May to October is known to be the active season of Crocodile Lizards in China (Huang et al. 2008; Ning et al. 2006). We visited all known Crocodile Lizard localities in northeast Vietnam in the Tay Yen Tu Nature Reserve (NR) in Bac Giang Province, Yen Tu and Dong Son - Ky Thuong NRs in Quang Ninh Province (Le and Ziegler 2003; Hecht et al. 2014; van Schingen et al. 2014a). All three NRs are part of the last remaining contiguous Evergreen Tropical Broadleaf Rainforest in northeast Vietnam, which has been extensively cleared in the region (Tordoff et al. 2000; BirdLife International. 2014. Sourcebook. Available from http://www.birdlife.org [Accessed 23 September 2013]). Northeast Vietnam is characterized by a monsoon tropical climate with cool winters (minimum temperature of coldest month about 12° C) and summer rains (Nguyen et al. 2000). The flora of this region belongs to the South-Chinese floristic unit and north Vietnam also shares close zoogeographic affinities with adjacent southern China (Zhu et al. 2003; Ziegler et al. 2008).

Data collection.—We conducted night excursions between 1845 and 2230 because Crocodile Lizards are diurnal and perch above water during the night (Huang et al. 2008; Ning et al. 2006; van Schingen et al. 2014b; Zollweg and Kühne 2013). We surveyed seven streams (two in Tay Yen Tu, two in Yen Tu and three in Dong Son-Ky Thuong NRs) inhabited by Crocodile Lizards, each with a team of four members. Every night we surveyed upstream for 3.45 h, covering 650–3,500 m of the stream length. We surveyed the streams in Tay Yen Tu NR three to five times, while other streams could only be surveyed once or twice due to inaccessibility and climatic constraints. We conducted 14 night surveys in habitats of Crocodile Lizards. We georeferenced each lizard we found with a GPS unit (Garmin GPSMAP® 64s, Garching, Germany) and measured 24 abiotic parameters characterizing the microhabitat and perch site of Crocodile Lizards. We measured water and air temperatures and air humidity with a digital thermometer and hygrometer (Exo Terra, PT2470, Hagen, Germany), and O_2 saturation, concentrations of nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) / ammonia (NH_3), phosphorus (PO_4^{3-}), iron (Fe^-), silicate (SiO_2), carbonate hardness (KH), and total hardness (GH) of water (Testlab 25502, Joachim Böhme Ludwigshafen (JBL), Neuhofen, Germany) to determine the water quality of each surveyed stream. The pH was determined with a digital pH meter.

We documented the stream sections at the perch sites of Crocodile Lizards, which we classified as pool, riffle, run, or waterfall. We characterized pools by slow flow velocity and small substrate sizes (Fig. 1), run sections by medium depth, medium flow velocity, and smooth flowing waters, and we defined riffles as shallow sections with high flow velocity and large substrate rocks (Jowett 1993). We measured the flow velocity with a digital flow meter (Windaus ZMFP126-S, Clausthal-Zellerfeld, Germany). To characterize the resting perch of Crocodile Lizards, we recorded perch height in cm (distance between perch and water surface or ground), perch diameter in mm, vertical distance from perch to shore in cm, water depth in cm, stream width in m, percentage vegetation coverage above perch, perch substrate (as branch, liana, bamboo, shrub, fern, rock, forest floor and water), and stream substrate (as loam,

FIGURE 1. Habitat of *Shinisaurus crocodilurus* in Vietnam. (A). Macro-habitat. (B and C). Typical microhabitat with backwater pool. Arrow indicates a Crocodile Lizard (*Shinisaurus crocodilurus*). (Photographed by Mona van Schingen and Marta Bernardes).

sand, gravel, cobbles). Definitions of water hardness (KH) follow US Geological Survey standards for the water hardness classification (USGS Water-Quality Information. Available from http://water.usgs.gov). We obtained interpolated annual temperature data for the localities from the Worldclim-Global Climate Data (WorldClim. 2013. Global Climate Data. Available from www.worldclim.org [Accessed 7 June 2013]; Hijmans et al. 2005). For comparing the habitat selection of different age classes, we measured the snout-vent lengths (SVL) of the lizards with a digital caliper to the nearest 0.1 mm. Based on these measurements, we categorized lizards into different age classes: < 100 mm = juvenile; 100–140 mm = sub-adult; and > 140 mm = adult (see van Schingen et al. 2014b). We caught lizards by hand and subsequently released them on the same perch.

Statistical analyses.—We performed a One-Way ANOVA combined with a Tukey posthoc test to test for differences of habitat parameters among localities by age. We used Barlett's test to verify homogeneity of variances and the Shapiro-Wilk test to verify normal distribution. If required, we log transformed data to meet assumptions of normality and constant variance. We used a chi-square test to examine whether perch preferences differed among localities. We tested correlations between environmental parameters with a Pearson's rank correlation. We further applied a principal component analysis (PCA) of 12 selected abiotic factors describing the perch site (pH, O_2-saturation, concentrations of NO_3, NO_2, NH_4/ NH_3, PO_4, KH total hardness and GH, altitude, perch height, perch width, the perch's vertical distance to the shore and canopy cover) to detect subordination of factors describing the habitat selection of Crocodile Lizards. Statistical analyses were performed with the program PAST (Hammer et al. 2001) and for all tests, $\alpha = 0.05$.

RESULTS

Habitat characterization.—We found Crocodile Lizards exclusively within first order streams, often close to the stream source, where the streams were shallow and narrow. Stream habitats were densely vegetated, mainly by broad-leafed trees and scattered bamboo, while macro-algae were mostly absent within the streams (Fig. 1). All streams were slow to medium

TABLE 1. Abiotic parameters of streams inhabited by *Shinisaurus crocodilurus* in northeast Vietnam in the Tay Yen Tu Nature Reserve (NR) in Bac Giang Province and in Yen Tu and Dong Son - Ky Thuong NRs in Quang Ninh Province. Values for canopy cover and flow velocities are approximate.

Parameter	Tay Yen Tu NR	Dong Son – Ky Thuong	Yen Tu NR	Total
pH	4.5–5	6.66–7.37	5.43–5.58	4.5–7.37
NO_2 [mg/l]	< 0.01	< 0.01	< 0.01	< 0.01
NO_3 [mg/l]	4–5	< 0.5–5	1–5	< 0.5–5
NH_3/NH_4 [mg/l]	< 0.05–0.1	< 0.05	< 0.05	< 0.05–0.1
PO_4 [mg/l]	0.05–0.1	< 0.02	< 0.02	< 0.02– 0.1
Fe [mg/l]	< 0.02–0.05	< 0.02–0.05	< 0.02	< 0.02–0.05
O_2 [mg/l]	8	6–10	8	6–10
SiO_2 [mg/l]	3–6	5–6	1.2–6	1.2–6
KH [d°]	1–2°	1°–2°	1°–2°	1°–2°
GH [d°]	< 1°	1°–2°	< 1°–1°	< 1°–2°
Stream width [m]	1–3	5–6	1–8	1–8
Stream depth [cm]	13–40	18–34	5–73	5–73
Canopy cover [%]	50–100	80–100	0–100	0–100
Flow velocity [m/s] (max)	0–0.47 (1.67)	0–0.3 (1.45)	0–0.15 (1.74)	0–0.47 (0–1.74)
Substrate type	Sand > Gravel	Loam > Gravel	Gravel > Sand	Gravel > Sand > Loam
Humidity [%]	78–86	87–88	85–88	87–88
Elevation [m] above sea level	330–505	180–330	728–847	180–847

in water flow, being shallow (5–73 cm) and with relatively narrow width (1–8 m; Table 1). The river bed was dominantly composed of sand, gravel, and some boulders from numerous riffle zones with big truncated tree branches residing in the water. Furthermore, many streams contained several small waterfalls followed by

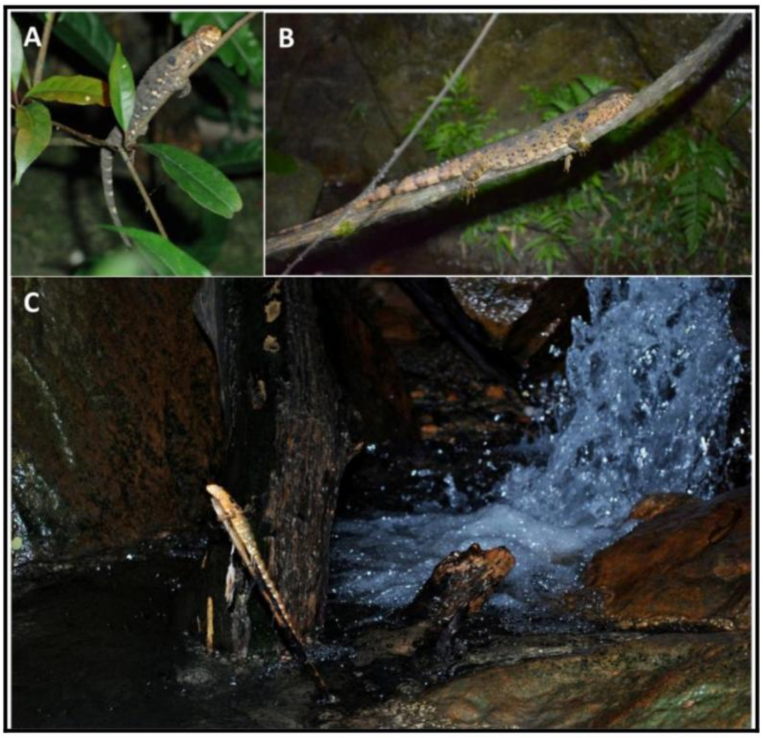

FIGURE 2. Resting perches of Crocodile Lizards (*Shinisaurus crocodilurus*) in Vietnam. (A). Juvenile. (B). Adult. (C). Sub-adult above backwater pool (Photographed by Mona van Schingen and Marta Bernardes).

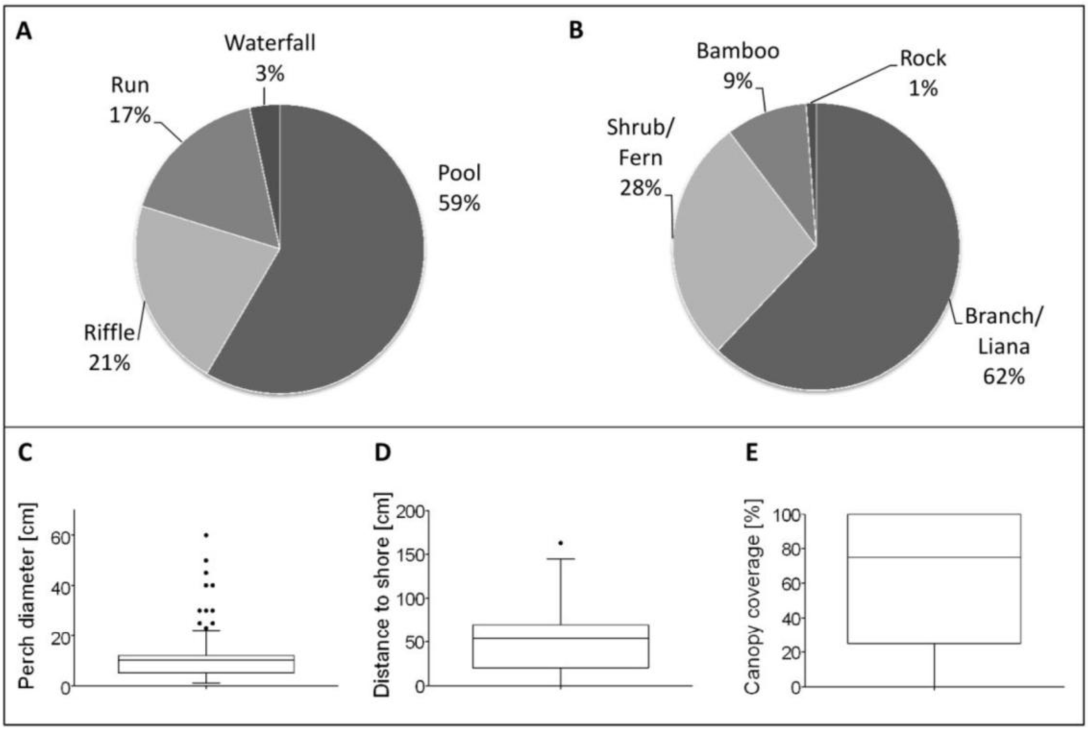

FIGURE 3. Perch selection of Crocodile Lizards (*Shinisaurus crocodilurus*) in Vietnam. (A). Stream section selection. (B). Substrate selection. (C-E). Box plots of perch characteristics (perch width, distance to the shore, canopy coverage).

backwater pools (Fig. 1B-C).

The streams inhabited by Crocodile Lizards were further characterized by oxygen-rich and soft waters with low nutrient concentrations of nitrate and phosphate (Table 1). Chemical parameters were similar among the three NRs, except for pH. Among the three surveyed sites, pH values were 4.50–5.00 in Yen Tu NR, 5.43–5.58 in Tay Yen Tu NR, and 6.70–7.40 in Dong Son - Ky Thuong NR (Table 1). The increase in pH from acid to relatively neutral waters was negatively correlated with the altitude of the surveyed streams (r_s = -0.873; P < 0.001), suggesting that streams at lower elevations had higher pH values. While we observed a high proportion (92.3%) of sub-adults and adults along the pH gradient from 4.5–7.4, we only found juveniles at pH values between 5.4–5.6. The yearly temperature at each locality was approximately 11–23° C, without high fluctuations.

Habitat selection.—We made 94 observations of 62 individual lizards (40 from Tay Yen Tu, 12 from Yen Tu, and 10 from Dong Son - Ky Thuong). We found that Crocodile Lizards preferred to rest directly above the water body or the stream bank but never above the ground soil. We encountered lizards most frequently resting above backwater pools (59%, Fig. 2C, Fig. 3A), with low numbers of lizards found above riffle or run sections (21% and 17%, respectively), and we found only a few lizards above small waterfalls (3%; Fig. 3A). We further found that Crocodile Lizards almost exclusively rested within the vegetation. We observed only one lizard resting on a rocky cliff and never saw lizards on the forest floor or in the water during night. We observed that the majority (about 62%) of lizards rested on branches and liana (Fig. 2A-B) and fewer rested on shrubs (28%; Fig. 3B). Adults commonly occupied liana and bamboo (about 30%) where we never found juveniles. We found juveniles frequently on ferns. The diameters of the resting perches were relatively small ranging from 1–120 mm (mean = 13.1 ± 15.7 mm, n = 91; Fig. 2A, 3C). Resting perches were located 0–163 cm from shore (mean = 51.8 ± 36 cm, n = 92, Fig. 3D), and this distance was not different among age classes. The distance of resting perches to the shore for juveniles was positively correlated with water depth (r_s = -0.38, P = 0.017), which was not the case for sub-adults or adults.

The mean canopy coverage above resting individuals was 60.66 ± 37.6 % (n = 83, Fig. 3E). Sub-adults and adults preferred resting sites with significantly higher canopy cover compared with juveniles ($F_{2,86}$ = 14.27, P < 0.001), and sub-adults exclusively preferred sites with dense coverage. The heights of the resting perches ranged from 11–210 cm above water (mean = 101.33 ±

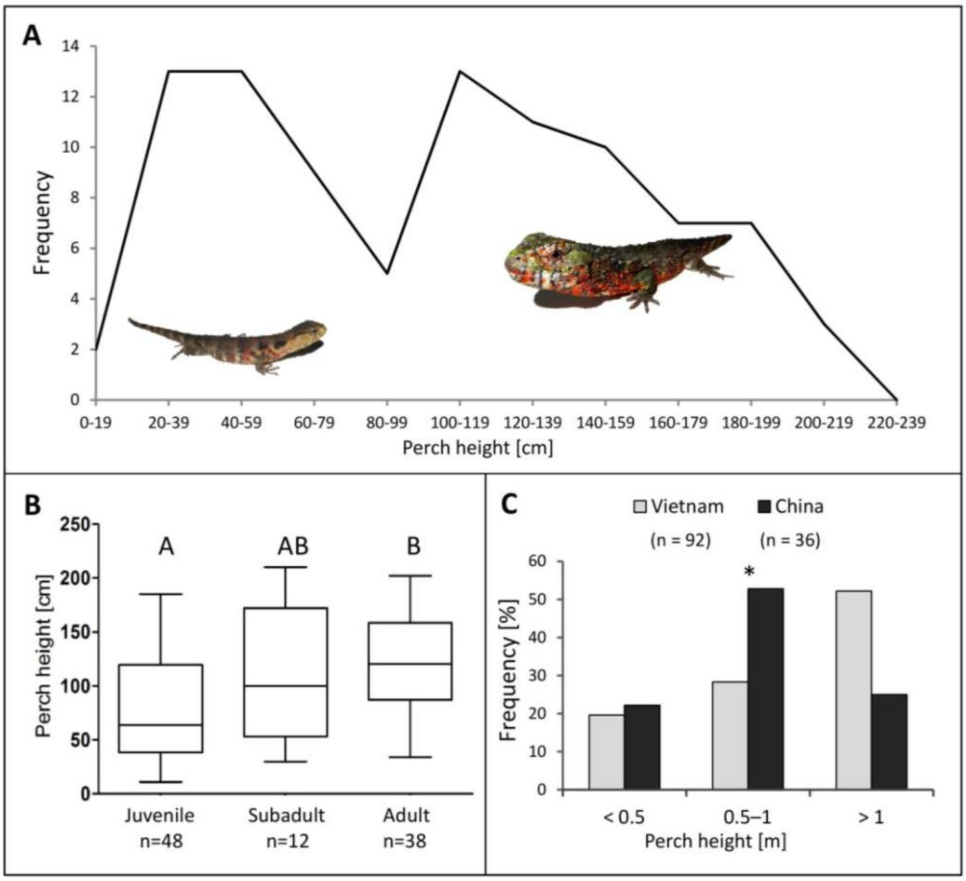

FIGURE 4. Perch heights of Crocodile Lizards (*Shinisaurus crocodilurus*). (A). Frequency histogram. (B). Spatial distribution of different age classes. (C). Spatial distribution of Vietnamese and Chinese individuals. Data for Chinese individuals from Ning et al. (2006).

53.3 cm, n = 92). There were two peaks of preferred perch heights at 20–59 cm and 100–119 cm above water (Fig. 4A), and adults occupied significantly higher perches than juveniles ($F_{2,89} = 5.60$ $P = 0.005$, Fig. 4B). The perch height was further positively correlated (r_s = 0.460; $P < 0.001$) with the canopy coverage. Our PCA with 12 abiotic factors describing the perch site of Crocodile Lizards revealed the first principal component (PC) to explained 88.6% of the overall variance. Principle Component 1 was strongly positively correlated with altitude (Table 2, Fig.5).

DISCUSSION

Habitat characterization.—The three surveyed sites differed significantly in elevation, each with a small altitudinal range, which is comparable with subpopulations in China (Zhao et al. 1999). We found that the factor altitude explained most of the distribution of Crocodile Lizards, but we do not assume that elevation is the ultimate factor determining their occurrence. We further found that elevation was correlated with both pH and stream width. In this context the small altitudinal ranges of each subpopulation compared to the whole species distribution range (200–1100 m: Huang et al. 2008; van Schingen et al. 2014a) emphasizes the importance of the need by this species of specific habitat conditions and a small realized ecological niche. The ecological niche appears to be restricted to very small sections of clean and remote streams. We found that Crocodile Lizards are strongly associated with mountainous streams mostly in untouched tropical broadleaf forests (Yen Tu and Tay Yen Tu NRs) with some occurrences in intermixed bamboo forests (Dong Son-Ky Thuong NR). This corresponds to habitat preferences of Crocodile Lizards in China (Wu et al. 2007). While annual habitat temperatures remain moderate and relatively constant, temperatures in Chinese habitats show comparatively high annual fluctuations (Zhao et al. 1999).

We further found that stream habitats generally were of soft and oxygen-rich waters, with low nutrient

TABLE 2. Factor loadings of first four principal components (explaining 99.97% of the total variance) in microhabitat selection of *Shinisaurus crocodilurus* in the Tay Yen Tu Nature Reserve (NR) in Bac Giang Province, Vietnam, Yen Tu and Dong Son - Ky Thuong NRs in Quang Ninh Province, Vietnam, and at a site in northeast Vietnam.

Factor	PC 1	PC 2	PC 3	PC 4
Perch width [cm]	-0.00937	-0.00070	-0.00019	0.00914
Perch height [cm]	0.03358	0.88150	0.04590	-0.46860
Altitude [m]	0.99900	-0.03836	0.01934	0.00140
Distance to shore [cm]	-0.01095	0.17940	0.88770	0.42380
pH	-0.00196	0.00415	-0.00161	0.00210
Canopy cover [%]	0.02430	0.43470	-0.4577	0.77480
O2 [mg/l]	0.00155	0.00571	-0.00394	0.00735
NH4 [mg/l]	0.00012	0.00015	$1.47E^{-5}$	0.00018
NO2 [mg/l]	$-4.57E^{-6}$	$1.64E^{-5}$	$-6.97E^{-6}$	$6.44E^{-6}$
NO3 [mg/l]	-0.00467	-0.01211	-0.00152	-0.01159
PO4 [mg/l]	-0.00027	-0.00021	-0.00056	-0.00037
GH [°d]	0.00378	0.00093	$1.16E^{-5}$	0.00288
KH [°d]	0.00677	0.00879	-0.00246	0.01162

concentrations and few macro-algae growth. These are typical characteristics of headwaters in mountain streams (Brehm and Meijering 1996). Stream widths ranged from 1–8 m and depth was 5–73 cm. In China, streams inhabited by Crocodile Lizards were slightly smaller and more shallow during summer (83.3% of lizards were found in streams with depth below 30 cm: Ning et al. 2006; Zollweg 2011a). However, stream depth is always variable depending on rainfall and season. The flow velocity of inhabited streams was generally slow to medium. We observed Crocodile Lizards only at sections of slow or without any flow velocity similar to observations in China (Ning et al. 2006).

We found that pH values were higher at lower elevations, which could have been due to the increased nutrient inputs from the riparian zone and the buffering capacities of the soils (Haines 2011). Juveniles of Crocodile Lizards were abundant (92% of all observed juveniles) at slightly acid sections with mean pH of 5.6 in Tay Yen Tu NR. Habitats in China are situated in limestone forest, where pH values are more basic (Michael Zollweg, pers. com.) due to the limestone (Cravotta and Trahan 1999), while most habitats in Vietnam are situated in granitic forest. Only Dong Son - Ky Thuong NR is located at the border to a limestone area, which might also explain higher pH values in these streams. Therefore, we do not think that the rock type is

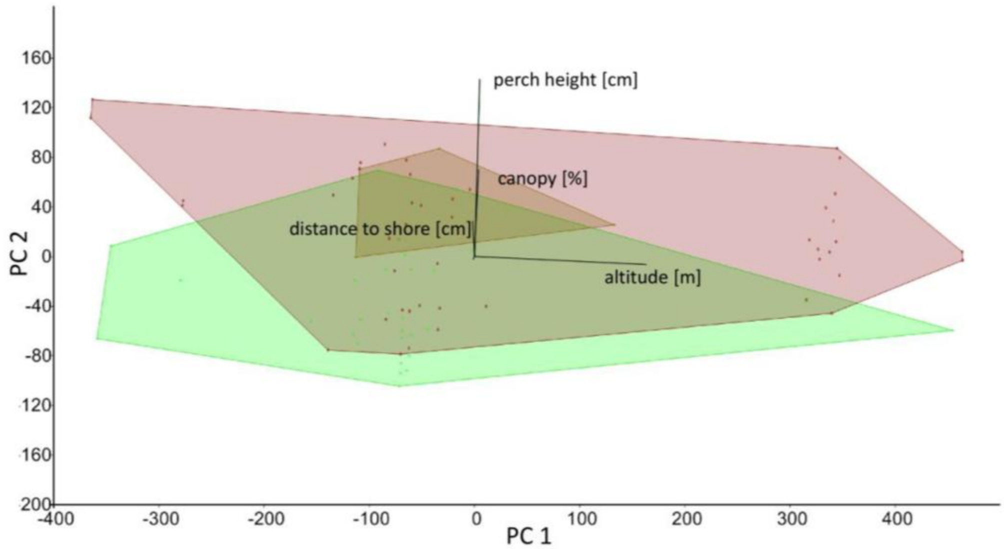

FIGURE 5. Biplot of the first and second principal components using 12 factors describing the perch selection of different age classes of Crocodile Lizards (*Shinisaurus crocodilurus*) (displayed with convex hulls). Adults (red), subadults (orange), and juveniles (green). Factors are presented as lines, whose lengths represent explanatory power of variance.

an essential factor for the habitat choice of Crocodile Lizards.

The high water quality might be another important habitat character for Crocodile Lizards because highest abundances of these lizards have been recorded in streams in Tay Yen Tu NR, where we also found abundant indicator species for high quality water, such as water-penny beetles of the family Psephenidae and stoneflies (Arnett and Thomas 2002). All inhabited streams were oxygen rich and depleted of phosphorous and nitrogen, indicating a low anthropogenic eutrophication of stream habitats. Thus, the water quality might also be an indirect factor indicating the influence of human settlements, which has already been reported as a restricting factor of the species occurrence for China (Ning et al. 2006). Accordingly, we found lowest abundances of Crocodile Lizards in Dong Son - Ky Thuong NR, where the distance to human settlements was least (see also van Schingen et al. 2014b and van Schingen et al. 2015). We also considered other biotic variables such as competitors or predators affecting the abundance and survival success of Crocodile Lizards (Irschick et al. 2005). Few Crocodile Lizards were found at sites with abundant syntopic semi-aquatic reptiles (such as the Water Dragon, *Physignathus cocincinus*, or the colubrid snake *Sinonatrix aequifasciata*).

Habitat selection.—We confirmed the assumption that different age classes of Crocodile Lizards have different perch choices. Juveniles occupied perches with significantly lower heights than those of sub-adults and adults. Similar observations have been reported for gekkonids (Snyder et al. 2010). We assumed that climbing up by lizards for shelter is associated with energy costs. We even assumed that juveniles have to feed more regularly than adults and therefore have to quit their perches more frequently. Thus, the reasons for the use of lower perches by juveniles might be regarded as a trade off to reduce the energy costs associated with climbing. However, this hypothesis needs to be tested.

Another explanation for a segregation of resting perches might be the reduction in interactions between conspecifics, as suggested by Irschick et al. (2005). Besides the height of perches, we found vegetation differences between juveniles and adults, with juveniles preferring ferns, while sub-adults and adults prefer branches and lianas, which also were more densely covered than the vegetation in which we found juveniles. While appropriate observations are lacking for other lizards, comparable spatial niche segregation between adults and sub-adults has been observed in the American Alligator, *Alligator mississippiensis* (Webb et al. 2009).

We also found inter-population differences in perch heights between Crocodile Lizards from Vietnam and from China. Individuals encountered in Vietnam occupied significantly higher perches (mostly higher than 1 m, up to 2.1 m) than Crocodile Lizards from Guangdong Province, China, where the majority of lizards were observed on perches 0.5–1 m above the ground (Ning et al. 2006). We assumed that this difference might be a result of the respective water depth below the perch, which was generally shallower in China. Because Crocodile Lizards jump into the water instead of climbing down to forage (Mona van Schingen, pers. obs.) or to escape (Huang et al. 2008), we assumed that perch height might be limited by water depth to prevent injuries while jumping into the water.

Implications.—The stenoecious habitat specialization and sedentarism of the Crocodile Lizard makes the species in particular prone to extinction in view of the acute ongoing habitat destruction and fragmentation. Habitat quality is steadily decreasing due to coal-mining activities causing the pollution of inhabited streams (van Schingen et al. 2014b; van Schingen et al. 2015). We observed that especially these streams, which are characterized by numerous backwater pools and appear necessary to provide the preferred resting sites for the species, were affected. To cope with local habitat destruction due to agriculture purpose, agreements with respective local farms helped to maintain at least core zones of important habitats intact in the Daguishan Nature Reserve in China. Further, a breeding station was constructed in 2003 and a first trial of releasing lizards back into habitat took place (Long et al. 2007, Zollweg, 2011b, 2012). These efforts have already led to a stable and an even slightly increasing subpopulation size within the Daguishan Nature Reserve in 2011 (Zollweg 2012) and would serve as an example for protection activities in Vietnam. In this context we developed a management program for Crocodile Lizards in Vietnam including the long-term monitoring of wild subpopulations. The present ecological study provides information to improve captive breeding in general and the development of a stable reserve population at the Me Linh Station for Biodiversity (see Ziegler 2015; Ziegler and Nguyen 2015). This study adds baseline data to identify suitable sites for a restocking program in Vietnam, which is planned for the near future. We also initiated an awareness campaign, including workshops, lectures, participation at conferences, and articles and poster to inform Vietnamese about this species. This has been done in close collaboration with the Forest Protection Department (FDP) of Tay Yen Tu NR to improve habitat conservation (see van Schingen et al. 2015; Ziegler 2015; Ziegler and Nguyen 2015).

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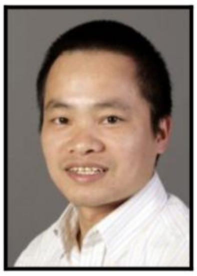

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Chapter 3: Potential distribution and effectiveness of the protected area network for the crocodile lizard Shinisaurus crocodilurus in Vietnam

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Potential distribution and effectiveness of the protected area network for the crocodile lizard, *Shinisaurus crocodilurus* (Reptilia: Squamata: Sauria)

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Abstract. The crocodile lizard, *Shinisaurus crocodilurus* Ahl, 1930, is a monotypic taxon, restricted in occurrence to southern China and northern Vietnam. Wild populations are presently suffering tremendous declines, mainly due to illegal poaching, habitat destruction, and fragmentation, which already led to the extinction of populations in Guangxi and Hunan provinces in China. In order to accelerate the discovery of so far unknown populations of *S. crocodilurus* and to identify suitable priority areas for conservation strategies, we determined the species' potential distribution using correlative species distribution models (SDMs) based on locality records and a set of satellite-based environmental predictors. In addition, we evaluated the coverage of the species' potential distribution with designated protected areas according to IUCN standards. The resulting SDM revealed potentially suitable habitats to be scattered and disconnected while being very small in size. Moreover, present coverage with nature reserves is extremely poor, underlining the urgent need for improved habitat protection measures and potential population restoration of *S. crocodilurus*.

Key words. Shinisauridae, Diploglossa, Conservation planning, Habitat suitability modelling, Species distribution modelling, Southeast Asia, Vietnam.

Introduction

The crocodile lizard, *Shinisaurus crocodilurus* Ahl, 1930, is the only living representative of the monotypic family Shinisauridae, and despite its striking appearance, it was only described relatively recently (Hu et al. 1984, Zhang 1991). The species usually is found along slow-flowing rocky streams in montane evergreen forests. The altitudinal range of this species was reported to reach from 200 to 1,500 m in China and from 400 to 800 m in Vietnam. (Zhao et al. 1999, Le & Ziegler 2003, Huang et al. 2008). So far, the occurrence of *S. crocodilurus* has been confirmed from Guangxi and Guangdong provinces in southern China while a couple of populations in Hunan and Guangxi provinces have already been extirpated (Huang et al. 2008, Zollweg & Kühne 2013, Z. Wu pers. comm.). In northern Vietnam, the species has been reported from the contiguous nature reserves Tay Yen Tu in Bac Giang Province and Yen Tu in Quang Ninh Province (Hecht et al., 2014, Le & Ziegler 2003, Ziegler et al. 2008, Nguyen et al. 2009). However, a variety of anthropogenic hazards have caused severe population declines within the last decades, reducing estimated population densities in China from 6,000 to 950 individuals between 1978 and 2008 (Zhao et al. 1999, Mo & Zou 2000, Huang et al. 2008). Illegal poaching for the international pet trade, traditional medicine and food represents the main driver fuelling the ongoing population decline, while habitat degradation, electro-fishing and fishing with poison also contribute to the species' demise (Huang et al. 2008). The Vietnamese populations are currently also threatened by habitat loss and alterations caused by intensive coal mining and illegal timber logging (Ziegler et al. 2008, M. van Schingen pers. obs.). The species' small body size combined with its striking appearance makes *S. crocodilurus* a desired target for poachers supplying the international pet trade (Le & Ziegler 2003, Huang et al. 2008). Therefore, the already heavily diminished populations of *S. crocodilurus* will likely continue to decline in China as well as in Vietnam if no immediate preventative conservation measures are initiated. While the species has not been assessed by the 'IUCN Red List' yet, it was assigned to Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and classified as a Category-I species under the 'Wild Animal Protection Law' in China (Huang et al. 2008, CITES 2013). This species has also been proposed to be included in the checklist of protected species in Vietnam (Nguyen 2011).

Correlative species distribution models (SDMs) have been used successfully to reveal potentially suitable habitats and investigate the effectiveness of protected areas (e.g., Araújo et al. 2004, Araújo et al. 2007, Hannah et al. 2007, Rödder & Schulte 2010, Rödder et al. 2010). The poikilothermic species' strong dependence on environmental conditions (e.g., water, ambient temperature) (Zhao et al. 1999, Ning et al. 2006, Wang et al. 2009) in combination with a preference for specific microhabitat characteristics renders *S. crocodilurus* an ideal taxon for performing species distribution modelling approaches. Thus, it is the aim of the present paper to predict the potential distribution of *S. crocodilurus* by applying SDMs and to identify potentially suitable habitats to guide further field exploration as a basis for improved protected area management planning.

Methods

We performed SDMs based on locality records and a set of environmental predictors that combine environmental variables and remote sensing data. We compiled a total of 20 occurrence records, partly from our own field research in northern Vietnam and southern China, as well as from literature (Huang et al. 2008). We computed a set of twelve environmental predictors based on temporal transformations of remote sensing data, using the *dismo* and *raster* packages for *Cran R* (Hijmans & van Etten 2012, Hijmans et al. 2012, R Core Team 2012). A set of pre-processed remote sensing variables derived from MODIS sensors of two NASA satellites (spatial resolution = 30 arc sec; temporal resolutions: MOD11A2 = 8-day averages; MCD43B4 = 16-day averages [Mu et al. 2007, Scharlemann et al. 2008]) was obtained from the EDENext project (imagery produced by the TALA Research Group, Oxford University using methods described in Scharlemann et al. 2008). The raw remote sensing dataset comprised monthly averages of the enhanced vegetation index (EVI), the normalized vegetation index (NDVI), and day- and nighttime land surface temperatures, collected between 2001 and 2005. The derived environmental predictors comprise variables describing annual averages as well as seasonal variability (Tab. 1).

We computed pairwise coefficients of determination based on Spearman rank correlations to assess co-linearity. A subset of twelve variables was selected with $R^2 < 0.75$, which was clipped to the spatial extent of the species' geographical range. We modelled the potential distribution of *S. crocodilurus* using the *biomod2* package v. 2.1.15 (Thuiller et al. 2013) for *Cran R*, applying the following algorithms: 'Generalised Boosting Models' (GBM), 'Multivariate Adaptive Regression Splines' (MARS), 'Generalized Linear Models' (GLM), 'Generalized Additive Models' (GAM), 'Classification Tree Analyses' (CTA), 'Artificial Neuronal Networks' (ANN), 'Surface Range Envelopes' (SRE), and 'Maxent'. The models were trained using a randomly selected subset of the species' occurrence records (80%), while the remaining 20% were used to analyse model performance with five iterations per algorithm, applying the 'receiver operating characteristic curve' (ROC) (Swets 1988), 'Cohen's Kappa', and the 'True Skill Statistic' (TSS) (Allouche et al. 2006). We used 1,000 randomly created pseudo-absence records within a circular buffer of 50 km, encompassing each presence record for model building. Based on the SDMs, we computed an ensemble integrating all SDMs with ROC > 0.7 ranked according to their performance. The final ensemble was projected onto a rectangular area slightly larger than the area covered by the species records to highlight potentially suitable habitats in northern Vietnam and southern China. As a presence/absence threshold, we selected the minimum score observed in the species' records. Areas characterized by environmental conditions exceeding those available within the 50 km buffer enclosing all species records were excluded from projections as extrapolations beyond the training range of the ensemble, as these would likely increase the uncertainty factor.

Potential habitat suitability for *S. crocodilurus* as well as the coverage with designated protected areas according to IUCN standards (categories I, II, IV, V, VI; IUCN 2013) were evaluated across the study area in order to reveal potentially suitable habitat yet unexplored for the occurrence of the species and to ease the future management planning of reserves. We obtained protected area shape files from the World Database of Protected Areas (IUCN, UNEP-WCMC 2013). To characterize the realized and potential niche of the species, we extracted all environmental variables from the existing species records as well as from the available environmental background within the 50 km buffer and computed density estimates using the *sm* package for *Cran R*.

Results

New population record

As a result of our recent field research in northern Vietnam in 2013, another population of *S. crocodilurus* was discovered in the Dong Son – Ky Thuong Nature Reserve on the eastern side of Yen Tu Mountain in Quang Ninh Province. The new population is distant by about 40 km from the known subpopulations in Vietnam (Bac Giang and Quang Ninh provinces) and 380 km from the closest subpopulation in China (Guangxi Province). We could also extend the known altitudinal range of the species in Vietnam (from

Table 1. Environmental variables and derived variables sets used for SDM development.

Abbreviation	Set variable	Derived variable
ED15078_bio10	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temperature	Mean Temperature of Warmest Quarter
ED15078_bio11	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temperature	Mean Temperature of Coldest Quarter
ED15078_bio2	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temperature	Mean Diurnal Range (Mean of Monthly max–min Temp.)
ED15078_bio3	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temperature	Temperature Isothermality (BIO2/BIO7) (* 100)
ED15078_bio4	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temperature	Seasonality of Temperatures
ED15078_bio5	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temperature	Maximum Temperature Warmest Month
ED15078_bio7	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temperature	Temperature Annual Range (BIO5-BIO6)
ED1514_bio1	MODIS V4 Band 14 Synoptic Months: Normalised Difference Vegetation Index (NDVI)	Annual Mean NDVI
ED1514_bio7	MODIS V4 Band 14 Synoptic Months: Normalised Difference Vegetation Index (NDVI)	Annual Range of NDVI
ED1515_bio1	MODIS V4 Band 15 Synoptic Months: Enhanced Vegetation Index (EVI)	Annual Mean EVI
ED1515_bio5	MODIS V4 Band 15 Synoptic Months: Enhanced Vegetation Index (EVI)	Maximum Monthly EVI
ED1515_bio7	MODIS V4 Band 15 Synoptic Months: Enhanced Vegetation Index (EVI)	Annual Range of EVI

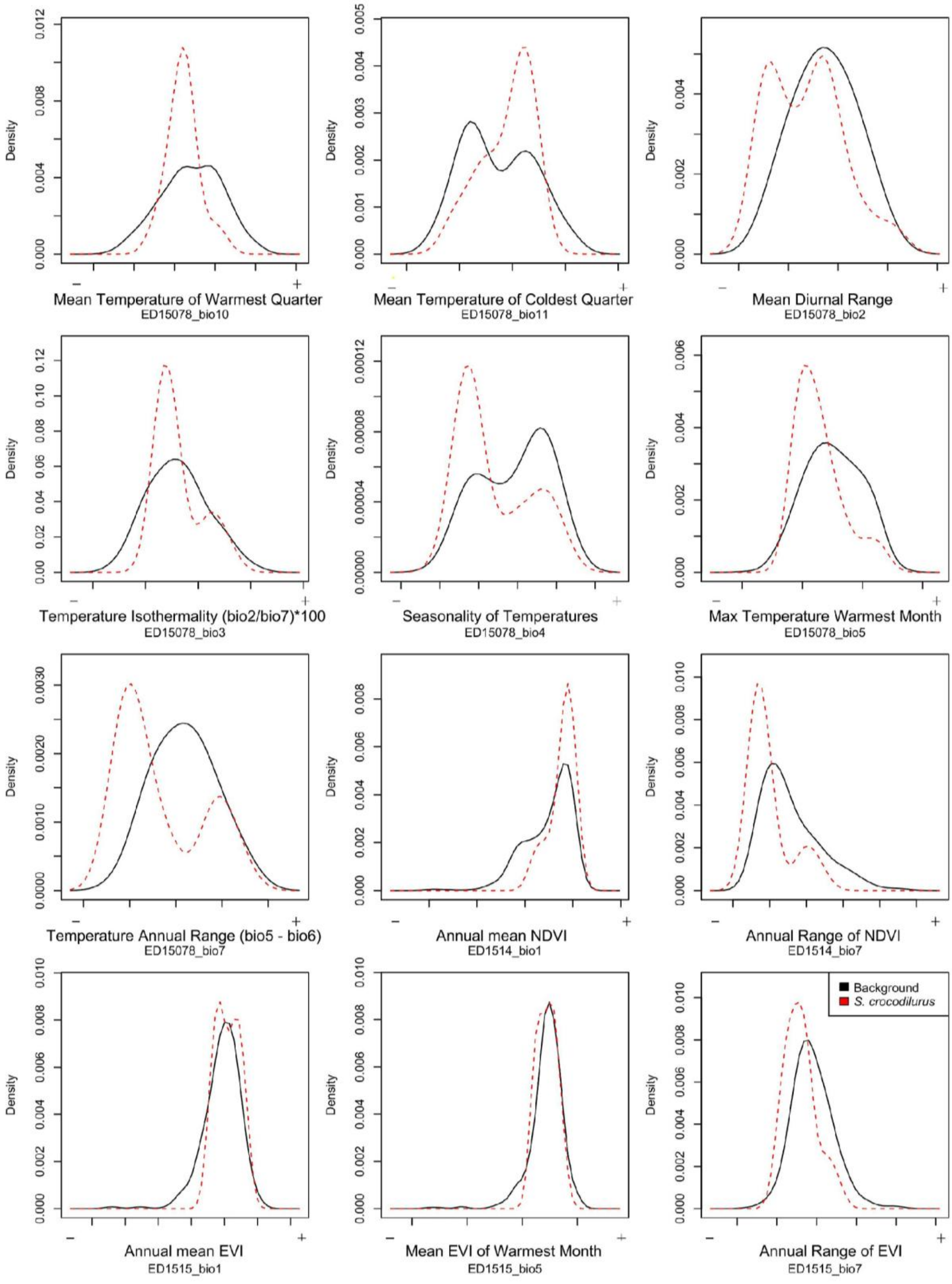
400 to 800 m, see Le & Ziegler 2003, Ziegler et al. 2008) by discovering individuals occurring from 180 m a.s.l. in the Dong Son – Ky Thuong Nature Reserve to 850 m a.s.l. in the Yen Tu Nature Reserve, revealing a similar altitudinal range compared to Chinese populations (from 200 to 1,500 m, see Huang et al., 2008, Zhao et al. 1999).

Realized and potential niche

With respect to all univariate comparisons, *S. crocodilurus* occupied an environmental niche nested in the overall available niche space (Fig. 1). The comparisons revealed only slightly smaller spans of the realized niches compared to the available niches, but different density maxima (Fig. 1), as *S. crocodilurus* occupies areas with relatively low NDVI scores compared to the overall NDVI range, indicating its close dependence on intact vegetation. Furthermore, *S. crocodilurus* inhabits areas with an annual temperature range that is relatively constant without extreme maxima. The overall relatively low annual temperature range is consistent with mountainous habitats.

Potential distribution

With 'excellent' ROC values being obtained for the ensemble (ROC_{test} = 0.996, Kappa = 0.239, TSS = 0.957), the model shows a strong ability to discriminate between suitable and unsuitable habitats. The variable ED1514_bio7 (Annual Range of NDVI) had the strongest effect on the model (52%) as measured by permutation importance, followed by ED15078_bio7 (Temperature Annual Range) (50%), ED15078_bio4 (Seasonality of Temperatures) (41%), ED15078_bio10 (Mean Temperature of Warmest Quarter) & ED15078_bio3 (Temperature Isothermality) (38%), ED15078_bio2 (Mean Diurnal Temperature Range), and ED1515_bio5 (Maximum Monthly EVI) (37%), whereas the remaining variables contributed on average less than 35% to the final model. The ensemble revealed only small proportions of the study extent to provide suitable habitats (China: 1.12%; Vietnam: 4.29%; Fig. 2). Only a fraction of the selected study region was found to be covered by protected areas in Vietnam (17.29%) while only 6.31% of the study region was found to be covered by reserves in China. Furthermore, only a fraction of habitat deemed suitable is presently located within designated protected areas (1.74% in China and 0.15% in Vietnam; Fig. 2). The model suggests potential additional occurrences of *S. crocodilurus* to exist, amongst others, in the Shiwandashan Nature Reserve (SNR) in southern China. According to our model, the SNR situated between the confirmed localities in southern China and northern Vietnam represents the largest contiguous area of potentially suitable habitat in China (Fig. 2). Small fragmented areas with high predicted probabilities for the occurrence of *S. crocodilurus* are scattered across northwestern Vietnam, but are presently not protected.



gure 1. Comparisons of density distributions of the realised bioclimatic space of *Shinisaurus crocodilurus* with the potential avail-le space along 12 environmental variables. Note that derived variables comprise relative scores specific to the study area. Therefore, ıly qualitative units are shown.

4

Discussion

Density estimates across the 12 environmental gradients revealed different characteristics of the available vis-à-vis realised niche, indicating that *S. crocodilurus* is a habitat specialist. Denoted already by Ning et al. (2006), the vegetation index proved to be a determinant for the occurrence of *S. crocodilurus*. In accordance with previous studies (Zhao et al. 1999, Wang et al. 2009), temperature-related variables revealed a strong contribution to the SDMs, as the species occupies habitats characterised by low temperatures as well as a low diurnal and annual temperature range. These microhabitat conditions are also characteristics of mountainous habitats. Our model revealed several spots covered by potentially suitable habitats to be situated in northwestern Vietnam. To date, the species has not been confirmed to occur in this area, and the Red River might serve as a geographical barrier, restricting the species' distribution to northeastern Vietnam. However, SDMs are not able to identify geographical barriers and the accessibility of a potentially suitable habitat so that this hypothesis has to be verified by further field surveys. The small size of potentially suitable habitats combined with heavy fragmentation and poor coverage with designated protected areas underlines the urgent need for significant improvements of the existing reserve network to increase effectiveness for the conservation of *S. crocodilurus*. Therefore, potentially suitable habitats with high detection probabilities should be surveyed for occurrences of the rare species. Due to its limited dispersal capacity, its close dependence on water (Zhao et al. 1999, Le & Ziegler 2003, Zollweg & Kühne 2003, Zheng & Zhang 2004, Ning et al. 2006, Huang et al. 2008), and a rather sedentary lifestyle, we expect locations situated in close proximity or between confirmed populations, such as the SNR in southern China or the Khe Ro Sector within Tay Yen Tu Nature Reserve in Vietnam, to be most promising. Such areas might represent important stepping stones for the species. The few existing reserves presently holding populations of *S. crocodilurus* (Tay Yen Tu, Yen Tu, and Dong Son – Ky Thuong nature reserves in northern Vietnam and the Luokeng, Daguishan, and Linzhouding nature reserves in southern China) need to be subjected to significant law enforcement to reduce the collection of individuals to a minimum and prevent electro-fishing and fishing with poison. Moreover, these protected areas should be considered for status elevation to prevent further habitat loss and fragmentation. The alarmingly rapid population declines observed recently throughout the species' distributional range highlights the urgency of an assessment for the IUCN Red List, which is currently undertaken by the IEBR and the Cologne Zoo, as well as the need for a zero quota on the commercial trade of wild-caught specimens. Not only the status, but also the size of the remaining populations should be analysed or re-analysed contemporarily to clarify whether scientifically coordinated population restoration is required besides improved habitat protection measures.

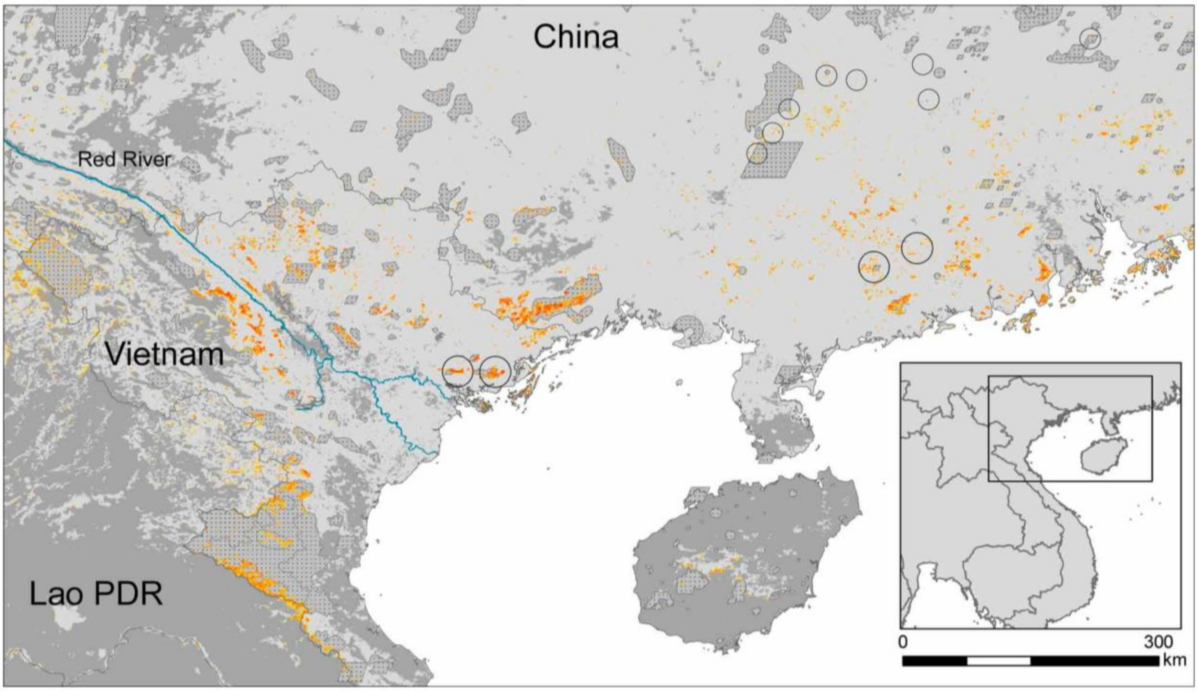


Figure 2. Occurrence records of *Shinisaurus crocodilurus* are displayed as black circles, with potential habitat suitability ranging from low (yellow) to high (red), coverage with designated reserves (stippled polygons), and the course of Red River (blue). For dark grey areas, no predictions could be made, as environmental conditions exceed the training range of the SDM. Only vague locality information is displayed in order to protect remnant populations.

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Chapter 4: Discovery of a new crocodile lizard population in Vietnam: population trends, future prognoses and identification of key habitats for conservation

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Discovery of a new crocodile lizard population in Vietnam: Population trends, future prognoses and identification of key habitats for conservation

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Abstract: The crocodile lizard, a globally endangered species with a restricted range in southern China and northern Vietnam, is an increasingly demanded species in the international pet trade. Poaching activities brought the species to the brink of extinction, while ongoing habitat destruction represents an additional peril to the species. Especially the Vietnamese population is extremely small with a preliminary estimation of less than 100 individuals. According to predictions of the species potential distribution, we conducted targeted field surveys to search for further populations in Vietnam in order to update the species' conservation status. We could prove the practical and efficient applicability of this theoretical model by the discovery of a new population from a predicted forest site near the international border between China and Vietnam. Based on monitoring of the Vietnamese population from 2010 to 2015 we further provide an overview about current population trends, which revealed dramatic local declines of more than 50% of effective population sizes and the species' extirpation at a third of all known sites. In addition, we predicted future scenarios of suitable habitats and compared these results with actual forest cover in order to define key habitats for effective conservation measures.

