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

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Abstract

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

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

Resumen

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

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

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Introduction

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

Accounting for approximately only 10 % (460,000 km²) in Southeast Asia, karst ecosystems are still considered an 'ark' of biodiversity, containing an extraordinarily high level of endemism (Day and Urich, 2000; Clements et al., 2006; Luo et al., 2016; Tolentino et al., 2020). Giving an example of the gekkonid genus *Cyrtodactylus*, comprising approximately 300 species mostly native to South East Asia, the karstic habitats (which far out–number all others) are not simply refugia but rather a mosaic of unique micro–niches forcing speciation in disjunct outcrops (Clements et al., 2006; Grismer et al., 2020). However, unsustainable human activities (particularly limestone quarrying) are endangering the biodiversity of karst ecosystems in Southeast Asia (Clements et al., 2006; Luo et al., 2016; Grismer et al., 2020).

The Huulien Tiger gecko, *Goniurosaurus huuliensis* Orlov, Ryabov, Nguyen, Nguyen and Ho 2008, was originally described from the Huu Lien Nature Reserve (NR) in Lang Son Province, northern Vietnam (Orlov et al., 2008; Nguyen et al., 2009; Nguyen, 2011). Being a habitat specialist, this endemic tiger gecko is only found in evergreen forests on karst formations (Orlov et al., 2008). Thus, *G. huuliensis* is particularly susceptible to the increase of anthropogenic threats that lead to habitat fragmentation and degradation. Additionally, Ngo et al. (2019a, 2019b) documented that a large number of wild tiger geckos have been illegally harvested for both local and international pet trade. Climate change may also have potentially negative impacts on wild populations of *G. huuliensis*

in the future (Ngo et al., 2021a). Consequently, the species was recently listed as Critically Endangered (CR) in the IUCN Red List (Nguyen, 2018), and included in CITES Appendix II and the Vietnam Government's Decree No. 06/2019/ND–CP (Group IIB) in 2019 (Ngo et al., 2019b). Although the tiger gecko is dramatically affected by severe threats, a comprehensive assessment of population status of the target species is still lacking.

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

Material and methods

Field surveys

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

We conducted surveys during the non–hibernation season of *Goniurosaurus* in April and August 2019, June 2020 in Lang Son Province, and April 2021 in Thai Nguyen Province. To guarantee the highest

detection probability for the nocturnal species (Orlov et al., 2008; Nguyen, 2011) and to limit the bias of observers, excursions were carried out between 8 p.m. and 5 a.m. of the next day. Each survey in each transect was conducted with at least two researchers, together with a ranger and a local person.

Population assessment

Due to its small body size and distribution within remote areas (Orlov et al., 2008; Ngo et al., 2021a), estimating population size of *G. huuliensis* based on direct records is deemed problematic. Huang et al. (2008) used statistical data from a 'capture mark–recapture' approach, developing the 'invisibility rate' method to estimate the population size. This method has been applied for other lizards in Vietnam, namely *Cnemaspis psychedelica*, *Cyrtodactylus gialaiensis*, *Goniurosaurus catbaensis*, *Physignathus cocincinus*, and *Shinisaurus crocodilurus* (van Schingen et al., 2014, 2016; Ngo et al., 2016a, 2019a; Nguyen et al., 2018; Gewiss et al., 2020; Luu et al., 2020). Accordingly, all encountered individuals of *G. huuliensis* were captured and individually marked with passive integrated transponder (PIT) tags (ISO FDX–B Glastransponder, 1.4 x 9 mm, Germany). In newly captured individuals, a microchip was injected under the skin on the left side of the body behind the shoulder. All captured and recaptured individuals were identified with a transponder–reader (Breeder Reader IC, Planet ID GmbH, Germany) (van Schingen et al., 2014; Ngo et al., 2019b). Each transect was repeatedly surveyed in intervals of at least two days. Coordinates and elevations of all capture localities were recorded using a GPS device (Garmin GPSmap62s, WGS84 datum) and are available on request to the authors. Geckos were captured by hand and subsequently released at the same sites after taking measurements and marking.

