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First record of *Lepidodactylus lugubris* (Duméril & Bibron, 1836) (Squamata, Gekkonidae) from Hainan Island, China

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Abstract

Lepidodactylus lugubris is known from tropical Asia, Oceania, and Latin America, but in China it was previously known only from Taiwan Island. In this paper, we report a new herpetofaunal record based on one specimen collected from Wanning, Hainan, China, which conforms to *L. lugubris* on both morphological and molecular data. Our finding brings the total species of the family Gekkonidae in Hainan to six.

Key Words

morphology, mourning gecko, ND2, new record, non-native

Introduction

The genus *Lepidodactylus* Fitzinger, 1843, currently contains 45 species that are distributed from Southeast Asia to Indo-Australia and Oceania, the majority of which are endemic to, or with narrow-range in, tropical islands (Uetz et al. 2022). By contrast, the most wide-ranging species of this genus is undoubtedly the mourning gecko *Lepidodactylus lugubris* (Duméril & Bibron, 1836). It has spread to tropical Asia, Oceania, and Latin America (introduced) from tropical Indo-Pacific islands (where it is thought to be native) in past decades, due to parthenogenetic reproduction and resistance of eggs to desiccation and saltwater spray (Cuellar and Kluge 1972; Brown and Duffy 1992; Bauer and Henle 1994; Hoogmoed and Avila-Pires 2015; Lapwong and Juthong 2018; Urra et al. 2020). The parthenogenetic lizard is comprised of several diploid and triploid clones that originated by hybridization between *Lepidodactylus moestus* (Peters, 1867) and a species that was undescribed until recent research concluded that it was possibly *Lepidodactylus pantai* Stubbs, Karin, Arifin, Iskandar, Arida, Reilly, Bloch, Kusnadi & McGuire, 2017 (Radtkey et al. 1995; Fujita and Moritz 2010; Ineich 2015; Karin et al. 2021). *Lepidodactylus lugubris* is a nocturnal species usually found near the coast, inside houses or on trees (Señaris et al. 2017; Behm 2018; Nania et al. 2020). Its spread is often thought to be possibly related to human activity, such as artificial transportations and cargo shipping (Kraus 2009; Nania et al. 2020). In China, *L. lugubris* was previously known only from Taiwan Island as a controversial native species (Ota 1986; Lee et al. 2019).

During our field survey from April to December 2021, a gekkonid lizard with a small and elongated body was collected in Hainan Island, China. Morphological

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comparison and molecular analysis indicated that this individual belongs to the species *Lepidodactylus lugubris*. Herein, we report this new herpetofaunal record from Hainan Island, China in detail.

Materials and methods

Field surveys were conducted in Shimei Gulf, Wanning City, Hainan Province. The specimen was euthanized and then fixed in 10% buffered formalin, later transferred to 75% ethanol. It is deposited in Shenyang Normal University (**SYNU**), Shenyang, China. Liver tissue sample was preserved in 95% ethanol for molecular analysis.

Genomic DNA was extracted from liver tissue using a DNA extraction kit (Tiangen Biotech Co., Ltd, Beijing). The mitochondrial fragment of the NADH dehydrogenase subunit 2 gene (ND2) sample was sequenced using primer rMet-3L (5'- ATACCCCGACAATGTTGG-3') and rAla-1H (5'- GCCTTAGCTTAATTAAAGTG-3') (Jonniaux and Kumazawa 2008). The PCR procedure was performed with an initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 40 s, 53 °C for 40 s and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. PCR products were purified with spin columns and then sequenced with a forward primer using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA). Sequencing was performed on an ABI Prism 3730 automated DNA sequencer by Wuhan Tianyi Huiyuan Bioscience and Technology Inc.

Measurements were taken following Zhao et al. (1999) and Kraus and Oliver (2020). All external measurements were made using digital calipers to the nearest 0.1 mm: **SVL** (from tip of snout to vent); **TrL** (trunk length from posterioredge of forearm insertion to anterior edge of hindleg insertion); **TL** (from vent to tip of tail); **TW** (maximum tail width); **HL** (head length from tip of snout to anterior margin of ear opening); **HW** (maximum head width); **HH** (maximum head height); **FA** (forearm length from central base of palm to elbow); **CS** (crus length from central base of heel to knee); **Ear** (maximum ear diameter); **EE** (shortest ear-to-eye distance); **EY** (greatest eye diameter); **EN** (eye-naris distance from anteriormost point of eye to posteriormost point of naris); **SN** (snout length from anteriormost point of eye to tip of snout); **IN** (internarial distance between centers of nares); **EE** (shortest ear-to-eye distance); **RW** (maximum rostral width); **RH** (maximum rostral height); **MW** (maximum mental width); **ML** (maximum mental length); **T4L** (length of the fourth toe from terminal lamellae to the beginning of the web between T3 and T4); **T4W** (maximum width of the fourth toe); **T4lamellaeL** (length of the series of lamellae on the fourth toe); **T3T4webL** (length of webbing between T3 and T4 from base of this webbing to its centre of emargination) and **T4T5webL** (length of webbing between T4 and T5 from base of this webbing to its center of emargination). In addition, scalation features include supralabials, infralabials, lamellae under T1 and T4, and divided lamellae under T4. Bilateral scale counts are given as left/ right.