Keywords: Niche modeling, new population record, climate change, priority areas for conservation, population dynamics, conservation planning

INTRODUCTION

The crocodile lizard (*Shinisaurus crocodilurus*), which was originally described by Ahl (1930) from small isolated areas in southern China and only relatively recently discovered from North Vietnam by Le & Ziegler (2003) (see also Ziegler *et al.*, 2008), is currently at the brink of extinction (van Schingen *et al.*, 2015a). Its outstanding color patterns and primeval appearance make the species evermore desired in the international trade. Since the 1980s, Chinese specimens are being internationally traded resulting in dramatic population declines to only 950 individuals in 2008 (Huang *et al.*, 2008; van Schingen *et al.*, 2015a). Individuals of the much smaller Vietnamese population just recently entered the international pet trade in amounts that are not sustainable for wild populations (van Schingen *et al.*, 2015a). While the heavily diminished wild populations are steadily shrinking the international demand for new bloodlines, especially from Vietnam is rising (van Schingen *et al.*, 2015a). In addition, ongoing habitat destruction currently imperils all populations of the ecologically highly specialized species (Huang *et al.*, 2008; van Schingen *et al.*, 2014b, 2015b). A previous study revealed an effective population size – defined as number of mature individuals – of only less than 100 individuals in Vietnam, distributed among three separated subpopulations (van Schingen *et al.*, 2014b). However, the appearance of relatively high numbers of allegedly Vietnamese specimens in the trade indicate that potentially unknown subpopulations must exist, which are probably still only known by local collectors (van Schingen *et al.*, 2015a). Due to the unsustainable trade in *S. crocodilurus* the species was listed on CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix

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II and recently as Endangered on the IUCN (International Union for Conservation of Nature) Red List of Threatened species (Huang *et al.*, 2008; Nguyen *et al.*, 2014). Facing the current alarming conservation status, the discovery of further potential subpopulations and the identification of key habitats is crucial for improved conservation measures. A first niche model approach predicted that the overall suitable habitats of *S. crocodilurus* are rare, fragmented, and poorly covered with protected areas (van Schingen *et al.*, 2014a). Species distribution models (SDMs) represent an increasingly applied tool to predict the potential distribution and to identify suitable habitats of species (e.g., Rödder *et al.*, 2010; Rödder & Schulte, 2010). However, these models are principally theoretical and have been rarely proven valid in the field. One aim of the present study was to use the predicted potential distribution of *S. crocodilurus* as a base to search for further occurrences in order to verify the approach and provide updated information on the population and conservation status of the species. We further provide an overview about current population trends of Vietnamese crocodile lizards based on population monitoring over the recent years.

At present, climate change is recognized as one of the major forces biasing species distribution and it is assumed to imperil numerous tropical lizard species, due to their narrow temperature tolerances (e.g., Araújo & Rahbek, 2006; Beaumont *et al.*, 2007; Sinervo *et al.*, 2012; Huey *et al.*, 2012; Tewksbury *et al.*, 2012). Crocodile lizards are semi-aquatic habitat specialists adapted to remote and densely vegetated, clean streams within evergreen broadleaf forest and regarded as dependent on annual moderate cool temperatures (Ning *et al.*, 2006; van Schingen *et al.*, 2014a, 2015b). Thus, *S. crocodilurus* is likely being affected by climatic change and habitat alteration. In China, Li *et al.* (2012) already projected that all suitable habitats for the crocodile lizard will vanish by 2080. Thus, another aim of the present study was to predict future scenarios of habitat suitability in order to identify key habitats for *S. crocodilurus*, which remain in the future. Since SDMs alone cannot incorporate every single factor to predict habitat suitability, we combined results of a Maxent model with detailed vegetation data to identify, which of the predicted suitable habitats still comprise intact and connected broadleaf forest. Based on this approach we aim to define priority areas for improved conservation measures and provide recommendations on where 1) nature reserves need to be upgraded, 2) newly established, or 3) forest corridors must remain to allow genetic exchange between populations, and 4) sites are most suitable for future restocking / release programs.

METHODS

Population status: According to our previous niche model approach (van Schingen *et al.*, 2014a) one of the largest areas of connected suitable habitats was predicted to be situated in the border region of China and Vietnam. However, no field research focusing on the species has been carried out in this region so far. Based on our preliminary predictions we conducted a field survey in the border area between China and Vietnam in June 2015 in order to verify the accuracy of SDMs and update the current population status of *S. crocodilurus* in Vietnam.

To evaluate population dynamics and the current status of the crocodile lizard in Vietnam, we analyzed population data from all known localities [Tay Yen Tu Nature Reserve (NR) (N 106.81168°, E 21.17190°), Bac Giang Province; Yen Tu (N 106.70006°, E 21.16716°) and Dong Son-Ky Thuong NRs (N 107.11962°, E 21.15358°), Quang Ninh Province], collected during field surveys in 2010, 2013, 2014 and 2015 (see van Schingen *et al.*, 2014b, 2015a for methods). All surveys had been conducted by our team during the rainy season between May and July. Since 2010 each individual with a snout-vent length (SVL) > 100 mm had been permanently marked by a passive integrated transponder (PIT) tag for individual identification and long term population monitoring (see van Schingen *et al.*, 2014b for details). Individuals were categorized in age classes based on SVL (for methods see van Schingen *et al.*, 2014b, 2015b). Methods conform with IUCN Resolution 4.015 (Guidelines regarding research and scientific collection of threatened species). Data of in total 52 night surveys were included in this analysis (Table 1). Population sizes were estimated according to Huang *et al.* (2008) and van Schingen *et al.* (2014b), with a slightly modified formula given as: $N = \Sigma\ [m(1 + i/x)]$. Hereby N is the total population size, m is the total number of observed individuals along one stream including all surveys within one season, i is the "invisible rate" index ($i = [\Sigma(b_n-a_n)]/\Sigma\ a_n$, where a_n is the number of observed individuals in stream n during the first survey and b_n is the total number of observed individuals in stream n) and x is the number of surveys in transect n during one survey. For the invisibility rate i we used the rate calculated by van Schingen *et al.* (2014b) to make the data comparable. A Chi²-test was applied to test for temporal differences in population structure using PAST version 2.17 (Hammer *et al.*, 2001). Significant difference was declared for $p < 0.05$.

Modeling: Based on 41 occurrence records and a set of environmental factors, we predicted the future potential distribution of *S. crocodilurus* with Maxent (Phillips *et al.*, 2006). All occurrence records from northern Vietnam were compiled during own field surveys between 2013 and 2015, while records from southern China were derived from literature (Huang *et al.*, 2008; Huang *et al.*, 2014; van Schingen *et al.*, 2014b) or were provided by researchers (Z. Wu in *litt.*, 2013). For minimizing effects of spatial autocorrelation and to ensure the independency of the records, we only included the two outermost occurrences of

Table 1. Summary of observed, estimated and recaptured *Shinisaurus crocodilurus* in Vietnam between 2010 and 2015.

	Tay Yen Tu		Yen Tu			Dong Son-Ky Thuong			Hai Ha (new)	Total
Variable	Site 1	Site 2	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	
2010										
Total (observed)	4	9	*	*	*	*	*	*	*	13
Mature (obs.)	3	4	*	*	*	*	*	*	*	7
Juveniles (obs.)	0	2	*	*	*	*	*	*	*	2
Night surveys	5	4	*	*	*	*	*	*	*	9
Total (estimated)	5	12	*	*	*	*	*	*	*	17
Mature (est.)	4	5	*	*	*	*	*	*	*	9
2013										
Total (observed)	7	33	3	8	*	2	4	5	*	62
Mature (obs.)	3	11	2	8	*	2	2	3	*	31
Juveniles (obs.)	2	21	1	0	*	0	1	0	*	25
Recaptures	1	1	*	*	*	*	*	*	*	2
Night surveys	3	5	1	2	*	1	1	1	*	14
Total (estimated)	10	42	7	13	*	5	9	12	*	98
Mature (est.)	4	14	5	13	*	5	5	7	*	53
2014										
Total (observed)	9	25	0	7	7	1	1	1	*	51
Mature (obs.)	5	6	0	0	1	1	0	0	*	13
Juveniles (obs.)	3	18	0	7	5	0	0	0	*	33
Recaptures	3	3	0	0	*	1	0	1	*	8
Night surveys	2	3	0	1	1	1	1	1	*	10
Total (estimated)	15	36	0	16	16	2	2	2	*	91
Mature (est.)	8	9	0	0	2	2	0	0	*	22
2015										
Total (observed)	27	25	0	0	20	0	4	5	12	93
Mature (obs.)	10	10	0	0	4	0	2	1	1	28
Juveniles (obs.)	10	13	0	0	15	0	1	4	11	54
Recaptures	6	7	0	0	1	0	2	1	*	17
Night surveys	6	6	0	1	2	1	1	1	1	19
Total (estimated)	33	31	0	0	34	0	9	12	28	147
Mature (est.)	12	12	0	0	7	0	5	2	2	41

*Asterisks indicate that respective site was not known at time point

one site in the analyses (Jennings & Veron, 2011; Jennings *et al.*, 2013). Since *S. crocodilurus* is heavily dependent on specific climatic conditions and restricted to certain altitudinal ranges (van Schingen *et al.*, 2014a, 2015b), we used the following 19 bioclimatic variables: BIO1 "Annual Mean Temperature", BIO2 "Mean Diurnal Range" (Mean of monthly [max temp - min temp]), BIO3 "Isothermality" (P2/P7) (*100), BIO4 "Temperature Seasonality" (standard deviation *100), BIO5 "Max Temperature of Warmest Month", BIO6 "Min Temperature of Coldest Month", BIO7 "Temperature Annual Range" (P5-P6), BIO8 "Mean Temperature of Wettest Quarter", BIO9 "Mean Temperature of Driest Quarter", BIO10 "Mean Temperature of Warmest Quarter", BIO11 "Mean Temperature of Coldest Quarter", BIO12 "Annual Precipitation (year)", BIO13 "Precipitation of Wettest Month", BIO14 "Precipitation of Driest Month", BIO15 "Precipitation

Seasonality" (Coefficient of Variation), BIO16 "Precipitation of Wettest Quarter", BIO17 "Precipitation of Driest Quarter", BIO18 "Precipitation of Warmest Quarter" and BIO19 "Precipitation of Coldest Quarter" obtained from the WorldClim global climate database (www.worldclim.org assessed in July 2015; Hijmans *et al.,* 2005), and an elevation layer derived from the Consortium for Spatial Information (CGIAR-CSI. Available from http://srtm.csi.cgiar.org, assessed in July 2015; Reuter *et al.,* 2007) as environmental predictors. The potential distribution was predicted for the current and future (2020, 2050 and 2080) situations, while the resolution of bioclimatic layers decreased from 800 m (present time) to 8000 m for future prognoses. We reduced the resolution of originally 3 m of digital elevation data to fit with bioclimatic data. Suitable habitats for *S. crocodilurus* were modeled using 10033 points (9992 background points and 41 present records) to determine the Maxent distribution. The model was trained with a randomly selected subset of the species' presence records (80%), while the remaining records were used to evaluate the model performance. The study extent for predictions was selected according to van Schingen *et al.* (2014a), covering the whole distribution range of the species. Besides specialization to climatic conditions and a distribution to a restricted elevation range, the crocodile lizard is further associated to undisturbed forest (e.g., Huang *et al.,* 2008; Ning *et al.,* 2009; van Schingen *et al.,* 2014b). Thus, we combined the results of the Maxent predictions with actual vegetation coverage, which has been classified from Landsat 8 satellite images (LC81260452014364LGN00, and LC81260462014364LGN00, resolution of 30 m), derived from the United States Geological Survey (USGS), available from http://glovis.usgs.gov (accessed July 2015). We calculated the "effective suitable area" consisting of closed forest coverage and suitable habitats according to Maxent predictions for the species' distribution range in northern Vietnam. This prediction was only made for the current situation, since no future prognoses for vegetation data are existent. The vegetation maps were classified by ERDAS with supervisor classification and all maps were created using ArcGIS software by Esri.

RESULTS

New population: According to predictions by SDMs we discovered a new population of *S. crocodilurus* in Hai Ha District, Quang Ninh Province close to the Chinese border (around N 21.196146°, E 106.882572°). This population represents the northernmost record of the species in Vietnam, about 60 km distant from the closest Vietnamese population in Dong Son-Ky Thuong Nature Reserve, and is situated outside of any protected area. Accurate locality data are not presented to prevent targeted poaching in this area. We observed a total of 12 individuals along a single forested lowland stream at low elevations between 131 and 198 m above sea level (a.s.l.). With respect to elevation, this finding represents the lowermost record of *S. crocodilurus* in general (vs. 200-1500 m a.s.l. in China and 180-850 m a.s.l. in Vietnam, see van Schingen *et al.,* 2014a). The habitat mostly resembled habitat sites in Dong Son-Ky Thuong NR, characterized by relatively broad lowland streams within mixed broadleaf and bamboo forest (van Schingen *et al.,* 2015b). We found all animals resting on branches, mostly above backwater pools, which is accordant to previous observations at known sites (van Schingen *et al.,* 2015b). The accompanying herpetofauna was similar to habitats within the Yen Tu Mountain range and Dong Son-Ky Thuong NR, and represented by high abundances of *Quasipaa* sp., *Sinonatrix* sp. and *Sphenomorphus cryptotis*. We observed only a single young adult (Fig. 1A) with a snout-vent length of 142 mm, while the remaining 11 individuals were juveniles (Table 1, Fig. 1B). Furthermore, we only encountered animals along one single stream in the area. Our interviews with local villagers revealed that crocodile lizards had been very abundant throughout the whole region until two years ago. Apparently, the population dramatically decreased due to poaching for the pet trade. Numerous local villagers are allegedly hunting the lizards for sale to traders from Hai Phong City or Hanoi. According to local villagers, only adult individuals are being collected for the trade, since juveniles cannot be sold. Meanwhile, it is allegedly extremely difficult even for the locals to still find the species in the wild.

Population trends: During four years of field research in 2010, 2013, 2014 and 2015 in Vietnam, we encountered a total of 192 different individuals of *S. crocodilurus*. From 2013 onwards, we annually recaptured 2 to 17 individuals marked in the year(s) before. Of the individuals marked in 2010 we only recaptured two lizards in 2013, whereof one individual was still recaptured in 2015. In 2010 we only surveyed Tay Yen Tu NR, while we discovered new sites in Yen Tu and Dong Son-Ky Thuong NRs in 2013 (Table 1). In total we discovered nine different sites inhabited by *S. crocodilurus* until 2015. Since the last field survey in 2015, we found that crocodile lizards were probably extirpated from three (one third) of these sites (Table 1).
Our study revealed an overall increase in total population size to currently approximately 147 individuals distributed over all known occurrences in Vietnam (Fig. 2A). However, regarding the effective population size – considering only mature individuals – the estimated wild population slightly decreased from 2013 to 2015 to about 41 individuals (Fig. 2A).
Comparing different regions, Tay Yen Tu NR contained the largest known subpopulation with currently about 64 estimated total individuals, whereof we considered 24

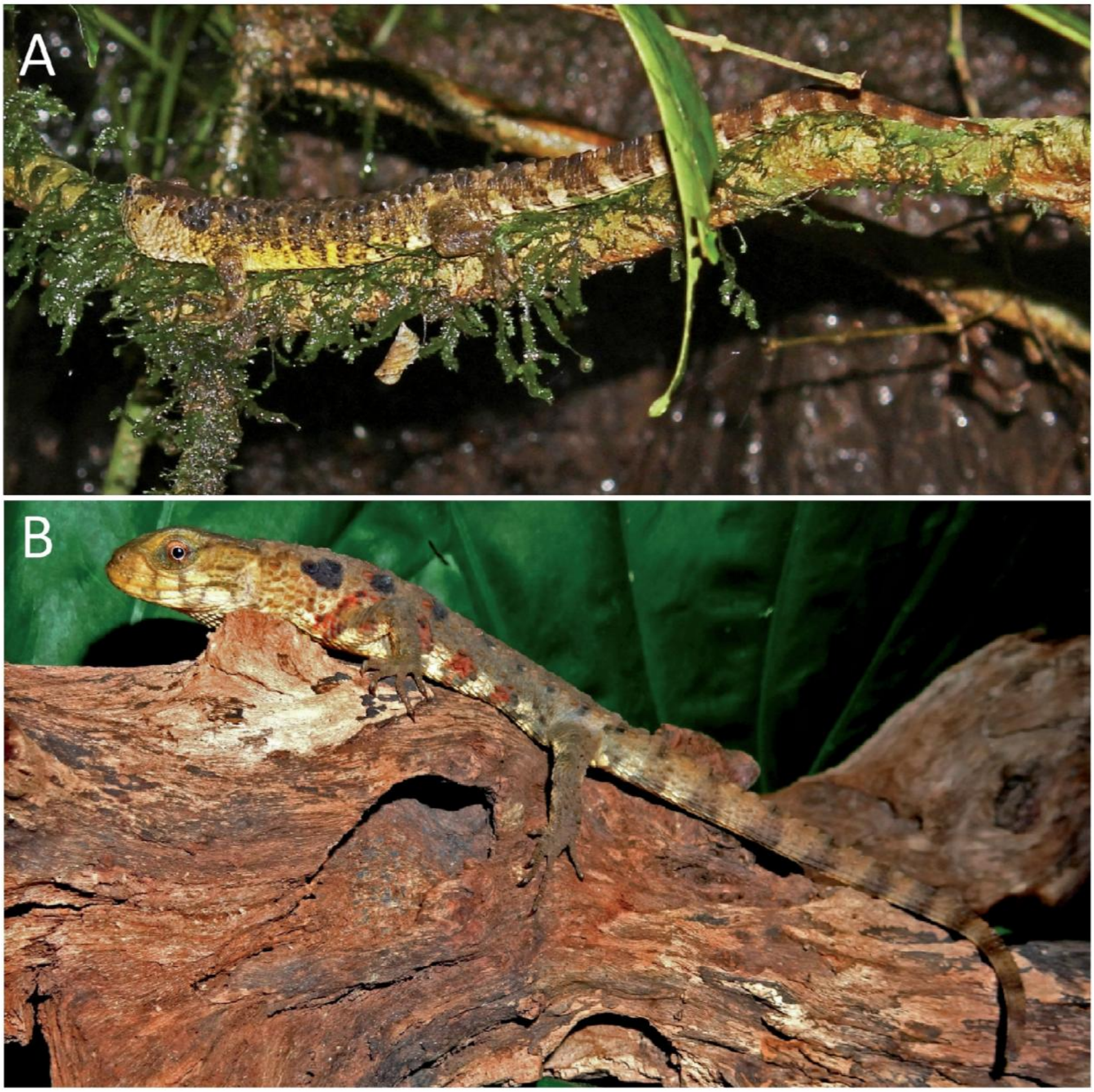

Fig. 1. New population of *S. crocodilurus* from Hai Ha District, Quang Ninh Province, Vietnam. (A) Adult in the habitat. (B) Juvenile. Photos C.T. Pham and M. van Schingen.

to be mature. We estimated the other three populations to consist in total of 21 to 34 and regarding mature individuals of two to seven mature individuals (Table 1, Fig. 2B). In fact we only encountered one to four mature individuals in 2015 each in Yen Tu NR, Dong Son-Ky Thuong NR and the new population at the border with China, respectively. Only in Tay Yen Tu NR the effective population size almost doubled throughout the last five years, while the other populations experienced a 57 to 60% decrease in mature individuals between 2013 and 2015, respectively (Table 1, Fig. 2C). Furthermore, a significant change in the population structure was recorded between 2010 and 2015 (Chi^2=18.53, df=6; p=0.005; Fig. 3). We found that mature individuals represented more than half (54%) of the population still in 2010, while the relative portion of this group decreased to 30% in 2015 (Fig. 3).

Suitable habitats: Of the environmental predictors the "Mean Diurnal Range" (27.1%), "elevation" (19.8%) and "Annual Mean Temperature" (12.4%) had highest relative contributions on the Maxent model predicting current suitable habitats. With respect to future prognoses the predictor "Mean Diurnal Range"

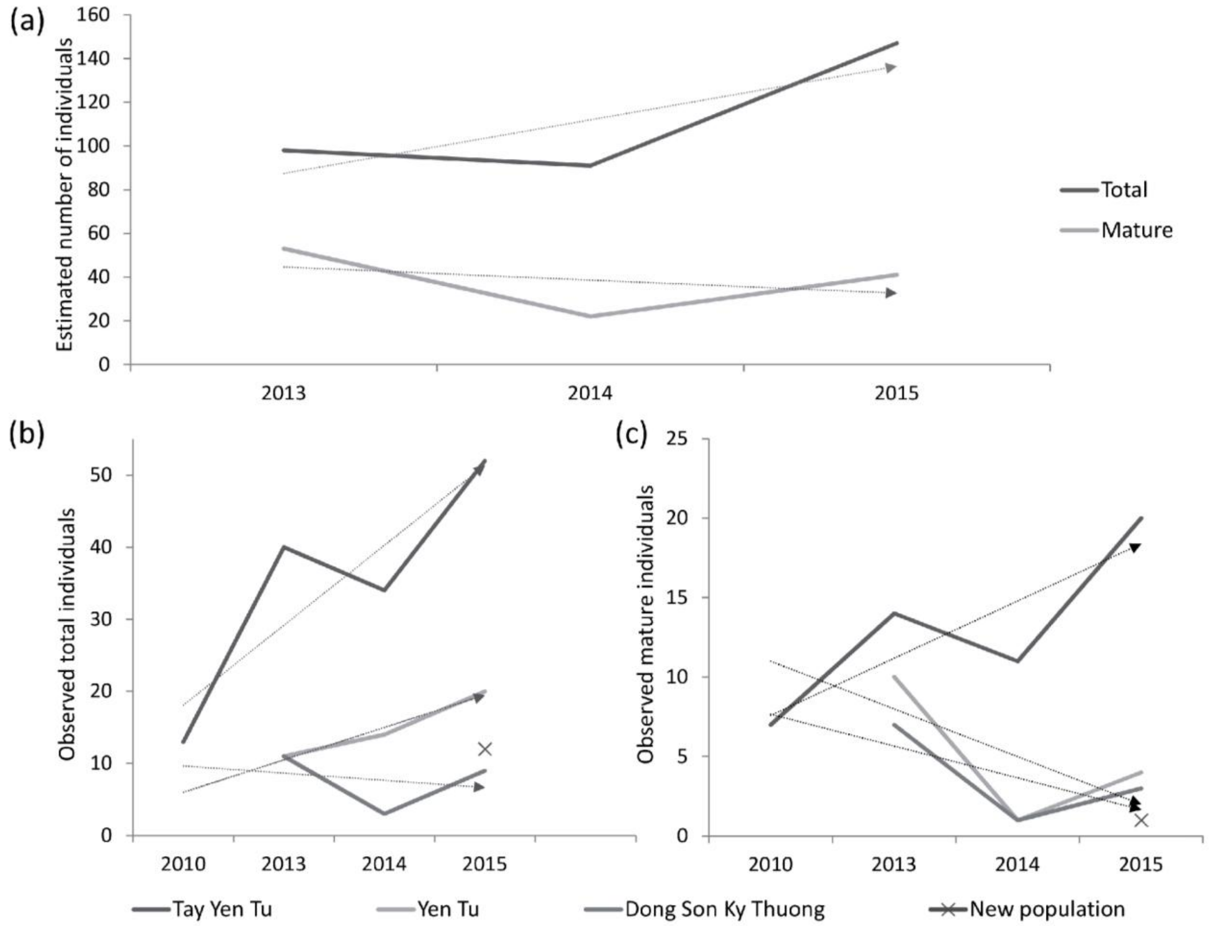

Fig. 2. Population trends of *S. crocodilurus* in Vietnam from 2010 to 2015. (A) Estimated total population sizes in Vietnam. (B) Observed total subpopulation sizes. (C) Observed effective subpopulation sizes. Arrows indicate trend lines.

gained in importance and remained the predictor with the highest relative contribution (39.3%, 30.5% up to 45.2%) in 2020, 2050 and 2080, respectively. Accordingly, response curves of the respective predictor indicate a restricted tolerance of *S. crocodilurus* to high diurnal temperature amplitudes and a strong dependence on constant and moderate temperature ranges. The relative contributions of other parameters only increased for later scenarios of the model, e.g. the "Mean Temperature of the Coldest Quarter" did not contribute to the present and the 2020 model, while this predictor became more important for the predictions from 2050 to 2080. In comparison to the bioclimatic predictors, the contribution of the factor "elevation" remained relatively constant (6.9 to 12.4%) throughout all predictions. Variables reflecting temperature data generally revealed higher contributions to the Maxent distribution than variables referring to precipitation, independent of the projected time period.

The model revealed only small proportions of the studied area to provide suitable habitats, which were further assumed to decrease from 5% to less than 0.3% between 2020 and 2080 (Fig. 4A-D). Our model suggested that the only remaining contiguous area of highly suitable habitat in Vietnam encompasses the Yen Tu Mountain Range, adjacent Dong Son-Ky Thuong NR and extends to the northeastern border with China (Fig. 4D). The areas with highest suitability throughout the whole distribution range were projected to be situated in Vietnam.

Combining the Maxent results with vegetation data, the effective suitable area was predicted to encompass about 263 km² (4.2%), 1253 km² (20.11%), 200 km² (3.2%) and 945 km² (15.2%), respectively of the distribution range in northern Vietnam, ranked from high to low suitability (Fig. 5). Accordingly, forests are already heavily fragmented, especially around present occurrence records. Core regions with intact forest coverage still exist in some parts of the Yen Tu Mountain Range, Dong Son-Ky Thuong NR. One of the major areas of contiguous forest expands from the eastern border of

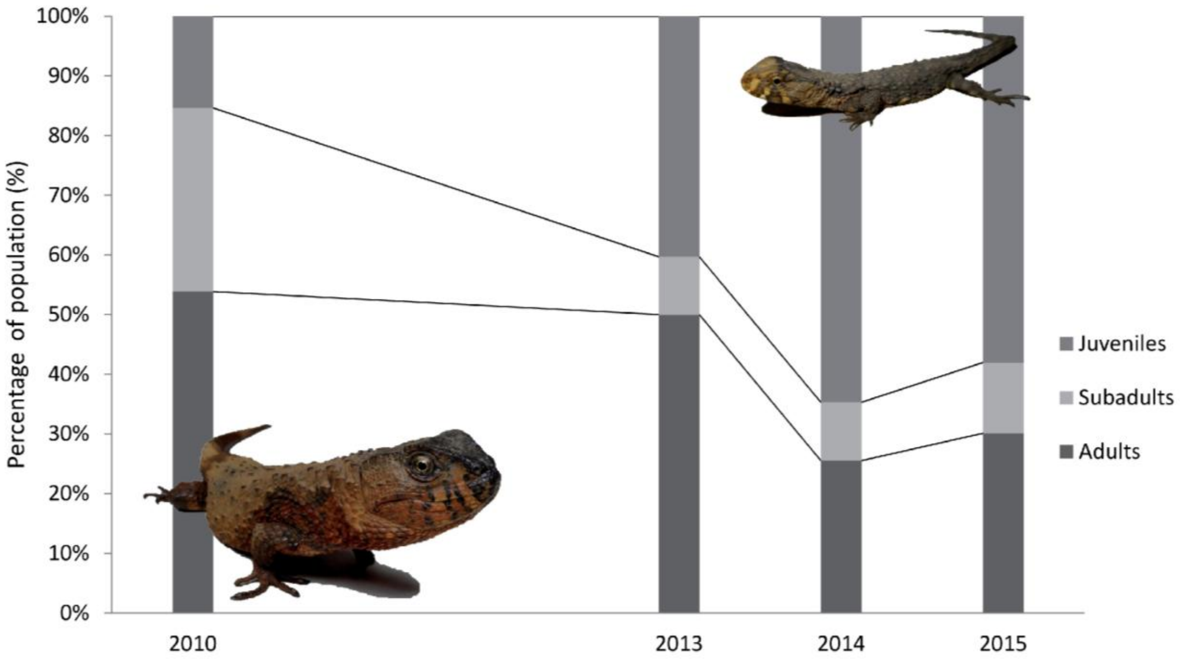

Fig. 3. Observed population structure of Vietnamese *S. crocodilurus* from 2010 to 2015. Photos M. van Schingen.

Tay Yen Tu NR and the western border of Dong Son-Ky Thuong NR, which forms an important corridor between the two reserves still inhabiting *S. crocodilurus*. Another major area of high suitability is situated in Hai Ha District in the Northeast of Quang Ninh Province, near the border to China. This region currently lies outside of any designated protected area (Fig. 5).

DISCUSSION

New population: The discovery of a new *S. crocodilurus* population in Vietnam based on predictions by SDMs (van Schingen *et al.*, 2014b) underlines the practical applicability and reliability of such theoretical models as useful tool to plan field research. This newly recorded population might represent one explanation of the noticeable high amounts of Vietnamese crocodile lizards currently present in the trade (van Schingen *et al.*, 2015a). Local villagers confirmed that adult crocodile lizards were heavily collected for the trade throughout the region in recent years. While *S. crocodilurus* reportedly was still relatively abundant some years ago, since recent years adult crocodile lizards were heavily collected for the trade throughout the region. These reports fit with our observations of almost exclusively juveniles. We assume the (former) presence of further *S. crocodilurus* subpopulations at the border region of Vietnam and China, which is promoted by SDMs (van Schingen *et al.*, 2014b) and statements of local villagers.

Since the new population is situated in-between the known Vietnamese and Chinese populations, a detailed genetic study of the population along with two others might provide important knowledge about the species' evolutionary history and phylogenetic relationships of the populations. The new population likely represents a kind of "missing link" between the known Vietnamese and Chinese populations. Such an analysis is currently being conducted by our team in order to determine if *S. crocodilurus* in China and Vietnam are indeed genetically differentiated. The results will be essential for designing appropriate measures, for both in-*situ* and *ex-situ* conservation, for this rare, unique, and endangered species.

Population trends: The overall increase in estimated crocodile lizards from 2010 to 2015 most likely resulted from the simultaneous increase in known sites, newly discovered populations and numbers of excursions. Evidence was provided by an increase in encountered juveniles over the last years, concurrent with a dramatic decrease in mature individuals (even though new populations were included in the estimations). Mature individuals are crucial for a stable and reproducing population (Reed *et al.*, 2003). *S. crocodilurus* reaches sexual maturity only after 2-4 years, and adults give birth only once a year (Zhao *et al.*, 1999; Bever *et al.*, 2005; Yu *et al.*, 2009). Since juveniles are also more sensitive to diseases, environmental changes and have a higher risk of mortality (Bever *et al.*, 2005; Zollweg & Kühne, 2013), we expect the present lack of mature individuals to cause a rapid decline in genetic diversity

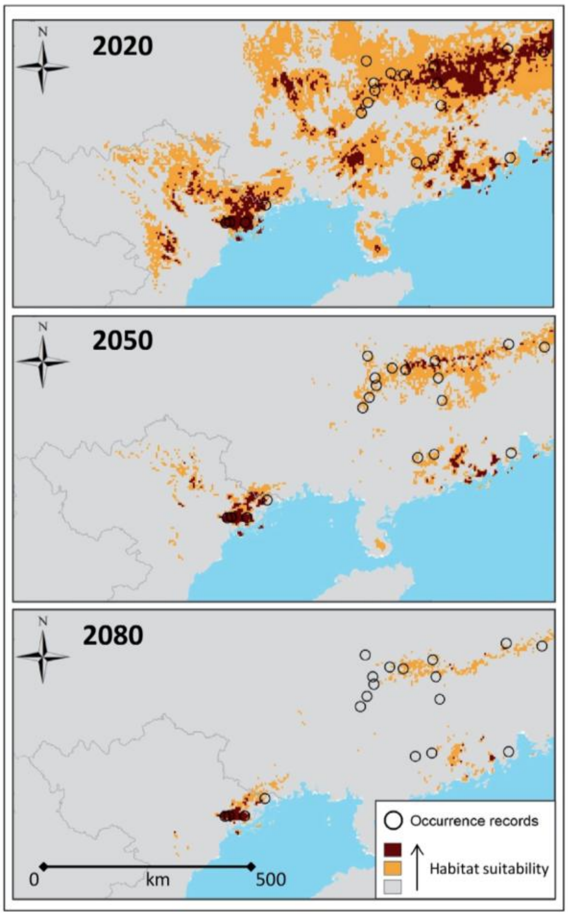

Fig. 4. Predicted suitable habitats for *S. crocodilurus* in the period between 2020 to 2080, based on bioclimatic data and elevation. Habitat suitability increases from yellow to dark brown.

and lead to local extinction. According to our data, crocodile lizards probably vanished from one third of the known sites already two years after discovery. The present scenario elucidates the severity of the current extinction risk, and how promptly local populations can cease. We assume that this observed local loss of mature lizards resulted from targeted poaching rather than from other threats, which would affect juveniles and adults in the same manner. In addition, our interviews with local villagers confirmed the frequent targeted poaching of only adult individuals for the pet trade.

Despite potential further populations, only known by local collectors, are not included in these estimates, our data clearly revealed current dramatic local population declines and repeated cases of local extinction. Chinese populations suffered from similarly severe local population declines of up to 90% and local extinctions (Huang *et al.*, 2008). But these declines were recorded within a time period of 26 years, while we observed comparable patterns in Vietnam within 1-2 years only. Because crocodile lizards are strongly sedentary and have specialist ecological requirements, they are particularly prone to local extinction (van Schingen *et al.*, 2014b). The aforementioned lack of adult individuals and specialized life history traits further impair the population recovery from anthropogenic pressures. Cases of local species extirpation due to poaching shortly after discovery have already been recorded in the region for other enigmatic and range restricted lizards, such as the Tiger Gecko *Goniurosaurus luii* (e.g., Stuart *et al.*, 2006). Vietnam is currently recognized as major consumer and exporter of wildlife (e.g., Nghiem *et al.*, 2012; Sodhi *et al.*, 2010). Illegal trade in Vietnamese crocodile lizards has been already frequently recorded, also in European markets (Kanari & Auliya, 2011; van Schingen *et al.*, 2015a). The high international demand of crocodile lizards is currently fueling the pressure on wild populations in magnitudes that are currently – and probably never were – sustainable, emphasizing the need of straight and enforced conservation measures (van Schingen *et al.*, 2015a).

Priority areas and recommendations for conservation: Li *et al.* (2012) already projected that all present habitats of *S. crocodilurus* in China will vanish until 2080. Accordingly, our model revealed an alarming, almost 95% loss of suitable habitats for *S. crocodilurus* from 2020 to 2080 throughout its distributional range. Only less than 0.26% of the study extent was predicted to still comprise suitable habitats in 2080, whereof most important regions were situated within the Yen Tu Mountain range and the border region between Vietnam and China. However, the prediction did not consider vegetation coverage, since future prognoses of habitat destruction are quite difficult. Based on the current high deforestation rate (Sodhi *et al.*, 2010) and the strict dependence of the species on undisturbed evergreen broadleaf lowland forest (e.g., Huang *et al.*, 2008; Ning *et al.*, 2006; van Schingen *et al.*, 2015b), we assume that the effective suitable area for *S. crocodilurus* will be smaller than the predicted calculation assumes. This particular forest type is considered as especially vulnerable and fast disappearing in Southeast Asia (Meijaard & Sheil, 2008) and has already been substantially cleared in northern Vietnam (Tordoff *et al.*, 2000), with some fragmented remains extending from the Yen Tu region to the Chinese border in Quang Ninh Province. In spite of the isolated and small population sizes, the preservation of forest corridors between localities seems a beneficial strategy to promote genetic exchange between subpopulations, besides upgraded local habitat protection at sites where *S. crocodilurus* occurs (Tewksbury *et al.*, 2002; Christie & Knowles, 2015). Based on our data, we recommend the maintenance of a corridor connecting contiguous Yen Tu and Tay Yen Tu NRs with Dong Son-Ky Thuong NR (within the

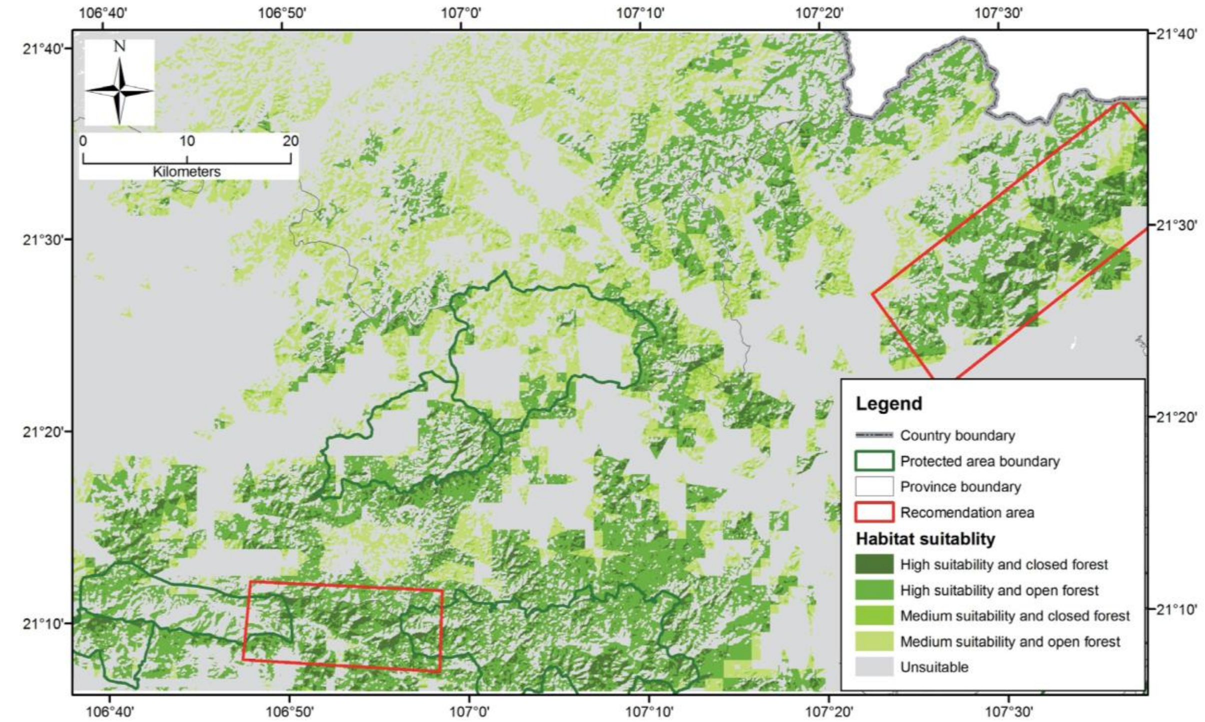

Fig. 5. Predicted suitable habitats throughout the distribution range of *S. crocodilurus* in northern Vietnam, using combined vegetation, bioclimatic and elevation data. Red squares indicate recommended priority areas for habitat conservation (bottom left: proposed corridor to link two existing reserves; top right: proposed reserve to be established in the future).

following coordinates: N 21.443772°, 107.378751°; N 21.532099°, E 107.684383°; N 21.359082°, E 107.441705 and N 21.608404°, E 107.612774°; Fig. 4). According to our present predictions and van Schingen *et al.* (2014b), the border region between Vietnam and China represents a further important area of high suitability, affirmed by the new population record. Interviews with local villagers gave evidence for a relatively broad distribution of the species in this region, which additionally contains another large area of contiguous and intact forest. However, this area is poorly studied and lies outside any protected area. This area probably possesses a high biodiversity value and further field research is required in order to evaluate its priority status for the establishment of a potential new nature reserve. Since the area was predicted to remain highly suitable still in 2080, it potentially also comprises suitable sites qualifying for a release program once the sites are sufficiently protected and should be considered for the establishment of a new reserve (N 21.170357°, E 106.794988°; N 21.127556°, E 106.874795°; N 21.154382°, E 106.974255° and N 21.196146° E 106.882572°).

Our previous SDSs proved successful in predicting sites of occurrence of *S. crocodilurus*. The existence of additional populations can be inferred from current trade patterns (van Schingen *et al.,* 2015a). Based on new satellite information, targeted surveys would be feasible to discover additional extant subpopulations. In face of the high extinction risk due to poaching, we recommend the immediate planning of further field research in areas of predicted habitat suitability, with the focus on the border area of Vietnam and China.

The dimension of impacts of climate change on tropical lizards are controversially debated (Tewksbury *et al.,* 2002; Sinervo *et al.,* 2012; Tewksbury *et al.,* 2012). *S. crocodilurus* represents a basal lineage of lizards, which experienced climatic shifts since the Cretaceous (Zhao *et al.,* 1999; Zollweg & Kühne, 2013). Currently, the two extant subpopulations are living under different climatic conditions in China and Vietnam. Chinese crocodile lizards are known to hibernate during the cold months, but a respective behavior is not studied for Vietnamese lizards which are exposed to more constant and moderate temperatures throughout the year (van Schingen *et al.,* 2015b). As a first step to explore the ability of *S. crocodilurus* to adapt to climatic changes, we recommend basic research on thermobiology and hibernation behavior in Vietnam.

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Chapter 5: Will climate change affect the Vietnamese crocodile Lizard? Seasonal variation in microclimate and activity pattern of Shinisaurus crocodilurus vietnamensis

Will climate change affect the Vietnamese crocodile lizard? Seasonal variation in microclimate and activity pattern of *Shinisaurus crocodilurus vietnamensis*

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Abstract

Climate change is considered to negatively affect vertebrate biodiversity, especially tropical lizards due to their narrow temperature tolerances. The crocodile lizard *Shinisaurus crocodilurus*, an ecologically highly specialized species, occurs only in isolated relict populations in South China and North Vietnam, and is currently facing extinction. In order to assess the variation and optima of the temperature niche in its natural habitat in Vietnam, we developed a data logger backpack system for the Vietnamese crocodile lizard, which is also the first of its kind for semi-aquatic lizards. *S. crocodilurus vietnamensis* showed a strong temperature selection of 24.21 ± 1.14 °C (min-max: 21.88-30.88 °C) at natural habitat sites, revealing a rather narrow temperature amplitude compared to the fundamental ambient temperature niche. Strong avoidance of temperature extremes in its environment indicated active thermoregulation and a strong dependence on intact vegetation for shading and constant cool streams. We further provide first insights into seasonal variation in

microhabitat use. In addition, the hibernation of *S. crocodilurus vietnamensis* was studied based on daily observations over a period of eight months at captive facilities at the Me Linh station for Biodiversity, Vinh Phuc Province, Vietnam.

Keywords: Thermal niche; conservation planning; data logger; pilot study; *in-situ* natural history research

Introduction

Global biodiversity loss due to anthropogenic pressures belongs to the current earths' most serious crises, with habitat destruction and climate change being considered to represent the most severe stressors (Kannan & James 2009; Pereira *et al.* 2010). The effects of global climate change will be likely exacerbated by anthropogenic pressures like habitat perturbations, habitat fragmentation, or environmental pollution that may initiate an extinction vortex (Fagan and Holmes 2006). Climate change may become a critical factor in particular for the survival of tropical lizards, due to their low vagility, high dependence on specific environmental conditions (Böhm *et al.* 2013), and narrow temperature tolerance (Tewksbury *et al.* 2008). The rather limited phenotypic plasticity and inability to respond to climate change by range shifts or local adaptation was predicted to cause a 35 to 40% extinction risk in range restricted tropical lizards by 2080 (Sinervo *et al.* 2010). Especially when combining the effects of climate change on physiology of lizards with indirect impacts on species interactions, community structure and ecosystem function, climate change is expected to become a significant problem in adaptation processes and survival of specialized lizards (Parmesan 2006; Schweiger *et al.* 2008; Tewksbury *et al.* 2008).

The crocodile lizard *Shinisaurus crocodilurus* represents the only living representative of the family Shinisauridae - a highly conserved and independent evolutionary lineage - and is adapted to specific forested aquatic habitats (van Schingen *et al.* 2014a). Fossil evidence suggests a broad distribution range of the ancient shinisaurids throughout Europe, North America and Asia (Conrad 2006; Conrad *et al.* 2014; Klembara 2008), of which nowadays only few isolated relict populations of *S. crocodilurus* remain in South China and North Vietnam (van Schingen *et al.* 2014a). Based on slight genetic and morphological differences, the extant populations recently revealed to represent two different conservation and taxonomic units (namely *Shinisaurus crocodilurus vietnamensis* in northern Vietnam and the nominate form, *S. c. crocodilurus* in southern China) (van Schingen *et al*. 2016b). Topical research also revealed different climatic conditions in the habitats of Chinese and Vietnamese crocodile lizards, namely relatively moderate and constant annual temperatures in Vietnam vs. high annual fluctuations in temperatures with minima below 0 °C during winter in China (van Schingen *et al.* 2015b, 2016b, c; Zhao *et al.* 1999; Zollweg & Kühne 2013). In addition, crocodile lizards are habitat specialists, strongly associated to remote freshwater ecosystems within intact tropical lowland evergreen broadleaf forests (e.g., van Schingen *et al.* 2015b). Based on captive individuals from China few data on the thermoecology of crocodile lizards are available (Wang *et al.* 2008, 2009), but respective knowledge is entirely lacking for Vietnamese crocodile lizards. It seems not unlikely that Vietnamese populations, which are adapted to low temperature fluctuations in their habitat, might therefore be especially vulnerable to changing climatic conditions. Therefore, the present study aimed to provide a detailed characterization of the microclimate and light intensity in *Shinisaurus* microhabitats at different seasonal scales as well as to assess the actual temperature selection of *S. crocodilurus vietnamensis* in natural habitats in order to assess its vulnerability to global climate change.

In this context we developed a data logger backpack system suitable for small sized semi-aquatic tropical lizards for the *in-situ* investigation of preferred temperature ranges. This system gave us also insights into the daily activity pattern of *S. crocodilurus vietnamensis* in its natural habitat during the non-hibernation season. Additionally, we assessed the seasonal variation in activity and habitat use of *S. crocodilurus vietnamensis* during a long-term monitoring of captive animals in semi-outdoor facilities at the Me Linh Station for Biodiversity, Vinh Phuc Province, North Vietnam.

Methods

Backpack system

To investigate natural temperature preferences as well as daily activity patterns of wild crocodile lizards in Vietnam we developed a backpack system inspired by Fisher & Muth (1995), Richmond (1998), Van Winkel & Ji (2014), and Warner *et al.* (2006). Due to the small size of crocodile lizards we used Thermochron iButton® (Model DS192H-F5#) as data loggers to record selected temperatures, since they are small in size (17.35 mm in diameter by 6.76

mm), light and waterproof. The weight of the whole backpack, including data loggers, ranged between 4 and 4.36 g, corresponding to 3 to 5% of the whole body mass of the lizards. In comparison, we recorded weights of about 3.65 – 4 g in neonates of *S. crocodilurus.* Numbers of offspring of *S. crocodilurus* generally represent around seven individuals, with reported cases of up to 15 offspring (Zollweg 2009; Zollweg & Kühne 2013). Thus we considered the weight of the backpack appropriate.

We chose CoPoly® (7.5 x 450 cm black, M+H VET s.r.o. Czech Republic), an elastic and waterproof veterinarian material to construct the backpack. We slightly adjusted the design of Gerner *et al.* (2008) to prevent the detaching of the data logger from the backpack, which was glued (Hold-it Soft Lure Superglue, Savange Gear) onto the central pad of the backpack. Before fitting onto the animals, the data loggers were activated. After activation, a small stripe of CoPoly® (1 cm in diameter) was wrapped around the data logger and the folded backpack. To fit the backpack on the animals, the part with the logger was placed dorsally between the shoulders, the shoulder straps were laid around the animals' necks, crossed ventrally, put up behind the forelegs and attached on the dorsal surface of the backpack with few cyanoacrylate superglue (Hold-it Soft Lure Superglue, Savange Gear). Contact of glue and lizard skin was prevented. It was important to ensure, that the legs of animals could move unrestrained and the straps fitted well without being too tight.

Data collection

Field research was conducted during May and June 2015, which is the non-hibernation season, in Tay Yen Tu Nature Reserve (NR), Bac Giang Province, North Vietnam. In addition further field surveys to collect environmental data at microsites during hibernation season took place in January 2016. Five animals with a snout-vent length (SVL) > 140 mm and a weight above 80g were captured during night surveys in May 2015 from two different streams in Tay Yen Tu NR. Data loggers were attached to the animals using the a.m. backpack system. The measuring interval of data loggers was every two minutes to ensure the capturing of all events of temperature changes. The animals were kept for one night at the station to adapt them to the backpacks. After detailed validation of their unrestricted mobility and normal behavior, animals were released at the same spot of capture.

Figure 1. ***Shinisaurus crocodilurus vietnamensis*** **with temperature data logger.**

After a period of four to 17 days, animals were recaptured to remove the backpack and assess the recorded data. As reference environmental microsite temperature profiles, we placed further temperature data loggers (iButton®), recording temperatures in intervals of five or ten minutes, within the water of each streams. In addition HOBO data loggers (HOBO Pendant® Temperature/ Light Data Logger (UA-002-64) Oneset®), recording air temperature (°C) and light exposure (lx) in intervals of one minute in summer and five minutes in winter were placed on a typical resting perch of *S. crocodilurus vietnamensis* covered with vegetation and on a control branch in about two meters distance, which was less covered by vegetation. Environmental data loggers were recording data over a period of two weeks in summer and about another 24 hours in January at the same spots in order to assess seasonal differences in microclimate.

Furthermore, long-term data on activity and perch selection of captive crocodile lizards within three semi-outdoor enclosures was recorded at the Me Linh Station for Biodiversity, Vinh Phuc Province, North Vietnam (see Ziegler et al. 2016). The enclosures had ground areas of about $2m^2$, $6m^2$ and $7m^2$ and heights of 140-180 cm and were similarly equipped with a small waterfall, a water part occupying at least 50% of the area and a land part with earth to dig in, leaves, rocks and branches reaching over the water body as well as bamboo tubes to hide (for details see Ziegler et al. 2016). Over a period of eight months the numbers of exposed and hidden animals, as well as the occupied substrate type (categorized as branch, hole in earth, stone or bamboo, enclosure wall of concrete and water) were recorded daily, together with the air temperature at the time point of observations. Observations were done during morning, when animals were usually active. All a.m. substrate types were present in each enclosure included in this study.

Data analyses

In order to test for differences (temperature and light intensity) between branches occupied by crocodile lizards and surrounding branches we used a two-sided t-test. Homogeneity of variances was tested with Bartlett's-test. For comparison of light exposure at different spots, we exclusively used data recorded during day (from 7 h to 19 h), since there is no illuminance during night. Water temperatures of streams were compared using a Welch Two Sample t-test. In order to test for differences between selected temperatures of animals, water and air temperatures, we applied a One-way ANOVA combined with a Tukey post-hoc test.

We herein defined "activity" as a warming or cooling event of *S. crocodilurus* that can be detected by a temperature change between two following measures of data loggers, which exceeds natural gradual changes in environmental temperatures between two time points. In order to identify the maximum natural environmental temperature change between two records, we calculated the temperature difference between each consecutive time point of environmental data loggers. We identified two limit values of ± 0.125°C in the water and ± 0.3°C in the air, assuming that changes in selected temperatures of animals that exceed these values must result from the animals' activities. Thereby, negative changes indicate a warming and positive changes indicate a cooling event. For better overview we built time groups to assess the daily activity pattern. Thereby all events within one hour assembled to the following full-hour group, e.g., an activity at 4:49 h belonged to the time group of five h. Overlaying histograms enabled the differentiation of warming and cooling events.