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

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

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

$$Ň = \Sigma [m*(1 + (i/x))]$$

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

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

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

$$Ň_S / A_S = Ň / A_F$$

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

$$Ň_{max.} = (Ň \times A_K) / A_F$$

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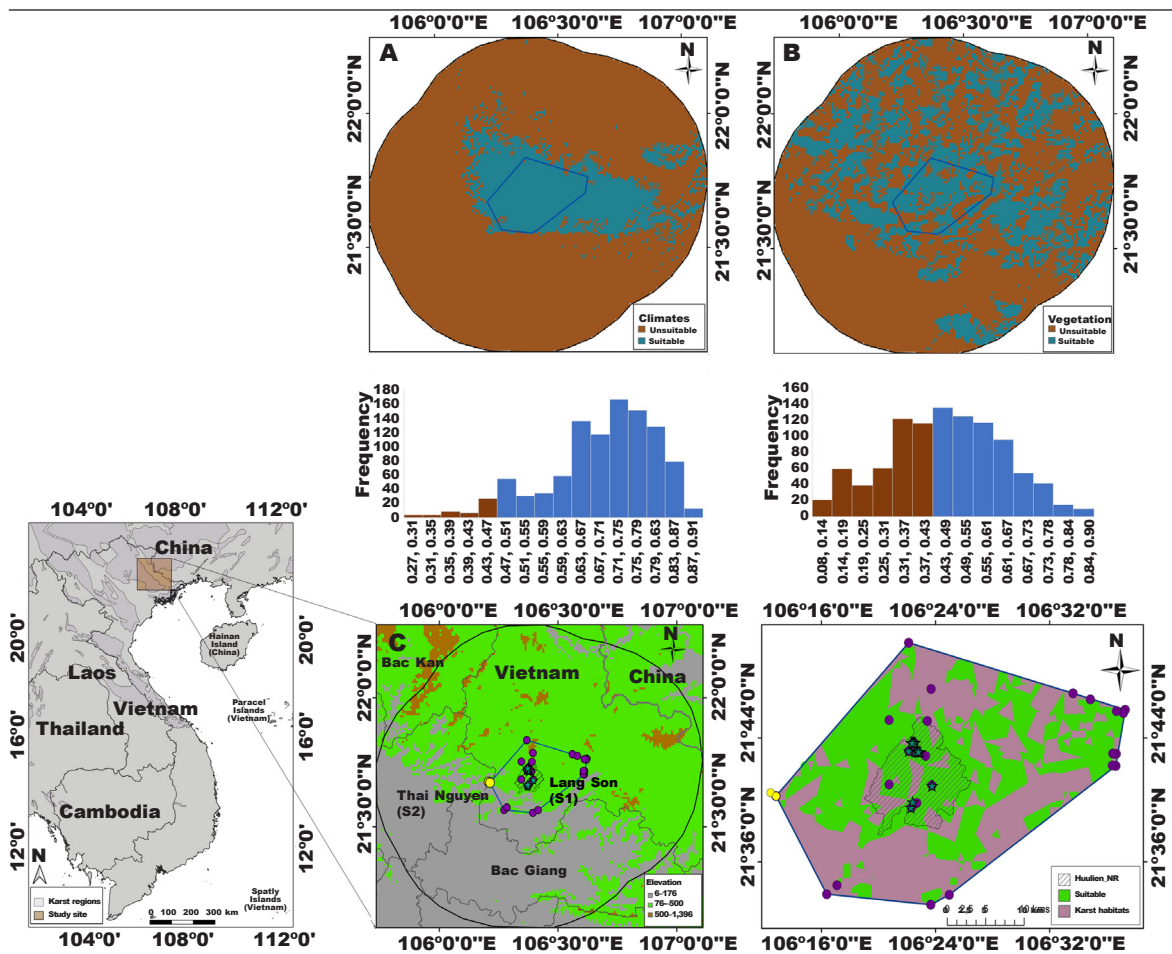
Fig. 1. Projected potential distribution of *Goniurosaurus huuliensis* in northern Vietnam: A, the climate model; B, the vegetation cover model, including histograms of suitable probability within the extent of occurrence (brown columns as unsuitable and blue columns as suitable); C, the suitable elevations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

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

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

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

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

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

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

Table. 1. (Cont.)

Transect	T1	T2	T3	T4	T5	T6	Total
Maximum abundance: survey area 32 km²							
Total obs.	5	5	17	10	2	14	53
$\check{N}$	11	11	38	9	4	39	112
$\check{N}$ Huu Lien NR (85 km²)							298
$\check{N}_S$ (408 km²)							1,428
$\check{N}_{max}$ (805 km²)							2,818