All analyses and graphics were performed with R version 3.2.5 (R Core Team 2016) using its base, car and ggplot2 packages (Fox and Sanford 2011, Wickmann 2009).

Results

Seasonal variation in microhabitat characteristics

The temperature profile of shaded branches, occupied by *S. crocodilurus vietnamensis*, significantly differed from those of surrounding, more sun exposed branches (t = 60.995, df = 28846, P < 0.001 in summer, see Fig. 2).

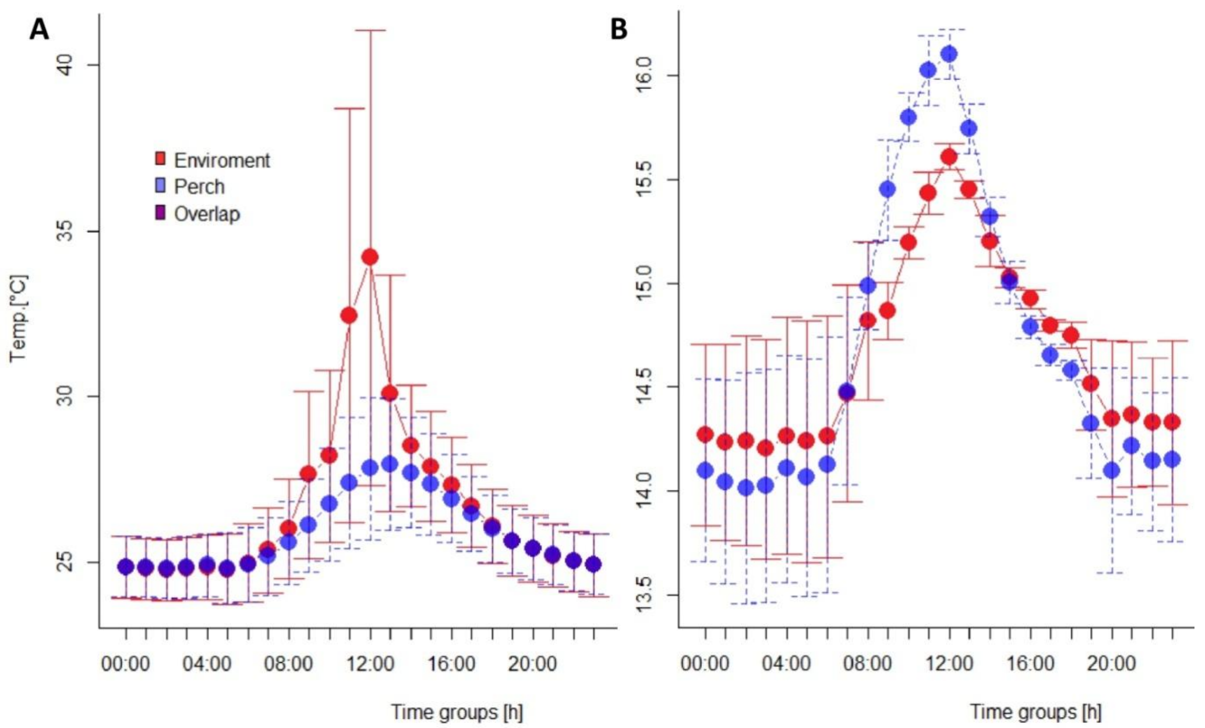

Figure 2. Hourly daily temperatures (C°) recorded by thermochron iButton data logger on shaded and open branches during A) summer and B) winter within natural habitat sites of *S. crocodilurus* in Vietnam. Means are represented with standard deviation.

We found this difference to be more pronounced during summer than in winter. In summer, the total recorded temperature spectrum within the habitat of *S. crocodilurus vietnamensis* ranged from 21.95 to 45.45°C (mean 26.7°C), while temperatures at perches were found to only range between 22.14 to 31.27°C (mean 25.9°C) and thus being more moderate without high fluctuations (Fig. 2, Table 1). We recorded the highest mean perch temperatures of 28 °C around 1 pm and a subsequent smooth and continuous decrease until 10 pm. During night, temperatures remained relatively constant (24 to 26°C). During winter, air temperatures between 13.36 to 16.33 °C were recorded (mean = 14.5°C). Water temperatures of inhabited streams were constant and ranged between 22.5 and 24.5 °C during summer and 17.1 and 17.5°C during winter.

Table 1. Microhabitat parameters of *Shinisaurus crocodilurus* in Vietnam during summer and winter.

	Summer		Winter	
	Min-Max	Mean+-sd	Min-Max	Mean+-sd
Water temperature [°C]	22.38-24.5	22.94+-0.4	17.12-17.5	17.29+-0.06
Shaded branch temp. [°C]	22.14-31.27	25.89+-1.69	13.36-16.33	14.51+-0.73
Open branch temp. [°C]	21.95-45.45	26.68+-3.45	13.56-15.66	14.55+-0.56
Shaded branch illumination [lx]	10.8-38580	739+-969.78	0-88	9.728+-19.65
Open branch illumination [lx]	10.8-242500	19380+-41474.32	0-128	28.91
Daily hours of illumination	5:00-18:00		6:00-17:00	

Regarding light exposure, the perch sites of *S. crocodilurus vietnamensis* were with up to 138,580 lx significantly less illuminated than to more open spots where we recorded up to 242,500 lx (t = 58.25, df = 15011, P < 0.001) during summer. Around noon highest light exposures were reached, which were in mean below 2,000 lx at the perch and between 24,276 and 90,000 lx at open branches (Fig. 3).

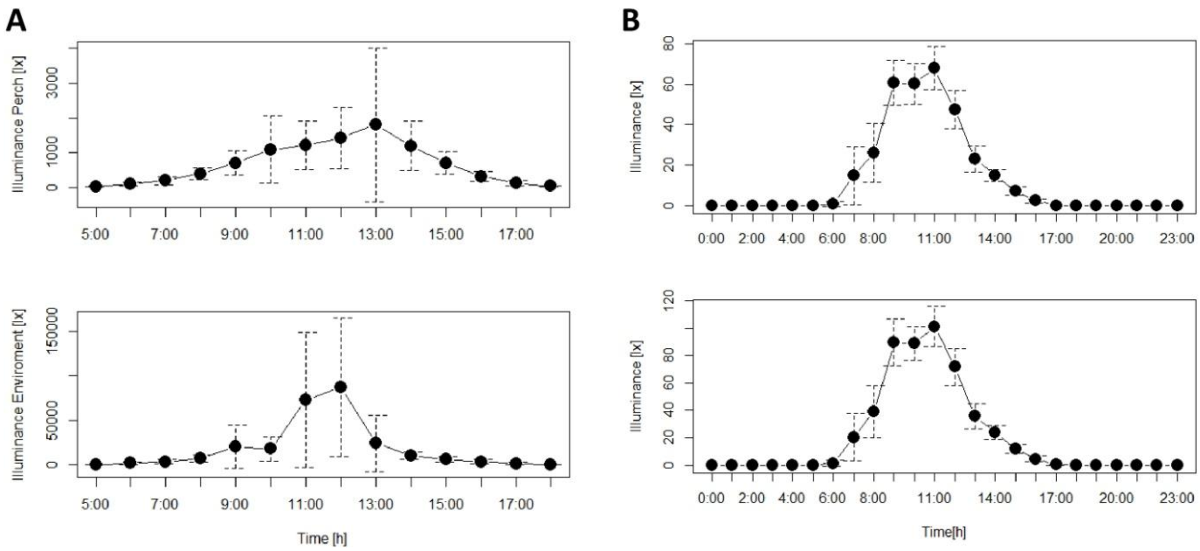

Figure 3. Hourly daily light exposure recorded by HOBO Onset data logger on shaded and open branches during A) summer and B) winter within natural habitat sites of *S. crocodilurus* in Vietnam. Means are represented with standard deviation.

We recorded light exposure from 5 h until 18 h during May and June. In January, we recorded distinctly fewer light exposure with only up to 120 lx around noon. Light exposure was recorded from 6 h to 17 h.

Selected temperatures of S. crocodilurus vietnamensis

(min-max: 21.88-30.88°C) and only rarely reached up to 30°C in two of the four animals. Mean selected temperatures significantly differed from ambient temperatures ($F_{7,\ 24368}$ = 1375, P < 0.001) by being generally lower (mean 26.7°C min-max: 21.95-45.45°C), but were above mean water temperatures (mean 22.97°C min-max: 22.38-24.5°C) (Fig. 4; Fig. 5). Furthermore, selected temperatures of animals revealed significant differences ($F_{3,\ 7899}$ = 3859, P < 0.001) indicating individual preferences and differences in behavior as well as time spent within the water. Furthermore, the amplitude of temperatures selected by *S. crocodilurus vietnamensis* was distinctly smaller (realized niche) than the amplitude of environmental temperature (fundamental niche) (Fig. 5) suggesting active thermoregulatory behavior of *S. crocodilurus vietnamensis*.

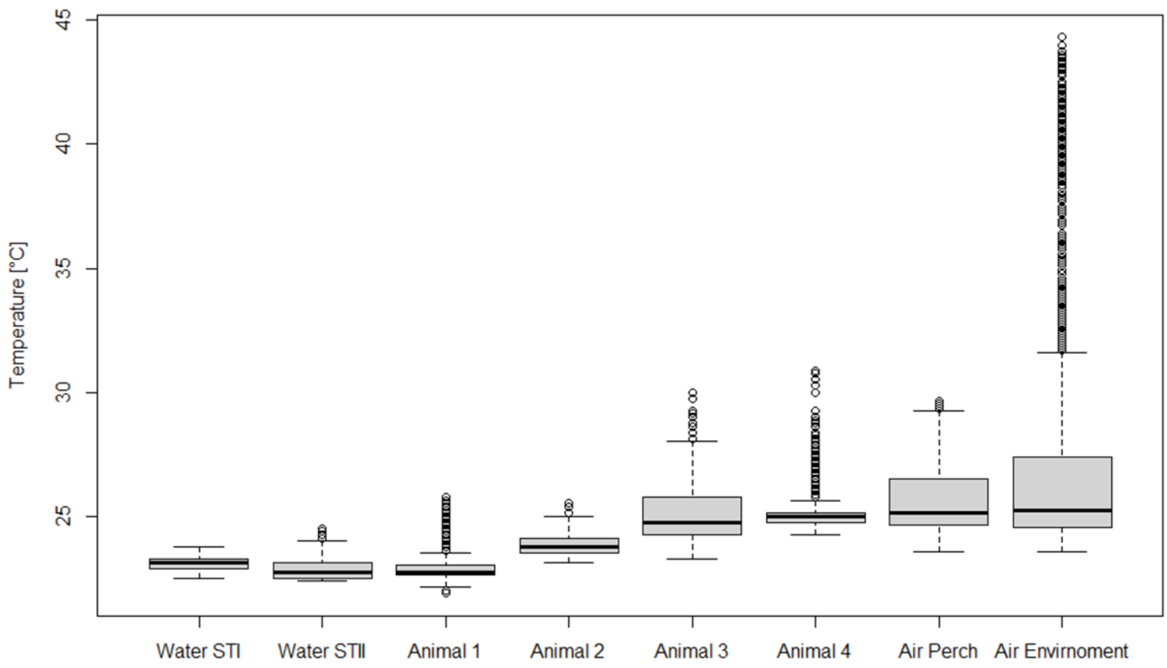

Figure 4. Boxplots temperatures recorded by data loggers attached to *Shinisaurus crocodilurus*, placed within water and on shaded and open branches within the natural habitat of the species during several days in May and June 2015.

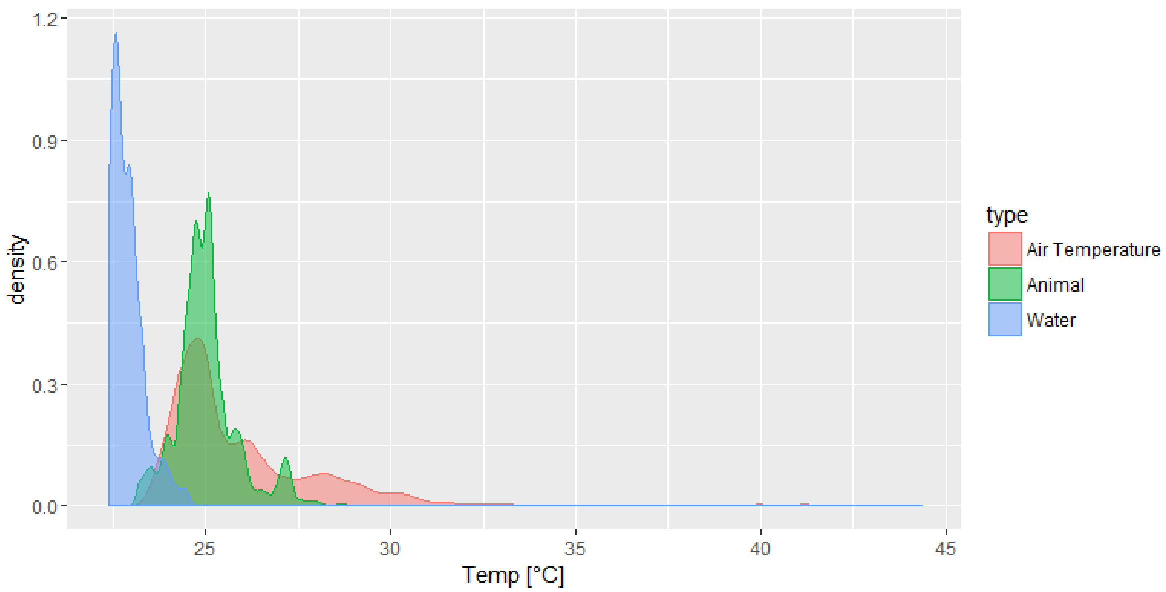

Figure 5. Kernel densities of recorded water temperatures, environmental temperatures and selected temperatures by *Shinisaurus crocodilurus vietnamensis* within one natural habitat in May and June 2015.

Daily activity pattern

First activities of *S. crocodilurus vietnamensis* within natural habitats were found to occur around 5 h related to sun rise, while last activities were detected at 20 h - 22 h (Fig. 6) after sunset. A peak of activities of all individuals was detected from morning to noon between nine and 12 h, while activities subsequently steadily declined (Fig. 6).

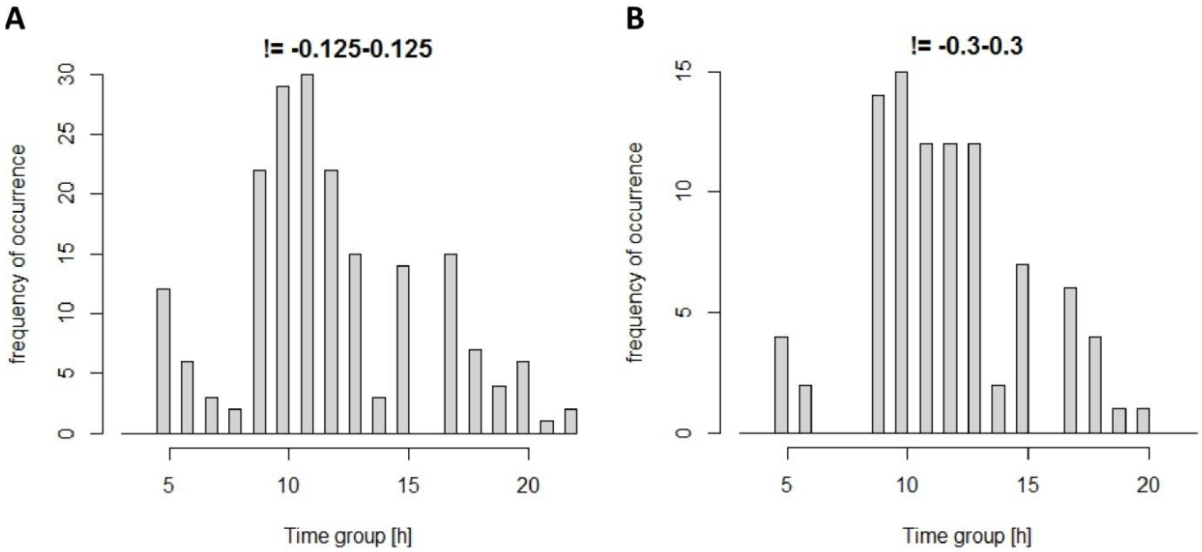

Figure 6. Cumulative histogram of detected distinct temperature changes of data loggers attached to *Shinisaurus crocodilurus* indicating daily activities defined as warming or cooling event. Activities were identified based on a temperature change of A) 0.125 °C and of B) 0.3 °C.

No activities were recorded between 22 h and 4 h. Observations during repeated field surveys confirmed these results. We never found animals, except some juveniles on their previous night perches after 6 h, while we still found some animals sleeping or awake on their night perches before 6 h. Of each investigated animal, the frequency of daily activities differed between each day. Our data further showed that days of especially high frequency of activities were followed by a day without any or only few recorded activities in some specimens.

Seasonal variation in microhabitat use

Based on long-term observations in captive breeding facilities, we found that the seasonal habitat use of *S. crocodilurus vietnamensis* differed significantly, viz., animals were generally found more exposed during summer and more hidden and inactive during winter (Fig. 7A). In August, 100% of the animals were found exposed, while this percentage slightly decreased but still exceeded 50% until November and subsequently dropped below 20% from December to February (Fig. 7).

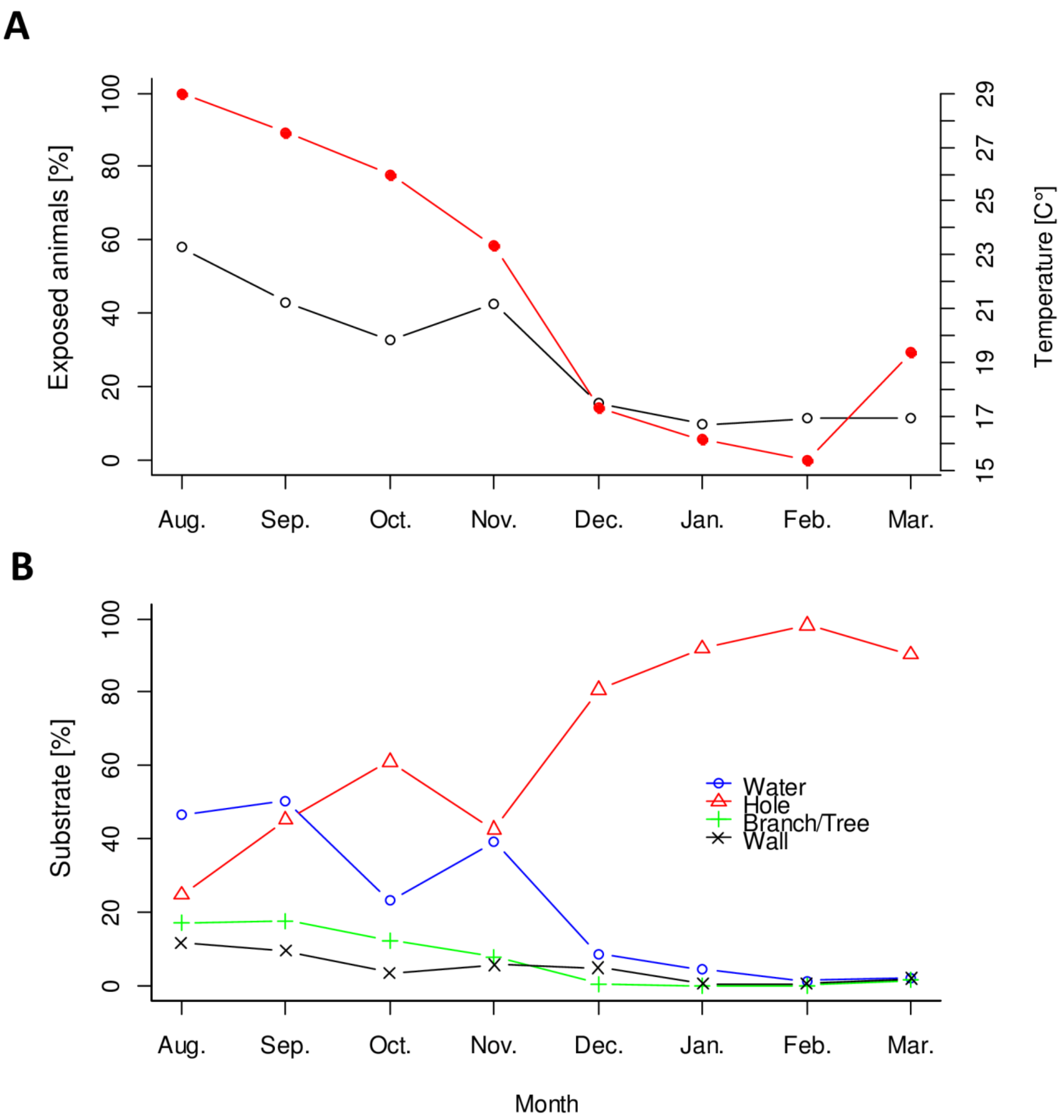

Figure 7. Seasonal shift in activity and substrate use of *Shinisaurus crocodilurus vietnamensis* in semi-outdoor enclosures. A: Monthly percentages of animals found exposed during day (red closed circles) and average monthly temperatures at time of observation (black open circles); B: Monthly percentages of different substrate types occupied by animals during day at time of observation (Blue circle = water, red triangle = hole, green cross = branch, black x = wall).

This pattern corresponded with respective recorded mean monthly temperatures, which decreased from about 23°C in August to around 17°C from December until March. In March an increase of activity and exposed animals to about 35% was observed. Corresponding to the percentage of animals being exposed or hidden also the occupied substrate type differed monthly. *S. crocodilurus vietnamensis* was frequently found on branches and within the water during August, while these substrates lost importance during the following month (Fig. 7B). In parallel, animals more frequently hid within different kind of holes in winter and were almost exclusively found inactive within holes or bamboo tubes from December to February.

Discussion

Method

The present study is the first of its kind *in-situ* testing a backpack system for medium sized semi-aquatic tropical lizards. Our approach appeared to be suitable to gain first insights into temperature selection and activity patterns of *S. crocodilurus vietnamensis* within the natural habitat. Due to the low weight and small size of the data loggers as well as the flexible material of the backpack the animals seemed not to be negatively affected by this method assuming their behavior to rather represent their natural behavior. In addition, this approach has a balanced price-performance ratio. However, this method appeared to be only suitable for strictly sedentary species, since no UHF/VHF transmitter were used in order to recapture animals, because the attachment of further tools would negatively impact carrying comfort, practicability and weight of backpacks as well as drastically increase costs.

Microhabitat

The microclimate at natural habitat sites is generally poorly studied in lizards, even though most lizard species are specialized to specific environmental conditions (Sinervo et al. 2010; Tewksbury et al. 2008). This study showed that the environmental parameters temperature and light exposure significantly differ between vegetated spots occupied by *S. crocodilurus* vietnamensis and more open spots in distance of only few meters in summer. The diurnal temperatures at perches were found to be cooler and with lower temperature fluctuations compared to surrounding spots. Similar pattern was recorded for light intensity, which was lower at spots occupied by lizards in comparison to adjacent more open spots (Table 1). Accordingly, we could show that animals avoided extremely high temperatures of up to 45°C measured in the sun and only rarely and shortly resided at spots with temperatures up to 30°C. Accordant with our previous assumption, the amplitude of selected temperatures of lizards (realized niche) was more narrow than the fundamental ambient temperature niche, indicating the adaptation of *S. crocodilurus vietnamensis* to cool and constant temperatures and thus its vulnerability to global warming. Mean selected temperatures rather were between mean air and water temperatures, suggesting the active thermoregulatory behavior of *S. crocodilurus vietnamensis* and thus its dependence on constantly cool water.

Overall annual temperatures in northeastern Vietnam are relatively moderate without high fluctuations, especially inhabited streams were characterized by constant water temperatures of around 23°C in summer and 17°C in winter (Table 1). In contrast, the climate at Chinese habitat sites in Guangxi and Guangdong provinces is characterized by high annual temperature fluctuations, hot summers and cold winters with minimum temperatures occasionally reaching -4°C (van Schingen et al. in press; Zhao et al., 1999; Ziegler et al. in prep.). Thus, we expect different adaptations to respective climatic niches in Chinese and Vietnamese crocodile lizards and probably also among geographically distinctly separated subpopulations in China. Since *S. crocodilurus crocodilurus* is exposed to higher temperature fluctuations in China, we assume that *S. crocodilurus vietnamensis* might become even more vulnerable to climate change. However, to verify these assumptions similar experiments need to be conducted in Chinese habitats and populations in the future.

Recent future climate predictions already revealed a dramatic decrease in suitable habitat sites for the Vietnamese crocodile lizard (van Schingen et al. 2016a). Herein, *S. crocodilurus vietnamensis* was found to be strongly dependent on intact vegetation coverage to avoid high temperatures, which make it especially vulnerable to the current increasing levels of habitat destruction and forest clearance at microhabitat sites. The species ability to cope with ongoing habitat degradation and climatic changes further depends on its mobility and ability to migrate to alternate habitat sites, which is currently under investigation in the frame of a separate study.

Activity pattern

Our study confirmed the diurnal activity of crocodile lizards, which appeared to be directly linked to light exposure, with first detected activities shortly after sunrise. These findings are in conjunction with previous studies, showing that crocodile lizards more distinctly react to visual than to olfactorial triggers (Jiang et al. 2010). A peak of activities in S. crocodilurus vietnamensis was recorded in the morning and around noon, which is in accordance with other observations in captivity (Zollweg & Kühne 2013; pers. obs.). Corresponding to the reported low metabolism of crocodile lizards (Jiang et al. 2010), we recorded some days without any activity of *S. crocodilurus vietnamensis*. During the last years of population monitoring within habitats of *S. crocodilurus vietnamensis* (van Schingen et al 2014a, b, 2015a, b, 2016a) several animals were repeatedly found completely covered with soil after a period of absence of several days or weeks during summer, indicating that Vietnamese crocodile lizards occasionally also spend periods inactive within the soil, even during the non-hibernation season. If this behavior can be interpreted as thermoregulatory performance needs to be further investigated. According to Long et al. (2007) Chinese crocodile lizards spend most of the diurnal time inactive (97.74%), while only about 1.55% and 0.12 % accounted for moving and feeding, respectively during summer. Referring to these findings we suggest that most recorded activities during our study accounted for thermoregulatory purpose.

On the temporal scale, we could show a seasonal shift in occupied substrate types as well as in activity in general. While animals were generally found to perch exposed during summer, most animals were hiding inactive within holes in winter from December to February. Animals frequently resided within the water during summer, but were almost exclusively terrestrial in winter. These findings indicate a strong influence of climate on metabolism and annual activity of *S. crocodilurus vietnamensis*. We also found that animals occasionally had short periods of activity during the hibernation season between December and February and inactive periods of several days during summer (s.a.). While animals were found to hibernate in holes in captivity, hibernation sites in the natural habitat could not have been identified during field surveys in winter 2016 so far (van Schingen et al. 2016b).

Outlook

In conclusion, our study revealed the strong association of *S. crocodilurus vietnamensis* with specific environmental and climatic conditions as well as intact microhabitat sites. Facing the current increasing diverse pressures on wild population, caused by habitat destruction, climate change and poaching, not only improved in situ conservation but also ex situ measures are of urgent need. The herein provided thermal data in concert with additional ecological data resulted from comprehensive field research is intended to be used as baseline for the improved design of keeping facilities within the framework of a conservation breeding program which will be dealt with in detail separately.

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2.2 Impacts of trade and implications for conservation

Chapter 6: Trade in live reptiles and its impact on reptile diversity: the European pet market as a case study

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Review

Trade in live reptiles, its impact on wild populations, and the role of the European market

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Reptile diversity

A B S T R A C T

Of the 10,272 currently recognized reptile species, the trade of fewer than 8% are regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the European Wildlife Trade Regulations (EWTR). However, the International Union for Conservation of Nature (IUCN) Red List has assessed 45% of the world's reptile species and determined that at least 1390 species are threatened by "biological resource use". Of these, 355 species are intentionally targeted by collectors, including 194 non-CITES-listed species. Herein we review the global reptile pet trade, its impacts, and its contribution to the over-harvesting of species and populations, in light of current international law. Findings are based on an examination of relevant professional observations, online sources, and literature (e.g., applicable policies, taxonomy [reptile database], trade statistics [EUROSTAT], and conservation status [IUCN Red List]). Case studies are presented from the following countries and regions: Australia, Central America, China, Galapagos Islands (Ecuador), Germany, Europe, India, Indonesia (Kalimantan), Islamic Republic of Iran, Japan, Madagascar, Mexico, New Zealand, the Philippines, South Africa, Sri Lanka, Vietnam, Western Africa, and Western Asia. The European Union (EU) plays a major role in reptile trade. Between 2004 and 2014 (the period under study), the EU member states officially reported the import of 20,788,747 live reptiles. This review suggests that illegal trade activities involve species regulated under CITES, as well as species that are not CITES-regulated but nationally protected in their country of origin and often openly offered for sale in the EU. Further, these case studies demonstrate that regulations and enforcement in several countries are inadequate to prevent the overexploitation of species and to halt illegal trade activities.

Contents

1. Introduction

1.1. Background

The concept of species conservation has been complemented by broader approaches to environmental challenges, such as the controversial "ecosystem services" concept (Costanza et al., 1997; Adams, 2014). However, in view of the increasing numbers of globally threatened species (IUCN, 2015), the protection and sustainable conservation of species demands more attention than ever (Pimm et al., 1995; Dirzo and Raven, 2003; Ceballos et al., 2015). Despite comprehensive efforts to prevent biodiversity loss (Proença and Pereira, 2013), success has been limited, as the decline and extinction of species is generally the result of a combination of synergistic processes (Brook et al., 2008; Crandall, 2009). Lenzen et al. (2012) linked biodiversity loss to the increased cultivation and export of agricultural products in tropical regions, identifying top foreign consumers as responsible for the decline of species. However, other anthropogenic threats contribute to direct and abrupt population declines, which may decimate species in otherwise intact habitats. For example, the targeted harvest and trade in species that have economic value in both the country of origin and the country of import is an immense threat (TRAFFIC, 2008).

Collection of targeted species occurs both legally and illegally (Rosen and Smith, 2010). Demand for rare species stimulates illegal trade (Courchamp et al., 2006; Wyler and Sheikh, 2013), which ultimately can lead to the over-harvesting of those species (Cooney et al., 2015). Although determining reliable figures is difficult, given the nature of illicit activities, the value of the legal and illegal wildlife trade has been

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estimated to be US$20 billion (Wyler and Sheikh, 2013) to US$323 billion (TRAFFIC, 2014). In 2008, the black market in wildlife trade was estimated to be the fourth most lucrative crime after trafficking in drugs, arms, and human beings (TRAFFIC, 2008).

The over-exploitation of reptiles in the pet trade has been discussed since the late 1960s (Lambert, 1969; Spellerberg, 1976). More recent studies have addressed the unsustainable exploitation of additional reptile species (Gibbons et al., 2000; Schlaepfer et al., 2005) and reported that intentional harvest is the second largest threat to the survival of many reptile species (Böhm et al., 2013). Popular literature has even profiled illegal reptile trade activities, its scope, and the people involved (Christy, 2008; Smith, 2011). Reptiles currently represent the second most species-rich vertebrate class after birds in the international pet trade (Bush et al., 2014). Engler and Parry-Jones (2007) stated that in 2005, the European Union (EU) was the top global importer by value of live reptiles for the pet trade (valued at €7 million) and reptile skins (valued at €100 million). In quantity the EU ranks second (18.2%) after the United States (56.1%) in live reptile imports (Robinson et al., 2015).

Species extinction processes that are causally linked to trade activities remain relatively undocumented (Jenkins et al., 2014). However, it is evident that certain species are particularly vulnerable to over-collection, such as long-lived species with long generation times, those with low fecundity, (e.g., tortoises and large lizards) (Reznick et al., 2002; Baling et al., 2013), and species that are rare and endemic to islands and specific habitats (Webb et al., 2002). The IUCN Red List assessments provide a summary of the current species status based on expert knowledge. However, the IUCN faces many challenges in these efforts as it is in large part a volunteer based organization, thus these assessments are at times out of date (e.g., Brown et al., 2013; Siler et al., 2012). Further, re-assessments of many species are carried out infrequently, resulting in many countries, such as the Philippines, remaining behind in updating the conservation status of species (e.g., Brown et al., 2013; Siler et al., 2012), including information on taxonomy and genetics (e.g., Siler et al., 2011, 2014). Nevertheless these assessments provide the best documentation of the status of species and changes therein. These species classifications, however, do not necessarily translate to national or international protection and species not assessed by the IUCN Red List may also be threatened. Formal protection must be achieved by additional entities. The information within the Red List assessments can aid in this effort by increasing capacity and reducing uncertainty in species' knowledge (see Hoffmann et al., 2008), but it is international Conventions that must create appropriate regulations and national authorities that must enforce them. Below we present an overview of the current status of reptiles, the various ways in which reptiles are traded, and the applicable regulations.

1.2. Global reptile diversity and international legal frameworks

As of August 2015, a total of 10,272 species of reptiles have been described: 193 amphisbaenians (Amphisbaenia), 6145 lizards (Sauria), 3567 snakes (Serpentes), 341 turtles (Testudines), 25 crocodilians (Crocodylia), and one tuatara (Rhynchocephalia) (Uetz and Hošek, 2015).

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) was enacted in 1975 and regulates the international trade in threatened and potentially threatened species (live specimens, parts, or derived products) to prevent over-exploitation and thereby ensure legal, sustainable, and traceable trade. The convention is implemented through an export and import control licensing system, to which currently 182 parties, including the EU, are bound. This system monitors trade in >35.000 species of flora and fauna (of which 793 are reptile species). These species are listed in three Appendices (see below), each having various prerequisites that underpin permitting trade.

Appendix I: lists species threatened with extinction; commercial trade is not permitted; presently 80 reptile species are included (https://www.cites.org/sites/default/files/eng/disc/species_02.10.2013.pdf - accessed 21 November 2015).

Appendix II: lists species that may become threatened with extinction, in case international trade is not regulated; trade is permitted in accordance with an export permit; however a trade in App. II species needs to ensure that such export will not be detrimental to the survival of that species (https://www.cites.org/eng/disc/text.php#IV - accessed 21 November 2015); presently 673 reptile species are included (see above source).

Appendix III: lists species that are currently being monitored in a given country that needs help to protect from over-harvesting; trade in these species is allowed following proper permitting; 40 reptile species at present are included (see above source).

Every two to three years there is an opportunity for CITES Parties to submit proposals for adding, removing, or transferring species in the Appendices. While consensus on these proposals is sought, many decisions are made by vote. The international role of CITES is to ensure that international trade in specimens of wild animals and plants does not threaten their survival, and, thus, the convention is positioned at the interface of biodiversity and trade interests (https://www.cites.org – accessed 20 November 2015).

The EU implements CITES via the European Wildlife Trade Regulations (EWTR), which includes additional control mechanisms, for example the suspension of imports of wild-sourced species (http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R0736andfrom=EN - accessed, 21 Nov. 2015). The three CITES Appendices are roughly aligned to the EWTR Annexes A–C. One non-CITES reptile species is listed in EWTR Annex A (Latifi's viper (*Montivipera* [*Vipera*] *latifii*) (Section 3.3.1)), and five in Annex B (e.g., the turquoise dwarf gecko [*Lygodactylus williamsi*] [see Section 3.2.1]). In addition, 23 non-CITES species are included in Annex D, which is a monitoring list to ensure sustainability, e.g., the five-keeled spiny-tailed iguana (*Ctenosaura quinquecarinata*) (http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R1320andfrom=EN - accessed 21 November 2015).

With only 7.7% of the world's reptile species listed in the CITES Appendices and 7.9% listed in the EWTR Annexes, the vast majority (92%) is not listed, and therefore trade in these species is not monitored or regulated at the international level, particularly within the EU.

1.3. EU imports of live reptiles over the last decade

Within the period 2004–2014, the EU member states officially reported the import of 20,788,747 live reptiles (CITES and non-CITES species) (Eurostat, 2015). With 6,101,040 live specimens, Germany was by far the largest importer within the EU, followed by Great Britain (3,469,109), Spain (2,912,171), Czech Republic (1,899,420), and Italy (1,780,546).

According to Eurostat (2015) the top 15 countries of origin for the EU live reptile imports are USA (13,083,406 specimens), China (1,181,561), Vietnam (1,038,065), Tanzania (835,423), El Salvador (611,643), Togo (570,475), Uzbekistan (451,691), Ghana (428,983), Indonesia (407,214), Egypt (351,176), Hong Kong (176,986), Taiwan (148,804), Madagascar (113,626), Guyana (90,964), and Benin (87,333). Ten out of these 15 countries of origin are within biodiversity hotspots, indicating a high proportion of endemic and threatened species: Vietnam, Tanzania, El Salvador, Togo, Ghana, Indonesia, Hong Kong, Taiwan, Madagascar, and Benin (Myers et al., 2000).

1.4. Reptiles assessed by the IUCN red list

Forty-five percent (n = 4669) of the world's reptiles have been assessed for the IUCN Red List (IUCN, 2015); of these, 180 have been classified as Critically Endangered (CR), 361 as Endangered (EN), and 403 as Vulnerable (VU). Of those assessed, at least 1390 species are threatened by "biological resource use" either as a primary or contributing threat. Among these, 25% (n = 355) of species are threatened by

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"intentional use (species is the target)" and 194 of these species (14%) are not included in the CITES Appendices. Although some species are collected for other purposes (e.g., some Pythonidae spp., Varanidae spp. and *Tupinambis* spp. are harvested intensively for the leather industry), most species are in fact collected for the international pet trade. Further, many species known to be sought after in the international pet trade did not show up within the aforementioned query of the IUCN Red List as they have not yet been assessed, such as the Solomon island skink (*Corucia zebrata*) and caiman lizards (*Dracaena* spp.). Additional species affected by the international pet trade, such as the Philippine forest turtle (*Siebenrockiella leytensis*), the pancake tortoise (*Malacochersus tornieri*), or the Natal midlands dwarf chamaeleon (*Bradypodion thamnobates*), also did not appear in the query as their assessments are out of date as discussed above (see IUCN, 2015).

2. Methods

The primary methodological approach for this review was extensive, global-scale consultation with experts who have relevant, long-term experience addressing reptile trade issues. These experts include: scientists, officers of conservation agencies, conservationists, and enforcement and customs officials. Contributing authors provided examples of unsustainable and illegal trade activities regarding the collection of, and international trade in, live reptiles within their regions of expertise. This information was supported by a comprehensive review of the published literature as well as anecdotal unpublished information that was made available by relevant administrative bodies. Information available for non-CITES species in trade (e.g., Annex D species of the EWTR) was filtered from the IUCN Red List database. Additional data sources including grey literature, reptile fairs, wholesaler and retail shop lists, and species' lists of private collectors were included. Furthermore, data extracted from several online databases (see below) augmented the results and conclusions of this review:

- CITES Trade database (http://trade.cites.org/): Searches entered for all reptile taxa in Appendices I–III for the year range 2004–2014 using trade terms "live" and "eggs (live)" and the relevant taxa (genus or species). Queries were structured to capture data regarding major exporting and importing countries, species, quantities, and sources
- EU Wildlife Trade Regulations (EWTR): All reptile species listed in Annexes A–D. (http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R1320andfrom=EN - accessed 21 November 2015)
- IUCN Red List (http://www.iucnredlist.org): All reptile species listed in any of the threat categories (IUCN, 2015)
- The Reptile database (http://www.reptile-database.org/): this reference provided the taxonomy applied (Uetz and Hošek, 2015).

3. Results

3.1. Legal but unregulated trade

Rarity in the wild or limited availability in the pet market (e.g., remote or hard to access habitats, low export numbers) makes a species especially attractive to private collectors (Brook and Sodhi, 2006; Courchamp et al., 2006; Hall et al., 2008). Accordingly, some traders have specialized in species that are threatened, were only recently described, rediscovered, or are not yet scientifically described (see Section 3.1.2).

3.1.1. Exploitation of threatened non-CITES species

Species that are classified as threatened by the IUCN Red List or national Red Lists (http://www.nationalredlist.org/home/about/ - accessed 1 April 2016) but are not yet covered by CITES are highly attractive for the international pet trade, especially those with distinct color patterns or special biological features. An analysis of non-CITES reptiles in the European pet trade with IUCN Red List status CR, EN, or VU resulted in 75 threatened species being identified for sale. Some of these non-CITES species are; however, nationally protected in their country of origin (see Section 3.2.1); others remain unprotected despite their precarious conservation status. Among these sought-after species are e.g., Orlov's viper (*Vipera orlovi*), CR and endemic to a small area in Caucasus, Russia, with <250 mature individuals left (Tuniyev et al., 2009a; Zazanashvili and Mallon, 2009); the party gecko (*Paroedura lohatsara*), CR and endemic to northern Madagascar (Raxworthy et al., 2011); the large-headed gecko (*Paroedura masobe*), EN and endemic to the north-eastern part of Madagascar (Rosa et al., 2011); the leopard fringe-fingered lizard (*Acanthodactylus pardalis*), VU and native to Egypt and Libya (Böhme and El Din, 2006); and a variety of geckos from New Caledonia, such as the live-bearing greater rough-snouted gecko (*Rhacodactylus trachycephalus*) (Bauer et al., 2012). Although Parker's snake-necked turtle (*Chelodina parkeri*) is classified by IUCN as VU and has only a limited distribution, Indonesia has set a collection quota of 150 specimens from West Papua and Papua each (Natusch and Lyons, 2012). This species is sold in Europe for approximately €400 each. For many other species, collections remain completely unregulated.

3.1.2. Exploitation of newly discovered or rediscovered species

Field herpetologists and conservationists are increasingly alarmed that type localities are used by commercial collectors to exploit these populations (Diesmos et al., 2004, 2012; Stuart et al., 2006; Menegon et al., 2011; Yaap et al., 2012). The examples are numerous and it is believed that reporting new locality records of threatened and highly demanded species could be considered detrimental to species survival (e.g., Grismer et al., 2008; Razafimahatratra et al., 2010).

In 2010, Grismer et al. (2010) described the new psychedelic gecko (*Cnemaspis psychedelica*) endemic to the 8 km^2 island Hon Khoai in Vietnam. From 2013 onwards, this species has regularly been for sale in Europe, for approximately €2500–3000/pair (Altherr, 2014; see Section 3.3.3). Likewise, in 2012, Yaap et al. published details of the first evidence of the Borneo earless monitor lizard (*Lanthanotus borneensis*) in West Kalimantan. Shortly thereafter, dozens of *L. borneensis* were offered by traders from the Czech Republic and Germany (see Section 3.3.3). The description of *Varanus bitatawa* (Welton et al., 2010) was soon followed by reports of specimens offered in the pet trade, which were caught in violation of the Philippines' Wildlife Act (Sy, 2012). Most strikingly, the rare colubrid snake *Archelaphe bella chapaensis* had not been seen in Vietnam since its description in the 1930s and was thought to be extinct. In 2010, the species was rediscovered in northern Vietnam (Orlov et al., 2010). One year later, online advertisements were offering "farmed" specimens from Vietnam, with prices up to €1650/pair. Likewise the rediscoveries of Campbell's alligator lizard (*Abronia campbelli*) in 2009 (Ariano-Sánchez and Torres-Almazán, 2010) and *S. leytensis* in 2004, resulted in intense poaching for the international pet trade (see Sections 3.3.5 and 3.3.3, respectively). Meijaard and Nijman (2014) outlined how publicity of rediscoveries through scientific and public media can have a detrimental effect on the species. According to Stuart et al. (2006), immediately after being described, both the Roti island snake-necked turtle (*Chelodina mccordi*), and the gecko *Goniurosaurus luii* from south-eastern China became recognized as rarities in the international pet trade, with a market price of about €1500 each. They became so heavily collected that currently *C. mccordi* is nearly extinct in the wild (Shepherd and Ibarrondo, 2005) and *G. luii* has been extirpated from its type locality (Grismer et al., 1999). Given the risk of illegal collecting and trade, scientists refrained from publishing locality records of two new *Goniurosaurus* species (Yang and Chan, 2015). Matilda's horned viper (*Atheris matildae*), a rare arboreal snake from the Tanzanian southern highlands was described by Menegon et al. (2011). To avoid over-exploitation scientists withheld precise locality information; however, the species was offered by Austrian and Serbian citizens for approximately €500/specimen within the same year. Indeed, even with the concealment of the type

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locality (e.g., Nilson et al., 1990) taxa are being exploited due to the immense interest in these species, and a lack of national and international law (Türkozan in litt. to Stuart, in litt. to Auliya, Oct. 2011).

3.2. Illegal trade

Reptile trafficking affects both CITES-protected species and species that are protected only at the national level in their range state or country.

3.2.1. Non-CITES nationally protected reptiles in the EU market

The EU's regulation on wildlife trade (Council Regulation [EC] No. 338/97) essentially implements the protection of species native to the EU or listed by CITES. All other species can be freely traded within the EU — whether they are threatened or strictly protected in their native range. An analysis by Altherr (2014) documented that the most expensive species are among those that are not CITES-listed but are protected by national law in their country of origin, resulting in low abundance in trade and fueling market prices. Those species may fetch prices of up to thousands of Euro/pair. Some traders specialize in the sale of such species because the profit margin is high, similar to some CITES species; however, there is no legal risk once the animals are in the EU, to openly offer them for sale. Among such cases are agamids from Sri Lanka (see Section 3.3.3), the Borneo earless monitor lizard (*Lanthanotus borneensis*) (see Section 3.3.3), arboreal alligator lizards (*Abronia*) from Guatemala (Section 3.3.5), spiny-tailed iguanas (*Ctenosaura*), four-toed worm lizards (*Bipes canaliculatus*) endemic to Mexico (see Section 3.3.5), Brazilian galliwasps (*Diploglossus lessonae*), as well as thorny devils (*Moloch horridus*) and shingleback lizards (*Tiliqua rugosa*) endemic to Australia (Section 3.3.4), South African adders (*Bitis* spp.) (Warner, 2009), and Okinawan ground geckos (*Goniurosaurus kuroiwae*) endemic to the Ryukyu Archipelago in Japan (Altherr, 2014).

One well-documented case is *Lygodactylus williamsi*. This species is IUCN Red Listed as CR and has an Area of Occupancy of only 8 km^2 in Tanzania. According to officials of the Tanzania Wildlife Research Institute, neither collection nor export of this species has ever been authorized, but specimens are often exported by intentional mislabelling or intermixed with unprotected geckos (Flecks et al., 2012). It is estimated that within a 4.5-year period (Dec 2004–Jul 2009) approximately 40,000 specimens were collected for the pet trade, resulting in a severely depleted population (Flecks et al., 2012). After four years of internal debate, the EU as the main destination finally included *L. williamsi* within its Annex B.

3.2.2. CITES species

Although CITES is a powerful tool to reduce or even ban the international trade of threatened species, there are, of course, several criminal ways to circumvent law. Export quotas (see https://www.cites.org/eng/resources/quotas/index.php - accessed 1 April 2016), may be systematically exceeded as has been documented for several tortoise species from Indonesia (Shepherd and Nijman, 2007), the graceful chameleon (*Chamaeleo gracilis*) from Togo, the Usambara three-horned chameleon (*Trioceros deremensis*) from Tanzania, and the savannah monitor (*Varanus exanthematicus*) from Benin (UNEP-WCMC, 2010). Paperwork is also often manipulated such that CITES-protected species are mislabelled as unprotected species. For example, the rosette-nosed pygmy chameleon (*Rhampholeon spinosus*) from Tanzania is classified by IUCN Red List as EN (Tolley and Menegon, 2014). This is the only species of its genus listed within CITES yet it is exported by the hundreds or even thousands, in shipments of "assorted pygmy chameleons" without proper or correct documentation (Anderson, 2014).

A widespread method of evading CITES regulations is camouflaging illegally wild-caught individuals with fraudulent labelling indicating that they have been captive-bred. Thus, there is growing and strong evidence that some countries appear to not be upholding their specific commitments under CITES and exercise laundering through commercial breeding farms (see Shi et al., 2007; Nijman and Shepherd, 2009; Lyons and Natusch, 2011; D'Cruze et al., 2015). This is evident by the fact that there are serious discrepancies between the numbers of reptiles exported to the EU that are declared as captive-bred and the numbers of reptiles that breeding facilities are actually producing or have the capacity to produce. Several Philippine species, such as varanids (*Varanus olivaceus*, *V. bitatawa*, *V. cumingi*), the Philippine cobra (*Naja philippinensis*), and turtles (*S. leytensis*, *Cuora amboinensis*), are a prime example of this. In one case, involving *V. olivaceus*, a private zoo owner in the Philippines substantiated his claim of captive breeding success (to apply for Philippine government CITES export permits) to legally export the species to the USA (Lutz, 2006). However, according to Bennett (2014) hard evidence of captive breeding could not be provided. Private zoological parks in the Philippines are claiming wild-caught specimens as captive bred in their facilities. One such facility claimed to have bred >100 *S. leytensis* without any evidence (e.g., broken egg shells, photographs of hatchling with egg yolk still attached) (Sy, 2014) (see Section 3.3.3).

A survey by Nijman and Shepherd (2009) found that all specimens of the emerald tree monitor (*Varanus prasinus*) and Timor monitor (*Varanus timorensis*) exported by Indonesia in 2006 as captive-bred were in fact wild-caught. According to field data collected between 2009 and 2011, Lyons and Natusch (2011) suggested that 80% of green tree pythons (*Morelia viridis*) exported by Indonesia claimed as captive-bred were in fact from the wild. Large-scale laundering of this kind has also been documented for Madagascan chameleons, which were channelled via Lebanon and Kazakhstan to escape quotas for wild-caught specimens and temporary trade suspensions (Todd, 2011). Additional examples include the Princely spiny-tailed lizard (*Uromastyx princeps*) and the Omani spiny-tailed lizard (*Uromastyx thomasi*), both listed under CITES Appendix II since 1977. *Uromastyx princeps* has never been officially exported from its range countries (Somalia, Ethiopia and possibly Kenya); however, since 2008 specimens were exported as "captive-bred" from Mali and Switzerland. The only official export of *U. thomasi* from its sole range state Oman was in 2008, but those eight wild-caught individuals were re-labelled in Mali as "captive-bred" and re-exported to Austria the same year (UNEP-WCMC, 2015).

In other instances, smugglers bypass CITES permitting and conceal reptiles on their bodies, in suitcases, or in parcels (e.g., Rosen and Smith, 2010; Herbig, 2011). In September 2007, a US citizen was arrested for smuggling three Fiji banded iguanas (*Brachylophus bulabula*) in his prosthetic leg. He told investigators that he had previously sold this type of iguana for several thousand Euros. In September 2015, a Mexican citizen was arrested in the Galapagos Islands for attempting to smuggle eleven endemic land and marine iguanas, listed in CITES App. II, with an estimated black market price of several thousand Euro each, on his body (see Section 3.3.5). The use of "hired tourists," as couriers for smuggling reptiles across borders is similarly common. In February 2014, British custom authorities arrested two young Romanian women with thirteen Bahamas rock iguanas (*Cyclura rileyi*) in their suitcases. The women were only engaged as couriers and, when questioned by authorities, named a Swiss man, who had paid for their trip (Perry, 2014). The black market value of *C. rileyi*, listed in CITES Appendix I, was estimated to be several thousand Euros each. According to the Bahamas CITES Authorities, the illegal export of these CR species to Western Europe had already been successful, and specimens in Germany and Austria are likely of illegal origin (Bahamas, 2014). Nevertheless, claimed offspring from these smuggled founders have received CITES export permits from European countries, and there are strong suspicions that these original animals are being used as cover to obtain CITES permits for newly smuggled animals and thus laundering new individuals of the same species (Bahamas, 2014). These examples demonstrate that if a highly lucrative species is involved, any strategy is applied to circumvent national and international law.

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The main objectives of this review are to illustrate the scope of international pet trade in reptiles, document the current policies and assess its efficacy, and substantiate claims that over-exploitation and illegal trade activities are widespread globally. Further, this review is intended to inform policy makers that the targeted unsustainable legal and illegal trade in numerous reptile species poses a considerable threat to biodiversity and proper ecosystem functioning in many of the world's most biodiverse countries, megadiverse nations, and global biodiversity conservation hotspots (e.g., Mittermeier et al., 1998; Mittermeier and Mittermeier, 2004).

3.3. Regional case studies

In the following sections, case studies are summarized by geographic location. As a result of trade dynamics, and the varying levels of implementation, enforcement, and policy among regions, there is substantial heterogeneity in the data available rendering these case studies variable and complex.

3.3.1. Europe, Middle East and Central Asia

Distinguishing between and enforcing regulations pertaining to captive bred, versus wild caught specimens in the trade remains a substantial challenge for enforcement officials. Information from Europe, Middle East and Central Asia demonstrates how national and international regulatory measures are inefficient to halt the illegal trade of threatened and endemic species.

German federal law on nature protection and the Species Conservation Act have prohibited the capture and husbandry of native amphibians and reptiles for almost 30 years. Nevertheless, the illegal trade of native reptiles is on-going and increasing. This correlated largely to a dramatic rise in reptile keeping in the 1980s, which was facilitated by the Internet and reptile trade fairs (Velo-Antón et al., 2011; Schneeweiss et al., 2014). This illegal trade has caused substantial declines, most likely in populations of European pond turtles (*Emys orbicularis*) and certainly in a population of European green lizards (*Lacerta viridis*). Between 2010 and 2011, three individuals responsible for the sale of >10,000 wild and native reptiles and amphibians since 1990, were convicted, sentenced to probation and fined. Discrimination between legal captive-bred and illegal wild specimens remains difficult to effectively implement, and sale of these species is continuing and at times even promoted. In 2013, the German umbrella organization of pet shops (ZZF) portrayed *E. orbicularis* in a magazine as a suitable terrarium and outdoor species, without mentioning the precarious situation of the species in the wild and the threats caused by illegal collections (Anonymus, 2013). Due to the practical inability of enforcing the regulations, Schneeweiss et al. (2014) called for a complete ban on the trading of native amphibian and reptile species.

For decades the demand in live vipers for the pet trade has been established by a worldwide-specialized community (Auliya, 2003). Many European and central Asian vipers occur in small geographic areas, and this species group is distinctly threatened by the pet trade. There are a number of examples where skilful animal collectors remove the last few specimens of a locally threatened population or species. The IUCN Red List assessed nine European viper species that are partially threatened by the pet trade and two European viper species are regulated in CITES: Wagner viper (*Montivipera wagneri*) and the Meadow viper (*Vipera ursinii*).

Three species of vipers that have been particularly impacted by the pet trade: *Montivipera latifii* in the Alburz Mountains in Iran, *M. wagneri* in eastern Turkey, and the Caucasus viper (*Vipera kaznakovi*) (Tuniyev et al., 2009b). All three species are range-restricted, native to only small geographic areas in high mountain regions. While *M. latifii* was formerly over-exploited for its serum by the Hessarak Serum Institute in Iran, since 2014 it has been listed in Annex A of the EWTR (see above); however, it is still not listed on the Appendices of CITES. *Montivipera latifii* is not only illegally collected for domestic antivenin production but also collected for the international pet industry (Behrooz et al., 2015). *Montivipera wagneri* was depleted by the international pet trade (Nilson and Andrén, 1999), and, as a result, was listed in CITES Appendix II, in 1992. The situation is also very critical for the range-restricted *M. albicornuta*. This species is not present in any protected area, thus there is an urgent need to restrict or prevent the collection of this species for the pet trade (Nilson, 2009).

In terms of species richness and taxonomic diversity, Iran harbors one of the most remarkable reptile faunas within the western Palearctic region (Rastegar-Pouyani et al., 2015). In Iran, the illegal collection of herpetofauna for the national and international pet trade is a serious threat; of particular concern are the European pond turtle (*Emys orbicularis*), the Caspian turtle (*Mauremys caspica*) (Rastegar-Pouyani et al., 2015), and a variety of geckos. While Iranian leopard geckos (*Eublepharis angramainyu*) are native to Iran, Syria, Turkey, and Iraq, specimens in the European pet trade are often specified to originate from the Iranian Provinces of Khuzestan, Kermanshah, and Ilam. According to the Iranian CITES authorities, these geckos have been collected from Iranian provinces without permission (Mobaraki in litt. to Altherr, 2014). Depending on the intensity of the color pattern, specimens may fetch €200–1000 each. Traders selling these geckos in EU markets are from Austria, the Czech Republic, Ukraine, and the United Kingdom. Significant illegal trade for the international pet market is also well documented for the endemic Iranian Kaiser's mountain newt (*Neurergus kaiseri*) (Mobaraki et al., 2013).

3.3.2. Africa and Madagascar

The EU is one key player commercially exploiting reptile populations from western Africa. Members of almost all reptile groups from this region are harvested for the international pet trade. Trade dynamics in western Africa are complex, and illegal cross-border activities are prominent. In addition, the laundering of wild-caught animals, as captive bred or ranch stock is increasingly prominent. High endemism in reptile diversity is associated with increased illegal trade activities in this region. Micro-endemic species and populations have been detrimentally impacted by the international pet trade and national protection is insufficient to deter the international market, which is largely fuelled by smugglers. Information from this region demonstrates how endemic species are preferentially sought-after, resulting in severe declines from unsustainable commercial harvest.

The West African sub-region (mainly Benin, Togo, and Ghana) is recognized as the second most prolific reptile-exporting region in the world, after Central and South America (Harwood, 2003, http://trade.cites.org/ - accessed June 2014). Studies on the export patterns of these countries have been conducted by Buffrénil (1995), Jenkins (1998), Harris (2002), Harwood (2003), and Ineich (2006); and more recently Luiselli et al. (2012) studied trade dynamics of Pythonidae spp. Individuals from the EU, the USA, and Japan are key players in exploiting wild species from this area. Despite CITES regulatory measures the export of reptile species from western Africa remains prominent. Since 1990, Togo has been the world's leading exporter of the ball python (*Python regius*), resulting in 80,000 specimens being exported, during the period 2000–2010 (AFFO, 2001). This led CITES to impose an export quota for reptile species originating from Togo.

The most common species in this trade, destined for the international pet industry, are tortoises (e.g., *Centrochelys sulcata*, *Kinixys nogueyi*, *K. erosa*, *K. homeana*), aquatic turtles (e.g., *Pelomedusa subrufa*, *Pelusios niger*, *P. castaneus*, *Trionyx triunguis*), lizards (e.g., *Chamaeleo gracilis*, *Chamaeleo senegalensis; Varanus exanthematicus*, *V. niloticus*, *V. ornatus*), and snakes (*Calabaria reinhardtii*, *Python regius* and *Python sebae*). Other less common species in trade that have more unpredictable market demands include the puff adder (*Bitis arietans*), rhinoceros viper (*Bitis nasicornis*), fat-tail gecko (*Hemitheconyx caudicinctus*), West African carpet viper (*Echis ocellatus*), West African night adder (*Causus maculatus*), West African bush viper (*Atheris chlorechis*), western green mamba (*Dendroaspis viridis*), black-necked spitting cobra (*Naja nigricollis*), and Geyr's spiny-tailed lizard (*Uromastyx geyri*). We

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note that several of these species, whose distribution is Sahelian, such as the African spurred tortoise (*C. sulcata*) and *U. geyri*, are consistently present in the Togo market, despite not occurring in Togo (Segniagbeto, 2009). This suggests that these, and possibly other species, are smuggled across weak country border controls that facilitate trafficking. For instance, local reptile farmers claim that many specimens, native to the "forest zone" and exported from Togo, Benin, and Ghana, are in fact from southern Nigeria. This is the case for the Home's hingeback tortoise (*K. homeana*) and the serrated hingeback tortoise (*K. erosa*), both captured in the Cross River State (Nigeria) and exported from Benin.

Although regulated by quotas, the illegal cross-border trade remains a concern. CITES data revealed that wild sourced live specimens constitute the largest proportion of this trade. In addition, specimens sourced from ranching operations include various loopholes. There appears to be no facility in any of these three countries that demonstrate a well-established captive breeding operation for any species. Thus, it can be confidently assumed that all specimens documented by the CITES trade database that are "officially" declared as exports (as well as those that are non-officially exported) from West Africa are in fact wild animals. This type of trade has had a substantial impact on wild populations of *P. regius* from Benin and Ghana due to gravid female export. In the last 15 years fecundity and mean body size of females has decreased substantially. Average clutch sizes of 15–18 eggs have decreased to 10–12 eggs (at least n = 5 females examined each year). Body size has decreased from around 1.5 m in total length to below 1.4 m. These trends are also coupled with a verified decrease in several Nigerian populations. Wild populations of the Gaboon viper (*Bitis gabonica*) have also been negatively impacted due to similar and additional threats (Reading et al., 2010).

There is a consensus among reptile traders operating in Togo, Ghana, and Benin that the abundance of *K. erosa* and *K. homeana* has declined over the years (see Luiselli and Diagne, 2013; Luiselli et al., 2013).

The Western Cape Province is home to 153 reptile species and subspecies; approximately 37% of the reptiles found in South Africa (Turner et al., 2012). Twenty-two species are endemic to the province (Branch et al., 2006). Since 1974, all wild animals in the Cape Province have been classified as protected, and it is illegal to collect, transport, keep in captivity, or export these animals without permission. Nevertheless, some South African reptiles are in high demand in the international pet trade, including the South African girdled lizard genus *Cordylus*, which has been taxonomically split into several genera (e.g., *Smaug*, *Hemicordylus*, *Karusosaurus*, and *Ouroborus* [Stanley et al., 2011]). This demand has arisen essentially because these lizards are morphologically diverse; from the small Oelofsen's girdled lizard (*Cordylus oelofseni*) to the large-scaled girdled lizard (*Cordylus macropholis*) to the giant girdled lizard *Smaug giganteus*. This group also includes the armadillo girdled lizard (*Ouroborus cataphractus*), which, when threatened, characteristically rolls itself in a ball by grabbing ahold of its tail in its mouth to protect its vulnerable belly. All taxa of the previously recognized genus *Cordylus* have also been listed under CITES since 1981.

Girdled lizards and South African tortoises are regularly sold overseas in the pet trade, and the legal origin is often doubtful. While some South African provinces have allowed exports of wild-caught girdled lizards and of captive-bred tortoises, including angulate and leopard tortoises from the Limpopo Province in South Africa to France (V. Eagan, pers. comm.), CapeNature, the Western Cape provincial conservation agency, has recorded a number of poaching and smuggling activities. From 2001 to 2004, three Czechs, five Slovakian, and nine Japanese citizens were arrested and fined for smuggling tortoises and *Ouroborus cataphractus*. Following this series of convictions, smuggling ceased for several years. The next and latest case of reptile poaching in the region was in April 2012, when a German and a British citizen were found guilty of the illegal collection of 24 native reptiles, including geckos, snakes, and one tortoise. These cases demonstrate that illegal wildlife trafficking remains a serious and on-going challenge to reptile conservation in South Africa.

Many populations of Madagascar's most threatened reptiles have been extirpated or have declined in abundance and distribution due to the intense pressure from the illegal wildlife trade (e.g., Carpenter et al., 2004). The most heavily traded Madagascan reptiles are endemic tortoises (*Astrochelys radiata*, *A. yniphora*, *Pyxis arachnoides*), chameleons (*Furcifer* spp., *Calumna* spp.), and geckos (*Phelsuma* spp., *Uroplatus* spp., *Paroedura* spp.) (Todd, 2011). These reptiles are frequently captured even inside protected areas. The ploughshare tortoise (*Astrochelys yniphora*), one of Madagascar's trademark species, has been most detrimentally affected by illegal trade, and is now one of the rarest reptile species in the world. The imminent threat to this unique species is the smuggling of adult and juvenile specimens from the wild. The demand by tortoise hobbyists in Thailand, Malaysia, Singapore, Indonesia, the Philippines, China, Japan, Europe, and the United States directly fuels the smuggling activities and has destroyed thirty years of previously successful conservation efforts. In 1997, the Government of Madagascar created a new protected area, the Baly Bay National Park, to protect all remaining wild individuals of the species. However, the illegal collection of ploughshare tortoises from Baly Bay National Park has accelerated over the past 15 years, resulting in very rapid declines in the already depleted populations, and the species is now very close to being extinct in the wild (Pedrono, 2008). In total, <400 wild individuals exist, likely fewer than the number of individuals illegally kept outside of Madagascar. Numerous seizures of ploughshare tortoises both in Madagascar and overseas underscore the smuggling pressure on this species. In March 2013, fifty-four ploughshare tortoises were seized in Bangkok Airport just a day after the completion of the global wildlife trade conference, CITES. This seizure represents over 10% of the entire wild population. *Astrochelys yniphora* is listed in Appendix I of CITES, which does not permit any commercial trade. All specimens that have been exported from Madagascar have been collected inside the Baly Bay National Park and smuggled out of the country illegally due to their high value on the black market and widespread corruption at multiple levels within the government.

3.3.3. Asia

Although national protection and international enforcement measures have improved in this region, complex smuggling networks persist, representing a substantial challenge. Some island nations with range-restricted reptile species (e.g., Sri Lanka) have become the focus of illegal international trade, especially when endemic species are not CITES-listed. Despite national protection and formalized threatened species status assessments, increasing smuggling activities are incentivized by an established consumer base in Europe. South Asian regional dynamics demonstrate how illegal trade activities involves species unregulated by CITES.

With its insular geography and high levels of endemic herpetological diversity, Southeast Asian regional trade dynamics identifies challenges unique to this region. Rare endemic insular species that are protected in their native range, but not regulated under CITES (also see 3.1.4.) have increasingly become the focus of illegal trade, with Europe acting as a primary consumer and supplier to other countries. Illegal trade has become the primary threat to wild populations but national and international policies appear inefficient to deter trade. Complex trade routes and laundering (captive breeding fraud) involving wild-harvested specimens remains the most substantial challenge. These case studies emphasize the severity of the threat caused by international demand for range-restricted CITES and non-CITES species despite national and international regulations. Japan is one of the major worldwide consumers of live reptile species. The country harbours endemic species that have attracted attention in the international pet industry. Despite national protection, many of these species have been reported abroad.

Elevated legal threat status and rarity are the major incentives to illegally collect reptiles that are then laundered through established commercial "breeding facilities." In such circumstances, substantiated cooperation

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between illegal traders and the scientific community has been documented. A summary of East Asian trade patterns provides specific examples (island endemics or range-restricted endemics on the adjacent Asian continent) demonstrating the variability of smuggling activities, which have resulted in a massive and expanding international pet industry.

Although the Indian star tortoise (*Geochelone elegans*) faces numerous threats, the illegal collection of a substantial number of individuals for the pet trade is the primary threat to this species (Shepherd et al., 2004; Shepherd and Nijman, 2008; Vinke and Vinke, 2010). Enforcement is on-going with this species and many tortoises have been successfully seized at international airports; however, the demand for this species is great and a complex network of smugglers using other modes of trade dispersal remains very active. A high percentage of tortoises die throughout the smuggling process, and most of the confiscated tortoises are small and immature specimens, which may indicate a decline in the population of adults. It is difficult to quantify the effects and magnitude of this trade, but annual wild harvests are estimated between 5000 (Choudhury and Bhupathy, 1993) and 20,000 individuals (Sekhar et al., 2004). Most recently 55,000 specimens were collected at one trade location in India with the principal target markets being Thailand and China (D'Cruze et al., 2015). Collections and possible translocations of this magnitude not only adversely affect recruitment, but may also cause the loss of local adaptations and introduce new pathogens (Alacs et al., 2007).

The tropical island of Sri Lanka is characterized by a high level of endemic and unusually attractive forms of lizard fauna that have entered the European pet market and are exclusively exploited from their natural habitats. Sri Lanka is home to 21 species of agamids, including eight members of the endemic subfamily Lyriocephalinae, (*Lyriocephalus*, *Ceratophora*, and *Cophotis*) (Somaweera and Somaweera, 2009; Amarasinghe et al., 2015). Most of these species have highly restricted distributions and are only found in unique microclimatic pockets (Palihawardana, 1996; Pethiyagoda and Manamendra-Arachchi, 1998; Somaweera et al., 2015). Currently, at the national level, Erdelen's horn lizard (*Ceratophora erdeleni*), Karunaratne's horn lizard (*Ceratophora karu*), the leaf-nosed lizard (*Ceratophora tennentii*) and Knuckles pygmy lizard (*Cophotis dumbara*) are listed by the IUCN Red List as CR; the rhino-horn lizard (*Ceratophora stoddartii*), the rough horn lizard (*Candoia aspera*), and the pygmy lizard (*Cophotis ceylanica*) are listed as EN, and the hump-nosed lizard (*Lyriocephalus scutatus*) is listed as VU (Ministry of Environment, 2012).

All Sri Lankan reptiles are protected by the 1937 Fauna and Flora Protection Ordinance (FFPO), prohibiting any collection and export except for scientific purposes. In the past enforcement was greatly relaxed, thus some animals in the European pet trade may have originated from legal exports during this relaxed period (e.g., Bartelt et al., 2005). However, smuggling and the illegal trade of *L. scutatus* and *Ceratophora* spp. has continued in recent times, and several countries now offer these species occasionally, including Germany (Altherr, 2014), Japan (Hettiarachchi and Daniel, 2011; Karunarathna and Amarasinghe, 2013), and Taiwan (Shiau et al., 2006). Local media publicized several incidents of Sri Lankan reptiles and other species being sold in Germany (Hettiarachchi, 2010; Hettige, 2011), and incidents of Europeans arrested in Sri Lanka while having reptiles in their possession (Rodrigo, 2012). In 2012, German pet traders urged the Export Development Board of Sri Lanka to resume reptile and amphibian exports to meet the demand in the EU (Anonymous, 2010a; Fernando, 2010). However, this idea was severely opposed by environmental activists (Anonymous, 2010b; Paranamanna, 2011) and halted.

Since 2013 an alarming abundance of adult Sri Lankan agamids has been documented in European online advertisements; including most members of the subfamily Lyriocephalinae as well as the black-cheek lizard (*Calotes nigrilabris*) and the Sri Lankan kangaroo lizard (*Otocryptis wiegmanni*). This indicates that there have been several recent smuggling events. These smugglers are based primarily in Russia, Italy, and France, and advertised these animals at very high prices: *C. stoddartii* (~€1000/pair), *C. ceylanica* (~€2200/pair) and *L. scuttatus* (up to €1600/pair).

A Bornean endemic, the earless monitor lizard (*Lanthanotus borneensis*), has become a species in great demand, particularly popular in the European pet market (Altherr, 2014; Nijman and Stoner, 2014). Despite being protected in its three potential range states of Indonesia (Kalimantan), Malaysia (Sarawak), and Brunei Darussalam, the lack of regulations protecting the species in consumer states enables international trade.

Over a 17-month period beginning in May 2014, Stoner and Nijman (2015) documented the online sale of nearly 100 earless monitor lizards from 35 different traders in 11 countries. The findings illustrated the global nature of this trade, though not so prevalent as that in Europe (Nijman and Stoner, 2014). Trade is occurring both in and out of Europe and, consistent with trade dynamics in other high-value reptiles, Germany is among the most pervasive of consumer states. Traders have offered earless monitor lizards for sale on specialist websites and specifically mention the reptile trade fair "Terraristika" in Hamm, Germany, held quarterly each year, as a place where individuals can be obtained after they have been purchased online. The species has also been documented for sale online in the Czech Republic, France, the UK (originating from Germany), and the Ukraine. This concentration of trade on mainland Europe is further compounded by traders in the EU directly supplying the USA market, making Europe an important consumer as well as a significant supplier. This continuing supply may account for an observed fall in price. At its highest point, the species was offered for sale for as much as US$15,500 (USA) and €9600 (Germany) but more recently prices have dropped to €3000/pair.

When buyers in the United States purchase this species from European traders it circumvents the US Lacey Act, which would prevent them from legally obtaining individuals directly from range states where the species is protected. No legislation comparable to the US Lacey Act exists in the EU, and since the species is not listed in the Appendices of CITES, or the Annexes of the EU itself, there are no mechanisms to prevent the trade within the EU, regardless of the illegal sourcing from the range states (Altherr, 2014).

The estimated count of the earless monitor lizard in trade is suspected to be conservative and only reports on the number observed for sale online. More crucial is the paucity of data on the species occurrence; the impact of trade and instances of removal from the wild cannot be quantified. The authenticity of captive breeding claims that are circulating for this species on specialist online forums are still under question; however, individuals are certainly being taken from their natural habitat. In October 2015, the first seizure and arrest was made at the International Airport in Indonesia's capital Jakarta, when a German national was found in possession of eight earless monitor lizards. Each specimen was purchased from a trader in Kalimantan, Indonesian Borneo, allegedly for around €3 (Anonymous, 2015), resulting in a substantial mark-up for those operating at the consumer end of the trade chain. More recently in March 2016, 17 specimens were seized from a German national at Supadio airport in Pontianak, capital of West Kalimantan (Anonymous, 2016).

Siebenrockiella leytensis is the only endemic semi-aquatic turtle in the Philippines (Diesmos et al., 2004). Taylor described the species in 1920 based on two specimens (Taylor, 1920) and over the next 30 years few additional individuals were reported (Buskirk, 1989). Wild populations in Palawan were only rediscovered in late 2001 (Diesmos et al., 2004). Publication of the rediscovery of wild populations of this rare and endemic turtle in Palawan (Diesmos et al., 2008, 2012) triggered an immediate interest from turtle hobbyist, who obtained specimens to the detriment of this species just a few months later (Schoppe and Cervancia, 2009; Schoppe et al., 2010). Although the Philippine Republic Act Number 9147 (Wildlife Resources Conservation and Protection Act) prohibits the collection of Philippine wildlife without proper permits, it did not prevent wildlife collectors and traders from illegally procuring and trading *S. leytensis* on local and international wildlife markets. Illicit

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trade of *S. leytensis* in Metro Manila was first documented in 2004, when 300 specimens were detected in two private facilities in Quezon City and Pasay City. Specimens fetched prices of €2000 in the European pet market (Gavino and Schoppe, 2004; Diesmos et al., 2008). Illicit trafficking has been consistently documented within the period 2004–2010, including a seizure of >600 individuals in Taytay, Palawan (Diesmos et al., 2008, 2012) and a record seizure of >4000 specimens in June 2015 in a warehouse in southern Palawan (Sy, 2015; TRAFFIC, 2015). The international pet trade is the greatest threat to this species (Diesmos et al., 2008, 2012; Schoppe and Shepherd, 2013).

The crocodile lizard (*Shinisaurus crocodilurus*), formerly known only from southern China, and since 2003 also from a few sites in northern Vietnam, was recently included in the IUCN Red List (Nguyen et al., 2014). The species is currently on the brink of extinction due to severe habitat loss in its restricted distribution, together with massive over-exploitation of the already heavily diminished and isolated wild populations. Its prominent color patterns and primeval appearance have made the species more popular among hobbyists especially from Europe (van Schingen et al., 2015). Following the species' inclusion in CITES Appendix II in 1990, the international trade in *S. crocodilurus* switched almost entirely to allegedly captive bred specimens, although there is clear evidence for the illegal origin of numerous specimens (van Schingen et al., 2015). Smuggling of wild-caught individuals to Japan and via Cambodia to Thailand, the false declaration of specimens and dubious trade via Kazakhstan and Lebanon, as well as the covert sale at the reptile fair in Hamm, Germany, have been well documented (e.g., Kanari and Auliya, 2011; van Schingen et al., 2015; pers. obs.). Recent population estimates of the species revealed 950 individuals in the Chinese populations (Huang et al., 2008) and only 100 individuals remaining in northern Vietnam (van Schingen et al., 2014). Recently, interviews with villagers in Vietnam confirmed that the collection of these lizards for trade has been more frequent than originally thought, which already has resulted in extirpation at some sites (van Schingen et al., in press).

In 2010, Grismer et al. described *C. psychedelica*, characterized by remarkably bright colors, as endemic to the small island of Hon Khoai in southern Vietnam. The first advertisements of this new species appeared on European Internet platforms in 2013, even though export of wildlife from Hon Khoai is prohibited. At the reptile fair in Hamm (Germany), in June 2014, at least nine pairs of *C. psychedelica* were offered by online unconnected adverts (Altherr, 2014). In 2015, a German dealer offered specimens to be exported to the USA, and in the same year the first adverts from the USA appeared. From 2013 to 2015, a total of 21 offers of *C. psychedelica* were noted. Ten of the internet offers (between 2 and 16 specimens) derived from Russia, three from Germany, three from Spain, one (10 specimens) from Czech Republic, and one (4 specimens) from the USA. Prices, if specified, varied between €1500–3500/pair; juveniles declared as captive bred offspring were offered for €500 by one trader from Russia. Range-restricted populations are especially prone to extinction, thus poaching is assumed to have tremendous impacts on the species' survival. Due to the small area of the island and the overall easy accessibility there are few possibilities for population recovery in *C. psychedelica*. Furthermore, the species' assumed low reproductive rate limits its ability to recover from this level of harvesting.

Japan is home to 13 Testudines spp., 38 Lacertidae spp., and 41 snake species, some of which are introduced species (Herpetological Society of Japan, 2013). The country is also a major importer of live reptiles (Auliya, 2003). In 2013, Japan imported >317.000 specimens of reptiles from 52 countries (Japan Customs, 2014). Due to the archipelago's geographical position between Asia and North America, the domestic market reveals an enormous diversity of species offered in both pet shops and the online market, with more than half of the world's tortoise species being sold in Japan (Kanari, 2010). The largest supplier of live reptiles to Japan is the Asian continent. Since 1980, Japan has been a party to CITES, and the objectives of CITES are implemented through the Act on Conservation of Endangered Species of Wild Fauna and Flora of 1992, which regulates domestic trade in wild species. Japan also protects national reptile species for cultural reasons. The Act on Protection of Cultural Properties of 1950 was enacted for protection of culturally important species as Natural Monuments (Kanari and Xu, 2012). Despite policies in place, illegal trade in reptile species continues; in fact, reptiles are the most prominent species involved in smuggling activities, particularly at the Japanese border (Kanari, 2010). At the CITES CoP16 in 2013, Japan submitted the first ever proposal for a native species, the Ryukyu black-breasted leaf turtle (*Geoemyda japonica*), which is endemic to the central Ryukyu Islands. The proposal was due to the species' abundance in the international trade and illegal trade activities demonstrating the likelihood for its decline, (Yasukawa and Ota, 2008). For example in the EU specimens are sold for EUR1000–1900 (Altherr, 2014; https://cites.org/sites/default/files/eng/cop/16/prop/E-CoP16-Prop-34.pdf - accessed 1 April 2016). Although the species was already fully protected by Japan's National Act on Protection of Cultural Properties, the listing in CITES Appendix II strengthened trade monitoring at the international enforcement level.

The illegal trade of turtles in China has been relatively well documented (Cheung and Dudgeon, 2006; Gong et al., 2009; Zhou and Jiang, 2008). Shi et al. (2007, 2008) discuss the laundering of wild-caught turtles by a giant largely unregulated turtle-farming industry that trades illegally collected turtles as captive-produced fare. Accordingly, these facilities represent a major threat to wild turtles. Further, Zhou et al. (2008) document how news of the high prices offered for rare turtles in the pet trade has reached even remote mountain villages. The value placed on CR species by the pet trade represents the major threat for some species by presenting incentive to collect every individual. The illegal pet trade in wild caught turtles and general overharvesting preceded scientific study, resulting in poor or absent locality information for some species. Pan's box turtle (*Cuora pani*), for which the actual locality was not known for many years (Parham and Li, 1999), McCord's box turtle (*Cuora mccordi*), Yunnan box turtle (*Cuora yunnanensis*), Zhou's box turtle (*Cuora zhoui*) (reviewed by Parham et al., 2001, [see Table 1], Parham et al., 2004 [see Fig. 1c], and the yellow-margined box turtle (*Cuora flavomarginata*) (Fong et al., 2002) exemplify this. Linkages to the European market have been outlined in the CITES proposal listing the genus *Cuora* in Appendix II (see https://www.cites.org/eng/cop/11/prop/36.pdf - accessed 23 February 2016). The illegal pet trade in turtles is an exceptional case that includes the scientific community. Dalton (2003) outlines how a Chinese smuggler supplied taxonomists with exotic turtles, using a New York veterinarian as an intermediary. Resultant confusion included the establishment of pointless captive breeding programs for human-produced hybrids, wasted field efforts, and a general drain on conservation energies (Parham et al., 2001; Stuart and Parham, 2007).

3.3.4. Oceania

Despite national protection of all native reptiles in some nations (New Zealand, Australia), the unique morphology, behaviour, and rarity of the region's endemic reptiles drives a high international market value for its reptile fauna. Documented extirpations of native reptiles appear to be related to an Oceania-specific consumer market in Germany. Despite strong enforcement, the existence of legal loopholes and captive-breeding fraud are substantiated by the preponderance of Oceania endemics commercially available abroad. Regional patterns indicate that even strong enforcement may not sufficiently deter smugglers of wild-caught animals — if the international market value for rare, endangered, and protected species is high.

New Zealand has a unique and distinctive fauna due to its long history of geographic isolation (Daugherty et al., 1993). Its reptiles are highly sought-after by animal collectors, particularly the tuatara (*Sphenodon punctatus*), an ancient reptile that is the last remaining member of the order Rhynchocephalia, and the *Naultinus* geckos (nine species collectively referred to as 'green geckos'). *Naultinus* spp. are of special interest because they are, in contrast to most of the world's gecko species, diurnal and brightly colored. Both the green geckos and the

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tuatara are highly desired because of: (1) a lack of availability (all native New Zealand reptiles are protected by the Wildlife Act, which prohibits their sale and export); (2) unusual life-history traits, including viviparity, slow growth, low reproductive rates (Cree, 1994), and extreme longevity (tuatara and New Zealand geckos are known to have reached ages of 91 and 53 years, respectively); (3) uniqueness (99% of New Zealand's terrestrial reptiles are endemic); and (4) rarity: most of the native New Zealand lizard fauna is threatened or at risk of extinction (83%, or 82 out of 99 terrestrial species, including some undescribed taxa; Hitchmough et al., 2013). Even relatively common New Zealand gecko species, such as *Woodworthia* spp. and *Mokopirirakau* spp., are also targeted by wildlife collectors, and sold on the European pet market for high prices (e.g., €2200/pair of *W.* cf. *brunnea*, €5300/pair of *M. granulatus* and €3700/pair of *Naultinus elegans*).

Many reptile populations in New Zealand are already small, fragmented, and threatened by predation and habitat loss. The illegal collection of individuals from a wild population is yet another threat that compromises their long-term viability and may extirpate some populations. For example, a population of jewelled geckos (*Naultinus gemmeus*) on the Otago Peninsula declined from about 70 individuals in 1994 to just two individuals in 2008: a decline of >95%, in part caused by illegal collection (Lettink, 2010). Anecdotal information indicated that a German national was sending persons to collect geckos from a certain site until they were almost gone. Even the impact of a single poaching event can be severe, as illustrated by a recent case in which 16 jewelled geckos (including 11 females, nine of which were gravid) were collected from another site that contained ca. 70 individuals (Knox, 2010). If these animals had not been returned to the wild, this population would have lost almost half of its breeding females and a quarter of its residents (Lettink, 2010). Within a year of the release of these animals, in 2010, collectors again targeted the site, and an unknown number of animals were taken and exported from New Zealand. Some animals were subsequently found advertised for sale on a German website (geckos were identified from their unique dorsal markings, which had previously been recorded in a photo library). Since 2001, eight people, including six German nationals, have been convicted for illegal collection and possession of New Zealand reptiles. This is thought to represent only a fraction of those involved, with significant numbers of reptiles being illegally exported to satisfy the growing market.

Being one of the 17 mega-diversity countries, Australia is known for its herpetological diversity, with at least 917 recognized reptile species and approximately 93% endemism (Mittermeier and Mittermeier, 2004; Chapman, 2009). Export of wildlife is strictly regulated under the nation's key environmental law, the Environment Protection and Biodiversity Conservation Act 1999, which was enacted in July 2000. Commercial export of native animals may only be permitted for dead specimens from approved sources. No export of live reptiles is permitted (Department of the Environment, 2015). However, endemism, morphological peculiarities (e.g., *Tiliqua rugosa*, *Moloch horridus*), and the intense colors and patterns of many Australian species are highly attractive to overseas collectors. Indeed, several cases of illegal trafficking have been recorded in recent years, including arrests of the persons involved.

In February 2015, two Russian and two Czech citizens were arrested after trying to smuggle 157 reptiles and amphibians, including skinks, geckos, and pygmy pythons (*Antaresia* spp.) (ACBPS, 2015). The court case ended with a 12-month prison sentence (Menagh, 2015). Pygmy pythons may fetch up to €1200 Euro/pair in the EU market; online offers are made by traders from several European countries.

In October 2015, the Australian Border Force at the Perth International Mail Gateway confiscated fifteen native Australian lizards concealed in parcels bound for Slovakia, including ten spiny-tailed geckos (*Strophurus ciliaris*), three bearded dragons (*Pogona* sp.), and two thorny devils (*Moloch horridus*) (ABF, 2015). A pair of spiny-tailed geckos, offered by a German trader, fetches approximately €1000 Euros; black market prices for thorny devils are estimated at over €10,000.

From August to September 2007, 40 reptile retail shops were visited in the regions of Kanto, Kansai, and Chubu, Japan. Almost 50 reptile taxa originating from Australia were recorded, mainly consisting of freshwater turtles, geckos, and monitor lizards, including one adult Perentie monitor (*Varanus giganteus*) (Kanari and Auliya, 2011).

3.3.5. Central and South America

Conditions are optimal for illegal trade activities in this region due to the lack of enforcement and domestic infrastructure, and widespread poverty. Several endemic species and populations have been detrimentally impacted and partially extirpated by the illegal international pet trade. The occurrence of a global hot spot of reptile diversity and endemism in the region is associated with international demand for many of its unique reptiles. Illegal trade in iconic, highly endangered species (e.g., Galápagos iguanas) elaborate trade routes to countries like Switzerland and Germany via western Africa. These case studies demonstrate the broad extent of illegal trade networks that are created, when smugglers can reliably exploit conditions in which national regulatory measures fail as a result of limited resources and weak enforcement.

The reptile fauna of Central America is comprised of >550 species (Köhler, 2008), many of which are targeted for the pet trade. Several of these species have narrow ranges and are threatened with habitat destruction and harvesting for local consumption (e.g., Pasachnik, 2013). When the threat of international trade is added, extirpations can be expected. The most sought-after reptile species from Central America are the spiny-tailed iguanas (*Ctenosaura* spp.) (Cotí and Ariano-Sánchez, 2008), Hog Island pink boas (*Boa imperator*; formerly recognized as a subspecies of *Boa constrictor*) (Reed et al., 2007), several turtles (specifically *Trachemys* and *Kinosternon* spp.) (Schlaepfer et al., 2005), the Guatemalan beaded lizard (*Heloderma charlesbogerti*) (Ariano-Sánchez and Salazar, 2015), the arboreal alligator lizards (*Abronia* spp.) (Ariano-Sánchez and Melendez, 2009; Ariano-Sánchez and Torres-Almazán, 2010), and the arboreal pit vipers (*Bothriechis* spp.) (CONAP, 2011). The situation in Central America is optimal for illegal trade: there is a high demand for many species, a lack of enforcement, bribery is well accepted, and immense poverty causes people to take risks for little profit. In addition, border control and government communication between countries is lacking, and smuggling routes are already well established because of the drug trade. Guatemala has been identified as an important route into El Salvador, from which animals are sent to Europe along with legal shipments of green iguanas (TRAFFIC reports over 225,000 green iguanas imported into the EU from Central America from 2005 to 2006). Highly threatened species such as *A. campbelli*, *Ctenosaura palearis*, *Bothriechis aurifer*, and *Heloderma charlesbogerti* have been detected along this route. In Europe, the entrance ports are usually the Czech Republic and Spain. In 2009, five *H. charlesbogerti* that had followed this route were confiscated from a private zoo in Denmark. The remaining population of *H. charlesbogerti* is believed to be <500 individuals. From 1993 to 2003 at least 10% of the population was taken from the wild to supply the illegal pet trade in the USA and Europe (Ariano-Sánchez and Salazar, 2015). The use of international courier services has also been identified as a way to traffic animals to Europe. In 2009, three *Abronia vasconcelosii* were found inside a videotape and were intercepted at Gatwick airport in the United Kingdom. This parcel was in route from Guatemala to the Czech Republic. Guatemalan authorities have identified one Mexican (partially residing in Spain) and one Czech national as playing major roles in this trade route.

The impact that trade can have on natural populations is exemplified below. LEMIS, the Law Enforcement Management Information System of the U.S. Fish and Wildlife Service, reported 240 wild-caught *Ctenosaura palearis*, endemic to the Motagua Valley, Guatemala, taken for commercial purposes in 2008, allegedly unknown to Guatemalan

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authorities. Locals advised that 50–60 individuals were collected for export each month and that they have noticed a dramatic decline of *C. palearis* in the past 20 years (Ariano-Sánchez and Pasachnik, 2013). Researchers visiting these areas reported the extirpation of several of these populations following extensive surveys (Cotí and Ariano-Sánchez, 2008). In addition, the presumed extinct Guatemalan endemic, *A. campbelli*, was rediscovered in 2009 (Ariano-Sánchez and Torres-Almazán, 2010). Interviews with locals shortly after the rediscovery showed that 48 individuals were being kept in captivity waiting to be collected by a smuggler (paying US$5 each) following a previous agreement for export. Had the timing of the interviews been only slightly later, the status of this species may be very different.

In Honduras, an insular population of *Boa imperator* exhibits dwarfism as well as a unique pink coloration that has been prized in the pet trade (Reed et al., 2007). Between the 1970s and 1980s, thousands of snakes were harvested from the wild (Porras, 1999; Reed et al., 2007), and, within a decade, not a single adult boa could be found on these islands during a rapid survey (Wilson and Cruz Diaz, 1993). In 1993, the Honduras Coral Reef Foundation was created, and the area was legally declared a Marine National Monument in 2003. Through these actions poaching has decreased dramatically; however, illegal removal is still being reported and these animals are being confiscated.

Mexico ranks second in the world (after Australia) in regards to reptile species diversity. Uetz and Hošek (2015) report 943 species for Mexico with almost 60% endemic to the country (see Flores-Villela and García-Vázquez, 2014). The country has very restrictive laws regulating capture and export of wild-caught reptiles. However, due to limited resources and capacity for enforcement, the illegal collection of reptiles is pervasive, even inside natural protected areas (Lovich et al., 2009). Many Mexican endemic species are highly sought-after in the international pet trade, including the Isla Todos Santos mountain kingsnake (*Lampropeltis herrerae*) and the Santa Catalina Island rattlesnake (*Crotalus catalinensis*). Both species are (1) classified as CR by IUCN Red List, (2) have limited ranges (<100 km^2 for *L. herrerae* and 40 km^2 *C. catalinensis*), and hence are highly prone to over-collection for the pet trade (Grismer, 2002; Avila Villegas et al., 2007; Hollingsworth and Frost, 2007), and (3) categorized in Mexico as threatened species "A" (threatened) (SEMARNAT, 2010). In addition, lizards such as the endemic San Lucan banded rock lizard (*Petrosaurus thalassinus*) are being illegally collected by the thousands for the international pet trade (Grismer, 2002), and other Mexican endemics, including the four-toed worm lizards (*Bipes canaliculatus*, €2000), Creaser's mud turtle (*Kinosternon creaseri*), Herrara's mud turtle (*Kinosternon herrerai*), and the Jalisco mud turtle (*Kinosternon chimalhuaca*), are for sale in Europe.

Mexico is home to 18 species of arboreal alligator lizards, (*Abronia* spp.), of which 17 are endemic. According to the Official Mexican Norm 059 (SEMARNAT, 2010), five *Abronia* species are listed as "P" (in danger of extinction), seven as "A" (threatened) and two as "PR" (subject to special protection), which makes any collection without a permit illegal, and those classified as "A" can only be collected for conservation purposes. In addition, six of these *Abronia* species are classified as EN, and two as VU by the IUCN Red List. The only legally exported species in this genus is *Abronia graminea* (listed in EU Annex D) due to the presence of a small Mexican captive breeding program that has been allowed to legally export a few individuals. No legal export has been permitted for any other species of *Abronia* (Conabio 2013 in litt. to Altherr, 2013) yet *A. graminea* is the most commonly traded species of its genus, and *A. deppii*, *A. lythrochila*, *A. martindelcampoi*, *A. mixteca*, and *A. taeniata* can be seen regularly in the pet trade (Altherr, 2014). *Abronia martindelcampoi* can achieve a price of €1200 and *A. mixteca* €4000/pair. European traders offering *Abronia* spp. are most often from Spain, which is a known gateway for Latin American species to Europe.

Spiny-tailed iguanas (*Ctenosaura* spp.) are also targeted by smugglers, of which 11 species are native and nine are endemic to Mexico. None of the Mexican *Ctenosaura* spp. are protected by CITES, but all of them are covered by Mexican law, prohibiting exports without permits. Only two species (*Ctenosaura pectinata* and *Ctenosaura defensor*) have been exported legally since 2000, but none to the EU (SEMARNAT, 2015). Nevertheless, at least nine Mexican *Ctenosaura* spp. are available in the European pet market, with prices between €150 for *Ctenosaura similis* and €1340 for *Ctenosaura conspicuosa*.

Of >40 reptile species native to the Galápagos Islands (Ecuador), six terrestrial species are threatened (IUCN, 2015). The islands represent an attractive source of specimens for private collections and have been receiving increasing attention from illegal traffickers. Four smuggling cases occurred between 2010 and 2015, with captured smugglers from e.g., Germany being prosecuted. These cases involved the illegal export of marine iguanas (*Amblyrhynchus cristatus*), terrestrial iguanas (*Conolophus subcristatus*), and a giant tortoise (*Chelonoidis* sp.).

Regarding the *Conolophus subcristatus* event, molecular tools were used to unambiguously assign and return the four iguanas to the proper population (Gentile et al., 2013). In this case, molecular tools were also used to produce a forensic report that contributed to sentencing the illegal smuggler to four years in prison, as he was found guilty under Articles of the Ecuadorian Criminal Code. In 2014, the Galápagos National Park started a program aimed at detecting and uncovering the network of illegal traffickers operating in Galápagos.

The UNEP-WCMC CITES trade database (2015) confirms that Ecuador has never declared export of live specimens of *C. subcristatus* or *A. cristatus* for commercial trade. Nevertheless, two live land iguanas and three live marine iguanas were officially exported from Mali to Switzerland in 2011 and 2012, respectively, labelled as "captive-bred" and for private purposes. In 2014, the Swiss CITES management authority issued export permits to Uganda for these animals, which for all practical purposes laundered these animals into the pet trade, and opened the door for additional smuggling events, claiming captive breeding. As was predicted, in September 2015 a Mexican citizen was arrested in Ecuador for attempting to smuggle an additional 11 juvenile Galapagos Marine iguanas to Uganda, underlining the role of Uganda as a transit point or laundering location in this on-going operation.

4. Discussion

This review provides a new perspective on the global reptile pet trade as it brings together key expert testimony from around the world that cannot be found elsewhere in the literature. Additional regions could not be included directly in this review (e.g., the western and southern Mediterranean area, the Canary Islands, the United States, New Caledonia, and eastern Africa); however, it is evident that they are affected by legal and illegal reptile pet trade as has been reported previously (e.g., Klemens and Moll, 1995; Bauer and Sadlier, 2000; Litzgus and Mousseau, 2004; Bertolero et al., 2011; Bauer et al., 2012). For many cases highlighted herein this is their first exposure to widespread awareness, and consequently a call to action for scientific and management authorities worldwide.

More than 90% of the world's reptiles are not regulated by CITES and the EWTR. This implies that non-CITES species, nationally threatened or newly discovered taxa (that commonly represent endemic taxa of charismatic species groups that have triggered international demand) are not protected from over-exploitation unless. However, as the case studies highlight, many species controlled under current policies are also illegally traded to supply the international reptile pet market (e.g., Alacs and Georges, 2008; Shepherd and Nijman, 2008; Nijman et al., 2012; Wyatt, 2013; D'Cruze et al., 2015). The current law and enforcement afforded to this cause is weak, partially caused by limited capacity (Sellar, 2014), in comparison to similar illegal trade, such as in drugs, arms, and human beings. In many regions presented within the case studies, the lack of enforcement capacity is mainly owed to understaffed authorities (e.g., Baard and de Villiers, 2000). These issues combined result in persistent illegal trade activities, and the continued over-

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exploitation of many species. Species examples covered in the case studies provide strong evidence that endemic species (e.g., species landlocked in mountain regions or oceanic islands) are those most targeted, and that on-going offtakes (attracted by international demand) can lead to the extirpation of species. However, localized over-exploitation of wide-ranging species can also lead to increased fragmentation and a reduction in gene flow, as exemplified by the intense illegal collecting of the Egyptian tortoise (*Testudo kleinmanni*) (Perälä, 2003) and several insular populations of the green tree python (*Morelia viridis*) (Lyons and Natusch, 2011).

A significant number of reptile populations have already been severely decimated for the pet trade (e.g., Klemens and Moll, 1995; Ariano-Sánchez and Torres-Almazán, 2010; Horne et al., 2011; Flecks et al., 2012), and evidence is provided within numerous CITES listings of species for which the pet trade poses a major threat. *Chelodina mccordi* (see above), the Burmese star tortoise (*Geochelone platynota*), *Testudo kleinmanni* (see above), numerous Asian box turtle species (*Cuora* spp.), the Mangshan pit viper (*Protobothrops mangshanensis*, see Gong et al., 2013) and many others (see https://www.cites.org/eng/com/ac/index.php) exemplify this.

The case studies clearly demonstrate that rare, geographically isolated, or protected reptile species trigger smuggling activities, as these species procure high-prices on the black market due to their paucity in the trade (Brook and Sodhi, 2006; Hall et al., 2008; Lyons and Natusch, 2013). Since scientific descriptions of new species are used as signposts for smugglers, an increasing number of herpetologists refrain from publishing detailed localities and do not support 'uplisting' to a more restrictive CITES Appendix as a preventative measure to avoid making these species more valuable (Rivalan et al., 2007). This results in a lack of proper listing and potentially management efforts, but is a last resort given the lack of trade enforcement currently present.

Although some entities would prefer a complete ban on all reptile trading this is an unlikely and controversial approach. Thus, in the event that national harvests of selected species (that represent a wider distribution, and display favorable life history traits) are allowed it is imperative that this is done in a sustainable manner (Leader-Williams, 2002). There are numerous examples of species (e.g., micro-endemics, those with prolonged sexual maturity and low reproductive output) that will be negatively affected if their trade is permitted. In addition, species yet to be assessed by the IUCN Red List occur in trade. Decision makers are therefore advised to network with experts e.g., of the IUCN Global Species Programme and their specialist groups to gain trade-relevant information to understand its impact on the relevant species.

One of the major issues and challenges is the fact that most species involved in legal trade are confronted by numerous uncertainties not only related to the species' biological and ecological traits. This, in particular, relates to species listed in CITES Appendix II, which may only be exported if sustainably harvested, so that the viability of their populations, and thus their role in the ecosystem, can be maintained (Article IV - https://www.cites.org/eng/disc/text.php#IV - accessed, 21 Oct. 2015). However, in practice, the need for a Non-Detriment Finding is commonly not met due to the lack of biological and collection data (Smith et al., 2011), but also due to economic pressure or lack of political will (see Lyons and Natusch, 2011). A striking example of CITES Appendix II species, that are traded without any NDFs, are monitor lizards endemic to Indonesia. Among nearly 30 species, five are nationally protected and six have been allocated an annual export quota. In more than ten species, adaptive management measures are not in place, and these species in particular are endemic to small islands e.g., *Varanus obor*, *V. kordensis*, *V. macraei* or *V. boehmei*; all these species are involved in the international pet trade (http://trade.cites.org; Uetz and Hošek, 2015).

There are many challenges to the effective implementation of these provisions (Nash, 1993; Gomar and Stringer, 2011). A major prerequisite to achieve this condition is the monitoring of the populations in question (Henle et al., 2013) and the provisioning of scientific knowledge to overcome uncertainties in this regard. However, in reality even trade that is well established among many reptile species lacks fundamental knowledge of the species' population dynamics over time. It is opportune to develop methods for a precautionary approach ("precaution is one of the guiding principles of environmental laws in the EU"; Kriebel et al., 2001) to preserve biodiversity rather than to support national economies or at a minimum to balance the protection of biodiversity and economic growth. Two range-restricted species, *V. boehmei* (CITES Appendix II) and the non-CITES listed Philippine pit viper (*Trimeresurus mcgregori*), exemplify this issue. Both species are classified as data deficient (DD) in the IUCN Red List, and are in fact heavily impacted by the international pet trade (Bennett and Sweet, 2010; Koch et al., 2013; Sy et al., 2009).

EU authorities should be aware of frequency in which some traders fraudulently declare their animals to have been bred in captivity. This can in reality represent individuals taken directly from the wild or the offspring of a gravid female giving birth or laying eggs soon after a smuggling event. Further, even for the truly captive bred individuals that are nationally protected in their native range and for which legal exports have never been permitted, the question on the legality of their breeding stock remains. The EU authorities should take more care to verify the validity of captive breeding claims by cross-checking with authorities in the native range countries before permitting imports or issuing exports. Too often a lack of this thorough investigation into the original export paperwork from the country of origin has resulted in the laundering wild reptiles as captive-bred, farmed, and has been well documented (Kabigumila, 1998; Shi et al., 2004; Vinke and Vinke, 2010; Pedrono, 2011; Lyons and Natusch, 2011; Sy, 2014).

Europe, as one of the major consuming regions worldwide in the live reptile trade, needs to take responsibility for the conservation of species outside its range (Gruttke, 2004). The approach to determine national responsibility to conserve and protect species has been elaborated more recently, developing a National Responsibility Tool that "uses a GIS-based approach to determine the international importance of a species distribution area in a focal area" (Schmeller et al., 2014). This approach has already been explored for regions outside Europe and should be established globally to monitor the conservation status of reptiles involved in the pet trade.