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

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

Threat assessment

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

Results

Population status

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

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

To estimate the total population size of *G. huuliensis*, we extracted respective layers of the survey sites and calculated their areas according to the survey periods (15 km² in April 2019; 21 km² in August 2019; 12 km² in June 2020 in S1; and 11 km² in April 2021 in S2), the area of the Huu Lien NR (85 km²) and suitable habitats (408 km²) within the whole extent of occurrence (EOO, 805 km²) (table 1, fig. 1). The extracted karst area is equal to the EOO (table 1). The Huu Lien NR was estimated to harbor 142–289 indiv. (238 ± 67 indiv.). The population size ($\check{N}_S$) within the suitable habitats was estimated to range from 680 to 1,447 indiv. (1,157 ± 293 indiv.), whereas the maximum population size ($\check{N}_{Max}$) in the karst habitats can achieve up to 2,855 individuals (table 1). We further calculated a potential maximum population size based on the maximum number of animals within each
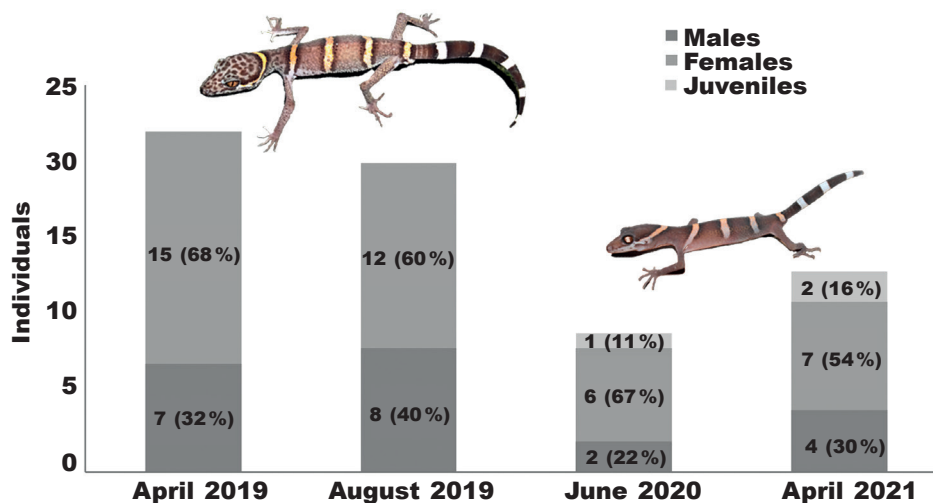
Fig. 2. Variations in the age and sex structures of *G. huuliensis* between survey periods in Lang Son (S1) and Thai Nguyen (S2) provinces, northern Vietnam (individuals observed but not caught were not included herein).

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

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

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

Anthropogenic impacts

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

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

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

Discussion

Population status

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

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

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

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

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

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

Threats

The species has been illegally over–harvested by local hunters in the Huu Lien NR in the past (Ngo et

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

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

Besides serving as preferred habitat and refugia for almost tiger gecko species under impacts of climate change, the karstic ecosystem containing complex topographies and unique micro–environmental conditions is considered the ancestral habitat of *Goniurosaurus* species (Grismer et al., 2021; Ngo et al., 2021b). Karst formations indeed are identified as the exclusive habitat of *G. huuliensis* (Ngo et al., in press); as such, its existence depends entirely on the integrity of karstic habitats. Similar to what is happening all over Southeast Asia, as documented by Clemens et al. (2006), the high rate of limestone quarrying is a primary threat to *G. huuliensis,* which has been extensively recorded in Lang Son and Thai Nguyen provinces, including habitats of the species in Huu Lien NR. As such, the newly undisturbed but

unprotected population of *G. huuliensis* in Thai Nguyen Province should be classified as at a low level of threat. To meet the local high demand for limestone products (e.g. cement), more cement factories could be built in Lang Son and Thai Nguyen provinces in the future, including the karst habitats of *G. huuliensis* and directly imperils untouched populations of *G. huuliensis*. Other human activities (namely logging activities, road construction, and pollution by plastic trash) have been observed within natural habitats of *G. huuliensis*, leading to forest fragmentation and degradation.

Conservation measures

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

Covering a total area of 85 km², the Huu Lien NR potentially harbors an estimated maximum number of 289 indiv. (accounting for approximately 15 % of the total population size). Based on this result, we strongly support a recommendation of Ngo et al. (2021a) that the Huu Lien NR should be considered as a 'center' for conservation programs to protect wild populations of *G. huuliensis*. However, approximately 85 % of the total wild individuals of *G. huuliensis* may be located outside the boundaries of the nature reserve. Without proper law enforcement and forest ranger patrolling in there, a plan to establish a species and karst habitat conservation area within the EOO is an adequate conservation solution to safeguard the wild populations of *G. huuliensis*. As such, priorities for the local economic development relative to karst formations (limestone quarrying) can be reassessed together with priorities

of biodiversity protection by relevant authorities. We highly recommend increasing research activities to promote the biodiversity value of karst ecosystems. Recently, many new species have been discovered from the karst habitats in the Indo–Burma biodiversity hotspot region, including tiger gecko species in China and Vietnam (Nguyen et al., 2009; CEPF, 2020; Ngo et al., 2021b). Globally threatened species, such as *G. huuliensis* should be highlighted as high–profile flagship species of karst ecosystems. The high value of biodiversity should be given with the priority of conservation policies to protect the karst landscape rather than favoring economic development.

Methodological limitations

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

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

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