Nationally protected species not regulated by CITES cannot be adequately protected from exploitation on the international market once illegally removed out of their native range, leaving the responsibility to combat this form of wildlife crime solely with the countries of origin (Vinke and Vinke, 2015). The EU, with its wealthy clients and lack of internal barriers, is considered a main destination for smuggled wildlife, including reptiles (Auliya, 2003; Engler and Parry-Jones, 2007; http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52016SC0038&from=EN - accessed, 22 March 2016), but has no legal basis to seize specimens, which were illegally obtained or to penalize smugglers discovered in their range states. In contrary, such nationally protected species are covered in the US by the Lacey Act, that e.g., "prohibits the import, export, acquisition, receipt, sale or purchase in interstate or foreign commerce of any fish or wildlife taken, possessed, transported, or sold in violation of any wildlife law or regulation of any state, or in violation of any foreign law" (Hoover, 1998). The US Lacey Act therefore enables US authorities to seize such animals and to place fines on related smugglers. In November 2015, a joint letter from 156 scientists, field biologists, and conservationists from 45 countries, called on the EU Commission to pass equivalent legislation. The decision is currently pending.

In July 2015, the Members of the EU joined a Resolution of the United Nations on confronting illicit trafficking in wildlife, to develop an EU Action Plan against Wildlife Trafficking. This EU Action Plan was launched February 2016 and aims to tackle wildlife trafficking (http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52016SC0038&from=EN - accessed, 22 March 2016). While the EU Action Plan does not provide for new legislation so far, a study of the EU

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Parliament on wildlife crime (published only a few days later), recommends that the EU "*should consider measures to curtail activities involving wildlife species protected by laws of their countries of origin (only); this may include new legislation, making import, sale, purchase and re-export of specimens, which have been captured, traded or exported in violation of laws in the country of origin a criminal act within the EU*" (http://www.europarl.europa.eu/RegData/etudes/STUD/2016/570008/IPOL_STU(2016)570,008_EN.pdf - accessed, 28 March 2016). It is noted that the American "US Lacey Act" provides "a simple and realisable model for such an approach".

Although not discussed in depth here, the mortality rate of reptiles from the point of harvest to the final destination is an additional concern. Studies indicate pre-export mortalities for reptiles of up to 13.3% in Togo (Harris, 2002) and up to 50% for chameleons in Madagascar (Brady and Griffiths, 1999). During international transport a mortality rate of 3.14–4% on average was recorded, with significantly higher losses in skinks (up to 14%) and chameleons (7%) (Steinmetz et al., 1998; Schütz, 2003). However, the impacts of reptile trade on biodiversity are not limited to the targeted reptile population but also contribute to the destruction of many habitats and thus a plethora of other species (Goode et al., 2004a). For example, trees are cut to collect arboreal reptiles (e.g., Philippine frugivorous monitor lizards, the Polillo false gecko [*Pseudogekko smaragdinus*], and *L. williamsi*, which inhabit palm-like *Pandanus* trees; Flecks et al., 2012), rock crevices are broken open, and rocks and logs are overturn on a regular basis (Hollingsworth and Frost, 2007; Goode et al., 1998, 2004b). Additionally, many of the species targeted are ecosystem engineers or keystone species in their respective ecosystems. For example, Rock Iguanas (*Cyclura*) help maintain and perpetuate native plant communities, such as dry forests, which are among the most endangered in the world. Thus depleting an area of such species may result in ecosystem degradation. It has been postulated that harvesting of species that inhabit very specific isolated ecosystems, such as on islands, accelerates the extinction even more than habitat destruction (e.g., Machado et al., 1985; Asian Turtle Trade Working Group, 2000).

5. Conclusions and recommendations

Herein we have showcased a plethora of cases in which legislation and enforcement are insufficient, and species and populations are being depleted because of wildlife trafficking. To address this, a National Legislation Project (NLP) has been established by CITES to analyse CITES relevant legislation in the member States (see https://cites.org/eng/legislation - accessed 2 May 2016). The data presented herein may be used by CITES in the aforementioned project but should also be used as a call to action for many countries, particularly those within the EU to step forward and control these activities such that global reptile diversity may be conserved. Managers and those involved in the NLP should pay specific attention to the following:

1. The legal and illegal trade in various reptile species, largely endemic to megadiversity countries and biodiversity hotspots, should be considered detrimental to their survival.
2. Numerous nationally protected species, often listed as threatened by the IUCN Red List because of illegal and unregulated trade activities, are not listed in the appendices of CITES.
3. Many species yet to be assessed by the IUCN Red List are heavily traded and also under the appendices of CITES.
4. No regulations are in place to monitor the unsustainable trade of range-restricted rare species that lack protection and appropriate listings.
5. Numerous species listed in CITES Appendix II, that reflect island endemics and/or are threatened, can be traded legally, and non-detriment findings have not been elucidated by the relevant scientific authorities.
6. There are limited resources in many regions that result in understaffed national authorities. This in turn provides the conditions necessary to circumvent national and international regulations. Better implementation of current regulations, including a checks and balances approach as well as strengthening of enforcement is necessary.

As a result of the conclusions drawn above, an EU-level approach is highly recommended to:

(i) shoulder the responsibility in trade of threatened and endemic species not listed in the CITES Appendices,
(ii) regulate trade of CITES Appendix II species when the status is uncertain, and
(iii) pass legislation, in order to protect non-CITES listed, but nationally protected species.

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Chapter 7: Last chance to see? A Review of the Threats to and Use of the Crocodile Lizard

Last Chance to See? A Review of the Threats to and Use of the Crocodile Lizard

Mona van Schingen, Ulrich Schepp, Cuong The Pham, Truong Quang Nguyen and Thomas Ziegler

The Crocodile Lizard, listed in CITES Appendix II and as Endangered in The IUCN Red List of Threatened Species, *is becoming ever more popular among hobbyists. Rising international demand for the species is exceeding available supply of captive-bred specimens, resulting in an increase in illegally sourced wild specimens on offer. Wild populations are at the brink of extinction due to habitat destruction and over-collection for the trade and for local use. It is estimated that fewer than 1000 individuals are presently distributed in small and isolated sites in southern China and northern Viet Nam. In view of the constant decline of diminished populations, any further trade in wild specimens is detrimental to the survival of the species. This study addresses the current status of the threats to and the trade in Crocodile Lizards and highlights the need for immediate measures to protect remaining populations from extermination.*

Introduction

The Crocodile Lizard *Shinisaurus crocodilurus* is the only living representative of the family Shinisauridae. The species was originally described by Ahl (1930) from southern China, where its range is restricted to a few isolated sites due to its high ecological specialization (Huang *et al.*, 2008). The outstanding colour patterns and primaeval appearance, as well as an interesting semi-aquatic lifestyle, have made the species a desired target for the international pet trade from the 1980s onwards, with a strong interest from specialized collectors. Within two decades, harvesting of the species had caused dramatic declines of wild populations in China (CITES, 1990; Huang *et al.*, 2008) before the first Vietnamese subpopulation was discovered in the Yen Tu Nature Reserve (NR), northern Viet Nam by Le and Ziegler (2003). Initial morphological and molecular comparisons revealed no significant taxonomic separation between the two extant subpopulations (Ziegler *et al.*, 2008). Recent field surveys on the population status and ecology of the species in Viet Nam led to the discovery of two further subpopulations in two adjacent nature reserves, viz. Tay Yen Tu NR and Dong Son-Ky Thuong NR (van Schingen *et al.*, 2014a).

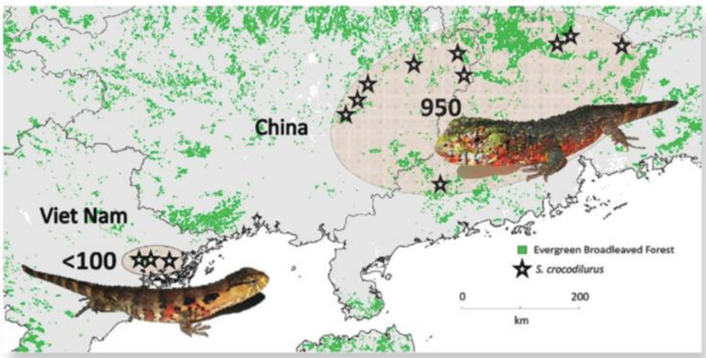


Fig. 1. Estimated wild population size of the Crocodile Lizard *Shinisaurus crocodilurus* in China and Viet Nam. Estimates derived from Huang *et al.* (2008) and van Schingen *et al.* (2014b), respectively. The habitats of *S. crocodilurus* are entirely surrounded by cultivated and agricultural land in Viet Nam; locality records derived from the authors' field surveys and from literature (van Schingen *et al.*, 2014a; Huang *et al.*, 2014).

Owing to multiple anthropogenic hazards, populations of the Crocodile Lizard are now facing extinction in the wild (Huang *et al.*, 2008; van Schingen *et al.*, 2014b). Besides habitat degradation, present at almost all known sites (Huang *et al.*, 2008; van Schingen *et al.*, 2014b), over-collection for consumption and the pet trade has been recorded as a severe threat to the species in China, while only little comparable information is available for the recently discovered Vietnamese subpopulations. The declining subpopulations in China were estimated at only 950 individuals in 2004 (Huang *et al.*, 2008); a similar study conducted in 2013 revealed the presence of fewer than 100 individuals in Viet Nam (van Schingen *et al.*, 2014b) (Fig. 1). In response to the international demand for the species (e.g., Nguyen *et al.*, 2004; CITES, 1990; Anon., 2014a), this study provides an analysis of the trade in Crocodile Lizards and a review and updated evaluation of threats as baseline information for improved conservation measures.

Distribution and Status

The Crocodile Lizard inhabits tropical evergreen broadleaf lowland forests in southern China (Guangxi Zhuang Autonomous Region, Guangdong Province) and northern Viet Nam (Bac Giang, Quang Ninh provinces) (Huang *et al.*, 2008; Le and Ziegler, 2003). It is particularly adapted to a specific forest ecosystem and individuals tend to rest at night on branches above pool sections of densely vegetated rocky streams (M. van Schingen, pers. obs.; Ning *et al.*, 2006; van Schingen *et al.*, in prep.), where they can be easily collected by poachers. The species can reach maturity after 13 months in captivity, but under natural conditions needs between two and four years (Yoshimi and Uyeda, 2011; Zollweg and Kühne, 2013). In addition, the period of pregnancy of lecithotrophic viviparous species, such as the Crocodile Lizard, is about nine to eleven months, which is comparatively long for reptiles (Zollweg and Kühne, 2013; Z. Wu *in litt.,* 17 June 2014). Large areas of habitat have been cleared in the species's range (Huang *et al.,* 2008; Le and Ziegler, 2003) which, in Viet Nam in particular, have been entirely surrounded by cultivated or agricultural land, which makes evasion of the species to other sites impossible. According to a niche model approach by van Schingen *et al.* (2014a), the actual and potential distribution of the species—considering climate and vegetation cover—is severely fragmented. Li *et al.* (2012) projected that all original habitats of the Crocodile Lizard in China will have vanished in 2081–2100 as a result of climate change.

Legislation

The species has been listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1990, which includes species not necessarily yet threatened, but which could become so if trade is not strictly controlled. Recently, the Crocodile Lizard was classified as globally Endangered in *The IUCN Red List of Threatened Species* (Nguyen *et al.*, 2014). Furthermore, it is included as a Category I species in the "Wild animal protection law" in China (Huang *et al.*, 2008), and at the end of 2013 the Ministry of Agriculture and Rural Development (MARD) proposed that the species be listed in the governmental decree of Viet Nam (T.Q. Nguyen, pers. comm.).

Methods

Evaluation of threats to and use of the species

Field surveys were conducted in Viet Nam between June and July 2013 and May and July 2014, determining the threats to the Crocodile Lizard by direct observations within the species's habitat viz. Yen Tu NR and Dong Son-Ky Thuong NR, Quang Ninh Province and Tay Yen Tu NR, Bac Giang Province. Nearly 80 villagers living in the surroundings of the nature reserves, and authorities of Quang Ninh and Bac Giang provinces, Son Don, Uong Bi and Ky Thuong districts and of the three aforementioned nature reserves were questioned in order to determine the general cognizance, perception and use of the species in Viet Nam. In addition, a literature survey was undertaken to evaluate the threats to and use of the species in China.

Analysis of trade

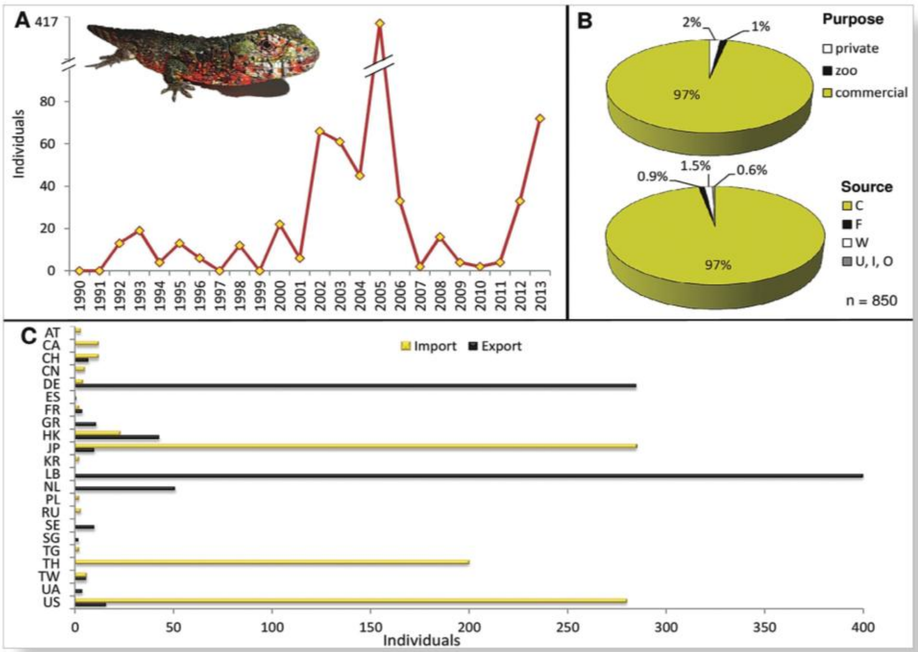
Trade data were obtained from the UNEP-WCMC CITES trade database (UNEP-WCMC, 1990–2013), which details all records of imports, exports and re-exports of CITES-listed species as reported by Parties. Data were available from 1990 to 2013. The analysis focused on the purposes "personal" (P), "commercial" (T), and "zoos" (Z), referring to live animals, since in the case of the Crocodile Lizard such trade is the most profitable. Internet platforms, reptile forums and Facebook pages were investigated to get an overview of the availability, demand, prices and evidence of illegal trade in this species. Four reptile fairs (three in Germany and one in Sweden) and 10 German pet shops were visited. Oral interviews were conducted with 26 dealers (20 from Germany, three from Sweden, two from the Czech Republic and one from Spain) on the respective reptile markets, 12 employees of pet shops that were visited, two zoo keepers (USA and Sweden) with experience in keeping Crocodile Lizards and 11 private keepers on their experiences in selling and keeping Crocodile Lizards, as well as to obtain information on origins and prices. A private keeper and two dealers of Crocodile Lizards in Viet Nam were contacted in writing. Data were collected mainly between August and December 2014. Names of interviewees are kept anonymous here for reasons of data privacy rights and internet links are not disclosed to prevent misuse.

Threats to the Crocodile Lizard and its Use in China

Literature survey

According to literature, consumption of Crocodile Lizards was traditionally believed to act as a cure for insomnia due to the long periods the animals spend motionless; they are also exploited for food (Herpin and Zondervan, 2006; Huang *et al.*, 2008; Nguyen *et al.*, 2014; Anon., 2014b). Li and Wang (1999) reported the sale of dried individuals in markets in China. While reports on any current use in traditional medicine were not found, cases of poaching for the pet trade are still being reported (Huang *et al.,* 2014; Kadoorie Farm & Botanic Garden, 2004; Zollweg, 2012). Interviews conducted by Huang *et al.* (2008) with 75 villagers living around the habitats occupied by Crocodile Lizards revealed that the majority (75%) had already hunted the lizard, but only 7.5% of those questioned had hunted the species for food or medicine (Huang *et al.*, 2008). The main motivation was to sell specimens to illegal traders for easy money (RMB10–1000~USD1.61–161.25) (Huang *et al.*, 2008).

The increasing application of electrofishing and use of poison for fishing are assumed to endanger the Crocodile Lizard in its aquatic phase (Huang *et al.,* 2008), and the sale of accidentally caught Crocodile Lizards on Chinese markets has often been recorded (Zollweg, 2011). In addition, the substitution of broadleaf forest for trees that produce more profitable timber contributes to the decrease of aquatic habitats, as do logging, water pollution from mining operations, and dam construction, which all change the natural water regime and degrade the species's habitats (Huang *et al.*, 2008; Huang *et al.*, 2014).



A: annual volumes of imports and exports of live specimens;
B: purposes of trade and source of animals *(C=captive bred, F=captive born, W=wild caught, U=unknown, I=confiscated or seized, O=pre-Convention)*;
C: Volumes of imports and exports of live specimens, per country *(AT=Austria; CA=Canada; CH=Switzerland; CN=China; DE=Germany; ES=Spain; FR=France; GR=Greece; HK=Hong Kong; JP=Japan; KR=Korea; LB=Lebanon; NL=Netherlands; PL=Poland; RU=Russian Federation; SG=Singapore; TG=Togo; TH=Thailand; TW=Taiwan; UA=Ukraine, US=USA)*

Fig. 2. International trade in Crocodile Lizards *Shinisaurus crocodilurus* from 1990–2013.
Source: UNEP-WCMC (1990–2013)

Threats to the Crocodile Lizard and its Use in Viet Nam

Literature survey and results of current survey

Crocodile Lizards soaked in rice wine were observed during the inspection of numerous local shops in Quang Ninh Province, Viet Nam, in 2013 (M. van Schingen, pers. obs.). A picture of a Crocodile Lizard preserved in alcohol was observed on the Facebook page of a Vietnamese pet shop, where the use of the species as a potency remedy was discussed.

Trade in live Crocodile Lizards in Viet Nam was recorded in 2002 at a tourist site (Yen Tu Temples, Quang Ninh Province) by Le and Ziegler (2003). At the time, some Crocodile Lizards were being offered as "baby crocodiles" to tourists for USD10–20 each. In May 2008, T.Q. Nguyen observed three specimens being offered for sale for USD5–6 at the same site. During recent field surveys, the authors discovered that cable cars had been installed to transport tourists to the top of the mountain where one, once remote, habitat of the Crocodile Lizard is situated. Recently employed forest rangers at this site have never seen a Crocodile Lizard, while some older rangers remembered that Crocodile Lizards had been frequently found at this site, as well as in lower regions of the mountains. Present surveys within these now easily accessible streams at the foot of the mountain revealed no presence of Crocodile Lizards. While 10 mature individuals had been recorded in 2013 in a stream at the top of the mountain, none could be found there in 2014.

Interviews with nearly 80 people in the remote villages situated within the species's habitats revealed a general ignorance about Crocodile Lizards and confusion with other lizards, as well as a lack of interest in this species. Only one farmer recalled cases of collecting Crocodile Lizards from nearby streams. Provincial authorities recognized the species from pictures, but assumed its extirpation from former localities.

According to recent field observations, the dramatic increase of habitat destruction and alteration as well as pollution are severe threats to the species in Viet Nam. Timber logging and slash-and-burn land clearance form a major threat to the species and coal mining activities were observed to cause drastic degradation of core habitats of the Crocodile Lizard. In 2014, local villagers were observed electrofishing in some habitat streams, which had not been the case the year before. At this site the rate of encounters with Crocodile Lizards dropped to three (one individual per stream) in 2014, compared to 11 during the same season in 2013.

Trade

Literature survey and results of current survey

Based on an interview with a reptile dealer, the first Crocodile Lizards appeared on the international pet market as early as 1982. Since 1985 an alarming rise in demand for Crocodile Lizards in the international pet trade has been recorded; specimens at that time fetched relatively high prices (e.g. DM995~USD595.63

Fig. 3. A distinctive primaeval appearance and striking colour pattern has resulted in an alarming rise in demand for Crocodile Lizards *Shinisaurus crocodilurus* by specialist collectors.

in Germany; CITES, 1990), although a pet shop in the USA was selling specimens for USD25 in 1987 (Hoffmann, 2006). While hundreds of specimens were legally imported from Hong Kong to Europe and the USA because the species had not been protected in the importing countries, the illegal sale of 3300 animals from Guangxi Autonomous Region, China, was reported between 1984 and 1986 (CITES, 1990). After being included in CITES Appendix II in 1990, the international trade in Crocodile Lizards suddenly switched almost entirely to specimens that were purported to be captive bred (~97%, UNEP-WCMC (1990–2013), Fig. 2). From 1990–2013, a mean of 39 ± 87 living individuals were annually recorded in international trade (Fig. 2); out of 850 animals, 97% were traded for "commercial" purposes and only 2% and 1% for "personal" and "zoo" purposes, respectively; the majority was imported by Japan (34%) and the USA (33%), followed by Thailand (23%) (Fig. 2). No exports from or imports to Viet Nam have been officially recorded (Fig. 2). A conspicuously high number (400) of allegedly captive-bred specimens was exported from Kazakhstan via Lebanon to Japan and Thailand in 2005, which makes Lebanon the major importer and re-exporter of Crocodile Lizards (Fig. 2). Kazakhstan has been a Party to CITES since 2000, whilst Lebanon acceded the Convention in 2013. Kazakhstan, as the country of origin, has not declared any imports or exports of Crocodile Lizards in its annual reports. Similar trade patterns involving a Kazakhstan-Lebanon connection have been observed in cases of trade in dendrobatid frogs and several reptile species, particularly from Madagascar (Nijman and Shepherd, 2009; 2011; Todd, 2011).

A case of definite trade with wild-caught individuals was confirmed by a German pet shop owner, who received three of reportedly numerous illegally imported specimens from China in 2003 from a dealer who was known for being involved in the fraudulent trade in reptiles. Furthermore, 104 Crocodile Lizards were seized at the border of Japan between 2007 and 2008 (Kanari and Auliya, 2011), and 19 individuals, collected in Viet Nam by a Vietnamese citizen, were smuggled from Cambodia to Thailand in 2014 (Robin des Bois, 2014).

Currently the trade in Crocodile Lizards has shifted almost entirely to the internet, partly via Facebook, which gives the dealer a reassuring level of security and control over the deal, especially when the legal origin of the specimens is doubtful. During the current research, the first internet offer (from the USA) was recorded in 2006 (USD700) on a reptile forum. There has subsequently been a conspicuous rise in offers and requests for this species, particularly on online reptile forums and in Facebook communities, especially in the USA and Germany. These mainly involve private individuals (81%) mostly offering their captive-bred offspring, but also pet shops and wholesalers (17%). Most of the observed entries (n=106) were from Europe (86%) (Germany 60%, Spain 5%, UK 4%, France 4%, Netherlands 3%, Belgium 2%, Slovakia 2%, Denmark 1%, Switzerland 1%, Russia 1% and Ukraine 1%), followed by the USA (10%) and Asia (Viet Nam 4%), but the origin in some cases was unclear. Crocodile Lizards are currently on offer for relatively high prices (e.g., ca USD1100, pet shop (USA), November 2013; juveniles for EUR490, pet shop (Germany), January 2015) on the internet and for a comparably low price (EUR150–300~USD174–348, BfN, *in litt.*, see also Bethge, 2014) at the reptile fair in Hamm, Germany. In December 2014, three Crocodile Lizards of unknown origin were observed by one of the authors at the reptile fair in Hamm in an unlabelled container, which was quickly concealed in a backpack once detected. Furthermore, even Crocodile Lizards reportedly originating from Viet Nam were observed at this reptile fair in 2014 being offered under the table (M. Zollweg, pers. comm., October 2014). Only since 2013 have Crocodile Lizards from Viet Nam been found being offered for sale on at least four different Vietnamese Facebook pages in Hanoi and Ho Chi Minh City; in 2014, one retailer in the country was offering specimens for export on his Facebook site (Fig. 3). While videos of several dozen captive adult lizards for sale were shown on Youtube.com, another dealer stated that he had almost 100 Crocodile Lizards from north Viet Nam for sale at his "farm". A hobbyist, keeping three wild-caught Crocodile Lizards from "the mountains of north Viet Nam", posted that there are many specimens available for sale and that retailers are allegedly highly interested in trading them on an international scale. Demand by hobbyists for Vietnamese specimens due to their more colourful appearance and for a supply of "fresh blood" for breeding has been frequently recorded on internet platforms.

Discussion

Considering the alarming status of the wild Crocodile Lizard population (Huang *et al.,* 2008; van Schingen *et al.*, 2014a; van Schingen *et al.*, 2014b), any collection of wild individuals is detrimental to the species's survival. This study shows that the trade in live animals has a highly detrimental impact on the species. Lack of comprehensive information on collection and use for traditional medicine in range countries means that it is not possible to assess with any certainty whether this is an additional threat, although the authors believe it is less significant than the live animal trade. Prices outside the range States remain lucrative (e.g. USD1100, pet shop (USA), 2013), leading to a growth of interest in selling to the international market. Specimens from Viet Nam have been on offer for export for USD180–350 (Facebook, 2014), while prices achieved in the national market seem to be rather low (USD5–25).

The shift in reported trade from wild-caught specimens to almost exclusively captive-bred specimens (>98%) after the species's listing in CITES Appendix II in 1990 is rather suspicious, since a very high mortality rate in captivity was reported at that time (CITES, 1990) and dealers of the species still state that the loss of a whole litter is commonplace due to the animal's sensitivity to stress, infection and inadequate water quality. Furthermore, dealers have confirmed that they still receive wild-caught specimens from China, mislabelled as "captive bred". Regarding the 400 allegedly captive-bred Crocodile Lizards exported from Kazakhstan to Lebanon in 2005, it is not far-fetched to conclude that such a trade pattern is a fraud to obtain "legal" CITES import permits for the laundering of smuggled animals into the trade. Besides the lack of established breeding facilities for such high quantities of an ecologically specialized species, it is further implausible that the alleged captive breeding group produced 400 hatchlings in 2005 and then suddenly stopped producing any offspring. Likewise, in Viet Nam, the large number of adult animals and the evident lack of proper enclosures—as illustrated in available pictures and videos—indicate that most specimens were wild caught, a fact confirmed in writing by a Vietnamese hobbyist. There is recent evidence for the covert sale of Crocodile Lizards from Viet Nam at the reptile fair in Hamm, Germany, even though reports on legal exports are lacking (M. Zollweg, pers. comm., November 2014). The present research shows that demand for the species exceeds supply, even though a few hobbyists successfully breed the species from time to time. The high interest of new bloodlines and morphs is currently increasing the pressure on wild populations, especially from Viet Nam. The remarkable increase in appearance of the species on relevant websites might also have triggered the increasing trade in Crocodile Lizards in Viet Nam. The aforementioned drop in encounters with adult individuals at some of the published habitat sites might be the consequence of locality data being misused by poachers. Experience in Viet Nam and China has demonstrated that only the more extensively monitored subpopulations are considered to be relatively secure and stable, indicating a positive effect of monitoring and research activities on wild populations.

Conservation Measures

For effective local conservation activities in Viet Nam, the authors' research team (Cologne Zoo, IEBR) initiated a comprehensive public awareness campaign. A brochure emphasizing the uniqueness of the last remaining lowland broadleaf forest ecosystem was created in order to support the conservation management, and to educate and raise awareness at the local authority level (Forest Protection Department (FPD), of Bac Giang Province, 2010). A poster (Fig. 4) was recently produced at the request of the FPD, highlighting the threats to this species within its remaining habitats and pointing out improved conservation measures; some 2000 copies have

Fig. 4. Poster developed for the awareness programme available in Vietnamese, German and English.

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been distributed among the respective nature reserves, and FPD's of Bac Giang and Quang Ninh provinces, high schools, universities, ranger stations, offices of communes, villages surrounding the nature reserves and the Me Linh Station for Biodiversity (see Ziegler, in press). A follow-up petition letter was sent to several agencies recommending, for example, the improvement of forest ranger work, the upgrade of the protection status of the species's habitat in Yen Tu Mountain area, the control of coal mining activities in the core zones of the nature reserves and the development of sustainable ecological and religious tourism in the region. In addition, the authors participated in local conferences, and held symposia and workshops in Hanoi and Ho Chi Minh City.

In China, agreements with local farmers have already helped to maintain at least core zones for Crocodile Lizards within the Daguishan NR and also a breeding facility for release programmes has been successfully established (Zollweg, 2012). Such a breeding programme was recently also initiated in Viet Nam at the Me Linh Station for Biodiversity, with promising preliminary results (Fig. 5; Ziegler, 2015). After the development of a stable captive population and based on comprehensive knowledge on the ecology and natural history of wild populations (e.g. van Schingen *et al.*, in prep.), a release and monitoring programme is planned to restock wild populations in Viet Nam, in accordance with criteria stipulated by the International Union for Conservation of Nature (IUCN, 2013).

Conclusions

The poaching of Crocodile Lizards in detrimental quantities has long been reported from China and over the last few years has also been recorded from the recently discovered and much smaller subpopulations in Viet Nam (Huang *et al.*, 2008, Le and Ziegler, 2003, Nguyen *et al.*, 2014). While wild populations of Crocodile Lizards are decreasing, international demand for the species is increasing and habitat destruction and degradation are expanding. Suitable habitats, especially in Viet Nam, are now restricted to a small area around Yen Tu Mountain and the number of wild Crocodile Lizards there is now very low. Due to its sedentary behaviour and specialization, the species's extirpation in the wild is predictable if forest protection is not drastically improved at these sites and illegal poaching curtailed. Since the trade in this species for hobbyist collection has only recently started in Viet Nam, immediate measures are required to prevent further collection of wild specimens.

Recommendations

Based on the evident harmful illegal trade in wild-caught specimens and to enable a more efficient control and prevention of poaching, a transfer of the species from CITES Appendix II to I is strongly recommended. Such an upgrade—which would be implemented in the EU by listing the species in Annex A of the Reg. EC 338/97—would in particular enable the CITES Management Authorities in the European Union, one of the major markets in the reptile and amphibian trade, to control and monitor the domestic EU trade. According to European law the commercial use of specimens of Appendix I (Annex A of Reg. EC 338/97) species is in general strictly prohibited. In most EU member States, such specimens must be registered with the relevant authorities and are subject to strict measures of certification and marking. This also applies to captive-bred specimens; their commercial use requires an official exemption certified by the respective Management Authority (European

Fig. 5. Juvenile Crocodile Lizards *Shinisaurus crocodilurus* bred at the Me Linh Station for Biodiversity in northern Viet Nam for a restocking programme in the species's original habitats in Viet Nam.

Commission, 2015). The CITES Standing Committee as well as all Parties to CITES should be urged to look very closely into the fraudulent claims of captive breeding (Lyons and Natusch, 2011) and enforcement efforts have to be increased, particularly into the apparent increase in online trade, which is partly taking place in closed systems provided by social media such as Facebook.

Based on the findings within the remaining natural habitats in Viet Nam, an upgrade of the existing reserves, the extension of the protected area network and improved ranger work at the sites where the species occurs is strongly recommended (van Schingen *et al.*, 2014b). Furthermore, in order to identify yet unknown sub-populations, field surveys should be conducted within suitable habitats based on the niche model approach (van Schingen *et al.*, 2014a), e.g. in the border region of China and Viet Nam, although publishing exact locality data should be avoided to prevent the misuse of such information. Due to minor differences in ecology between Crocodile Lizards in China and Viet Nam (van Schingen *et al.*, in prep.), a more comprehensive genetic comparison would clarify the conservation status and importance of single and extant subpopulations (van Schingen *et al.*, 2014b), which is also important for potential future hybridization in captivity. In order to evaluate the impact of the awareness-raising campaign, the recently established monitoring systems should be continued in the long term.

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Chapter 8: Can isotope markers differentiate between wild and captive reptile populations? A case study based on crocodile lizards (Shinisaurus crocodilurus) from Vietnam

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Can isotope markers differentiate between wild and captive reptile populations? A case study based on crocodile lizards (*Shinisaurus crocodilurus*) from Vietnam

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ABSTRACT

The international wildlife trade in allegedly "captive-bred" specimens has globally increased during recent years, while the legal origin of respective animals frequently remains doubtful. Worldwide, authorities experience strong challenges to effectively control the international trade in CITES-listed species and are struggling to uncover fraudulent claims of "captive-breeding". Forensic analytical methods are being considered as potential tools to investigate wildlife crime. The present case study is the first of its kind in reptiles that investigates the application of $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios to discriminate between captive and wild crocodile lizards from Vietnam. The CITES-listed crocodile lizard *Shinisaurus crocodilurus* is listed as endangered on the IUCN Red List mainly due to habitat loss and unsustainable exploitation for the international pet trade. Our results revealed significant differences in the composition of the two tested isotope systems between captive and wild individuals. Isotope values of skin samples from captive specimens were significantly enriched in ^{13}C and ^{15}N as compared to specimens from the wild. We also used the weighted *k*-Nearest Neighbor classifier to assign simulated samples back to their alleged place of origin and demonstrated that captive bred individuals could be distinguished with a high degree of accuracy from specimens that were not born in captivity. We conclude that isotope analysis appears to be highly attractive as a forensic tool to reduce laundering of wild caught lizards via breeding farms, but acknowledge that this potential might be limited to range restricted or ecologically specialist species.

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1. Introduction

Recorded international trade in CITES-listed reptile species from Southeast Asia between 1998 and 2007 accounted for at least 17.5 million animals, but real levels of trade are expected to be significantly higher (Nijman, 2010). Approximately 20% of the recorded trade volume is expected to have derived from captive-breeding or ranching facilities, which have been promoted because they may reduce harvesting pressure on wild populations and simultaneously support local livelihoods (UNEP-WCMC, 2014). Trade in specimens labeled as captive bred has significantly increased in recent years (UNEP-WCMC, 2014), but concerns have also been expressed by some Parties of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) over possible fraudulent captive-breeding claims as well as inaccurate reporting of trade in wild versus ranched or captive-bred specimens (CITES, 2014). Parties to CITES have acknowledged that illegal harvest and trade in wild-taken reptile specimens through captive breeding facilities undermines the rules of the Convention and may result in the trade becoming detrimental to wild populations. The CITES Secretariat has, therefore, commissioned a study on methodologies to differentiate between wild and captive-bred CITES-listed snakes in the trade, including its parts and derivatives (CITES, 2013).

One of the forensic methodologies of interest is stable isotope analysis, which potentially can play an important role in the investigation of wildlife crime through identification and profiling of tissue samples (Moncada et al., 2012; Voigt et al., 2012). The quantitative measurement of stable isotope ratios in metabolically inert tissue samples has potential for accurately determining the origin of respective organism due to the fact that the isotopic composition of certain elements, such as carbon and nitrogen, of a consumer reflects its diet and contains information on its respective local food web (Ehleringer and Matheson, 2007; Fry, 2006). In theory, this property can also be applied for the differentiation between wild and captive animals. Wild specimens generally feed on various prey taxa, which again might have lived on diverse isotopic sources namely different plant species and parts, reflecting a complex food web or certain geographic region. By contrast, captive animals are usually kept under a controlled feeding regime with few prey species, which are often grown on a constant isotopic source. First practical evidence for the successful applicability was found by Kays and Feranec (2011) to distinguish captive from wild wolves, whereas Ewersen and Ziegler (2014) used stable isotope analysis to discern morphometric misidentifications of Neolithic wolves and dogs.

The endangered crocodile lizard, *Shinisaurus crocodilurus*, is one of the species, which is mainly threatened by habitat loss and unsustainable exploitation of remaining and already heavily diminished wild populations, which are restricted to isolated sites in northern Vietnam andsouthern China (e.g., Auliya et al., submitted for publication; Huang et al., 2008; Nguyen et al., 2014 and van Schingen et al., 2014b, 2015a). The inclusion in CITES Appendix II in 1990 caused an almost entire shift of the international trade in crocodile lizards to allegedly captive bred specimens (van Schingen et al., 2015a). However, evidence suggests that illegal domestic and international trade in wild crocodile lizards is ongoing, which is contributing to declines of effective population sizes to about 100 individuals in Vietnam and 950 in China, while some subpopulations have already been extirpated (Huang et al., 2008; van Schingen et al., 2014b, 2015a). A lack of law enforcement capacity and skills results in limited trade controls for this species, the problem being further exacerbated by the majority of trade having shifted to online platforms in recent years. Furthermore, there is no scientific methodology in place that enables enforcement officers or investigators to determine for certain whether specimens claimed as being captive-bred were in fact reproduced in controlled conditions, and not simply taken from the wild (van Schingen et al., 2015a).

We herewith provide the first case study on lizards testing the applicability of isotopic markers in scales to discriminate between captive and wild individuals of the crocodile lizard from Vietnam. Scales are made of alpha and beta keratin and are metabolically inert since they were formed from the epidermis. The advantage of isotope analysis is the provision of a constant and long term signal on the species' diet, which makes it rather impossible for fraudulent captive-breeding farms to rapidly adapt isotopic signatures of target species via alteration of food (Rosenblatt and Heithaus, 2013; Warne et al., 2010). Therefore, we expected pronounced differences in the isotopic signatures between captive-bred and wild crocodile lizards, as well as differences in variance of isotopic signatures in wild compared to captive specimens. We aim to aid developing a conservation tool, which can be globally applied by e.g. exporting or importing CITES Parties to detect cases of mis-declaration of relevant specimens, if the legal origin is ambiguous. We also assess the accuracy of the methodology and evaluate its forensic potential and limitations while taking into account the effect of two environmental parameters (elevation and pollution) on isotopic signatures of wild individuals.

2. Methods

2.1. Study area

The study area encompassed all known *S. crocodilurus* localities in Vietnam, namely Tay Yen Tu Nature Reserve (NR) in Bac Giang Province as well as Yen Tu NR and Dong Son-Ky Thuong NR in Quang Ninh Province (Hecht et al., 2013; Le and Ziegler, 2003 and van Schingen et al., 2014a). The three sites are located within distances of 10–40 km of each other. All three NRs are part of the last remaining contiguous evergreen tropical broadleaf rainforest in northeast Vietnam, which has been extensively cleared in the recent past (Tordoff et al., 2000; BirdLife International, 2013). The dominant rock type in the region is granite, while Dong Son-Ky Thuong NR is situated in the border region to limestone dominated area. Furthermore, sampling sites were situated along an elevation gradient increasing from sites in Dong Son-Ky Thuong NR to Yen Tu NR (see van Schingen et al., 2015b). Northeast Vietnam is characterized by a monsoon tropical climate with cool winters (minimum temperature of coldest month ∼12 °C) and summer rains (Nguyen et al., 2000). The flora of this region belongs to the South-Chinese floristic unit and North Vietnam also shares close zoogeographic affinities with adjacent southern China (Zhu et al., 2003; Ziegler et al., 2008). According to van Schingen et al. (2014a) extant habitats for the crocodile lizard are heavily fragmented, small and poorly covered by protected areas. Furthermore, all habitats currently suffer tremendous degradation by coal-mining, forest deforestation and opening for the development of touristic and religious sites (van Schingen et al., 2014b, 2015a).

2.2. Sampling

Three test groups were categorized during the present study, namely "wild", "semicaptive" and "captive". Wild specimens were sampled from all the three known occurrence sites of *S. crocodilurus* in Vietnam (see 2.1). Semicaptive specimens originated from sites in Tay Yen Tu NR, but had been kept for at least three years within a conservation breeding program facility established between the Institute for Ecology and Biological Resources (IEBR) and the Cologne Zoo (Ziegler, 2015; Ziegler et al., 2015; Ziegler and Nguyen, 2015) at the Me Linh Station for Biodiversity, Vinh Phuc Province, in the north of Vietnam. Captive specimens were defined as offspring born at the Me Linh Station. Adult specimens at Me Linh are kept in small groups of three to four individuals in outdoor enclosures of about 2–7 m^2, respectively, while juveniles are kept in small groups or pairs within plastic boxes inside the station during the first months. Animals are fed once or twice a week with mainly beetle larvae and sometimes earthworms and crickets, while juveniles are fed more frequently.

The sampling in the field was carried out between June and July 2013 during the rainy season. Ten specimens from the wild population were captured by hand and immediately released after handling at the same site. Sampling occurred in four different streams, one from Yen Tu, two from Tay Yen Tu and one from Dong Son-Ky Thuong NRs. Only small tissue parts of the tail tip (∼0.5 cm) were taken and subsequently stored in 70% ethanol, since this tissue is capable of regeneration, and thus serves as a harmless sampling method in lizards (Comas et al., 2014; Struck et al., 2002; Takimoto et al., 2008). It had been reported, that there is no difference of $\delta^{15}N$ or $\delta^{13}C$ ratios among the tail and other body parts in lizards (Takimoto et al., 2008). Furthermore, three semicaptive specimens and eight captive born specimens were sampled in the same manner. Fast regeneration of tail tissue had been observed in captivity and during monitoring in the wild (van Schingen et al., 2014b, 2015a).

2.3. Isotope analysis

Scale samples were taken from the tail and analyzed at the accredited (DIN EN ISO/IEC 17025:2005) Agroisolab Facility for Stable Isotope Research in Jülich, Germany between April and September 2015. Samples were dried and cut into small aliquots with a scalpel. Subsamples of 1–4.5 mg were subjected to analysis by loading them into 4 × 6 mm tin capsules for carbon and nitrogen isotopic measurements. We used a Nu Horizon® continuous flow isotope ratio mass spectrometer. Results were reported relative to the Vienna Pee Dee Belemnite ($\delta^{13}C$) and atmospheric N_2 ($\delta^{15}N$), respectively and measured isotopic ratios (R) were expressed in δ units in the conventional permil notation, where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$. After every tenth sample the calibrated laboratory standard (leucine) was also measured. The laboratory standard was calibrated against a set of international standards (carbon: IAEA-CH-6, IAEA-CH-7; nitrogen: IAEA-N-1, IAEA-N-2). These IAEA standards were used regularly to cover the whole range of measurements. Any stretch-shift effect of the system was checked routinely in the calibration of the laboratory standard and no stretch-shift effect was detectable. Thus, the laboratory standard was only used for correction of the sample. The temperature of the laboratory was kept constant at 25 °C; linearity was checked and defined in an extra calibration run and was below 0.1‰ between $1 * 10^{-9}$A and $2 * 10^{-8}$A, so that no additional corrections were required. In order to assess precision of the analyses, we performed at least two replicate measurements for each sample, while carbon and nitrogen were measured concurrently. Analytical uncertainties, based on these replicate analyses were typically in the range of 0.1‰($\delta^{13}C$, $\delta^{15}N$), corresponding relative errors were 0.4% ($\delta^{13}C$) and 1.4% ($\delta^{15}N$). Isotopic ratios were given as means, respectively.

2.4. Statistical analysis

All statistical analyses were conducted using the R environment for statistical computing and graphics. Delta values were normalized and tested for normal distribution using the Kolmogorov–Smirnov test (Sokal and Rohlf, 1995). All *p*-values were greater $p = 0.05$ so that normal distribution of the stable isotopes was assumed. We built three classifiers, representing specimens from (i) wild populations, (ii) semicaptive origin, and (iii) bred in captivity (see 2.2). We were also interested whether the environmental factors elevation and pollution exert a statistical effect on isotopic composition in wild specimens. Therefore, we created a factor based on elevation of four studied streams and set the split in lowland and upland rivers at an altitude of 500 m asl. Two of the studied streams were polluted by discharge from an open coal mine: Thus, we created a second factor with the categorical values polluted and non-polluted. Levene's test (Sokal and Rohlf, 1995) revealed equal variances for the tested isotope ratios so that exploratory group testing was conducted with ANOVA.

Table 1
Isotopic ratios of nitrogen and carbon in wild, semicaptive and captive *Shinisaurus crocodilurus* from Vietnam. Isotope ratios are expressed as mean and standard deviation (sd). Stable isotope ratios (R) are expressed in δ units in the conventional permil notation where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$.

Sample	Class	Origin	$\delta^{13}C$(‰)	sd	$\delta^{15}N$(‰)	sd
Wild	SC1	Tay Yen Tu	−25.6	0.1	6.3	0.1
Wild	SC2	Tay Yen Tu	−24.7	0.1	6.3	0.1
Wild	SC3	Tay Yen Tu	−24.9	0.1	6.3	0.1
Wild	SC4	Tay Yen Tu	−24.6	0.1	4.5	0.1
Wild	SC5	Yen Tu	−23.5	0.1	6.6	0.1
Wild	SC6	Tay Yen Tu	−24.0	0.1	6.2	0.2
Wild	SC7	Yen Tu	−23.8	0.1	6.2	0.2
Wild	SC8	Yen Tu	−24.3	0.1	5.5	0.1
Wild	SC9	Tay Yen Tu	−25.6	0.1	5.3	0.1
Wild	SC10	Dong Son-Ky Thuong	−25.1	0.1	5.3	0.1
Semicaptive	SC11	Me Linh Station	−24.6	0.1	7.4	0.2
Semicaptive	SC12	Me Linh Station	−21.9	0.1	9.6	0.2
Semicaptive	SC21	Me Linh Station	−24.8	0.1	7.7	0.1
Captive	SC13	Me Linh Station	−23.8	0.3	9.3	0.2
Captive	SC14	Me Linh Station	−23.6	0.1	9.3	0.1
Captive	SC15	Me Linh Station	−23.9	0.2	8.7	0.1
Captive	SC16	Me Linh Station	−23.7	0.1	9.7	0.1
Captive	SC17	Me Linh Station	−23.4	0.1	8.7	0.1
Captive	SC18	Me Linh Station	−23.6	0.3	8.3	0.1
Captive	SC19	Me Linh Station	−23.9	0.4	9.2	0.1
Captive	SC20	Me Linh Station	−23.6	0.1	8.0	0.5

For the assignment simulations according to the origin of the classifier (i) wild, (ii) semicaptive, or (iii) captive-bred, we run multi-isotope testing. We applied the weighted k-Nearest Neighbor Classifier (Hechenbichler and Schliep, 2004) that assigns a sample to the classifier whose summed kernel densities is maximized among its k nearest neighbors, and which has been successfully tested for isotope ratios (Ziegler et al., in press). The basic rationale for the nearest neighbor rule, developed by Fix and Hodges (1989), is that samples with small Euclidian distance belong to the same class meaning that these samples are likely derived from the same place of origin. In order to address the problem of cutting natural variation due to limited sample size, we calculated the mean and standard deviation for each classifier to simulate 100 isotopic ratios per class. We randomly subdivided the data in a training set ($n = 200$) and a test set ($n = 100$). Our model revealed that misclassification is lowest at $k = 4$.

3. Results

3.1. Isotopic markers

We investigated the potential power of isotopic markers to distinguish the intermixing of captive-bred and wild specimens by analyzing ratios of $\delta^{15}N$ and $\delta^{13}C$ in wild (10 individuals), captive (8 individuals) and semicaptive (3 individuals), see Table 1. We found that the origin of samples had a strong effect on theisotopic ratios (Fig. 1). The mean $\delta^{13}C$ values of wild and captive specimens were −24.6‰ and −23.7‰, whereas the mean $\delta^{15}N$ values of wild and captive specimens were 5.9‰ and 8.9‰, respectively. Mean isotope values of skin samples from captive specimens were significantly enriched in ^{13}C (t-test; $t = 3.92$, d.f. $= 10.23$, p-value $= 0.003$) and ^{15}N (t-test; $t = 10.45$, d.f. $= 15.87$, $p -$ value < 0.001) as compared to specimens from the wild. Means of specimens in the semi-captive category were more similar to the means of the captive group ($\delta^{13}C$: −23.8‰; $\delta^{15}N$: 8.2‰). The standard deviation in both tested isotopic systems was lowest in the captive bred specimens ($\delta^{13}C$: 0.17‰; $\delta^{15}N$: 0.57‰). Standard deviation of the wild group was 0.72‰ for $\delta^{13}C$ and 0.66‰ for $\delta^{15}N$. The semicaptive group showed the largest standard deviation (sd $\delta^{13}C$: 1.62‰; sd $\delta^{15}N$: 1.19‰), which could be primarily attributed to sample SC12 from this group which differed by more than 2.2‰ in both isotope ratios from other samples of that group.

We created kernel density plots of the simulated data to better show the distribution of the isotope variables and superimposed the plots of the specimens of different origin (Fig. 2). We also conducted a global Kruskal–Wallis-test and found significant differences among the three groups of origin over the two stable isotopes ($p -$ values < 0.05). However, in the $\delta^{13}C$ signature there was some overlap between the wild and captive groups (Fig. 2a), whereas the $\delta^{15}N$ density curve hardly intersected between these two groups (Fig. 2b). The probability of the simulated semicaptive group showed a multimodal distribution in both isotopes, due to the combined effect of large variation of isotopic ratios and the small sample size ($n = 3$) of the original data (Table 1).

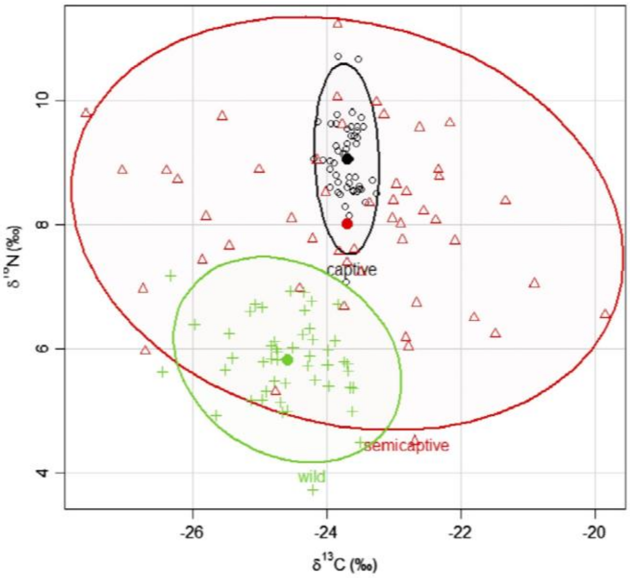

Fig. 1. Bivariate plot of simulated data ($n = 300$) with centroid and 95% confidence interval for each classifier (black circles—captive origin; red triangles—semicaptive origin; green crosses—wild origin). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

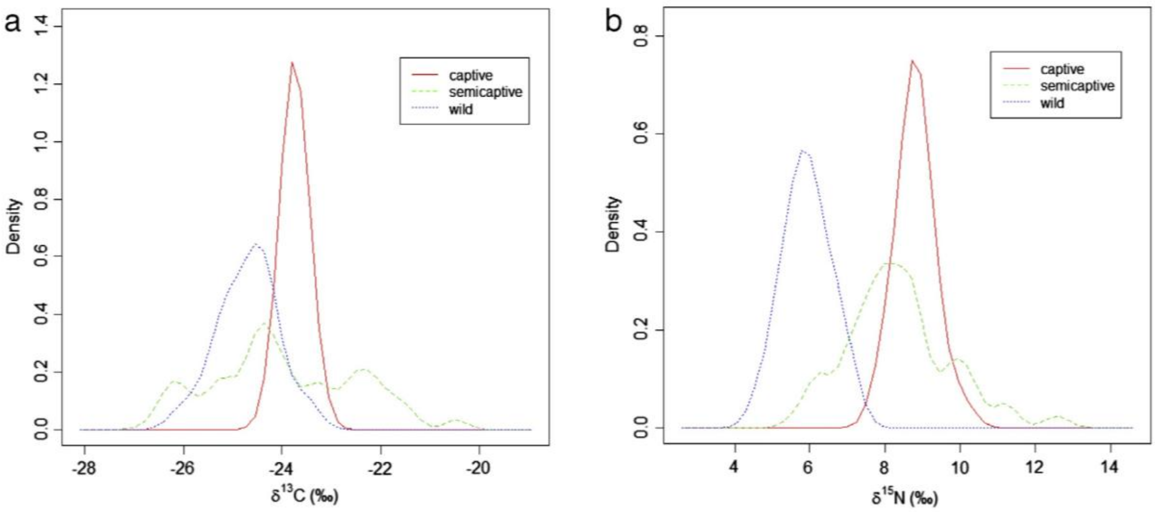

Fig. 2. Kernel density of simulated isotope ratios of (a) δ^{13}C and (b) δ^{15}N of captive (solid line), semicaptive (dashed line) and wild (dotted line) groups of *Shinisaurus crocodilurus* specimens.

3.2. Effect of environmental parameters

Judging by box plots (Fig. 3), displaying the potential effect of elevation and pollution at habitat sites on the isotope values of *S. crocodilurus*, there appeared to be a difference in δ^{13}C values for different elevations (Fig. 3a), while this difference is less pronounced between polluted and non-polluted streams (Fig. 3b). The ANOVA (Table 2a) for themain effect of elevation revealed a significant effect to the extent that δ^{13}C is enriched in lower elevations ($F = 7.38$, $p = 0.03$), but no significant effect of pollution on isotope ratios in *S. crocodilurus* ($F = 0.12$, $p = 0.75$). We did not detect any effect of elevation and pollution on δ^{15}N values (Table 2b).

3.3. Assignments

The test group was composed of randomly selected samples of wild ($n = 35$), semicaptive ($n = 33$) and captive ($n = 32$) origin. We defined accuracy as the proportion of correctly assigned samples divided by the number of total samples. Accuracy for the weighted *k*-NN rule differed in the source of origin (Table 3) and was highest among the captive group with almost 98%

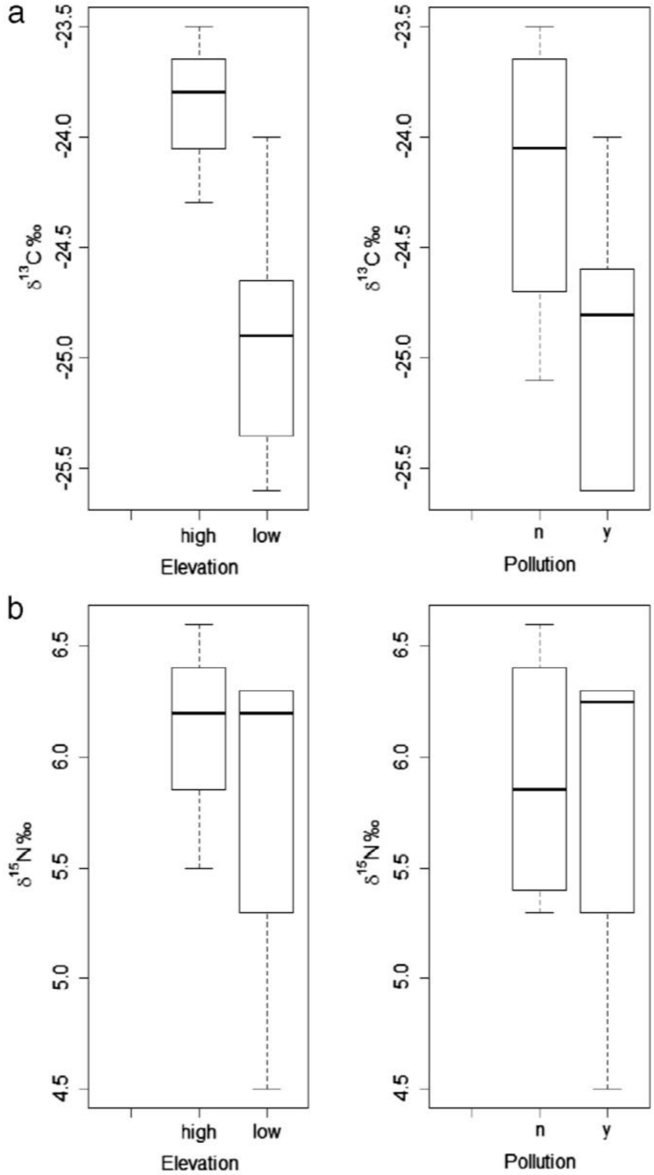

Fig. 3. Boxplots of isotope ratios of (a) $\delta^{13}C$ and (b) $\delta^{15}N$ of wild specimens ($n = 10$) of *Shinisaurus crocodilurus* grouped by elevation and exposition to pollution discharge (y—exposed; n—not exposed).

Table 2a
Analysis of Variance (ANOVA) for the model: "$\delta^{13}C \sim$ **elevation+pollution**".

Factor	DF	SS	MS	F	Pr(>F)
Elevation	1	2.368	2.368	7.3/8	0.03*
Pollution	1	0.034	0.034	0.10/	0.753
Residuals	7	2.247	0.321		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

of correct assignments. Percentage of correct assignments was lower in the semicaptive (89%) and the wild population (91%). However, differentiation between the captive and the wild group was 100% since no samples from the captive group were assigned to the wild population and vice versa. Six samples (17.1%) from the wild group were assigned to the semicaptives, while the test statistics assigned three semicaptives (9%) to the wild population.

Table 2b
Analysis of Variance (ANOVA) for the model: "δ^{15}N ∼ **elevation**+**pollution**".

Factor	DF	SS	MS	F	Pr(>F)
Elevation	1	0.268	0.268	0.541	0.486
Pollution	1	0.229	0.229	0.462	0.519
Residuals	7	3.468	0.495		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3
Results of weighted k-NN validation of test data ($n = 100$; $k = 4$). Accuracy = proportion of correctly assigned samples divided by the number of total samples. Origin as classifiers (no. of captive samples = 32; no. of semicaptive samples = 33; no. of wild samples = 35).

	Assigned to			Accuracy
	Captive	Semicaptive	Wild	
Captive	31	1	0	98%
Semicaptive	1	29	3	89%
Wild	0	6	29	91%

4. Discussion

While common dietary analyses are limited in representing the trophic niche integrated over time and thus form only "snapshots", recent studies developed a novel approach of measuring the trophic niche width based on the variance of stable isotopes of species (Bearhop et al., 2004). Particularly, the stable isotope ratios of nitrogen (δ^{15}N) and carbon (δ^{13}C) proved to be a useful tool to estimate the trophic position of a consumer within food chains, as the ratios of stable isotopes in consumers represent the ratios of its diet. Only few studies are yet performed on stable isotopes in lizards (Comas et al., 2014). Furthermore, the potential of stable isotope analyses as forensic tool to investigate wildlife crime has only recently been considered and is poorly tested in practical applicability as yet (Lyons and Natusch, 2006).

4.1. Isotopic variation

We found a relatively high variance in isotopic composition among wild individuals, reflecting the feeding on multiple prey species from terrestrial and limnic ecosystems with different isotopic signatures (Fig. 2). Preliminary studies on the natural prey spectrum of *S. crocodilurus* revealed a variation of almost 15‰ in δ^{15}N and 9‰ in δ^{13}C in stomach contents of *S. crocodilurus* from the wild (van Schingen, 2014). Based on stable isotope analyses of individual prey taxa, a high interspecific variance has been found within macroinvertebrate families from different habitat sites (van Schingen, 2014). We have shown that isotopic variance is smaller in captive crocodile lizards due to an increase in the homogeneity of diets resulting from a more restrictive human control over the feeding regime and the animals itself (Fig. 1). This has been shown in mammals (Kays and Feranec, 2011; Ewersen and Ziegler, 2014), but the concept can also be applied to ectothermic vertebrates whose diet-tissue equilibrium is usually attributed to growth rather than to metabolic turnover (Seminoff et al., 2006; Reich et al., 2008).

Stable isotope analyses are increasingly performed in ecology to elucidate patterns of food webs, ecological processes and systems viz. patterns of energy flow and nutrient cycling, food chain lengths, food web organization, short- and long-term diet patterns, habitat use, and animal movements (Milanovich and Maerz, 2012).

The enrichment in ^{13}C is commonly used as measure for the carbon source (primary producers) upon which the food web is based on and can strongly differ between ecosystems (Barrett et al., 2005; Bearhop et al., 2004; Briggs et al., 2012; Post, 2002) so that this isotopic marker appears to have some discriminatory power to distinguish captive from wild specimens. The intraspecific variation in wild specimens was conspicuously higher than the standard deviation in captive individuals, reflecting the higher variability of ^{13}C food sources in the wild. While we found relatively homogenous values in captive individuals, isotope compositions of semicaptives revealed several peaks (Fig. 2a). This rather large intraspecific variance might have resulted from individual differences in molting or the presence of regenerated tail tips, which differently reflect the "wild" signal among semicaptives.

We found that captive crocodile lizards were more enriched in ^{15}N than wild individuals (Figs. 1, 2b). The value of δ^{15}N increases about a certain amount along the food chain, which makes it a useful marker to estimate the trophic position of a consumer (Barrett et al., 2005; Bearhop et al., 2004; Briggs et al., 2012; Post, 2002; Rheinardt et al., 2013; Struck et al., 2002; Takimoto et al., 2008). Thus, the observed difference in ^{15}N probably resulted from prey taxa (beetle larvae, crickets) which fed on industrially produced powder and pellets containing remains of vertebrates, such as fish and poultry. In contrast, wild crocodile lizards were found to dominantly feed on saprophagous worms and insect larvae, which are situated at the bottom of the food web and thus are only sparsely enriched in ^{15}N (van Schingen, 2014, Zhao et al., 1999 and Ziegler et al., 2008). Additionally, enhanced δ^{15}N values in terrestrial organisms may also be caused by specific metabolic processes, which had been reported for tortoises (Struck et al., 2002). McCue and Pollock (2008) showed that starvation leads to higher δ^{15}N and lower δ^{13}C values in reptiles. However, this pattern has been only detected in excrements rather than in scale tissue and signs of starvation have not been found in neither wild nor captive specimens.

Regarding the impact of environmental factors on isotopic signatures, we found that lizards from higher altitudes (>500 m) were characterized by higher δ^{13}C values than lowland specimens (Fig. 3a). This effect can partly be attributed to an increase of δ^{13}C values in plant material by 1‰ per 1000 m elevation gradient (Anderson and Smith, 2002). In addition, the increase in δ^{13}C at higher altitudes might be due to reduced interspecific competition and associated increased trophic niche breadth which has been reported for gekkonids (Comas et al., 2014). Furthermore, the covariate altitude was found to be negatively correlated with the pH of the inhabited streams (van Schingen et al., 2015b), which might impact the isotopic composition and species assemblage of primary producers such as algae, hydrophytes or riparian plants (Hinga et al., 1994). Even though *S. crocodilurus* forages in riparian environments, the magnitude of the indirect effect of the species on the aquatic terrestrial linkage (Rheinardt et al., 2013) is unknown, but a question for future research to be approached with stable isotope analyses. Other influences on isotope variation should be considered such as ingestion of C_3 or C_4 plants by herbivores, climate, age effects and other impacts attributable to locations (Struck et al., 2002).

4.2. Potential for use in law enforcement

Our results revealed significant differences in the composition of the two tested isotope systems – namely carbon and nitrogen isotopic ratios – between all three investigated classes of origin. Our study gives first evidence for the capability of discriminating wild from captive bred lizards. Given the size of extant habitats and the small effective population size of about 100 individuals in Vietnam, we consider our sampling as representative. However, to address concerns on the comparatively small sample size ($n = 21$), we applied a simulation approach to avoid the risk of point estimates (Jackson et al., 2011). Primarily based on the nitrogen isotope signature, captive bred individuals could be clearly distinguished from the two remaining classes of origin with only one wrong assignment in our simulation model (accuracy: 98%). Thus, the risk of false accusations against a legitimate breeder or keeper of legal captive bred specimens is minimal. Furthermore, only one of 68 test cases (1.4%) of wild origin (both semicaptive and wild classes) would be wrongly assigned as captive bred. Therefore our established method could be an important tool for enforcement in efforts to reduce laundering of wild caught lizards via breeding farms. However, this method is assumably not applicable to food specialists, which forage on a limited range of prey taxa and need to be fed in a similar way in captivity.

The proposed methodology even works for specimens from the wild that were kept in captivity for at least three years so that isotopic diet-tissue equilibrium can be assumed (Revelles et al., 2007). This is in so far surprising since lepidosaurian reptiles cyclically renew and shed their epidermis, which takes place about once or twice a year in adult crocodile lizards. Our results, by contrary indicate the presence of metabolically inert keratin tissue in the tip of the tail that is not shed during progressive epidermal renewal. Thus, isotope analysis appears to be highly attractive as a future forensic tool to identify the origin of specimens. However, the present approach does not allow an immediate differentiation of wrongly labeled individuals from captive individuals by authorities such as custom officers, since some time for laboratory investigations under consistent conditions is required.

5. Conclusion

Species with vast distribution ranges often show considerable variation in isotope ratios, in which case provenance is difficult to establish using isotope analysis (Ziegler et al., in press; UNOCD, 2014). On the other hand, for range restricted species with distinctive isotopic signatures, stable isotope analysis can indicate most likely areas of provenance and reduce laundering of wild caught lizards via breeding farms that rely on alien feeding regimes. Our research only included the *S. crocodilurus* population from Vietnam, since it is much smaller than the Chinese population and restricted to a very small geographic range (van Schingen et al., 2014a,b, 2015a). Immediate conservation action and law enforcement are required to control illegal trade activities (Auliya et al., submitted for publication; van Schingen et al., 2014b, 2015a). The present study provides a reference framework against which specimens of ambiguous origin can be cross-checked. In a further step, populations from China as well as different captive populations should be sampled and analyzed in order to raise the effectiveness of this approach (Lyons and Natusch, 2006). To eliminate biases due to different sample preparation methods such as lipid extraction, the sample treatment should follow a consistent protocol and optimally be conducted at the same laboratory in order to achieve the highest comparability of results (Briggs et al., 2012). Even though nitrogen and carbon isotopes appeared to be most suitable in evaluating trophic positions and food sources, the investigation of multiple isotopic signatures might further increase the discriminatory power to distinguish captive and wild animals. Of particular interest to distinguish sources of origin are water isotopes since gradual rain-out of oceanic air masses moving inland often produces large isotopic effects on hydrogen and oxygen isotopic compositions over southeast Asia (Araguás-Araguás et al., 1998).

This study was intended to develop an approach which could be applied by respective authorities to uncover cases of mislabeling and illegal trade in specimens of CITES-listed reptile species. Although the sampling procedure might raise ethical concerns, the method is justified given the conservation status of many wild populations and has therefore been strongly recommended for endangered reptile species (Struck et al., 2002). Isotopic profiling can be very useful in answering specific compliance questions, eventually supporting a management regime that informs authorities and enforcement staff to focus and deploy law-enforcement efforts to uncover fraudulent claims of captive-breeding. Where knowledge of isotopic differences between metabolically inert reptile skin is important for conservation activities, the herein examined cost-effective isotopic markers and statistical methods might be adopted.

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2.3 Linking *in situ* with *ex situ* conservation

Chapter 9: Is there more than one Crocodile Lizard? An integrative approach reveals Vietnamese and Chinese Shinisaurus crocodilurus represent separate conservation and taxonomic units

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Is there more than one Crocodile Lizard? An Integrative Taxonomic Approach Reveals Vietnamese and Chinese *Shinisaurus crocodilurus* Represent Separate Conservation and Taxonomic Units

Gibt es mehr als eine Krokodilschwanzechse? Ein integrativer taxonomischer Ansatz zeigt, dass vietnamesische und chinesische *Shinisaurus crocodilurus* separate Schutz-, sowie taxonomische Einheiten darstellen

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Abstract

The Crocodile lizard *Shinisaurus crocodilurus*, the only living representative of the family Shinisauridae, is a habitat specialist adapted to remote freshwater habitats within evergreen broadleaf forests. Its current distribution is restricted to few small and isolated remnant occurrences in South China and North Vietnam. Multiple anthropogenic threats such as massive habitat destruction and unsustainable over-collection for the international pet trade brought the species to the brink of extinction. We herein employed an integrative taxonomic approach including comprehensive molecular comparisons based on fragments of mitochondrial genes (cytochrome b, partial *ND6*, and partial tRNA-Glu) in concert with in-depth morphological and ecological analyses in order to determine the status of the extant populations. Based on molecular, morphological, and ecological differences, we herein describe a new subspecies, *Shinisaurus crocodilurus vietnamensis* ssp. n., from Vietnam. Our findings emphasize the importance of improved *in situ* conservation measures in both countries, as both China and Vietnam harbor unique Crocodile lizard forms. We also recommend additional *ex situ* conservation measures, *i.e.*, separate conservation breeding management of the subspecies in order to maintain genetic integrity and adjust husbandry conditions according to detected differences in ecological niche occupation.

Keywords: Conservation units; Ecology; Molecular biology; Morphology; New subspecies; Shinisauridae

Introduction

Shinisaurus crocodilurus was described as new species, genus and family by Ahl (1930). Previously only known from southern China, it was subsequently reported from Vietnam by Le and Ziegler (2003). An initial preliminary examination of a potential taxonomic separation of the disjunct populations was conducted by Ziegler, Le, Vu, Hendrix, and Böhme (2008), but available data did not reveal unambiguous differences between samples from China and Vietnam. More recently, additional subpopulations have been discovered in Vietnam, all of which are distinct geographically from known Chinese populations (Hecht et al., 2013; van Schingen, Ha, et al., 2016; van Schingen, Ihlow, et al., 2014). Similar to the observed population decline in China, the Vietnamese subpopulations have decreased within recent years to less than 150 mature individuals (Huang et al., 2008; van Schingen, Ha, et al., 2016; van Schingen, Schepp, Pham, Nguyen, & Ziegler, 2015). Anthropogenic impacts such as habitat destruction and poaching for the international trade were found to pose main threats to the species, which led to its inclusion in CITES Appendix II and in the IUCN Red List as Endangered (Nguyen, Hamilton, & Ziegler, 2014). In contrast to earlier conclusions in Ziegler et al. (2008), recent ecological field studies – conducted by our working group in northern Vietnam revealed ecological differences between Vietnamese and Chinese populations; for example, in perch selection (van Schingen, Pham, et al., 2015). Based on these findings, questions arose necessitating more detailed information both on habitat use and taxonomic status. Our trade analyses also revealed that individuals from Vietnam have already appeared in both local and international pet trade (van Schingen, Schepp, et al., 2015), which might cause intermixing of individuals from distinct extant populations. To address the above issues, the present study aims to answer the following questions: Is/are there only one or different management unit(s), which should be maintained separately in conservation breeding programs? If so, how can they be identified when locality information is lacking? Herein we used an integrative taxonomic approach to assess intraspecific variation between the northeastern (Chinese) and southwestern (Vietnamese) populations of the *Shinisaurus crocodilurus* complex, based on morphometric ratios, molecular data (cytochrome b, partial *ND6*, and partial tRNA-Glu) and ecological divergence.

Material & Methods

Specimens Examined

Only adult specimens (SVL > 140 mm) were used for morphological examination. The following voucher specimens originating from Vietnam were examined, which are deposited in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam, the Vietnam National Museum of Nature (VNMN), Hanoi, Vietnam, and the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany: IEBR 3806-3810, VNMN 04744, ZFMK 83902-83903. The following specimens, deposited in the ZFMK were examined, which putatively originate from China: ZFMK 39424, 40557, 41332, 43788-43790, 44308, 44691, 46042, 57856, 63573, 73089 and 78803. Even though these specimens are derived from the trade without precise locality data, they had been deposited in the Museum before 2003, when the species had been reported for the first time from Vietnam, so that Vietnamese origin is unlikely. Furthermore some living specimens from the Me Linh Station for Biodiversity, Vinh Phuc Province, Vietnam, which originate from northern Vietnam with the following PIT tag numbers: 972273000155236, 97227300020654, 972273000200822, 972273000203267, 972273000200806 and 972273000156594 were also morphologically examined. Data from field surveys (van Schingen, Pham, et al., 2014, 2015), photographs, and tail tip tissue samples from living individuals from Tay Yen Tu NR, Bac Giang Province, Yen Tu NR and Dong Son-Ky Thuong NRs, Quang Ninh Province, Vietnam were also included.

Morphology

The following scalation characters were taken: Enlarged supralabials, adjacent rows of supralabials (below eye), enlarged infralabials, adjacent infralabial rows (below eye), enlarged collar scales, transversal ventral scale rows (from collar to cloaca), longitudinal ventral scale rows (between lateral folds), enlarged scales anterior to cloaca, tail whorls, and lamellae below fourth toe. For comparing morphometric characters the following measurements were taken with a caliper to the nearest 0.1 mm: HL (head length from tip of snout to posterior dorsal cranial edge), HL_b (from tip of snout to collar), HW_a (head width between posterior dorsal cranial edges), HW_b (head width at widest portion of head), HH (head height), CH_a (cheek height, from mouth to top of head at posterior edge), CH_b (cheek height measured above eye), Or (diameter of orbit), IN (inter-nare distance), NE (distance between nare and eye), E (eye diameter), EE (distance between eyes, measured anteriorly above snout), AG (distance between axilla and groin), SVL (snout-vent length), Fa (forearm length), Hl (hind limb length).

We performed a Principal Component Analysis (PCA) of nine selected morphometric characters describing the head morphology (HH/HL, HW_a/HL, CH/HL, CH_b/HL, Or/HL, IN/HL, NE/HL, E/HL, EE/HL) to detect differences in morphology. All characters were proportinated to the "head length". Statistical analyses were performed with the program PAST (Hammer, Harper, & Ryan, 2001) and for all tests, $\alpha = 0.05$. Afterwards a t-test was conducted for each ratio with GraphPad Prism (version 5.0 for Windows, GraphPad Software, La Jolla California USA), and www.graphpad.com was used to test for significant differences.

Molecular Methods

Fragments of mitochondrial genes, including cytochrome b, partial ND6, and partial tRNA-Glu were amplified by primer 1 and primer 2 from Huang, Wang, Linmiao, Wu, and Chen (2014). Three taxa were used as outgroups based on their phylogenetic relationships with *Shinisaurus crocodilurus* (Li et al., 2012). In addition to 14 sequences of *S. crocodilurus* from China obtained from GenBank, we sequenced five small tissue samples of the tail tip of *S. crocodilurus* from Quang Ninh and Bac Giang provinces from northern Vietnam (animals were subsequently released at the site of capture). Tissue samples were extracted using DNeasy blood and tissue kit, Qiagen (California, USA). Extracted DNA from the fresh tissue was amplified by PCR mastermix (Fermentas, Canada). The PCR volume consisted of 21 μl (10 μl of mastermix, 5 μl of water, 2 μl of each primer at 10 pmol/μl and 2 μl of DNA or higher depending on the quantity of DNA in the final extraction solution). The following temperature profile for PCR was used: 95 °C for 5 min to activate the taq; with 40 cycles at 95 °C for 30 s, 50 °C for 45 s, 72 °C for 60 s; and the final extension at 72 °C for 6 min.

PCR products were subjected to electrophoresis through a 1% agarose gel (UltraPure™, Invitrogen). Gels were stained for 30 min in 1× 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 Purification kit (Fermentas, Canada). Purified PCR products were sent to Macrogen Inc. (Seoul, South Korea) for sequencing.

Sequences generated in this study were edited using the program Geneious v.7.1.8 (Kearse et al., 2012). The sequences were aligned in BioEdit v7.1.3 (Hall, 1999) with default settings. Data were analyzed using maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP 4.0b10 (Swofford, 2001) and Bayesian analysis as implemented in MrBayes 3.2.1 (Ronquist et al., 2012). For MP analysis, heuristic analysis was conducted with 100 random taxon addition replicates using tree-bisection and reconnection (TBR) branch swapping algorithm, with no upper limit set for the maximum number of trees saved. Bootstrap support (Felsenstein, 1985) was calculated using 1000 pseudo-replicates and 100 random taxon addition replicates. All characters were equally weighted and unordered. For ML analysis, the optimal model for nucleotide evolution was determined using Modeltest 3.7 (Posada & Crandall, 1998). The analysis was conducted with a stepwise-addition starting tree, heuristic searches with simple taxon addition and the TBR branch-swapping algorithm. Support for the likelihood hypothesis was evaluated by bootstrap analysis with 100 pseudoreplications and simple taxon addition. We regarded bootstrap values of ≥70% as strong support and values of <70% as weak support (Hillis & Bull, 1993).

For Bayesian analyses, we used the optimal model determined by Modeltest with parameters estimated by MrBayes 3.2.1. Two simultaneous analyses with four Markov chains (one cold and three heated) were run for 10 million generations with a random starting tree and sampled every 1000 generations. Log-likelihood scores of sample points were plotted against generation time to determine stationarity of Markov chains. Trees generated before log-likelihood scores reached stationarity were discarded from the final analyses using the burn-in function. Two independent analyses were run simultaneously. The posterior probability values for all clades in the final majority rule consensus tree were provided.

Ecology

To investigate potential differences in hibernation (*e.g.*, temperatures) and thermal niches, field surveys were conducted in January 2016, which is the coldest month within habitat sites of *Shinisaurus crocodilurus* in Tay Yen Tu NR, northern Vietnam, where lizards had been most abundant (van Schingen et al., 2014b; van Schingen, Ha, et al., 2016; van Schingen, Pham, et al., 2014; van Schingen, Ziegler, et al., 2016). The two known sites in this area were each surveyed for diurnal and nocturnal activity. Air and water temperatures were recorded with dataloggers (HOBO Onset for air temperature, Thermochron iButton for water temperature). To assess differences in environmental factors between Chinese and Vietnamese populations we predicted suitable habitats separately for each population (China *versus* Vietnam) based on occurrence records, bioclimatic and elevation data by using Maxent. The occurrence records for the Vietnamese and Chinese populations were used to predict one set of environment factors for Vietnam and China (for details see van Schingen, Ha, et al., 2016; van Schingen, Ziegler, et al., 2016). Furthermore, we compared ecological characteristics obtained from previous studies (Hu, Jiang, & Zhao, 1984; Ning et al., 2006; van Schingen, 2014; van Schingen, Pham, et al., 2015; Werner, 2015; Zhao, Zhao & Zhuo, 1999; Zhu et al., 2002; Zollweg, 2012; Zollweg & Kühne, 2013) for Chinese and Vietnamese populations.

Results

Morphology

Morphological examinations revealed significant differences of seven morphometric factors associated with the head shape between Chinese and Vietnamese representatives, namely HH/HL ($t = 2.37$, df = 26, $p < 0.05$), CH_a/HL ($t = 3.28$, df = 24, $p < 0.005$), O/HL ($t = 7$, df = 19, $p < 0.0001$), IN/HL ($t = 4.71$, df = 26, $p < 0.0001$), NE ($t = 3.92$, df = 26, $p < 0.001$), E/HL ($t = 4$, df = 226, $p < 0.0001$), and EE/HL ($t = 3.56$, df = 21, $p < 0.005$). Vietnamese *Shinisaurus crocodilurus* are characterized by a relatively longer and more pointed

Table 1. Comparison of head morphology between adult specimens of the Vietnamese and Chinese *Shinisaurus crocodilurus*; Min. = minimum, Max. = maximum, SD = standard deviation (for further abbreviations see materials and methods).

Ratio	Vietnam (n = 7)			China (n = 14)		
	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD
HW/HL	0.47	0.85	0.59 ± 0.12	0.53	0.66	0.57 ± 0.04
HH/HL	0.50	0.61	0.56 ± 0.04	0.53	0.73	0.62 ± 0.06
CH_a/HL	0.31	0.34	0.33 ± 0.01	0.32	0.64	0.42 ± 0.09
Or/HL	0.23	0.27	0.25 ± 0.02	0.29	0.38	0.33 ± 0.02
IN/HL	0.16	0.19	0.17 ± 0.01	0.18	0.26	0.23 ± 0.03
NE/HL	0.22	0.33	0.28 ± 0.04	0.19	0.27	0.23 ± 0.02
E/HL	0.12	0.21	0.17 ± 0.03	0.20	0.28	0.25 ± 0.03
EE/HL	0.35	0.39	0.36 ± 0.02	0.36	0.53	0.43 ± 0.05

snout with a lower cheek and smaller orbits compared to Chinese individuals (see Table 1, Fig. 1). The PCA of nine selected characters of head morphology revealed the first Principal Component (PC1) to explain 100% of the variance between the investigated groups. PC1 was positively correlated with the factors E/HL, EE/HL, IN/HL, O/HL, CH_b/HL, HH/HL and negatively correlated with HW/HL, CH_a/HL, and NE/HL. The scatter diagrams of Vietnamese and Chinese Crocodile lizards are almost entirely separated (Fig. 2). With respect to coloration we did not find apparent differences between either Vietnamese or Chinese

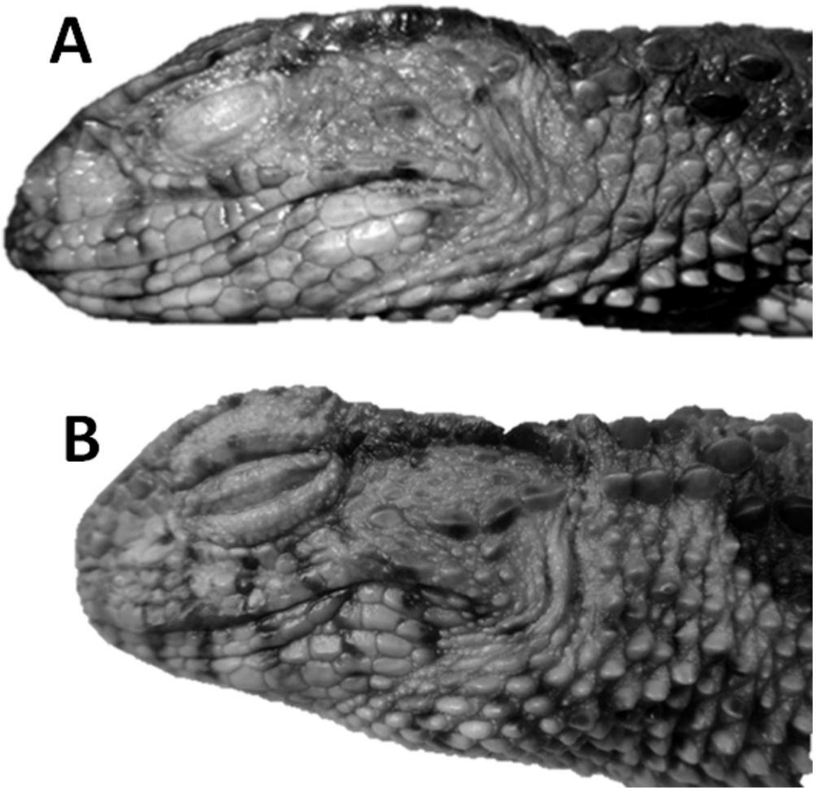

Fig. 1. Head shape variation in A: *Shinisaurus crocodilurus* (IEBR 3809) from Vietnam, and B: *S. crocodilurus* (ZFMK 44691) from China.

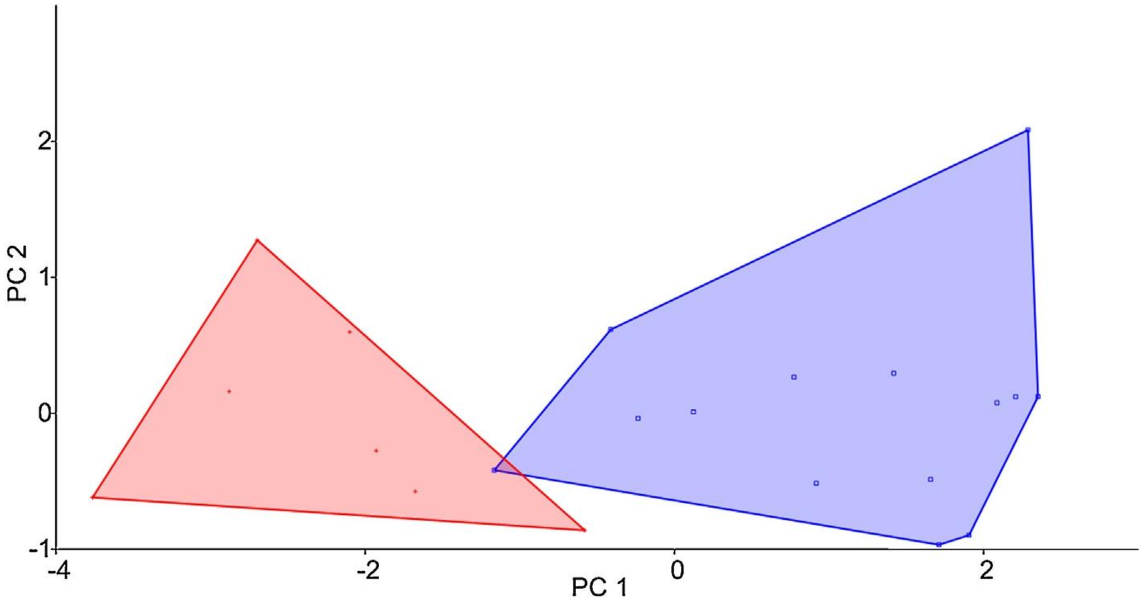

Fig. 2. PCA of nine selected morphometric characters describing the head morphology of *Shinisaurus crocodilurus*. Red: Vietnamese individuals and Blue: Chinese individuals.

specimens or between the subpopulations in Vietnam. Rather, we found a high variability in coloration and color pattern within each investigated site (Figs. 3 and 4).

Molecular Results

The final matrix consisted of 1364 aligned characters, of which 284 were parsimony informative. Maximum Parsimony analysis of the dataset recovered 36 most parsimonious trees with 689 steps (CI = 0.89; RI = 0.88; Fig. 5). Model TVM+G was selected by ModelTest 3.7 for ML and Bayesian analyses. In the ML analysis, the score of the single best tree found was 4647.14 after 14,136 arrangements were tried. Both Bayesian runs reached stationarity after 10,000 generations. All samples from Vietnam clustered within an independent clade with strong statistical support from all analyses (Fig. 5). The genetic divergence between sequences derived from the samples from Vietnam and those from populations in China was about 2.1–3.6% based on the mitochondrial genes. Two clades from China were about 2.5–3.7% genetically divergent from each other, although only one clade was strongly supported to be monophyletic by all analyses. The other clade received high statistical corroboration value only from the MP analysis (BP = 92%) (Fig. 5). The clades from China do not form a monophyletic group (Fig. 5).

Ecology

Comparing data provided by Hu et al. (1984), Zhao et al. (1999), Zhu et al. (2002), Ning et al. (2006), Zollweg (2012), Zollweg and Kühne (2013), van Schingen (2014), van Schingen, Pham, et al. (2015), and Werner (2015) with findings of the present study, *Shinisaurus crocodilurus* from both the northeastern and southwestern populations were found to differ in ecology and microhabitat occupation (Table 2). In China, the species

Fig. 3. Variation in color patterns of Vietnamese *Shinisaurus crocodilurus*: A: Adult male from Dong Son-Ky Thuong NR, Quang Ninh Province; B-D: Adult males from Yen Tu NR, Quang Ninh Province; E-G: Adult males from Tay Yen Tu NR, Bac Giang Province; H, J-L: Adult females from Tay Yen Tu NR; I: Adult female from Dong Son-Ky Thuong NR. Photos: M. van Schingen.

occurs in limestone mountains within evergreen broadleaf forests and bamboo forests or plantations (Zhu et al., 2002; Zollweg, 2012), while *S. crocodilurus* in Vietnam is only reported from granitic habitats within evergreen broadleaf forest, sometimes intermixed with bamboo (van Schingen, Pham, et al., 2015). The inhabited streams in China differ in generally being more narrow, having a more shallow water level, lower flow velocity and being more densely covered with vegetation (Ning et al., 2006; van Schingen, Pham, et al., 2015; see Table 2). While inhabited streams in China were mostly completely covered with thick vegetation (Zollweg & Kühne, 2013), we frequently observed Crocodile lizards in Vietnam in streams, which were only marginally covered with vegetation (van Schingen, Pham, et al., 2015). Furthermore, habitats of Chinese and Vietnamese populations differed in microclimate (a higher annual temperature amplitude and lower temperatures in winter with minimum temperatures reaching $-4\,^{\circ}$C in China [Zhao et al., 1999] *vs.* continuously moderate temperatures in Vietnam [van Schingen, Pham, et al., 2015]). In contrast to reports about Chinese specimens that initiate hibernation at temperatures between 8 and 11 °C and become active at constant temperatures of 15–18 °C (Hu et al., 1984; Zhao et al., 1999), we found Vietnamese specimens to hibernate in winter at air temperatures between 13.4 and 18.4 °C and water temperatures between 17.1 and 22.3 °C within the natural habitat. Also after several days of sun and air temperatures above 20 °C, *S. crocodilurus* was still found hibernating in January. In addition, we found differences in perch selection: Investigated specimens from Vietnam occupied significantly higher perches than Chinese individuals

Fig. 4. Variation in ventral color patterns of Vietnamese *Shinisaurus crocodilurus*: A-C: Adult males from Yen Tu NR; D: Adult female from Dong Son-Ky Thuong NR; E, I, J: Adult females from Tay Yen Tu NR, F: Adult male from Dong Son-Ky Thuong NR, G-H: Adult males from Tay Yen Tu NR. Photos: M. van Schingen.

(van Schingen, Pham, et al., 2015). Based on field surveys in Vietnam in 2013, 2014 and 2015, we observed only two of 192 different individuals to rest on rocks, while this substrate is reported to be more regularly occupied by Chinese individuals (Zollweg, 2015; Zollweg & Kühne, 2013). Dietary analyses on Vietnamese Crocodile lizards revealed their diet to mainly consist of terrestrial invertebrates, in particular oligochaete worms, followed by cockroaches and crickets, while vertebrates were generally not consumed (van Schingen, 2014; Werner, 2015). In contrast, Chinese Crocodile lizards were reported to commonly feed on small vertebrates such as fish, frogs, tadpoles and small lizards, and frequently aquatic invertebrates such as shrimps (Zhao et al., 1999; Zollweg, 2011; Zollweg & Kühne, 2013).

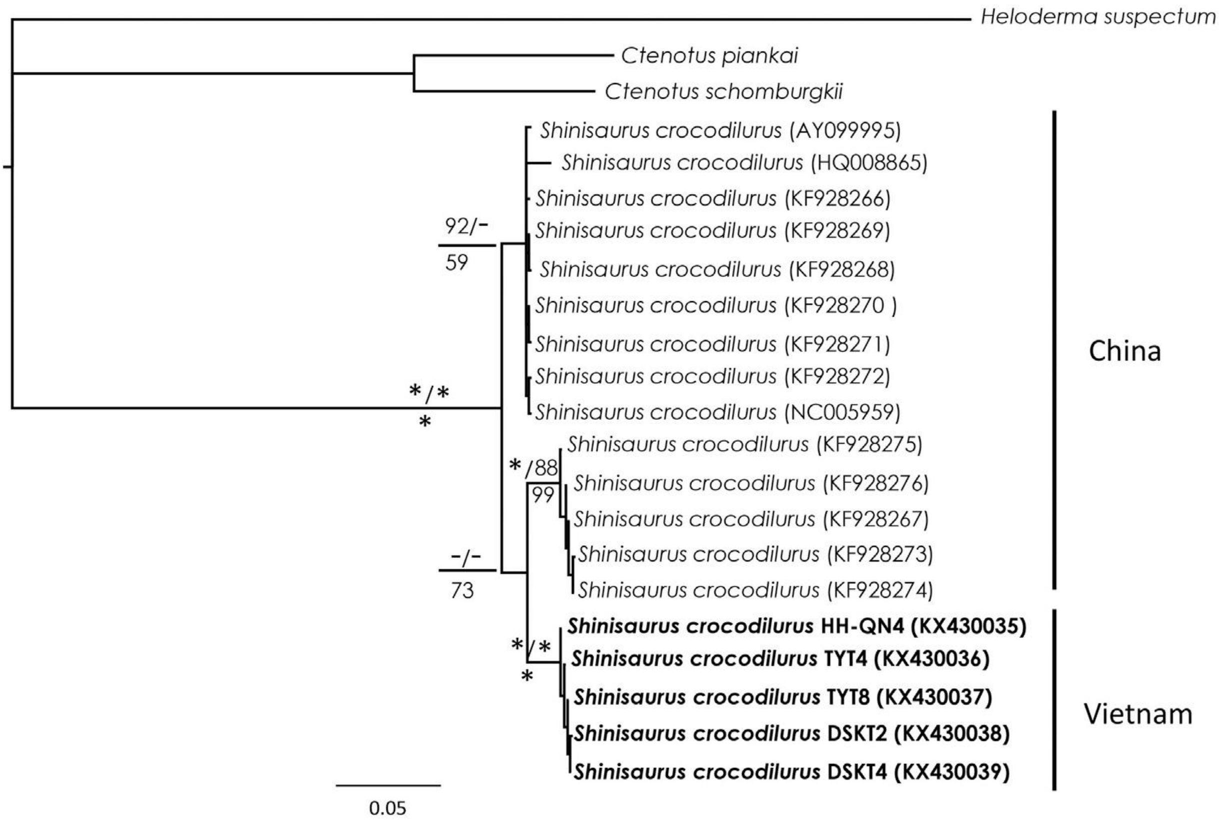

Fig. 5. Bayesian cladogram based on mitochondrial genes with statistical support values for major clades. Numbers above and below branches are bootstrap values of MP/ML analyses (>50%) and Bayesian posterior probabilities, respectively. Asterisk denotes 100% value. New samples of *Shinisaurus crocodilurus* from Vietnam, based on released individuals after samples were taken, are highlighted in bold. Abbreviations show field numbers of the samples. HH-QN: Hai Ha – Quang Ninh; TYT: Tay Yen Tu, DSKT: Dong Son-Ky Thuong.

Predicted Suitable Habitats

The prediction of suitable habitats based on climatic and elevation parameters revealed significant differences in niche parameters between Vietnamese and Chinese *Shinisaurus crocodilurus* (see Fig. 6). Suitable habitats hardly overlapped for both populations. The predicted suitable habitats primarily encompassed the known distribution ranges of each population. Suitable habitats for Vietnamese *S. crocodilurus* were centered almost exclusively in Bac Giang and Quang Ninh provinces, Northeast Vietnam, comprising known sites and expanding to the ocean (Fig. 6). Suitable habitats for Chinese *S. crocodilurus* encompassed a much wider area, exceeding the known distribution range, while areas of high suitability were small and isolated (Fig. 6). The climatic parameters with the greatest influence varied among localities. For Vietnamese populations, the main contribution to the Maxent distribution were "Annual Mean Temperature" (42.1%) and "Precipitation of Driest Month" (23.4%). For Chinese populations "Precipitation of Warmest Quarter" (55%) and "Temperature Annual Range" (26.4%) were the greatest contributors.

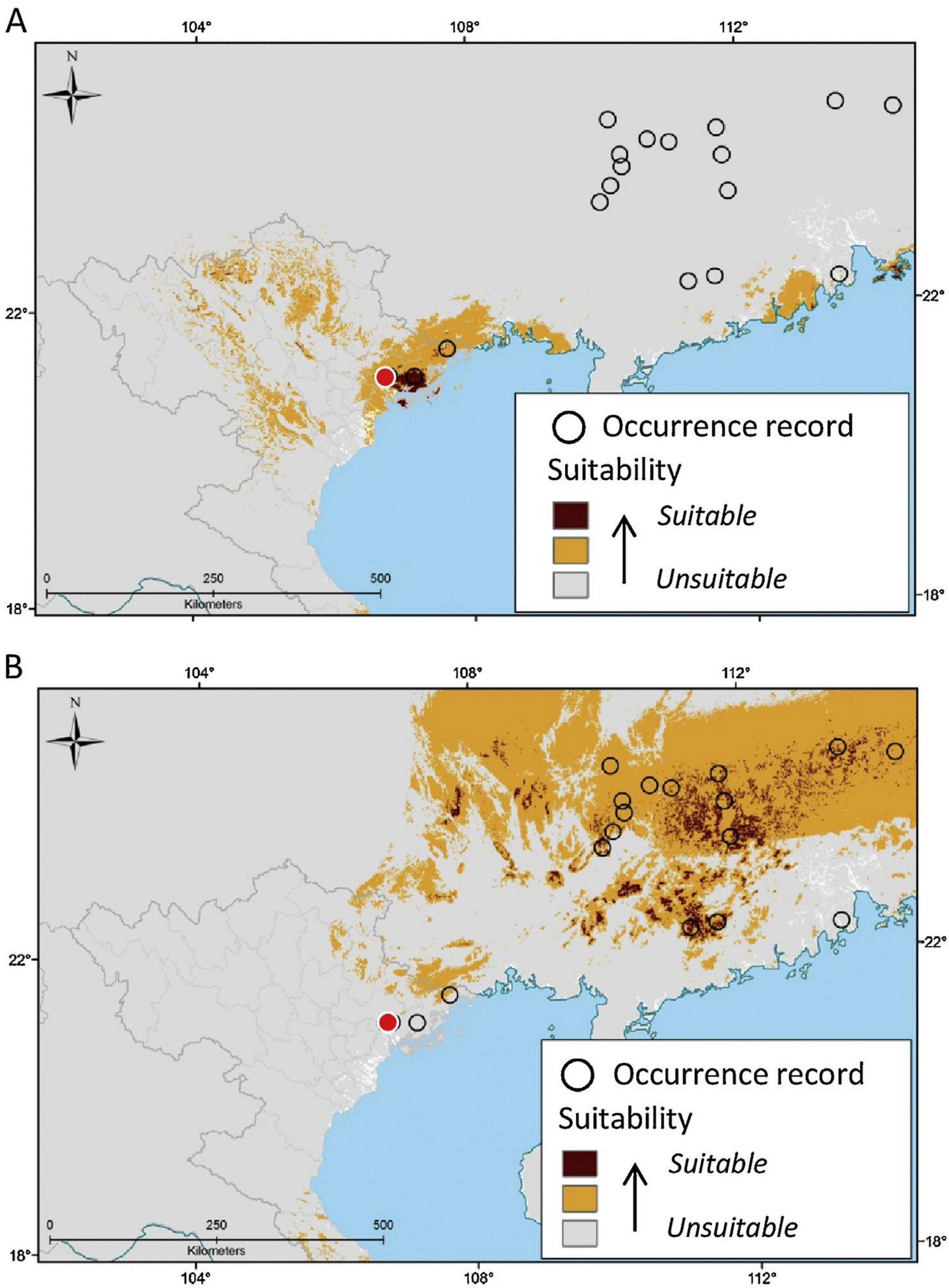

Fig. 6. Predicted suitable habitats of *Shinisaurus crocodilurus* from (A) Vietnam and (B) China based on climatic and elevation data. The red circle represents the type locality of the subspecies described herein.

Table 2. Ecological factors describing the habitat occupancy of *Shinisaurus crocodilurus* in Vietnam and China. Data of Chinese specimens were obtained from Ning *et al.* (2006) and from Vietnamese specimens from van Schingen, Pham, et al. (2015).

Ecological factor	Classification	Number		Ratio [%]	
		Vietnam	China	Vietnam	China
Perch height [m]	<0.5	18	8	19.6	22.2
	0.5–1	26	19	28.3	52.8
	>1	48	9	52.2	25
Perch diameter [cm]	<1	37	21	40.7	58.3
	1–2	40	12	44	33.3
	>2	14	3	15.4	8.3
Vertical distance to shore [cm]	<0	1	6	1.1	16.7
	0	12	30	13	83.3
	$0 \geq 1$	74	–	80.4	–
	>1	5	–	5.4	–
Vegetation coverage [%]	<30	23	0	27.7	0
	30–60	16	2	19.3	5.6
	>60	44	34	53	94.4
Vegetation type	Evergreen broadleaf	52	28	83.9	77.8
	Conifer broadleaf	0	4	0	11.1
	Bamboo/Broadleaf	10	4	16.1	11.1
Water velocity	Slow	22	26	35.5	72.2
	Middle	40	8	64.5	22.2
	High	0	2	0	5.6
Stream width [m]	<1	0	12	0	33.3
	1–2	18	14	42.9	38.9
	>2	24	10	57.1	27.8
Stream depth [cm]	<30	42	15	48.3	83.3
	30–60	43	3	49.4	16.7
	>60	2	0	2.3	0
Rock type	Granite	–	–	100	0
	Limestone	–	–	0	100

Integrative Approach

Based on morphological with molecular and ecological data suggests that the northeastern (Chinese) and southwestern (Vietnamese) *Shinisaurus crocodilurus* constitute distinct lineages. Ratio analyses revealed distinct differences in head morphology between the Vietnamese and Chinese populations. Molecular divergence is greater than intra-population variation levels seen in other groups, in particular because *S. crocodilurus* represents a very ancient and evolutionarily conserved clade (Bever, Bell & Maisano, 2005; Conrad, 2004, 2006; Hu et al., 1984; Zollweg & Kühne, 2013). Ecological comparisons between the northeastern and southwestern populations revealed distinctly different adaptation strategies between them. Böhme (1978) pointed to Kühnelt's principle of regional stenoecy, which emphasizes different ecological adaptations as the first indication for taxonomic separation at the subspecific level, even when morphological differences are not yet evolved or are only slightly developed. Subspecies has been a controversial concept among systematists primarily due to subjective criteria used to diagnose distinct populations under consideration (Barrowclough, 1982; Mallet, 2001; Wilson & Brown, 1953). In this case, populations of *Shinisaurus* from Vietnam have distinct morphological, molecular, and ecological features, and are geographically isolated from Chinese populations which all together strongly support taxonomic revision. However, we herein refrain from applying the specific rank to the populations because one of the two clades from China is not strongly supported as monophyletic by the mitochondrial markers employed in this study. More data are therefore needed to provide a definite solution for the taxonomic classification of Chinese populations. Based on the clear morphological, ecological, and genetic distinction of Vietnamese populations and the still unresolved relationship between Chinese populations we decide to take a more cautious approach and thus herein formally describe the Vietnamese population of *Shinisaurus crocodilurus* as a new subspecies.

Taxonomic Account

Shinisaurus crocodilurus vietnamensis ssp. n.

Holotype

Adult male, IEBR 3806 (TYT2012.1SC), collected on 02 July 2012 at an elevation of 407 m in Son Dong District, Bac Giang Province, Vietnam by Cuong The Pham (Fig. 7).

Paratypes

One adult male, IEBR 3807 (TYT2011.2), collected on 15 April 2011; and three adult females, IEBR 3808 (ML 2015.1SC), IEBR 3809 (ML 2014.2SC), IEBR 3810

Fig. 7. Male holotype (IEBR 3806) of *Shinisaurus crocodilurus vietnamensis* ssp. n. in life. Photo: C.T. Pham.

(ML 2014.1SC), collected on 03 July 2012 at elevations from 400 to 500 m in Son Dong District, Bac Giang Province, Vietnam by Cuong The Pham; adult female, ZFMK 83902, collected in 2005 in Yen Tu Mts., Quang Ninh Province, Vietnam, by Le Khac Quyet.

Diagnosis

Shinisaurus crocodilurus vietnamensis ssp. n. differs from the nominate form *Shinisaurus c. crocodilurus* from China by the following combination of morphological characters: a relatively lower ratio of head height (HH) to head length (HL) and cheek height (CH_a) to head length (lower head and cheek); a relatively higher ratio of eye-naris distance (EN) to head length and lower ratios of eye-eye distance to head length and internares distance to head length (snout longitudinally elongated and transversely narrower); and a relatively smaller ratio of orbital diameter (Or) to head length. For further morphological, molecular and ecological separation of the new subspecies from the nominate form see also the section "Comparisons".

Description of Holotype

Adult male; SVL 148.8 mm; head high and angular; two lateral edges of the head forming triangle anteriorly, posteriorly protruding in two convex tips; snout shorter than postorbital part of head, rounded and slightly pointed anteriorly; distance from nares to anterior edge of orbits about twice as long as distance from nares to snout; external nares within the middle of nasal scales, surrounded by five supranasals; upper jaw with a row of 21 (left)/20 (right) enlarged supralabials each; one further scale row above; rostral shield much smaller than mental shield; ventral fold from mental shield to middle of head; infralabials 11, edged below by five rows of smooth enlarged scales, extending from lateral to the ventral side of the head; remaining ventral head scales conical, keeled and posteriorly increasing in size; three enlarged dorsal orbital scales surrounded by two rows of smaller scales, the total number of small scales directly surrounding the enlarged dorsal orbitals is 21 (left)/20 (right); dorsal head scales rough; snout and dorsal head surface with numerous small scales; orbits oval, eyelids well developed, pupils round with two fine pointed tips above and below; a row of six enlarged scales on posterior cranial margin; on each side a row of five enlarged marginal scales between posterior dorsal cranial edge of head and dorsal orbital region; height of cheek about half the width of posterior dorsal cranial edges; cheek with an angled row of five enlarged scales, surrounded by small scales, originating from central cheek and stretching to dorsal cranial edges; a row of eight enlarged flat collar scales, some keeled; neck with numerous enlarged keeled osteoderms.

Body cylindrical and compact, tail laterally compressed; ventral scales smooth, enlarged, in 37 transversal rows from collar scales to cloaca; paravertebral scale rows 12; cloaca bordered by seven enlarged scales anteriorly and 11 enlarged scales posteriorly, outermost protruding; no precloacal pores; enlarged dorsal osteoderms in eight longitudinal rows and 26 transverse rows, surrounded by small rough scales; tail longer than snout-vent length (199 mm, tail tip lost); dorsal surface of tail with two rows of enlarged and posteriorly keeled osteoderms with one pair per tail whorl; ventral surface of tail covered by smooth, posteriorly keeled scales; limbs relatively short; dorsal surface of limbs covered with differently sized, keeled osteoderms; fingers and digits relatively long, manus phalangeal formula 4-3-2-5-1, pes phalangeal formula 4-3-2-5-1 for hindlimb digits; fore- and hindlimb digits slightly overlapped when adpressed.

Coloration (in alcohol): The dorsal coloration of head, abdomen, tail and limbs dark grayish brown; cranial edges darker; lateral cranium light reddish brown; mental region reddish brown; lateral cranium with six irregular black stripes radiating around the orbit and extending to the snout; lateral head, neck, and trunk ornamented with irregular black blotches; conspicuous lateral black patch on neck; lateral torso reddish; throat and ventral trunk are reddish brown, with the venter being somewhat lighter; tail reddish with 12 black bands; ventral limb surface similar to coloration of ventral trunk; anterior side of hind limbs with black stripes. For life coloration see Fig. 7.

Variation (based on preserved paratypes)

The male paratype IEBR 3807 differs from the male holotype in coloration by having a grayish blue mental region and the throat with yellowish blotches in between, and a yellowish venter with black lateral stripes extending toward. Whereas the male paratypes bear a color-contrasting mental and gular region, the female paratypes have a venter that is uniformly colored or with irregular pattern, *viz.*, distinct red blotches laterally on the trunk in IEBR 3809; the female paratypes differ further from the males in having yellowish instead of reddish venters; the lateral head surface of the female paratypes IEBR 3809 and IEBR 3810 is especially light; the female paratype IEBR 3808 differs from the holotype by having a very short tail with a regenerated portion (original portion 61.5 mm); in the female paratype IEBR 3810 the six bands radiating from the orbit are especially pronounced. Furthermore, the males had a relatively higher ratio of head length (HL) to the length from axilla to groin (AG) (males > 0.75 > females). For scalation and measurements see Table 3.

Comparisons

Morphologically, *Shinisaurus crocodilurus vietnamensis* ssp. n. differs from *Shinisaurus c. crocodilurus* by having an elongated snout: a longer distance between naris and eye NE/HL (0.28 ± 0.04 *vs.* 0.23 ± 0.02); by having a frontally thinner snout: a shorter inter-naris distance IN/HL (0.17 ± 0.01 *vs.* 0.23 ± 0.03) and a shorter distance between the eyes EE/HL (0.36 ± 0.02 *vs.* 0.43 ± 0.05); by having a lower ratio of HH/HL (0.56 ± 0.04 *vs.* 0.62 ± 0.06), a lower ratio of Ch_a/HL (0.33 ± 0.01 *vs.* 0.42 ± 0.09), and a lower ratio of Or/HL (0.25 ± 0.02 *vs.* 0.33 ± 0.02) (see Table 1 and Figs. 1 and 2).

Genetically, *Shinisaurus crocodilurus vietnamensis* ssp. n. differs from *Shinisaurus c. crocodilurus* by 2.1–3.6% in fragments of mitochondrial genes (cytochrome b, partial ND6, and partial tRNA-Glu) (Fig. 3).

Ecologically, *Shinisaurus crocodilurus vietnamensis* ssp. n. differs from *Shinisaurus c. crocodilurus* in occupying different climatic niches: moderate annual temperatures and observed hibernation behavior at 13–20 °C in *Shinisaurus crocodilurus vietnamensis* ssp. n. *vs.* a high annual temperature range (hot summers and cool winters, with sometimes less than 0 °C) and hibernation temperatures of 8–11 °C in *S. c. crocodilurus*; both subspecies

Table 3. Measurements (in mm) and morphological characters of the type series of *Shinisaurus crocodilurus vietnamensis* ssp. n. (for abbreviations see materials and methods).

Character	Male holotype (IEBR 3806)	Male paratype (IEBR 3807)	Female paratypes (IEBR 3808-3810)
			Min–Max
SVL	148.8	156.3	147.2–165.4
HL_a	37.2	36.1	32.3–37.2
HL_b	53.9	54.2	48.6–54.2
HW_a	21.5	21.5	16.8–27.7
HW_b	26.8	24.5	18.3–26.8
HH	20.9	21.1	16.8–21.2
CH_a	12.1	12.1	10.6–12.1
CH_b	12.2	12	11.2–13
EE	12.9	12.5	11.5–13.3
Or	9.6	9.7	7.5–9.7
IN	6.4	6.4	5.3–6.4
NE	12.3	10.4	7.7–12.3
E	6.3	6	5.6–7.4
TB	13.4	14	10.8–14
AG	58	71.5	67–78.4
FA	44.3	51.3	40.8–54
HiL	56	54.3	53.4–57.8
Gr-K	16.9	24.4	21.3–24.4
A-K	17.2	19	14.8–19
Longitudinal ventral rows	12	12	13–14
Transversal ventral rows	37	37	37–39
Supralabials	21	18	18–21
Adjacent supralabial rows	2	1	1–2
Enlarged infralabials	11	10	11–12
Adjacent infralabial rows	5	3	4–5
Enlarged collar scales	8	7	8–10
Scales before cloaca	7	8	6–8
Tail whorls	39	40	39–44
Lamellae below 4th toe	23	20	20–26
HL_b/AG	0.93	0.76	0.67–0.72
AG/TB	4.3	5.1	5.9–6.9

have non-overlapping predicted suitable habitats (see Fig. 6); *Shinisaurus crocodilurus vietnamensis* ssp. n. is adapted to granitic forests, while *Shinisaurus c. crocodilurus* occurs in limestone mountains; *Shinisaurus crocodilurus vietnamensis* ssp. n. exclusively occupies vegetation, while *Shinisaurus c. crocodilurus* also occupies rocks; *Shinisaurus crocodilurus vietnamensis* ssp. n. occupies higher perches (in average above 1 m *vs.* in average between 0.5 and 1 m in the nominate form); for further microhabitat differences (*e.g.*, vegetation coverage, stream width, water level, flow velocity) see chapter ecology and Table 2. *Shinisaurus crocodilurus vietnamensis* ssp. n. differs from *Shinisaurus c. crocodilurus* in preferred prey composition (preference of terrestrial invertebrates; vertebrates are generally not consumed by *Shinisaurus crocodilurus vietnamensis* ssp. n. *vs.* frequent feeding on aquatic prey and small vertebrates in the nominate form).

Etymology

The subspecies is named after the country of origin.

Distribution

Currently, *Shinisaurus crocodilurus vietnamensis* ssp. n. is only known from small and isolated sites in Quang Ninh and Bac Giang provinces, Northeast Vietnam (see Fig. 6).

Natural History

Shinisaurus crocodilurus vietnamensis ssp. n. is adapted to granitic freshwater streams within the evergreen broadleaf forest, with moderate annual temperatures without large fluctuations. For more detailed information see van Schingen, Pham, et al. (2015).

Discussion

While the preliminary molecular comparison between Chinese and Vietnamese *Shinisaurus crocodilurus* by Ziegler et al. (2008), which was based on a fragment of the 16S rRNA gene, revealed only minor differences (0.2%), the present study, including fragments of several other mitochondrial genes (cytochrome b, partial ND6, partial tRNA-Glu), showed distinct differences at the molecular level. Ziegler et al. (2008) included only one available sample as a representative for the Chinese form of *Shinisaurus crocodilurus*. This specimen originated from a zoo collection that derived from the trade with unknown origin. The low genetic divergence found by Ziegler et al. (2008) may be due to the selection of an unrepresentative sample. Although it is implausible, it cannot be excluded, that the analyzed specimen originated from China. China and Vietnam share a long border, which does not necessarily correlate with the subspecies boundary, as is further discussed below. Another possibility is the conservative nature of the rRNA 16S gene, which does not possess a mutation rate similar to faster evolving markers in the mitochondrial genome (Hixson & Brown, 1986).

The integrative taxonomical approach employed in this study clearly demonstrates that *Shinisaurus crocodilurus* consists of at least two separate taxa, which also represent distinct conservation units. In addition to the new taxon described herein from Vietnam, we found evidence for possible presence of two independently evolving clades from China, which have a similar range of mitochondrial genetic divergence. Nonetheless, based on our mitochondrial DNA markers, the monophyly of one clade received strong statistical support only from the maximum parsimony analysis. The two clades do not have a clear geographic barrier, and occur in sympatry in some areas (Huang et al., 2014). More studies using nuclear markers are, therefore, needed to determine if the populations from China constitute two distinct taxonomic groups.

Our results not only point to the importance of improved *in situ* conservation measures in both countries, as both China and Vietnam harbor each a unique form of the Crocodile lizard, but also to improved *ex situ* conservation measures. The establishment of separate conservation breeding programs are clearly needed, because the genetic data argue for distinct management units to maintain their genetic integrity. The detected differences in ecological niche occupation shown in this study further confirm the need for separate husbandry conditions.

Another important conservation issue is how to distinguish between both subspecies, when the origin is unknown, especially in the case of traded specimens. Besides the molecular analyses, which allow differentiation between the subspecies, there is recent evidence for another forensic approach, *i.e.*, using isotopic markers to potentially identify different origins of *S. crocodilurus* (van Schingen, Ziegler, et al., 2016). This method has succeeded in separating wild from captive individuals and would also certainly be able to differentiate between the two subspecies, since the isotopic composition of a consumer reflects its diet and habitat (Briggs et al., 2012), and *S. crocodilurus* occupies different habitats and microhabitats and consumes different preys in China and Vietnam, respectively. The detected differences in prey organisms are probably verifiable in different trophic levels of the two subspecies (Post, 2002). However, these assumptions would have to be tested in a separate study.

In addition, differences in head morphology were revealed to be suitable to get a first hint on the origin of respective individuals. However, we are not able to preclude that all Chinese populations are morphologically similar or that there exist no further morphometric differences between subpopulations with long geographic isolation. This possibility is likely since the distribution range of Chinese Crocodile lizards is much broader and fragmented than that of Vietnamese subpopulations. In fact, Zhao et al. (1999) reported different color patterns in Crocodile lizards between different localities in China.

As mentioned in the beginning of the discussion, there are still a couple of questions remaining, such as where the exact natural boundary between the two subspecies is situated. The present SDMs suggests the boundary lies near the Vietnamese-Chinese border, since there exists a large area of unsuitable habitat for both subspecies in southern China located in between the two populations, assuming that genetic exchange has already been limited for a long stretch of time. Another pending question is whether populations in Vietnam in fact represent a full species. However, such questions need to be addressed by further more comprehensive research.

Outlook

Owing to the dramatic population decline of *Shinisaurus*, particularly of the Vietnamese subspecies (van Schingen, Ha, et al., 2016; van Schingen, Schepp, et al., 2015), better informed conservation efforts are critically needed. Breeding programs have been established in China (Zollweg, 2012), and are currently being developed in Vietnam (Ziegler, 2015; Ziegler & Nguyen, 2015; Ziegler et al., 2016). However, our results suggest that conservation efforts should be carefully managed. For example, morphological and genetic screening of zoo stocks and specimens confiscated from the trade should be employed to maintain genetic integrity of captive lineages. We also recommend establishing scientifically coordinated studbooks at least for the Vietnamese subspecies, which has an alarming low estimated natural population size. Determining geographic origins of individuals kept in *ex situ* facilities outside of China and Vietnam seems to be an obvious necessity for *ex situ* breeding programs. Moreover, general husbandry of *Shinisaurus* could be improved by accounting for the taxon-specific ecological differences, including thermal conditions and trophic niche, reported herein (and in a forthcoming article).

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Zusammenfassung

Die Krokodilschwanzechse Shinisaurus crocodilurus, der einzige rezente Vertreter innerhalb der Familie Shinisauridae, ist auf abgelegene Fließgewässer innerhalb des immergrünen Tieflandfeuchtwalds spezialisiert und kommt nur noch in wenigen kleinen und inselartigen Reliktvorkommen in Südchina und Nordvietnam vor. Diverse anthropogene Bedrohungen, wie massive Habitatzerstörung und nicht nachhaltiges Absammeln für den internationalen Tierhandel, haben die Art mittlerweile an den Rand der Ausrottung gebracht. Im Rahmen eines integrativ taxonomischen Ansatzes haben wir neben einer umfassenden genetischen Analyse anhand von Fragmenten der mitochondrialen Gene Cytochrom b, partieller ND6 und tRNA-Glu außerdem detaillierte morphologische und ökologische Untersuchungen durchgeführt, um den Status der getrennten Populationen in Vietnam und China zu klären. Basierend auf den molekularen, morphologischen und ökologischen Unterschieden, beschreiben wir die Krokodilschwanzechsen aus Vietnam als neue Unterart, Shinisaurus crocodilurus vietnamensis ssp. n.. Unsere Daten unterstreichen die Wichtigkeit verbesserter in situ Schutzmaßnahmen in beiden Ländern, da China und Vietnam jeweils eine einzigartige Krokodilschwanzechsenform beherbergen. Wir empfehlen weiterhin verbesserte ex situ Schutzmaßnahmen, speziell das getrennte Erhaltungszucht-Management

der Unterarten, um deren genetische Integrität zu erhalten, sowie an die in dieser Studie aufgezeigten ökologischen Unterschiede angepasste Haltungsbedingungen.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.zoolgart.2016.06.001.

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Chapter 10: New insights into the biology and husbandry of Crocodile lizards including the conception of new facilities for Shinisaurus crocodilurus vietnamensis in Vietnam and Germany

Manuscript in preparation:

New insights into the biology and husbandry of Crocodile lizards including the conception of new facilities for *Shinisaurus crocodilurus vietnamensis* in Vietnam and Germany

Neue Erkenntnisse zur Biologie und Haltung von Krokodilschwanzechsen einschließlich der Entwicklung neuer Anlagen für *Shinisaurus crocodilurus vietnamensis* in Vietnam und Deutschland

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Abstract

The crocodile lizard (*Shinisaurus crocodilurus*) is a commonly kept reptile species. Recent integrative taxonomic research revealed the Chinese representatives to be morphologically, genetically and ecologically distinct from the Vietnamese populations, which occur in alarmingly low population sizes. All extant populations are threatened by habitat destructions and poaching for the pet trade. Thus it will be crucial to manage the Vietnamese and Chinese forms separately as different conservation units, both to maintain their genetic integrity and to adjust appropriate husbandry conditions within *ex situ* approaches. For this reason we provide a topical review of particular ecological adaptations of the newly described subspecies from Vietnam (*S. c. vietnamensis*), based on recent field work as well as husbandry experiences at the Me Linh Station for Biodiversity in North Vietnam. We further oppose our new findings to natural history data available for the nominate subspecies from China. Based on our current knowledge, we update existing minimum husbandry requirements and elaborate different husbandry parameters for both subspecies. Furthermore, we provide new approaches on the sex identification and introduce new husbandry models and facilities, respectively, for the Vietnamese subspecies both in the Me Linh Station for Biodiversity in North Vietnam and in the Cologne Zoo, Germany.

Key words: zoo biology; natural history; habitat parameters; hibernation; sex identification; conservation breeding; *Shinisaurus crocodilurus vietnamensis*

Introduction

The crocodile lizard (*Shinisaurus crocodilurus*) is a commonly kept reptile species both in Zoological Gardens and among hobbyists. Originally only known from southern China, the species was recently also proven to occur in Vietnam (Le and Ziegler, 2003). Latest integrative taxonomic research revealed the Chinese populations to be morphologically and genetically distinct from Vietnamese representatives, as well as occupying a different ecological niche (van Schingen et al., 2016b). As a consequence the importance of conservation breeding programs was highlighted, in particular for the newly described subspecies from Vietnam (*Shinisaurus crocodilurus vietnamensis*), also as population estimates from northern Vietnam were alarmingly low (van Schingen et al., 2014b, 2016a). To maintain the genetic integrity of the different subspecies within *ex situ* approaches, it will be crucial to manage the Vietnamese and Chinese forms separately as different conservation units. Also due to different ecological adaptations (van Schingen et al., 2015a, 2016b) different husbandry parameters have to be considered in terms of improved breeding projects. Thus, we herein provide a topical review of habitat requirements and use, based on our extensive continuous field work in North Vietnam during the last years and husbandry experiences with the Vietnamese subspecies at the Me Linh Station for Biodiversity (Ziegler et al., 2016). In addition, we oppose our in part still unpublished ecological data on *Shinisaurus crocodilurus vietnamensis* to literature data available for some Chinese populations. We critically discuss and update existing minimum husbandry requirements based on our current knowledge. Further we provide new data on the sex identification of crocodile lizards and introduce new husbandry models and facilities, respectively, for the Vietnamese subspecies both in the Me Linh Station for Biodiversity in Northern Vietnam and in the Cologne Zoo, Germany.

Material & Methods

Ecological field surveys took place during summer in May, June and July 2013, 2014, 2015 and 2016 in Tay Yen Tu Nature Reserve (NR), Bac Giang Province, Yen Tu NR, Dong Son-Ky Thuong NR and Hai Ha District, Quang Ninh Province, North Vietnam, as well as during winter in January 2016 in Tay Yen Tu NR. During these surveys microhabitat parameters both during summer and winter, habitat selection, thermal niche and activity patterns of wild *S. crocodilurus vietnamensis* have been extensively investigated (for detailed methods see van Schingen et al., 2014a,b, 2015b, 2016 a,b,submitted).

Experiences and observations on captive *S. crocodilurus vietnamensis* mainly originate from the Me Linh Station for Biodiversity in North Vietnam, where the species is kept since 2012 (Ziegler, 2015; Ziegler and Nguyen, 2015; Ziegler et al., 2016); before the subspecies has been kept in the Amphibian Station Hanoi (Ziegler et al., 2011).

Based on these previous studies and further unpublished observations, we reviewed the ecological requirements of Vietnamese crocodile lizards in order to give concrete recommendations for husbandry of this subspecies. We opposed our data from Vietnam with data available for China (after Huang et al., 2008; Hu, Jiang, and Zhao, 1984; Long et al., 2007a, 2007b; Long, 2008; Ning et al., 2006; Wang et al., 2008; Wang et al., 2009; Yu et al., 2006; Zhao, Zhao and Zhuo,1999; Zhao et al., 2006; Zhu et al., 2002; Zollweg, 2012; Zollweg and Kühne, 2013; and experiences of private keepers).

In order to determine sexes we used a combination of different characters, namely coloration, morphometry and depth of cloaca. We identified several characters qualifying to distinguish sexes in Vietnamese crocodile lizards, which is crucial for the building up of a conservation breeding, since *S. crocodilurus* has no distinct sexual dimorphism. Therefore we measured head length (HL, from tip of snout to anterior cranial edge), trunk length (AG =

axillary groin, trunk length between axillary and groin) and the widest diameter at cloaca (CL = diameter below cloaca at widest part) from different populations and adopted the probing technique to test its value for sex determination in crocodile lizards in Vietnam (PD = penetration depth of probe into cloaca). We generated quotients of selected metric characters (HL, AG, CL, PD) and applied an unpaired t-test to detect significant differences. F test was used to assess homogeneity variance, in case of different variances we applied Welch's t-test. All analyses were conducted using Graphpadprism version 5.0 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com to test for significant differences.

Results

1. Ecological niche of *Shinisaurus crocodilurus vietnamensis*

Habitat

We found *Shinisaurus crocodilurus vietnamensis* to occur in granitic evergreen broadleaf lowland forests in heights between 50 and 850 m a.s.l., while *Shinisaurus c. crocodilurus* is reported to inhabit limestone mountains within evergreen broadleaf forests, intermixed bamboo or shrubbery forests or mixed or conifer and mixed broadleaf forests at elevations between 200 to 1,500 m a.s.l. (Zhu et al., 2002; Huang et al., 2008, Zhao et al., 1999; Zhao et al., 2006). Unfortunately, suitable habitat sites are steadily shrinking e.g., due to forest clearance, illegal logging, the extension of coal-mining and are predicted to further dramatically decline due to the impact of climate change (van Schingen et al., 2016a).

In Vietnam, the riparian zones of inhabited streams are usually densely vegetated, mainly by broad-leafed trees, ferns, scattered bamboo and canes, while the canopy cover above the stream is generally not entirely closed. In Chinese habitats, streams are reportedly completely covered by thick vegetation, commonly in heights between 0.5 and 1 m (Ning et al., 2006; Zollweg and Kühne, 2013).

Even though *Shinisaurus crocodilurus vietnamensis* is adapted to running waters, we found that it prefers spots above backwater pools or sections of impounded water next to small waterfalls with almost no flow velocity (0–0.47 m/s) (van Schingen et al., 2015b). However, if densities of crocodile lizards along streams were high, we more frequently observed animals, especially juveniles, to also rest upon faster running sections. In Vietnam, animals usually rested above stream parts with depths between 5–73 cm and stream width of 1–8 m, while streams in China are reportedly more shallow, in mean about 10 cm and generally below 30 cm, and narrow, mainly between 1–2 m or even less (Ning et al., 2006; van Schingen et al., 2015, 2016b; Zollweg and Kühne, 2013).

In Vietnam, stream habitats are characterized as soft waters (GH <1°-2°) with a high water quality namely a high oxygen content (6-10 mg/l), low nutrient concentrations of nitrogen (NO_2 < 0.01 mg/l; NO_3 < 0.5-5 mg/l; NH_3/NH_4 < 0.05-0.1 mg/l) and phosphate (PO_4 < 0.002-0.1 mg/l) as well as no iron and copper contents (van Schingen et al., 2015; pers. obs., 2016). Thus, hardly any macroalgae were present in stream habitats. Furthermore, the water was found to range from neutral to relatively acid conditions with pH values between 4.5 to 7.37, while pH values of 6.5 were measured in Dayaoshan Nature Reserve, Guangxi, China (Long, 2008; van Schingen et al., 2015b).

Resting places were usually branches or ferns above the water body. Of 215 different animals we only observed two individuals sleeping on granite cliffs above the water, while all other animals had been found in different kinds of vegetation, but never on the forest floor. Juveniles were found to select ferns, shrubs and canes, while adult animals were usually found on tree branches, which were further more densely vegetated (van Schingen et al., 2015). Preferred perch heights of adults were, with a median height of about 119.3 cm above the water level, significantly higher as those of juveniles (median 63.5 cm) (van Schingen et al., 2015b). Similarly, van Schingen et al., (2015b) also found interpopulation differences, namely Vietnamese crocodile lizards occupying significantly higher perches (mainly above 1 m) than Chinese crocodile lizards (mainly between 0.5 and 1m). Chinese Crocodile Lizards also prefer plants, branches and shrubs above the water body, but were found to also spent about one third of the day in burrows characterized by high concealment with vegetation and shielded from sunlight, a depth of 18 to 132 cm (n=124), a distance to the stream of <0.5 m, heights of 0.3 m above the water body and high humidity (Long, 2008; Zhao et al., 2006). Such burrows may be deep and serpentine swallets, tree holes or rock shelters (Zhao et al., 2006).

Recent field research confirmed the diurnal lifestyle of crocodile lizards in Vietnam, which appeared strictly dependent on sunlight. First daily activities were usually recorded with sunrise, while a peak of activities was found during morning and noon (van Schingen et al., submitted). As it is the case for the whole night time, animals were found to also spend large periods inactive during day (van Schingen et al., submitted). Crocodile Lizards were also reported to be diurnal in China, but spending 98.5% of the day inactive on the perch above the water body or hidden during summer (Zhang 2006; Zollweg and Kühne, 2013).

Long-term field research revealed extremely small home ranges of the species (van Schingen et al., submitted); if not disturbed several animals were even observed to occupy the same branches as resting perches over several years. In China, first studies revealed home ranges of about 6.8-10.9 m² (n=4), while home ranges were positively affected by the size of respective backwater pools (Long et al., 2007b). Furthermore, individuals were found to disperse along streams, mostly only one adult specimen was observed per pool indicating a territorial behavior of the species. Adults males were observed to attacked each other having been placed together in captivity. However, exceptions were observed during field work for different age classes (juveniles, subadults and adults) as well as different sexes, which were occasionally found aggregated.

Climate

Recorded field temperatures at perch sites in Vietnam were ranging between 22.14 to 31.27 °C (mean 25.9°C) in May and June and between 13.36 and 16.33°C in winter (van Schingen et al., submitted). Water temperatures were more constant, ranging between 22.5-24.5°C in summer and 17.1 and 17.5°C in winter (van Schingen et al., submitted). Humidity was found to be usually high between 78 and 88 % (van Schingen et al., 2015b). While illumination values of up to 242500 lux have been recorded within the macrohabitat, only up to 38580 lux were reached at noon at perch sites of *S. crocodilurus vietnamensis* in summer (van Schingen et al., submitted). During winter light exposure was comparably low with recorded values of only up to 120 lux around noon. Days were also about two hours shorter in January compared to summer. Based on data loggers attached to the animals, the active avoidance of sun exposed spots with high temperatures could be recorded (van Schingen et al., submitted). Furthermore, it was shown that the occupied temperature niche of crocodile lizards was narrower compared to the environmental fundamental niche, with the animals temperatures being generally cooler compared to environmental temperatures. Crocodile lizards were found to actively control their temperature to keep their optimum and are thus strongly dependent on constantly cool water and adjacent terrestrial parts. In China, measured body temperatures of wild crocodile lizards lay with 20.4-22.55 °C slightly above substrate temperatures (18.4-21.5°C) in May (Wang et al. 2008). Wang et al. (2009) further demonstrated that animals selected temperatures between 22.5 and 28.3 °C out of a range of 18-50°C under laboratory conditions and that gravid females as well us one-year old juveniles had highest body temperatures.

Concerning climate, microhabitats in Vietnam and China differ by generally higher annual temperature amplitudes with up to 40°C in summer and minimum winter temperatures occasionally reaching -5.6 °C at some sites in China in contrast to continuously moderate temperatures in Vietnam (Long, 2008; van Schingen et al., 2016a; Zhao et al., 1999; Zollweg and Kühne, 2013). Additional predictions of suitable habitats based on climatic parameters revealed different regions to be suitable for *S. crocodilurus vietnamensis* and *S. c. crocodilurus*, respectively indicating different climatic adaptations of the two subspecies (van Schingen et al., 2016b).

Hibernation

Recent studies revealed that Vietnamese crocodile lizards also have a period of hibernation (van Schingen et al., 2016b). Observations in captivity indicate an almost strict inactive phase from December to February, while animals occasionally also have short periods of activity during winter (van Schingen et al., submitted). Field observations confirmed that *Shinisaurus crocodilurus vietnamensis* are hiding inactively during winter month, but also gave evidence for further occasional inactive periods during the summer month (van Schingen et al., submitted). Recent studies of captive crocodile lizards in Vietnam indicated that preferred spots for hibernation are holes within the earth or trees, while also first evidence for the digging into the soil derived from field observations (van Schingen et al., submitted). The frequent usage of burrows also during summer has been recorded for Chinese crocodile lizards (Long et al., 2007b; Zhao et al., 2006). While Vietnamese crocodile lizards were found exclusively terrestrial in captivity and in the wild during hibernation, hibernating crocodile lizards have been occasionally found within the water in China, which is also confirmed by numerous hobbyists (Zollweg and Kühne, 2013). However first evidence exist for the main usage of humid but dry burrows for hibernation in China (Zhao et al. 2006). Chinese crocodile lizards were reported to hibernate from October to April and initiate hibernation at temperatures between 8-11 °C and become active at constant temperatures of 15-18°C (Hu et al., 1984; Yu et al., 2006; Zhao et al., 1999; Zollweg and Kühne, 2013). We found that Vietnamese crocodile lizards initiated hibernation at mean temperatures of about 17.5 °C in December in captivity and were starting to get active without a pronounced increase in temperature in March (van Schingen et al., submitted). Field observations in Vietnam revealed that animals were still inactive at temperatures between 13.4°C and 20°C during January (van Schingen et al., 2016b). Overall, the hibernation period in Vietnamese specimens is shorter, not strict and occurs at generally higher average temperatures compared to conditions in China. However, periods of inactivity without feeding are apparently of need for the species.

1.4 Diet

First dietary analyses on *S. crocodilurus vietnamensis* revealed a preference for oligochaete worms, followed by cockroaches and crickets; while vertebrates were not found being consumed (van Schingen et al., 2016b; Werner, 2015). Chinese Crocodile lizards were reported to feed on a wider prey spectrum ranging from aquatic invertebrates such as shrimps, which are frequently consumed, to small vertebrates such as fish, frogs, tadpoles and small lizards (Zhao et al., 1999; Zollweg, 2011; Zollweg and Kühne, 2013). A stomach content analysis by Ning (2007) revealed that *S. c. crocodilurus* preferred earthworms of the genus *Pheretima* (34.78%), followed by Araneida (8.70%), Tettigoniidae (8.70%) and Cicadidae (8.70%) out of a various range of invertebrate prey species covering more than 20 families. Detailed studies of Long et al. (2007a) on the time budget of *S. crocodilurus crocodilurus* showed that feeding only accounted for about 0.12% of the diurnal time.

2. Adapted husbandry recommendations

The currently available German minimum husbandry requirements, which are based on information of Chinese crocodile lizards, are compiled in Table 1. In the subsequent Tables 2-6 we summarized the most important facts on *S. crocodilurus vietnamensis* both from natural habitats and captive facilities (treated in detail in the previous chapters) and opposed to respective data for the Chinese representatives, if available (see material and methods).

Table 1. The currently valid German minimum requirements („VDA & DGHT Sachkundenachweis") for *Shinisaurus crocodilurus* from South China.

Habitat requirements	**Stream shore dweller**
Enclosure size for 1.1 (L x W x H) in SVL	6 x 4 x 4 (thereof ½ water)
Ground temperature	20-25°C
Local basking spots	35°C
Social composition	1.1
Comments	hiding places under water and on land; climbing opportunities, partly wet substrate

Table 2. Macrohabitat parameters of *Shinisaurus crocodilurus vietnamensis* compared to *S. c. crocodilurus.*

	Shinisaurus c. vietnamensis	***Shinisaurus c. crocodilurus***
Forest type	Granitic evergreen broadleaf lowland forests	Limestone mountains within evergreen broadleaf or intermixed bamboo, conifer or shrubbery forests
Altitudinal range	50 to 850 m a.s.l.	200 to 1,500 m a.s.l.
Shore vegetation	Densely vegetated shore zones; canopy cover above water parts not entirely closed	Water body completely covered by thick vegetation in heights of 50-100 cm
Water body	Running freshwater habitats with pools	Shallow, slowly running freshwater habitats
General climate	Annual continuously moderate temperatures without high fluctuations	Higher annual temperature amplitudes and lower temperatures in winter with minimum temperatures occasionally reaching -5.6 °C
Relative humidity	78 to 88 %	Around 82-83 %
Illumination	up to 242500 lux	Data not available

Table 3. Microhabitat parameters within natural habitats of *Shinisaurus crocodilurus vietnamensis* compared to *S. c. crocodilurus.*

	Shinisaurus crocodilurus vietnamensis	***Shinisaurus c. crocodilurus***
Resting places	Branches or ferns above the water body; rarely granite cliffs above the water. Juveniles select ferns, shrubs and canes; adults on more densely vegetated tree branches (mean diameter about 1.3 cm)	Prefer plants, branches or shrubs above the water body; sometimes on cliffs; diameter of branches < 1 cm;
Preferred perch height above the water body	Average about 120 cm (up to 210 cm) for adults 60-65 cm for juveniles	Mainly between 50 and 100 cm for adults
Water body	Prefers spots above pools next to small waterfalls with almost no flow velocity; when densities are high, in particular juveniles can also be found resting upon faster running sections	Prefer pools; only one adult per pool
Water depth	5 - 73 cm	Around 10 cm (< 30 cm)
Stream width	1 - 8 m	1 - 2 m (or smaller)
Microclimate at perch sites	22.14 to 31.27 °C (mean 25.9°C) in May and June; 13.36 to 16.33°C in January	Data not available
Selected temperature	24.21 ± 1.14 °C (21.88-30.88 °C) *in situ*	22.5 - 28.3 °C under laboratory conditions
Illumination at perch site	up to 38580 lux at noon at perch sites in summer. During winter only up to 120 lux around noon. Photoperiod of 14 hours in summer and 12 hours in winter	Data not available

Table 4. Water parameters at habitat sites of *Shinisaurus crocodilurus vietnamensis*.

Parameter	**Value**
Water temperature	22.5-24.5°C (summer)
	17.1-17.5°C (winter)
General hardness	<1°-2° dH
Oxygen	6-10 mg/l
Nitrogen	NO_2 < 0.01 mg/l
	NO_3 < 0.5-5 mg/l
	NH_3/NH_4 < 0.05-0.1 mg/l
Phosphate	0.002-0.1 mg/l
pH value	4.5 to 7.37 (in comparison to 6.5 at one habitat site in China)
Iron and copper contents	Absent
Macroalgae	virtually lacking

Table 5. General behavior of *Shinisaurus crocodilurus vietnamensis* compared to *S. c. crocodilurus.*

	Shinisaurus crocodilurus vietnamensis	***S. c. crocodilurus***
Lifestyle	Diurnal, with first daily activities during sunrise (highest activities during morning and noon); extended inactive periods during day and whole night time	Diurnal; during summer spending 70% of the day inactive on perch and 28,5% hidden in burrow
Territoriality	Prefer same resting site (e.g., branch); small home ranges	Home range about 6.5-11 m² in habitat sites;
	usually only one adult per pool; occasionally different sexes or juveniles occur together with adults	Only one adult per pool in natural habitat sites
Temperature regulation	Dependent on constantly cool water and adjacent terrestrial parts to regulate temperature	Behavioral and physiological means
Occupied temperature niche	Animal's selected temperatures generally cooler compared to environmental temperatures, but warmer than water temperatures; active avoidance of high temperatures at sun exposed spots	Body temperatures of wild crocodile lizards slightly above substrate temperatures; gravid females and one-year old juveniles with highest body temperatures
Diet	Invertebrates, preference of earthworms	Invertebrates and small vertebrates

Table 6. Hibernation of *Shinisaurus crocodilurus vietnamensis* compared to *S. c. crocodilurus.*

	Shinisaurus crocodilurus vietnamensis	*Shinisaurus c. crocodilurus*
Location	Earth and tree holes are preferred hibernation spots in captivity; also first evidence for digging into the soil in the wild During hibernation exclusively terrestrial in captivity; similar observations in the wild	Mainly in humid, but dry burrows (earth holes, tree hollows, stone cracks), covered with vegetation; usually close to the stream and in low heights above water body; depth of 18-132 cm; hibernation within the water occasionally recorded in the field and frequently in captivity
Initiation	At mean temperatures of about 17.5 °C in captivity	At temperatures between 8-11 °C
Ending	Without pronounced increase in temperature in March; still inactive at temperatures between 13.4°C and 20°C in the field during January	At constant temperatures of 15-18°C in March or April
Duration	Hibernation period from December to March in outdoor enclosures in Vietnam	Hibernation period from October to April in Chinese habitats
	Generally shorter hibernation period, not strict and at higher average temperatures; periods of inactivity without feeding required.	Generally extended hibernation period, strict and at lower average temperatures
	inactive phase during winter (both in the wild and in captivity), occasionally short periods of activity during winter in captivity; occasional inactive periods during summer in the field.	

After current knowledge the following parameters seem to be necessary to reconstruct keeping conditions adapted to natural requirements; where possible, we have tried to distinguish between the different requirements of the Vietnamese and Chinese subspecies (for details we refer to the results chapters and Tables 2-6):

For terrarium construction, *S. crocodilurus* requires a forest stream habitat, with a densely vegetated shore zone. Compared with the nominate form, *S. crocodilurus vietnamensis* prefers less dense vegetation above the water part. We recommend larger terraria as indicated in the currently available minimum husbandry requirements, especially regarding the terrarium height, given the preferred perch height in *S. crocodilurus vietnamensis* of around 120 cm. Depending on that, water depth should be sufficiently deep, so that individuals that jump into the water from higher resting perches cannot get injured. Generally, the nominate form seems to require more shallow waters (ca. 10 cm, maximum water depth 30 cm), whereas *S. crocodilurus vietnamensis* also occurs in deeper water. Concerning water body size, *S. crocodilurus vietnamensis* inhabits distinctly wider streams than the nominate form. The water part should contain both running and still water sections. The water quality should be excellent, with high oxygen content. The terrarium should provide for sufficient high relative humidity. In particular *S. crocodilurus vietnamensis* requires plants and branches with different diameters above the still water parts. At least the Vietnamese representatives require a land part with substrate for digging. Hiding opportunities such as bamboo canes and cork tubes should be available both on land and in the water, also for hibernation. Direct and intense sun rays are avoided. In the winter season air and water temperature reduction is required. Air temperatures in summer should range between 21 and 30 °C. High annual temperature fluctuations are only found in China, the nominate form thus requires a stronger temperature decrease. In contrast, hibernation temperatures for *S. crocodilurus vietnamensis* should not fall below 13 °C. Hibernation period in *S. crocodilurus vietnamensis* lasts from December to March opposed to from October to April in the field for the nominate subspecies. Photoperiod should be shortened during winter, with less intense illumination.

3. Sex identification in *Shinisaurus crocodilurus vietnamensis*

3.1 Morphology

Morphological examination of adult Vietnamese crocodile lizards from different populations revealed significant differences between males and females in the ratios of head length to trunk length (males: 2.029 ± 0.05 vs. females: 2.25 ± 0.1; $t=2.22$, $df=25$, $p=0.036$) and diameter of the cloaca relative to the trunk length (males 4.64 ± 0.13 vs. 6.38 ± 0.34; $t=4.72$, $df=11$, $p=0.0006$). Thus, males have a relatively bigger head and shorter abdomen length compared to females, which was already reported for Chinese individuals (He et al., 2011). Furthermore, males can be distinguished from females by having a relatively bigger cloaca diameter compared to females (15.4 ± 2.2 in males vs. 12.7 ± 2.1 in females). The differentiation of sexes using the ratio between head width and head length, which was shown in Chinese crocodile lizards (Wölfel, 2003) is not applicable in Vietnamese crocodile lizards, since we found no significant differences ($t=0.37$, $df=25$, $p = 0.72$). Concerning coloration males usually had more bright color patterns. Generally, coloration in males was characterized by the ventral head and gular region being of contrasting color (red, grey, blue or yellow) compared to the ventral trunk. Especially bright yellow or blue gular regions were exclusively found in males. In contrast, brightly red gular coloration occurred in both sexes, however if present in females the red coloration usually extended throughout the whole ventral trunk or proceeded into an irregular or patchy pattern, without clear separation of coloration. Otherwise females were frequently more faintly colored. However, the color pattern as character to determine sexes should only be considered in adult specimens. Similar observations have also been made in Chinese individuals (e.g., Zollweg 2012; Zollweg and Kühne, 2013).

3.2 Probing

Probing of animals revealed to be a useful method to determine the sex of adult crocodile lizards. Regarding the penetration depth in relation to the trunk length we could prove significant differences between males and females (mean AG/PD 6.9 ± 0.2, n=34) compared to females (mean AG/PD 20.7 ± 2.15, n=21; t=8.114; df=53; $p < 0.0001$; Table7), implying already that penetration depth was distinctly deeper in males than in females. The mean penetration depth of the probe into the hemipenis and hemiclitoris pockets behind the cloaca, respectively, was 10.4 ± 1.16 mm in males and 4.5 ± 2.39 mm in females.

Table 7. Probing of Shinisaurus crocodilurus vietnamensis.

Sex	Axillar groin [mm]	Cloaca right [mm]	Cloaca left [mm]
female	85.5	5.0	6.0
female	91.0	2.0	3.0
female	89.5	7.0	4.0
female	75.0	2.0	3.0
female	69.5	2.0	3.0
female	57.5	2.0	3.0
female	77.5	10.0	7.0
female	82.1	5.0	6.0
female	83.2	6.0	8.0
female	79.5	0.0	4.0
female	84.2	6.0	5.0

male	79.0	8.5	10.1
male	71.8	11.5	11.0
male	78.8	11.0	9.0
male	70.0	11.0	10.5
male	72.0	11.5	11.5
male	72.5	11.0	12.0
male	63.2	9.5	9.0
male	65.8	7.0	11.0
male	54.0	10.0	11.0
male	62.0	9.0	10.0
male	67.5	11.0	11.0
male	68.1	10.5	10.5
male	84.6	8.0	11.0
male	74.2	11.0	12.0
male	79.8	11.0	10.0
male	71.8	11.0	12.0
male	73.9	10.0	10.5

4. Conception of new facilities

4.1 Me Linh Biodiversity Station, Vietnam

In the Me Linh Station for Biodiversity, a group of Vietnamese crocodile lizards is kept since 2012, after the removal from the Amphibian Station, Hanoi, where it was originally established in the previous years (see Ziegler et al. 2011). In Me Linh, three enclosures with ground areas of ca. 2m^2, 6m^2 and 7m^2 were built up between 2012 and 2014 (for details see Ziegler et al. 2016). Here, we already considered our field research based findings about ecological requirements of *S. c. vietnamensis*, namely sufficient enclosure height (140-180 cm), a high water quality and slow water flow via pumps / permanent fresh water supply, as well as sufficient hiding, climbing and resting opportunities both on land and in the water parts. Subsequent to the publication by Ziegler et al. (2016), we further decided to integrate boundaries made by stones and concrete into all enclosures in order to create land parts which can be filled with substrate, as the latest findings showed that *S. c. vietnamensis* seems to bury itself at times during hibernation.

As we decided to further prioritize and enlarge the crocodile lizard *ex situ* breeding project, new keeping facilities were developed in 2016. In total 8 enclosures (each four compartments arranged in two blocks) were set up on a concrete base measuring 4 x 5 m. The two enclosure blocks are placed in 95 cm distance to each other, the space in between serves as a working corridor. Also in here, the bases consist of concrete basins (90 cm in height); the tops are made by iron frames (110 cm high) covered with metal gauze, with each one large keeper door opening to the front. Each enclosure measures 200 x 100 x 200 cm (l x w x h). One pump (SERA pond pp 3000) per block is attached for creating a slow water flow, the water parts of the four compartments are connected by holes (covered with plastic light grid) to allow for water circulation. In addition, running fresh water is led in through a cascade (made by larger stones and concrete) in one corner of each enclosure to keep up the water quality, increase the oxygen content and to provide for different flow velocities, which also increases the choice / variety of resting places for the lizards. The four inner corners of the block are divided by ca. 35 cm high curved boundaries made of bricks and concrete as land parts, the water level is kept at ca. 30 cm. By this, every enclosure has a land part (ca. 35-40% of the enclosure ground area, maximum width ca. 185 x 45 cm) on the inner corner, a “stream” flowing around the land part, and a cascade at the outermost corner. Stones fixed with concrete on the outer side of the land walls serve both for resting places and for an eased water exit. The land parts are filled with a drainage layer of stones and gravel and a ca. 20 cm high substrate layer (natural soil from the station’s surroundings) covered with leaves. Furthermore, all land parts are equipped with living plants in different sizes as well as further climbing, resting and hiding opportunities like horizontally and obliquely arranged branches reaching from the land part above the water, stones, roots and bamboo canes; large stones in the water parts serve as resting places. The whole facility is covered by a roof made by corrugated acrylic glass as weather protection; suspensible roof openings provide for natural UV radiation. Hatchlings can be raised in glass terraria/ separate plastic (fauna) boxes inside an air conditioned room during the first months.

4.2 Cologne Zoo, Germany

In Cologne Zoo, a unit (700 x 265 x 238 cm l x w x h) for the keeping and breeding of Vietnamese crocodile lizards was established in 2016 in order to extend the reserve population to a second institution. The right side of the room is intended for the husbandry of breeding pairs. Here, we set up an enclosure of 600 x 80 x 200 cm (l x w x h), consisting of a large polypropylene basin (80 cm in height) and a top made by a steel construction with attached metal gauze (120 cm in height). The enclosure is divisible into five compartments, each 120 cm in width, giving the opportunity to keep in total up to five couples, or to provide wider enclosures for a lower number of animals. The dividing walls consist of each two parts: one plastic light grid board for the lower part (enabling water flow through the whole facility), which can be inserted in tracks in the basin, and acrylic glass slides for the upper part, which can be inserted into slots inside the metal construction. Every compartment is accessible via a keeper door opening to the front. Also in here, land parts for filling in of substrate were created, here made by water proof PVC-boxes (80 x 30 x 35 cm l x w x h) which were pasted up with plastic gauze and afterwards covered with stones and tile glue in order to provide climbable walls, water accesses and resting places. The water level inside the enclosure is variably adjustable through a rotatable overflow pipe; based on the present height of the land parts it is kept at ca. 30 cm. The floor of the basin is slightly beveled from the left to the right enclosure side; the water is led through a pvc pipe from the right side via a pump (AquaMedic DC Runner 5.0) into a filter (Biotec Screenmatic 12) at the left enclosure side and from there pumped back into the enclosure, creating a slow water circulation. In addition, fresh water can be led in from the front side of each compartment; here, cascades made of larger stones and concrete similar to the ones in the Melinh Station are planned to provide further natural enclosure structure. The water can also completely be discharged via a separate plug valve. The left side of the room is planned for the rearing of juveniles or keeping of surplus adults. Here, shelfs with terraria (two terraria of 120 x 56 x 75 cm, 6 terraria of 60 x 56 x 45 cm l x w x h) and one additional empty shelf where juveniles can be individually housed in plastic boxes, were constructed. Illumination for all enclosures will be provided via LED strips (basic illumination), mercury vapor lamps with low watt strength (temperature range and UV access) and UV compact lamps (UV access for juveniles). The basic room temperature is around 24-25°C, the room is provided with an air conditioner for being able to cool down the room temperatures to around 14 °C during hibernation period. In the back part of the room, it is intended to set up a “research area”, e.g., for examination of the species’ metabolism via treadmill experiments.

5. Discussion

Despite newest insights into the different habitat requirements of both subspecies of *Shinisaurus crocodilurus*, there still remain open questions at time. As was already discussed by van Schingen et al., (2016b), the geographic separating line between the Vietnamese and Chinese subspecies is not yet known. Currently we assume that it coincides with the border between China and Vietnam, but it cannot be excluded that the Vietnamese subspecies also stretches into southern China. It also cannot be excluded that China harbors more than one crocodile lizard form, which can only be resolved by comprehensive future morphological and molecular analyses of interpopulation variation. In the case that the Chinese populations represent different forms, the husbandry requirements for the Chinese populations would likewise have to be revised according to regional differences.

The fact that differentiation of sexes using the ratio between head width and head length, is applicable for Chinese crocodile lizards (Wölfel, 2003) but not for Vietnamese crocodile lizards, can be explained by the recent finding that Vietnamese and Chinese specimens represent separate taxa that differ in head morphology (van Schingen et al., 2016b). However, concerning sex identification, there exist several characters namely morphometric values, color pattern and probing, which if considered in combination enable a relatively reliable determination of sex. But these characters are only distinctly pronounced in adult individuals of about at least three years, which makes a proper sex determination of subadults impossible.

Because Vietnamese crocodile lizards recently have increasingly appeared in the international pet trade (Auliya et al. 2016., van Schingen et al., 2015a), the origin of captive individuals must be identified in the future to maintain the conservation units genetically pure, and avoid hybridization within potential conservation breeding for future restocking / release. A genetic screening provides for the best results (van Schingen et al., 2016b), but also head shape is helpful in giving first hints to distinguish between the subspecies. However, as previously already stated, the variation of the Chinese populations so far is only insufficiently studied, so that final conclusions cannot be made at time. Another helpful and promising approach might be the analysis of isotopic signatures. Based on that we were already able to distinguish between wild caught and captive crocodile lizards, which was the first case study of its kind for lizards (van Schingen et al., 2016c). Future analyses must prove, whether this method also is able to distinguish between different natural populations, which seems to be realistic, as habitats in Vietnam and China are different, with distinct, separate trophic networks. Last but not least the herein introduced husbandry models / facilities have to be tested in the long term.

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Zusammenfassung

Die Krokodilschwanzechse (*Shinisaurus crocodilurus*) ist eine häufig in Zoos und Privathand gehaltene Reptilienart. In einem integrativ taxonomischen Ansatz konnte kürzlich aufgezeigt werden, dass sich die chinesischen Vertreter morphologisch, genetisch und ökologisch von den vietnamesischen Populationen unterscheiden. Aktuellen Populationsschätzungen zufolge kommen insbesondere die vietnamesischen Krokodilschwanzechsen in alarmierend geringen Individuenzahlen vor. Sie sind durch Lebensraumzerstörung und Abfang für den Lebendtierhandel bedroht. Aus vorgenannten Gründen ist es wichtig, die vietnamesischen und chinesischen Formen als voneinander getrennte Schutzeinheiten zu behandeln, sowohl um ihre genetische Integrität zu erhalten als auch innerhalb von *ex situ* Schutzmaßnahmen angemessene Haltungsbedingungen zu bieten. In dieser Studie geben wir, sowohl basierend auf unseren jüngsten intensiven Freilandforschungen innerhalb der letzen Jahre, als auch Haltungserfahrungen in der Me Linh Biodiversitätsstation in Nordvietnam, eine aktuelle

Übersicht über die besonderen ökologischen Anpassungen der neu beschriebenen Unterart (*S. c. vietnamensis*) aus Vietnam. Weiterhin stellen wir unsere neuesten Ergebnisse ökologischen Informationen zur Nominatunterart aus China gegenüber. Wir aktualisieren die derzeit verfügbaren Mindestanforderungen für die Haltung von Krokodilschwanzechsen und arbeiten anhand des aktuellen Wissensstands verfügbare Unterschiede bezüglich der Haltungsparameter für beide Unterarten heraus. Zusätzlich stellen wir neue Informationen bezüglich der Geschlechtsunterscheidung bereit und stellen neue Haltungskonzepte bzw. –anlagen für die vietnamesische Unterart vor, die wir in der Me Linh Biodiversitätsstation in Nordvietnam und im Kölner Zoo in Deutschland aufgebaut haben.

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3. Discussion

3. 1 Conservation status and autecology of *S. crocodilurus* in Vietnam

3.1.1 Population trends and ecological niche

A preliminary population estimation presented in **chapter 1,** demonstrated a dramatically small population size of less than 100 mature crocodile lizards, resided to three subpopulations in Vietnam. With respect to the size of the total wild crocodile lizard population, the Vietnamese population exerts only a minor contribution, comprising approximately ten times less individuals compared to the Chinese population (see Huang et al. 2008). But also in combination, the total wild population size of the species lies with about 1100 individuals below the threshold of 5000 individuals, depicted by Traill et al. (2007), which is the minimum required size to maintain a stable population over a longer time period.

Furthermore, a first evaluation of threats depicted in **chapter 1** identified habitat destruction by illegal logging, forest clearance for agricultural purposes, coal-mining, pollution, tourism, electro-fishing as well as poaching to tremendously imperil *S. crocodilurus* in Vietnam, comparable to the situation in China (Huang et al. 2008). Regarding its much smaller size, the Vietnamese population appeared to be even more vulnerable than the Chinese population.

A first microhabitat characterization in **chapter 2** confirmed the strong ecological specialization of *S. crocodilurus* to clean, densely vegetated and remote stream, far from human settlements. The water was characterized as soft and depleted of nutrients, assuming that expanding agricultural land use and the increased utilization of fertilizers and pesticides as well as pollution caused by mining activities will dramatically reduce the quality of existing habitats. Furthermore, *S. crocodilurus* was found to prefer perches above pool sections behind waterfalls and only occurred within intact evergreen broadleaf forests, which have been already extensively cleared (Tordoff 2000). Juvenile and adult specimens were herein found to differ in spatial niche segregation. Microhabitat sites, suitable for all age classes of *S. crocodilurus* are thus extremely rare and isolated from each other.

According to the herein identified small population sizes, apparent threats and the species' special adaption to rare, threatened and isolated microhabitats, compiled in **chapter 1 - 2**, *S. crocodilurus* already meets the criteria to be ranked as "Endangered" on the IUCN Red List of

Threatened species, according to IUCN guidelines. Thus, the assessment of *S. crocodilurus* took place based on the present results and its inclusion on the IUCN Red List as globally "Endangered" recently succeeded as a first implication to upgrade the species' conservation status (Nguyen et al. 2014).

Field research between 2010 (Hecht et al. 2013) and 2013 (**chapter 3**) revealed the presence of three separated *S. crocodilurus* populations in Vietnam (Tay Yen Tu NR, Bac Giang Province, Yen Tu NR and Dong Son-Ky Thuong NR, Quang Ninh Province). In order to evaluate the total potential distribution range of *S. crocodilurus* and assess the probability of the presence of further unknown populations, species distribution models (SMDs) were applied in **chapter 3**. These estimates revealed suitable habitats to be small, isolated and poorly covered with protected areas. One still relatively large contiguous area of suitable habitat was predicted to be situated in the border region of Vietnam and China. Targeted field surveys, conducted in this region in 2015 and 2016, led to the discovery of a new population, reported in **chapter 4.** This discovery presents an important finding, because even though SDMs are a meanwhile commonly and broadly applied tool in zoology and climate change research, they are only scarcely proven in practice. The present finding demonstrates the reliability of such models in forecasting the presence of species. Furthermore, this newly recorded population presents the most eastern Vietnamese population, geographically situated in between known populations from China and Vietnam and might represent an important component to understand the evolutionary history of recent crocodile lizards.

According to local villagers the newly discovered population - which reportedly had been extensive and individual rich still some years ago - almost completely vanished since the last 1-2 years. The most likely cause was the reported massive over-collection by locals to sell *S. crocodilurus* for about 1€ per animal to reptile traders. Starting with only collecting adults some years ago, villagers reportedly also collect juveniles meanwhile, due to the present lack of mature individuals (Anon. ref., pers. com.). Accordingly, we only encountered juvenile or subadult specimens in this area during surveys in 2015 and 2016. Similar scenarios were observed at all remaining occurrence sites in Vietnam during latest surveys in 2016. An evaluation of long-term population data, compiled during annual population monitoring (**chapter 4**), revealed that the portion of mature individuals steadily decreased all over the places. Since juvenile crocodile lizards have a naturally higher risk of mortality and are especially sensitive to stress and diseases and environmental changes (Bever et al. 2005; Zollweg and Kühne 2013) and *S. crocodilurus* reaches maturity only after 3-4 years (Bever et al. 2005; Yu et al. 2009; Zhao et al. 1999), it can be assumed that the lack of mature individuals will cause a rapid decline and even collapse of local populations as well as a genetic bottleneck (Kuo and Janzen 2004). Results of **chapter 4** even revealed, that crocodile lizards already completely extirpated from one third of former localities in Vietnam within a time frame of few years. Similar drastic local population declines of up to 90% have been observed in Chinese populations too, but within a period of 26 years (Huang et al. 2008).

In addition, latest field surveys in 2016 - not included in the presented estimates in **chapter 4** yet - revealed that the situation at all localities became even worse. The former most intact and suitable habitat sites, located in Tay Yen Tu NR, were almost completely destroyed due to expanding coal-mining activities, illegal logging, pollution of streams and the change of the stream courses in order to build new logging and mining roads. Furthermore, increasing numbers of mining workers are staying within the reserve and thereby - by own admission - live on natural resources, including crocodile lizards. According to recent unpublished interviews with local villagers and staff of the Forest Protection Department (FDP) of Tay Yen Tu NR, religious tourism is planned in Tay Yen Tu NR, similar to the situation in adjacent Yen Tu NR on the other side of the mountain, where *S. crocodilurus* already extirpated from unprotected sites. Planned activities in Tay Yen Tu NR include the building of five pagodas along the mountain, the construction of roads and cable cars to facilitate the accessibility for tourists and the development of infrastructure such as hotels to host high numbers of tourists in the near future (FPD Tay Yen Tu pers. com. 2016).

In summary the **first hypothesis**, stating that wild *S. crocodilurus* populations are extremely small, in decline and imperiled by numerous anthropogenic stressors, could be herein affirmed, highlighting a strong conservation concern. Also the **second hypothesis**, postulating that SDMs suit to forecast the presence of unknown *S. crocodilurus* populations could be confirmed.

The evaluation of long-term GPS data obtained from marked *S. crocodilurus* individuals between 2010 and 2015 further enabled a first estimation of home range sizes within natural habitats. Preliminary results of the unpublished Master thesis of Barthel (2015), embedded in this thesis, indicated home ranges of less than 100 m^2. Subadults were found to cover longest distances in short periods, while adults appeared to occupy the same perch over years (pers. obs.). Long et al. (2007) also found extremely small home ranges of 6.5 m^2 - 11.6 m^2 in captive Chinese specimens after their release into the wild. Due to the apparent strong sedentarism of *S. crocodilurus*, it can be assumed, that the species' ability to migrate to alternative sites in cases of habitat destruction or alteration is extremely low.

Regarding microhabitats in Vietnam, two syntopic, aquatic and ecological-similar lizards, namely *Physignathus cocincinus* and *Sphenomorphus cryptotis*, were frequently observed to co-occur with *S. crocodilurus* along the same streams (pers. obs.). Knowledge on niche segregation and potential concurrence between species aids to understand speciation processes and might also become crucial whenever it comes to the planning of conservation translocations. Thus, the spatial and trophic niche segregation has been investigated for the three species in the frame of two unpublished bachelor theses (Sahl 2015; Werner 2015), embedded in this study. Preliminary results revealed significant differences in small-scale spatial distribution between the three species. Temporally, *S. cryptotis* is mostly nocturnal (pers. obs.), while *P. cocincinus* (e.g. Bauer and Jackman 2008; pers. obs.) and *S. crocodilurus* (e.g. Zollweg and Kühne 2013; pers. obs.) are diurnal species. In addition, dietary and stable isotope analyses of respective species indicated distinct trophic niches. This partial niche segregation in space, time and diet is assumed to enable the coexisting of these species according to Gause's "Competitive Exclusion Principle" (e.g., Gause 1973; Navarro et al. 2013; Pianka 2000).

3.1.2 Impacts of climate change

Suitable habitats for *S. crocodilurus* have been shown to be small and fragmented in **chapter 3** as well as steadily shrinking due to multiple anthropogenic stressors in **chapter 2** and **chapter 4**. However, the impact of future climate change has not been included in the evaluation of habitat suitability so far. Li et al. (2010) projected that all suitable habitats for *S. crocodilurus* will vanish by 2080 as consequence of global warming in China. A similar decrease of suitable habitat sites to only 0.3 % throughout the whole species distribution range by 2080 was predicted in **chapter 4,** as a result of global climate change. However, to better evaluate the impacts of climate change and the potential of species to adapt to environmental changes, knowledge on animal physiology and temperature selection plays an additional crucial role (Parmesan 2006; Schweiger et al. 2008; Sinervo et al. 2010; Tewksbury et al. 2008). Even though most lizard species are specialized to specific environmental conditions, data on temperature selecttion is lacking for most species, as is data on microclimate at habitat sites (Sinervo et al. 2010; Tewksbury et al. 2008). In order to assess the temperature selection of *S. crocodilurus* within natural habitat sites in Vietnam, a backpack system attaching temperature data loggers on crocodile lizards was developed in **chapter 5,** which was also the first of its kind study in aquatic lizards in general. This technique proved to be successful to gain first insights into the thermal niche and activity pattern of *S. crocodilurus*. Due to the low weight and flexible material of the backpack, animals probably represented natural behavior. This approach further convinced with a balanced price-performance ratio. However, this method probably only suits for strong sedentary species with predictable resting places, as it is the case for *S. crocodilurus*. For other, more mobile or concealed living species, the method would have to be amended, i.e. by integrating a UHF/ VHF transmitter, reducing carrying comfort and drastically increasing costs for this method. First results of the present study indicated the species' selection of cool and constant temperatures, the avoidance of high temperatures at sun exposed spots as well as the active thermoregulatory behavior of *S. crocodilurus* and its dependence on shadowed areas and constant cool water. According to suggestions by Sinervo et al. (2010), namely that especially tropical lizards are vulnerable due to their narrow temperature tolerances, *S. crocodilurus* showed a very small realized temperature niche, compared to the fundamental niche (**chapter 5**). Since suitable microhabitats for *S. crocodilurus* represent only relict regions and forested areas with cool climate have been extensively cleared throughout the species distribution range, *S. crocodilurus* is assumed to be highly vulnerable to climate warming. Even though *S. crocodilurus* might tolerate higher temperatures for a short period, climate warming will likely restrict its time of activity, decrease its reproductive behavior and thereby might likely result in local extinctions, as predicted by Sinervo et al. (2010) for numerous other lizards.

The combination of findings in **3.1.1** and **3.1.2** affirmed the **third hypothesis**, suggesting *S. crocodilurus* to be a habitat specialist, adapted to cool temperatures and being prone to become negatively affected by climate change.

The results of this thesis further demonstrated, that geographically separated crocodile lizard populations are exposed to different climatic and microhabitat conditions in China and Vietnam (**chapter 2, 4** and **9**). Since Vietnamese representatives are i.e. exposed to more constant and cooler temperatures (vs. annual fluctuations with extremely cold winters and hot summers in China), it is likely that they are even more vulnerable to climate change in comparison to Chinese congeners. However, this hypothesis has to be validated by similar research on temperature selection and microclimate in Chinese populations.

3.2 The role of trade in *S. crocodilurus* and implications for conservation

International trade is recognized as second largest threat to numerous reptile species (Böhm et al. 2010) with international demand - especially for rare species - particularly stimulating the illegal harvest (Courchamp et al. 2006; Wyler and Sheikh 2013). **Chapter 6** provided a review of the impacts of trade in live reptiles on wild populations with a focus on the EU, which plays a major role in the global reptile trade and thus needs to take over responsibility for the conservation of species outside its range (Gruttke 2004). Between 2004 and 2014, a number of 20,788,747 live reptiles (CITES and non-CITES species) were officially imported by the EU member states, whereof Germany had been with 6,101,040 living specimens the major importer within the EU (Auliya et al. 2016; Eurostat 2015). Numbers of illegally traded animals are expected to be much higher. Vietnam and China were shown to belong - together with the USA - to the top three countries of origin with respect to live reptile imports into the EU. Many case studies presented in **chapter 6** emphasized that regulations and enforcement to prevent overexploitation of species and to restrict illegal trade activities are inadequate in many countries. Common ways to undermine CITES regulations are the mislabeling of CITES-protected species as unprotected species (Anderson 2014) or the widespread use of "hired tourists" as carriers to smuggle specimens across borders. Another common method is the mislabeling of illegally wild caught specimens as captive-bred specimens, wherefore commercial breeding farms are sometimes used to launder high numbers of specimens (D'Cruze et al. 2015; Lyons and Natusch 2011; Nijman and Shepherd 2009). **Chapter 6** demonstrated that numerous reptile populations have already been severely diminished by poaching to supply the pet market (e.g., Ariano-Sánchez and Torres-Almazán 2010; Flecks et al. 2012; Horne et al. 2011; Klemens and Moll 1995). It was shown that especially endemic, rare, geographically isolated or protected species are most targeted by smugglers and gain especially high prices on the black market (Brook and Sodhi 2006;

Cooney et al. 2015, Hall et al. 2008; Lyons and Natusch 2013). Stuart et al. (2008) i.e. reported a case of the extirpation of a Tiger Gecko at its type locality due to over-collection shortly after its original description. Accordingly, concrete evidence for their vulnerability and presence in the European reptile market was recently found for two further enigmatic and only recently described Vietnamese island endemic species, namely *Goniurosaurus catbaensis* and *Cnemaspis psychedelica* (Ngo et al. 2016a, b), which led to the recent inclusion of both species on the IUCN Red List as "Endangered" (Nguyen et al. 2016 a, b).

Chapter 7 provided a review addressing the threats to *S. crocodilurus* with a main focus on actual trade impacts on the species. Due to its special adaptation, prominent color patterns, primeval appearance and convenient size, crocodile lizards became ever more popular also among hobbyists especially from Europe. In 1982, crocodile lizards were found to appear on the international pet market with Germany being one of the first importing countries. A drastic rise in the demand for the species was already recorded since 1985. At that time, specimens already fetched relatively high prices of 995 DM, which equals approximately 500 EUR in Germany. Since *S. crocodilurus* had not been protected in the importing countries, hundreds of wild caught specimens were recorded to be imported from Hong Kong to Europe and the US. Besides, the illegal sale of 3300 animals from the type locality in Guangxi Autonomous Region, China had been reported between 1984 and 1986. Such high numbers of traded animals were already at that time identified as threat to *S. crocodilurus* and were linked to first observed declines of wild populations. As consequence, *S. crocodilurus* was included in CITES Appendix II in order to better control the trade in the species (CITES 1990). Since then, the international trade in *S. crocodilurus* promptly shifted to allegedly captive bred specimens, even though concrete evidence for the illegal origin of specimens existed in numerous cases. Cases of illegal trade in wild caught specimens were still repeatedly reported by the detection of illegal smuggling, false declaration of specimens or the covert sale - also of Vietnamese specimens - at the reptile fair in Hamm, Germany (Kanari and Auliya 2011; Robin de Bois 2014). However, such cases of seizures only represent "the tip of the iceberg" of the total illegal trade activities. The sale of *S. crocodilurus* on local markets and pet shops has been also repeatedly observed in Thailand, China and Vietnam in high numbers until 2016 (Auliya in lit., Ngoc pers. com., Nguyen pers. obs.). Meanwhile, the trade in *S. crocodilurus* outside its range states was found to have almost entirely shifted to internet platforms, partly via Facebook, what makes illegal activities particularly difficult to control. Results of the trade analysis in **chapter 7** showed, that by far the most adverts on international internet platforms came from Germany. It was further demonstrated that the demand for crocodile lizards is meanwhile drastically exceeding the offer of captive bred specimens. Pet shop prices still remain high, depending on age, coloration and sex (e.g. up to 1100 USD per adult in the US or 490 EUR per juvenile in Germany), which makes the international business in *S. crocodilurus* especially attractive for local dealers in the countries of origin. Even though no Vietnamese crocodile lizards officially left Vietnam to date, evidence exists for the presence and retail of numerous Vietnamese specimens in Germany. According to recent unpublished interviews with local villagers and traders from Vietnam, *S. crocodilurus* is - since recently - collected everywhere to sell specimens for around one EUR per animal to traders in cities, from where they are particularly sold as "allegedly captive farm bred" for several hundred Euros. Most of the collected animals are currently exported to China (Anon. ref., pers. com.). A recent visit of an alleged breeding farm assumed the wild origin of stocked specimens and further uncovered extremely bad health conditions of offered animals, which were stored in high numbers within little boxes without food until sale or shipment.

Meanwhile, over-exploitation was found to dramatically imperil all wild crocodile lizard populations in Vietnam, while the species has already been extirpated from several localities (**chapter 5, 6, 7**). Based on own observations in 2016, the only single site, still harboring several adult animals, was a short stream section, which is fenced-in and highly protected by rangers, because it contains a freshwater reservoir supplying the surrounding villages. As side effect, *S. crocodilurus* profits from the high control mechanisms within this area. In this context, the **fourth hypothesis** claiming the distinct imperilment of wild *S. crocodilurus* in Vietnam, due to unsustainable international trade in the species, was confirmed.

Since Germany was found to play a major role in the trade in *S. crocodilurus* also from Vietnam, the country needs to take over responsibility for the species within its range state.

While authorities are globally experiencing strong challenges to effectively control the international trade in CITES-listed species and struggle to uncover fraudulent claims of "captive-breeding", forensic analytical methods are being increasingly considered as potential tools to assess wildlife crime (**chapter 8**; Lyons and Natusch 2015). Thereof, stable isotopes were recently suggested as potential approach, because isotopic ratios in animal tissue reflect its diet and specific ecosystem and thus are considered for being suitable to differentiate between the source ("wild" vs. "captive") or the origin (geographic origin) of specimens (Fry 2006; Lyons and Natusch 2015; Moncada et al. 2012; Satterfield and Finney 2010; Voigt et al. 2012). Few case studies already indicated the successful use of isotopic markers to differentiate between wild and captive mink and wolves (Hammershøj et al. 2005; Kays and Feranec 2011), between wild and farmed fish and crustaceans (Dempson and Power 2004; Carter et al. 2015), or to trace the origin of traded elephant ivory (Ziegler et al. 2016), while this novel method is generally poorly tested yet (Lyons and Natusch 2006). Lyons and Natusch (2015) suggested the potential of this method to discriminate between farmed and wild snakes.

Chapter 8 provides a pilot study assessing the suitability of $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios as tool to discriminate between legally captive bred and illegally wild caught specimens, for the first time in reptiles exemplary on the crocodile lizard in Vietnam. Herein, significant isotopic differences were found between wild and captive-born *S. crocodilurus* specimens, underlining the high suitability of isotope analyses as forensic tool to reduce the laundering of wild caught lizards. However, the potential of its application appeared to be limited to range restricted or ecologically specialist species and requires a reference data base of all wild populations (see also Ziegler 2016), which was the case for Vietnamese crocodile lizards. Hence, this method proved to be highly useful in the present case and provides an important reference framework against which *S. crocodilurus* specimens with unknown origin can be cross-checked. Accordingly, the present pilot study affirmed the **fifth hypothesis**, claiming that isotopic markers suit as novel forensic tool to identify the source of *S. crocodilurus* specimens, due to dietary differences.

As follow-up study, it is recommended to extend the reference framework including respective analyses of Chinese populations in the future. In addition, the investigation of further isotopes should be considered to increase the potential discriminatory power to differentiate wild from captive specimens. Ziegler (2016) discussed the potential applicability of isotopic markers as a chemical imprint of legal captive-bred individuals in commercial breeding farms (i.e. *Python* farms for skin production) to prevent the laundering of wild animals. However, preliminary results of a recent pilot feeding experiment with *Varanus* specimens indicated the detectability of ^{15}N glycine - which had been supplemented to the diet - already after two weeks in the skin of juveniles, due to their high rate of epidermal renewal (Ziegler 2016). In consequence, this novel approach needs to be tested for

suitability in different taxonomic groups as well as in different age classes to conclude on its general applicability in wildlife forensics, which is currently in process (Dittrich pers. com.; Natusch pers. com.; Ziegler 2016).
Besides isotope analyses, the use of genetic analyses such as the investigation of microsatellites has been extended from its application in human forensics to a variety of animal species (e.g., Andreassen et al. 2012; Kraus et al. 2014; Mucci et al. 2014; Schury et al. 2014) and was also proposed as potential tool to discriminate between captive and wild reptiles (Lyons and Natusch 2015). The applicability of this approach in order to identify the origin of *S. crocodilurus* specimens will be discussed in **3.3.1**.

In addition, the permanent marking of individuals with transponders during annual population monitoring (**chapter 1, 4**) further enables to identify specimens of wild origin in Vietnam, which illegally entered the trade and thus presents another control mechanism. The application of PIT tags was also proposed by Lyons and Natusch (2015) as potential methodology to differentiate between captive and wild CITES-listed snakes.

To restrict further export of *S. crocodilurus* specimens from theirs range states and to control and exacerbate the trade in the species in order to decrease the pressure on wild populations, *S. crocodilurus* was uplisted from CITES Appendix II to I (CITES 2016) after concordant approvement at the seventeenth Conference of the Parties (CoP17) in South Africa in October 2016. The respective proposal referred, inter alia, to the data compiled in **chapter 1 - 7**, which provided strong arguments for the fulfillment of *S. crocodilurus* of respective criteria (see also Ziegler and Nguyen 2015). As consequence the export and import as well as any commercial acquisition, sale or swap of wild *S. crocodilurus* specimens will be generally prohibited worldwide in all 183 CITES member states.

3.3 Linking in situ with ex situ conservation

3.3.1 Taxonomy and biogeographic patterns

In view of the heavily diminished populations and ongoing declines especially in Vietnam, knowledge on genetic diversity and taxonomic relationships between Chinese and Vietnamese representatives became in particular crucial - not only to understand evolutionary and biogeographic patterns of this ancient lineage - but also for the planning of urgent conservation measures. In **chapter 2** slight differences in perch selection as well as in microhabitat characteristics have been elucidated between Vietnamese and Chinese *S. crocodilurus*, assuming potential divergent ecological adaptions among geographically extant populations. Accordingly, SDMs, applied in **chapter 9**, predicted different habitats and regions to be suitable either for Vietnamese or Chinese representatives, which are exposed to different climatic conditions as well as slightly different stream properties, vegetation and rock types, respectively. Such different abiotic and biotic conditions in combination might exert different selective pressures on geographically separated populations and thus promote divergent evolution (e.g., Jaffe et al. 2015; Losos 1994; Schluter 2009). Pursuing that question, a detailed morphological examination, presented in **chapter 9**, identified several characteristics in head morphology, that differed significantly between Chinese and Vietnamese representatives, whereas respective characters were found to be identical among all Vietnamese populations. While differences in scalation evolve rather randomly, characters of body proportions are known to underlie selective pressures and have been frequently recorded between lizard species or populations that were exposed to different environmental conditions (e.g., Jaffe et al. 2015; Grizante et al. 2012; Husak and Rouse 2006; Kohlsdorf et al. 2008; Losos 1994). In view of the strong morphologic conservatism and comparably slow evolutionary rate of *S. crocodilurus*, these new findings indicate a probable taxonomic differentiation between geographically separated populations as consequence of ecological divergence. In the sense of integrative taxonomy, a comparison of mitochondrial DNA had been additionally applied in **chapter 9** in order to support or refute that assumption. Even though a previous genetic comparison by Ziegler et al. (2008), using 16S DNA, indicated minor differences of only 0.2%, the herein conducted analysis, using fragments of three other mitochondrial genes (cytochrome b, partial ND6 and partial tRNA-Glu), revealed genetic differences of 2.1-3.6 % between Chinese and Vietnamese populations. The minor molecular difference in the previous study by Ziegler et al. (2008) might have resulted from the conservative nature of the investigated 16S gene, possessing a lower mutation rate than faster evolving mitochondrial markers (Hixson and Brown 1986). Additionally, only a single specimen of a zoo collection - coming from the trade with unknown origin - had been analyzed by Ziegler et al. (2008) as representative for the Chinese *S. crocodilurus.* The investigated specimen might have not been representative, i.e. if collected close to the border, since the Chinese-Vietnamese border does not necessarily represent the taxonomic boundary of *S. crocodilurus* taxa. It can't even be completely excluded that the respective specimen originated from Vietnam.

In comparison, Vietnamese subpopulations were completely genetically identical. In contrary, Chinese populations clustered into two different phylogenetic groups with interspecific divergence of a similar magnitude. However, the monophyly of one group received no statistical support by Maximum Likelihood and Bayesian analysis. Based on the combination of genetic, morphological and ecological differences between extant populations, the Vietnamese population was subsequently described as own subspecies *S. crocodilurus vietnamensis* as first cautious taxonomic action (**chapter 9**). In view of the strong conservatism and high similarity of extant *S. crocodilurus* with 50 million year old fossil relatives, the present genetic, morphological and ecological differences might be even ranked as more important, considering a taxonomic split on the species level, pending additional and more detailed molecular analyses in the near future. However, the present first results affirmed the **sixth hypothesis**, stating that extant *S. crocodilurus* populations represent several taxa, namely at least two subspecies.

Already now, the present taxonomic separation in **chapter 9** involves tremendous consequences regarding the conservation status of *S. crocodilurus* and drastically increases the conservation concern and value of the Vietnamese subspecies as well as the need of immediate conservation actions. Concomitant follows the split into two different conservation units, implying the amendment and development of a separate conservation management program.

In order to assess the still unresolved phylogenetic relationships among subpopulations, Huang et et. (2014) already investigated 19 microsatellite loci and the same fragment of mitochondrial DNA as in **chapter 9** of eleven wild and two captive *S. crocodilurus* populations in China (Figure 6). They identified three main genetic groups divided into two different clades (Figure 7), accordant to our findings in **chapter 9**. The genetic separation of these three groups had been shown to mainly correspond to their geographic distribution (Figure 6, Figure 7). Hence, these groups were also proposed by Huang et al. (2014) as different management units, defined by significant divergence in mitochondrial or nuclear loci and significant differences in allele frequency distributions.

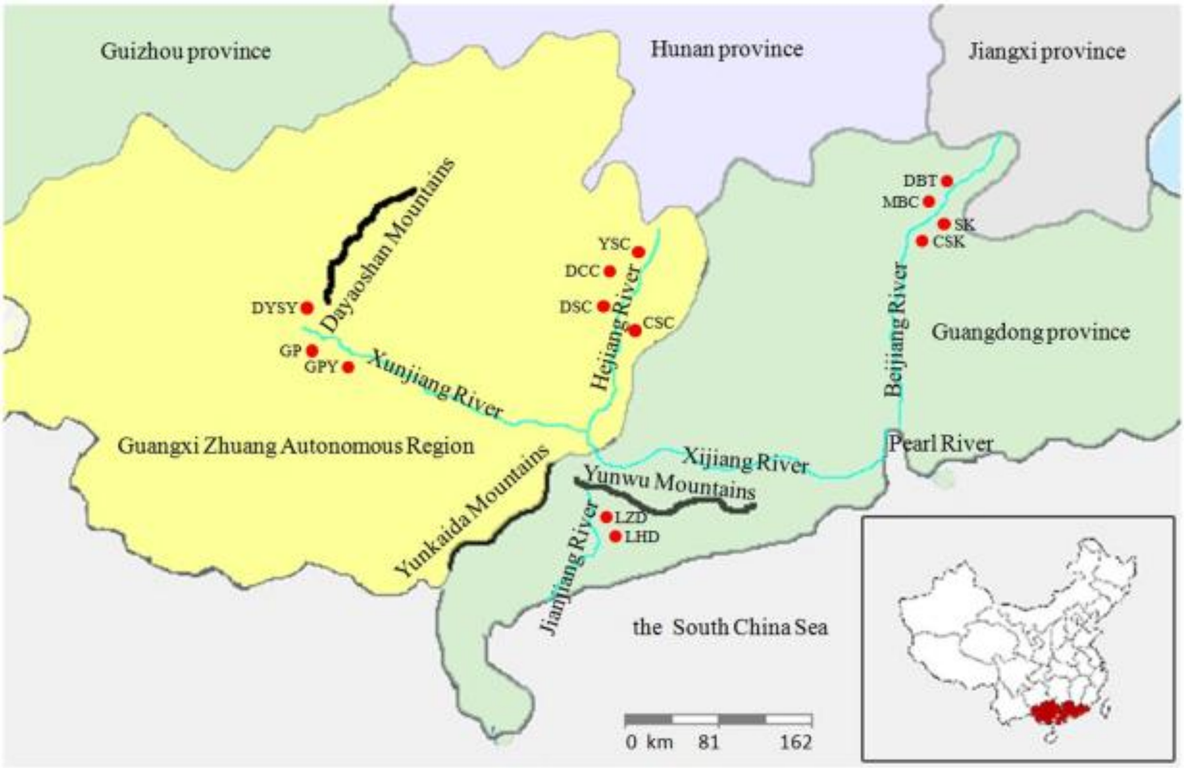

Figure 6. Map of sampled *S. crocodilurus* populations (red dots) in China. Obtained from Huang et al. (2014).

Huang et al. (2014) further demonstrated that all groups and populations showed a low genetic diversity and that *S. crocodilurus* is extensively inbred in China. Thus, enforced protection of all three units was strongly recommended by Huang et al. (2014).

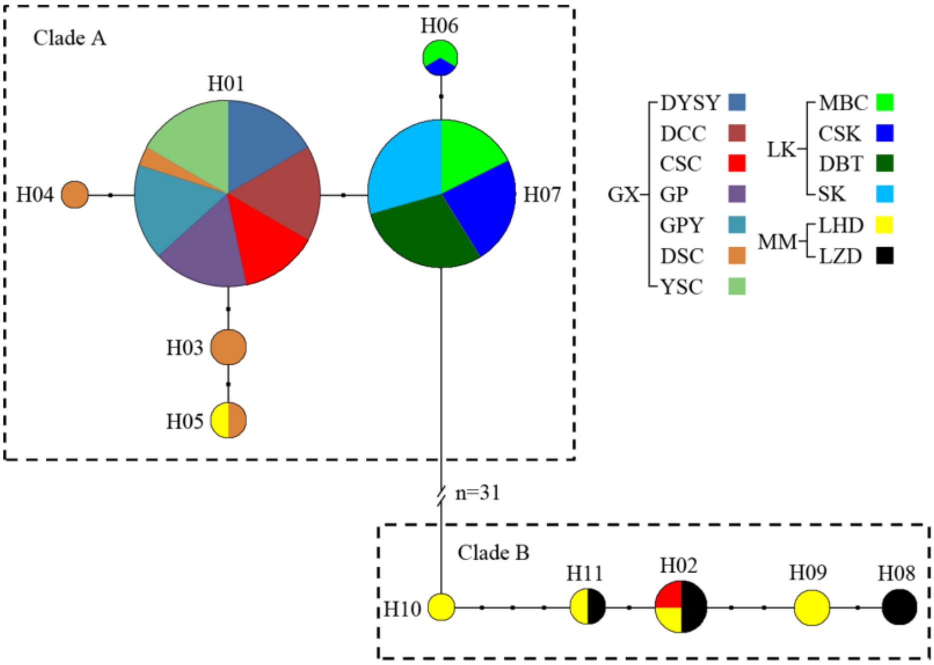

Figure 7. Median-joining network showing phylogenetic relationships among populations. Haplotypes shown as circles with size indicating number of individuals. Mutational steps between haplotypes are represented by a small black dot. Colors represent populations. Obtained from Huang et al. (2014).

A first microsatellite analysis in order to assess the genetic diversity among Vietnamese populations as well as to further investigate the relationship between Chinese and Vietnamese populations, was recently performed by our *Shinisaurus* working group according to the study of Huang et al. (2014), by comparing tissue samples of Vietnamese specimens from all known localities collected during own surveys with respective data on Chinese specimens obtained from GenBank. Preliminary results confirmed the presence of two clades among Chinese representatives and indicated one clade consisting of all Vietnamese populations, between which no genetic divergence was found so far. Further more comprehensive analyses are in process in order to obtain unambiguous data to conclude on a potential taxonomic split on the species level.

Similar to the situation in China, preliminary results assume that also Vietnamese populations are highly inbreeding, emphasizing the need of improved conservation and a managed conservation breeding program (see **3.3.2**).

Regarding biogeographic patterns of *S. crocodilurus* populations, Huang et al. (2014) suggested that the basal divergence between the two Chinese clades might have resulted from the development of river systems and mountains, since populations of clade A were distributed along the Pearl river system and populations of clade B were found along the Jianjing River system (Figure 6, Figure 7). Both clades were separated by the Yunwu Mountains, which might represent a geological barrier. Mountain ranges or river systems have already considered to represent evolutionary barriers for other lizards in Southeast Asia (e.g., Chen et al. 2014; Luu et al. 2015). Geologically, Dayaoshan Mountain, located at the intersection of the north-south species transition, had been an important pathway of animal migration and - due to the lack of an ice sheet during the Quaternary glacial period - an ancient species refuge with a rich biodiversity in the past (Huang et al. 2014). Genetic analyses of Huang et al. (2014) revealed that the most frequent and widespread haplotypes (H01 and H07; Figure 6) were found in Guangxi Province and Luokeng, Guangdong Province, assuming that an initial population expansion might have started from there. Considering localities of fossil representatives (Conrad et al. 2014), which have been found northwards of the current distribution of crocodile lizards in China, but lack to the South of Vietnamese populations, a former population expansion of *S. crocodilurus* from China towards Vietnam might be assumed. To explain the limited distribution range of the species throughout

Vietnam, the Red River might be considered as geological barrier restricting the distribution of *S. crocodilurus* westwards. However, the most likely restricting factor might represent the distribution of intact and connected evergreen lowland broadleaf forest, which is - in Vietnam - only found throughout the distribution range of *S. crocodilurus* (visible in **chapter 5**). The application of SDMs in order to predict past scenarios of climate and vegetation coverage might aid to a better understanding of evolutionary patterns of *S. crocodilurus.*

3.3.2 Conservation management

Previous findings presented in **chapter 1-9** emphasized the high vulnerability of *S. crocodilurus* - especially in Vietnam - to a combination of various threats. Since poaching and habitat destruction remain difficult to control, *ex situ* measures turned out to become especially important. Bridging the gap between *in situ* research and *ex situ* measures as well as the collaboration of researchers with zoological gardens herein proved to be a particular efficient strategy for conservation. The present study emphasized the importance of microhabitat analyses and basic research on niche adaptation in order to plan and implement conservation breeding programs. This study revealed a specific thermal niche of *S. crocodilurus*, which has tremendous impacts on its reproduction success in captivity and is crucial for adequate husbandry and to reduce mortality in captivity.

The taxonomic split of *S. crocodilurus* in at least two subspecies strongly argues for the establishment of different conservation breeding programs to retain a stable reserve populations in captivity and maintain the genetic integrity of each taxon. In China, such a breeding program was already established and is achieving increasing success (e.g., Zollweg 2012; Zollweg and Kühne 2013). Accordingly, a respective conservation breeding program was recently also initiated in the Me Linh Station for Biodiversity, Vinh Phuc Province, Vietnam by the support of the Cologne Zoo and the Institute of Ecology and Biological Resources, Hanoi (Ziegler and Nguyen 2015; Ziegler et al. 2015). In addition to already existing facilities, a new *Shinisaurus* house containing eight further semi-outdoor enclosures was constructed in the beginning of 2016, according to the specific habitat requirements of *S. crocodilurus* compiled in **chapter 2**, **6** and **10** (Ziegler 2016; Ziegler and Nguyen 2016). In **chapter 10**, updated recommendations for keeping conditions, amended for Vietnamese crocodile lizards, were compiled and provided. Ideally, future reproduction success and surplus offspring might form a solid base for a restocking of wild populations after IUCN guidelines.

As initial step, the selection and combination of proper breeding pairs is required. However, sexing remains challenging since *S. crocodilurus* so far has no distinct sexual dimorphism (Zollweg and Kühne 2013). In **chapter 10** a set of different morphological characters was identified, that - if regarding all characters in combination - gives a reliable evidence for the sex of Vietnamese crocodile lizards and should provide a reference work for other zoos and keepers. On this basis, enclosures in Me Linh Station for Biodiversity were stocked with potential fitting couples of *S. crocodilurus* in August 2016. In order to establish a geographically separated reserve population, another keeping unit is currently constructed in the Cologne Zoo (**chapter 10**). In addition, the establishment of a studbook, separately for Vietnamese and Chinese crocodile lizards, is urgently recommended to manage the breeding of the two subspecies, maintain the genetic integrity, prevent the inbreeding of Chinese and Vietnamese specimens and to adapt husbandry according to different conditions at natural habitat sites. That approach has already been recommended to the European Association of Zoos and Aquaria (EAZA) (Ziegler and Nguyen 2016). Such a studbook would imply the genetic screening of all specimens apparent in zoos or private hand in order to identify their origin. Even though the determination of precise localities of specimens is not possible based on the in **chapter 9** and **3.3.1.** applied genetic approaches, mitochondrial DNA revealed to be suitable to differentiate between Chinese and Vietnamese specimens, which will be crucial to assess the origin of seized or traded specimens and would be also essential to build the base for such breeding program or studbook. In order to test this in practice, a preliminary investigation of mitochondrial DNA of several *S. crocodilurus* derived from the trade in Vietnam was carried out by our *Shinisaurus* working group and confirmed that all tested specimens originated from the country. Since saliva samples are sufficient, this approach further represents a non-invasive method, which does not involves large efforts of sample acquisition.

As a first premise for the planning of future restocking activities of *S. crocodilurus*, the identification of suitable areas as well as their guaranteed protection is obligate. Based on the combination of predicted habitat suitability by SDMs and actual forest coverage data, derived from satellite images, priority areas for conservation were herein identified together with recommendations for the establishment of new reserves and the maintenance of forest corridors, connecting already existing reserves with *S. crocodilurus* populations (**chapter 4**). This information aims to inform local authorities and decision makers with the important scientific knowledge to implement measures for habitat protection. Public relation work represent another major part of successful conservation. In this context, further awareness raising efforts are continued on both, the official and political level as well as on the popular or educational level, including the implementation of environmental education programs (e.g., Ziegler 2015; **chapter 8**). Secondly, potential interactions between different syntopic species need to be understand in order to prevent competition with or repression of native species after translocation. Preliminary investigations already indicated the ecological niche segregation between *S. crocodilurus* and most common sympatric species (**3.1.1**), which supports the positive process of a release. Thirdly, the species migratory behavior impacts the success of release programs. Since *S. crocodilurus* was found to be extremely sedentary in Vietnam as well as in China after a first trial of a release (**3.1.1**), the species is considered to be suitable for a restocking, because released animals would probably stay in the target area.

4. Conclusion

The present thesis provided essential baseline knowledge on the so far unknown ecological adaptation and strong habitat specialization of *S. crocodilurus* in Vietnam. The herein developed backpack system with temperature data loggers turned out to be applicable to gain first insights into the thermal niche of Vietnamese *S. crocodilurus*, namely a narrow realized niche of constantly cool temperatures indicating the species' vulnerability to global warming. Accordingly, suitable habitats were predicted to tremendously decrease in the near future as consequence of climate change. SDMs were additionally successfully proven in practice to serve as useful tool in predicting the presence of *S. crocodilurus* populations. However, overall wild populations were estimated to be dramatically small and found to steadily decline since the last recent years. Besides habitat destruction, overexploitation to supply the international trade was found to represent a major threat to the species. Pursuing that problem, this thesis demonstrated the applicability of isotopic markers to distinguish between the sources (legal captive bred vs. illegal wild caught) of *S. crocodilurus* specimens and thereby for the first time of a reptile species in general. Based on these first promising results this novel approach could aid to better control the illegal trade in *S. crocodilurus* and might have the potential to be amended for other species and become a future tool addressing wildlife crime.

Even though a previous genetic comparisons revealed no distinction between extant crocodile lizards from China and Vietnam, the herein applied integrative taxonomical approach revealed different ecological adaptations as well as morphological and genetic differences between Vietnamese and Chinese *S. crocodilurus*, resulting in the split into two subspecies. This finding demonstrated that the examination of multiple species traits might still gain new insights, even in species that are already longtime under investigation. The taxonomic split was associated with a separation into two conservation units, entailing dramatic consequences on the conservation status of each unit.

Furthermore, combined results of ecological research and population estimates have been used to upgrade the conservation status of *S. crocodilurus* by including the species on the IUCN Red List as "Endangered" as well as by its uplisting from CITES Appendix II to I at the last conference of the parties (CoP17) in Johannesburg, South Africa, in October 2016. Furthermore, this research provided the scientific basis for an urgently required conservation breeding program in Vietnam with the future goal of a restocking program, emphasizing the strong linkage between *in situ* research with *ex situ* measures for effective conservation management. This thesis clearly demonstrated the suitability of a various spectrum of novel forensic and theoretic tools in order to enforce conservation activities. However, it also highlighted the necessity of basic research in order to provide a solid base for advanced techniques and the enforcement of legislations. Thus, the present study might serve as a good example on how to combine ground research with modern, multidisciplinary methods as well as with the involvement of zoological gardens in order to improve species conservation, which remains challenging in view of the global biodiversity crisis.

5. Outlook

A major future goal should be the further development of forensic methods as well as the establishment of broadly applicable novel tools in order to more efficiently identify, control and sanction the illegal global wildlife trade. As follow-up study to the herein presented pilot study assessing the applicability of carbon and nitrogen isotopes to determine the source ("captive" vs. "wild") of *S. crocodilurus* specimens, the suitability of further isotopic or elemental markers should be investigated. In this context, it would be also crucial to identify the required time, until changes in a specimens diet result in different isotopic signatures of its skin, in order to evaluate in how far this method might even be misused to launder wild caught specimens as captive bred ones. Therefore, long-term feeding experiments with isotopic labeled food would be most helpful. A first respective study by Ziegler (2016) indicated, that isotopic signatures change rapidly in the skin of juvenile *Varanus* specimens after feeding isotopic labeled diet, due to their rapid epidermal renewal cycle. In order to evaluate the general practicality of this method, its application needs to be tested on further species and groups, which is currently under investigation in separate case studies, i.e. on snake skins (Natusch pers. com.), monitor lizards (Ziegler 2016) and frog legs (Dittrich pers. com.). It is further recommended to also test the potential of isotopic markers to identify precise regions of origin of *S. crocodilurus* specimens. Genetic methods, namely mitochondrial and microsatellite analyses, turned out to be suitable to identify the country of origin in the species, but failed to distinguish geographically close situated populations (**see 3.3.1**). Possibly, isotopic markers might suit to distinguish between populations or even between animals from different streams and - if applied for trade specimens - might ideally help to detect the presence of unknown occurrences.

In view of the dramatic conservation status of *S. crocodilurus* and its extremely small and declining wild populations in conjunction with multiple acute anthropogenic pressures, the discovery of such potential further unknown populations as well as the confirmation of the presence of the species in inaccessible terrains would be essential for further conservation implementations. However, expeditions to search for *S. crocodilurus* populations are frequently restricted by the inaccessibility of habitats, especially in remote and mountainous areas as well as by climatic constrains.

eDNA as tool to detect unknown populations?

Environmental DNA (eDNA) analyses represent another relatively novel tool in ecological research, which enables to detect the presence or absence of a target species based on small traces of its DNA (i.e. from feces, skin or saliva) in humid environment such as water or moist substrate, where DNA remains relatively stable. Especially small fragments of mitochondrial DNA - which is present in numerous copies within each cell - can still be detected with high probability. Using species specific primers, the presence or absence of an aquatic or semiaquatic species can be theoretically detected by analysing water samples. This has the advantage that animals don't need to be captured or seen to identify its presence, especially in inaccessible habitats as well as in case of small populations or shy species. In practice, such eDNA monitoring was already successfully applied to detect different amphibians, fishes and even mammals in aquatic habitats (Ficetola et al. 2008; Jerde et al. 2013; Taberlet et al. 2012), resulting in new insights into distribution patterns and even abundances of respective species (Takahara et al. 2012).

Thus, we aim to investigate the suitability of eDNA as tool to detect *S. crocodilurus* populations in Vietnam, which would be the first trial testing this method for reptiles in running waters and for semiaquatic lizards in general (van Schingen et al. 2016).

Some groundwork, such as the primer design, its testing on functionality and their specifity for *S. crocodilurus*, as well as some sampling at habitat sites in Vietnam already took place.

Modeling extinction probability of S. crocodilurus

Sinervo et al. (2010) predicted the local extinction of 39% of worldwide lizard populations due to consequences of climate change, if considering thermo-physiological traits of lizards. Thereby, species specific operation temperatures as well as maximum and minimum tolerated temperatures (critical temperatures) were considered to determine the ability to stand climatic alterations. The hours of restriction (h_r), defined as time spent in thermal refuges, are assumed to play an important role, since extinction is predictable if h_r exceeds a threshold time period (Sinervo et al. 2010). Even though species might be able to tolerate increased temperatures for a while, essential behaviors such as foraging or reproduction might be limited due to climate warming, causing extinctions in the long-term. Sinervo et al. (2010) developed temperature profiles of species to include thermophysiological data in species SDMs to predict their extinction risk. Likewise, the prediction of the extinction risk of *S. crocodilurus* on a spactial scale, by including thermal physiological data to previous SDMs, would aid to identify sites for potential restocking. The selection of suitable sites for a planned future translocation based on thermal suitability has already found to be important in Tuatara, which need thermal specific micro-sites for nesting (Mitchell et al. 2008). Besides choosing sites only based on security aspects, Mitchell et al. (2008) successfully used the thermal modeling as tool to identify sites for a conservation translocation program in these highly vulnerable Tuataras. Accordingly, a similar approach should be considered for *S. crocodilurus* in the near future.

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Subpublications and Record of Achievement

Part 1: Autecology and conservation status of *S. crocodilurus* in Vietnam

Chapter 1: **van Schingen, M.**, Pham, C.T., Thi, H.A., Bernardes, M., Hecht, V.L., Nguyen, T.Q., Bonkowski, M. and Ziegler, T. (2014). Current status of the Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam with implications for conservation measures. *Revue Suisse de Zoologie.* 121(3): 1-15.

The author of the present thesis contributed substantially to the planning of the study. Field surveys and data collection, data analyses, preparation of graphics as well as the writing of the manuscript draft were carried out by the author. The author was also the leading author regarding an application to receive funds from EUAC for that study.

Chapter 2: **van Schingen, M.**, C.T. Pham, H.A. Thi, T.Q.Nguyen, M.Bernardes, M. Bonkowski and T. Ziegler, T. (2015). First ecological assessment on the endangered Crocodile Lizard *Shinisaurus crocodilurus* Ahl, 1930 in Vietnam: Microhabitat characterization and habitat selection. *Herpetological Conservation and Biology*. 10(3): 948-958.

The author substantially planned the study, prepared and conducted the data collection in the field. Data analyses, creation of graphics and writing of the paper draft was performed by the author.

Chapter 3: **van Schingen, M.**, Ihlow, F., Nguyen, T.Q., Ziegler, T., Bonkowski, M., Wu, Z. and Rödder, D. (2014). Potential distribution and effectiveness of the protected area network for the Crocodile Lizard *Shinisaurus crocodilurus* AHL, 1930 (Reptilia: Squamata). *Salamandra* 50(2): 71-76.

Field research, data gathering and preparation were conducted by the author, while main parts of the SDMs were performed by Flora Ihlow and Dennis Rödder. The two first authors prepared the manuscript draft.

Chapter 4: **van Schingen, M.**, Ha, Q.Q., Pham, C.T., H.Q., Le, T.Q., Nguyen, Q.T., Bonkowski, M. and Ziegler, T. (2016). Discovery of a new crocodile lizard population in Vietnam: Population trends, future prognoses and identification of key habitats for conservation. *Revue Suisse de Zoologie*. 123(2): 241-251

The author contributed by the substantial planning of the study, the conduction of field surveys, evaluation and processing of longterm population data, creation of graphics as well as by the leading production of the manuscript. Q.Q. Ha performed niche modeling analyses using Maxent.

Chapter 5: **van Schingen, M**., Barthel., L., Pham, D., Pham, C.T., Nguyen, Q.T., Ziegler, T. and Bonkowski, M. (submitted). Will climate change affect the Vietnamese crocodile lizard? Seasonal variation in microclimate and activity pattern of *Shinisaurus crocodilurus vietnamensis. Tropical Ecology.*

The author planned, prepared and organized the study and co-supervised the embedded Master Thesis of Leon Barthel. The development of a backpack system with data loggers, the field work and data collection was carried out by Leon Barthel and the author together. The author helped and instructed Leon Barthel during data analyses and provided the paper draft.

Part 2: Impacts of trade and implications for conservation

Chapter 6: Auliya, M., Altherr, S. Ariano-Sanchez, D., Baard, E.H., Brown, C., Cantu, J.-C., Gentile, G., Gildenhuys, P., Henningheim, E., Hintzmann, J., Kanari, K., Krvavac, M., Lttink, M., Lippert, J., Luiselli, L., Nilson, G., Nguyen, T.Q., Nijman, V., Parham, J., Pasachnik, S.A., Pedrono, M., Bauhaus, A., Rueda, D., Sachnez, M.-E., Schepp, U., **van Schingen, M.**, Scheeweiss, N., Segniagbeto, G.H., Shepherd, C., Stoner, S., Somaweera, R., Sy, E., Türkosan, O., Vinke, S., Vinke, T., Vyas, R., Williamson, S. and Ziegler, T. (2016). Trade in live reptiles and its impact on reptile diversity: the European pet market as a case study. *Biological conservation.* 17 pp.

The author provided formulated data and helped to prepare parts of the manuscript.

<u>Chapter 7:</u> **van Schingen, M.**, Schepp, U., Pham, C.T., Nguyen, T.Q. and Ziegler, T. (2015). Last chance to see? Threats to and use of the Crocodile Lizard. *Traffic Bulletin*. 27: 19-26.

The author conducted a literature review, a trade recherche via internet platforms and using trade databases and communicated with dealers on reptile fairs and in pet shops. The author performed data analyses, created graphics and provided main parts the paper draft.

<u>Chapter 8:</u> **van Schingen, M.**, Ziegler, T., Boner, M., Streit, B., Nguyen, T.Q., Crook, V. & Ziegler, S. (2016). Can isotope markers differentiate between wild and captive reptile populations? A case study based on crocodile lizards (*Shinisaurus crocodilurus*) in Vietnam. *Global conservation and Biology*. 6: 232-241.

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Part 3: Linking *in situ* with *ex situ* conservation

<u>Chapter 9:</u> **van Schingen, M.**, Le, M., Ha, Q.Q., Pham, C.T., Nguyen, T.Q. and Ziegler, T. (2016). Is there more than one crocodile lizard? An integrative taxonomic approach reveals Vietnamese and Chinese *Shinisaurus crocodilurus* to represent separate conservation and taxonomic units. *Der Zoologische Garten* 85: 240-260 doi:10.1016/j.zoolgart.2016.06.001.

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The author edited and reviewed own data, conducted a literature review and helped Thomas Ziegler to prepare the manuscript draft and generation of tables. Thomas Ziegler and the author conducted the sexing of animals in Me Linh Station for Biodiversity, Vietnam.

Erklärung (gemäß § 4 Abs. (1) Nr.9)

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen - , die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von unten angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von apl. Prof. Dr. Thomas Ziegler und Prof. Dr. Michael Bonkowski betreut worden.