Phylogenetic analysis

Twenty-one sequences from seven known *Lepidodactylus* species plus one out-group sequence from the gekkonid *Hemiphyllodactylus huishuiensis* which was used to root the tree were obtained from GenBank and comprised the dataset (Table 1). Sequences were aligned in Clustal X 2.0 (Thompson et al. 1997) with default. The alignment was checked and manually revised if necessary. Gaps/ Missing Data Treatment use the complete-deletion option in MEGA 6.06, substitutions to included: Transitions + Transversions option (Tamura et al. 2013). We ran Jmodeltest v2.1.2 (Darriba et al. 2012) with Akaike and Bayesian information criteria on the alignment, resulting the best-fitting nucleotide substitution models of GTR + I + G. Phylogenetic analysis was constructed using Bayesian inference (BI) in MrBayes 3.2.4 (Ronquist et al. 2012). Two independent runs with four Markov Chain Monte Carlo simulations were performed for ten million iterations and sampled every 1000 iterations. The first 25% of samples were discarded as burn-in. Convergence of the Markov Chain Monte Carlo simulations was assessed with PSRF ≤0.01 and ESS (effective sample size) value > 200 using Tracer 1.4 (http://tree.bio.ed.ac.uk/software/tracer/). Uncorrected pairwise sequence divergences utilizing the ND2 gene were calculated using MEGA 6.

Results

The ML and BI analyses resulted in identical topologies (Fig. 1). Uncorrected pairwise sequence divergences are reported in Table 2. As illustrated by the phylogenetic result, the *Lepidodactylus* sample from Hainan, China is clustered together with other *Lepidodactylus lugubris* samples and exhibit robust monophyletic lineage with strong support (BPP 1.00) and low intrapopulational genetic differentiation (0.13–0.76%; Table 2). Thus, we regard this specimen as representing a new herpetofaunal record from Hainan Island, China.

Taxonomic account

Lepidodactylus lugubris (Duméril and Bibron, 1836)

Specimen examined. SYNU210417 (adult female) was collected by Ming-Hong Huang, Hao-Tian Wang, Xian-Chun Qiu and Pi-Peng Li on 1 May 2021 from Shimei Gulf, Wanning City, Hainan Province, China (18°40'01"N, 110°17'16"E; at an elevation of 5 m).

Morphological description. Morphological characters of the specimen agreed well with the Duméril and Bibron's original description (translated by Amarasinghe et al. 2009) and subsequent description of Zhao et al. (1999) in Chinese.

Table 1. Localities, voucher information, and Genbank accession numbers for all specimens used in this study.

Species name	Locality	Specimen voucher	Genbank accession number	References
Ingroup				
Lepidodactylus aureolineatus	Mindanao, Philippines	ACD6367	MG780700	Oliver et al. 2018
Lepidodactylus aureolineatus	Mindanao, Philippines	ACD6368	MG780701	Oliver et al. 2018
Lepidodactylus balioburius	Batan, Philippines	KU314019	MG780706	Oliver et al. 2018
Lepidodactylus balioburius	Batan, Philippines	KU314020	MG780707	Oliver et al. 2018
Lepidodactylus christiani	Negros, Philippines	ABTC32655	MG780711	Oliver et al. 2018
Lepidodactylus herrei	Leyte, Philippines	RMB4330	JQ173539	Siler et al. 2012
Lepidodactylus herrei	Leyte, Philippines	RMB4331	JQ173540	Siler et al. 2012
Lepidodactylus lugubris	Shimei Gulf, Wanning, Hainan, China	SYNU210417	ON416995	This study
Lepidodactylus lugubris	Sulawesi, Indonesia	RMB1436	MG780756	Oliver et al. 2018
Lepidodactylus lugubris	Daru Islandn, Papua New Guinea	CCA16060	MG780750	Oliver et al. 2018
Lepidodactylus lugubris	Daru Islandn, Papua New Guinea	CCA16127	MG780751	Oliver et al. 2018
Lepidodactylus lugubris	Woodlark Island, Papua New Guinea	BPBM39154	MG780747	Oliver et al. 2018
Lepidodactylus lugubris	Luzon, Philippines	ACD2589	MG780730	Oliver et al. 2018
Lepidodactylus lugubris	Luzon, Philippines	ACD2593	MG780731	Oliver et al. 2018
Lepidodactylus lugubris	Masbate, Philippines	KU302816	MG780752	Oliver et al. 2018
Lepidodactylus lugubris	Ticao, Philippines	KU302817	MG780753	Oliver et al. 2018
Lepidodactylus lugubris	Singapore	ZRC24847	JN393944	Heinicke et al. 2011
Lepidodactylus lugubris	Mole Island, Solomon Island	ABTC50415	MG780728	Oliver et al. 2018
Lepidodactylus lugubris	Suriname	MVZ247594	JX515614	Heinicke et al. 2012
Lepidodactylus pantai	Kei Kecil, Indonesia	MVZ: Herp: 295037	MZ189429	Oliver et al. 2018
Lepidodactylus pantai	Kei Kecil, Indonesia	MVZ: Herp: 295038	MZ189430	Oliver et al. 2018
Lepidodactylus planicaudus	Mindanao, Philippines	ACD1606	MG780773	Oliver et al. 2018
Outgroup				
Hemiphyllodactylus huishuiensis	Huishui, Guizhou, China	NJNUh00729	KU519707	Yan et al. 2016

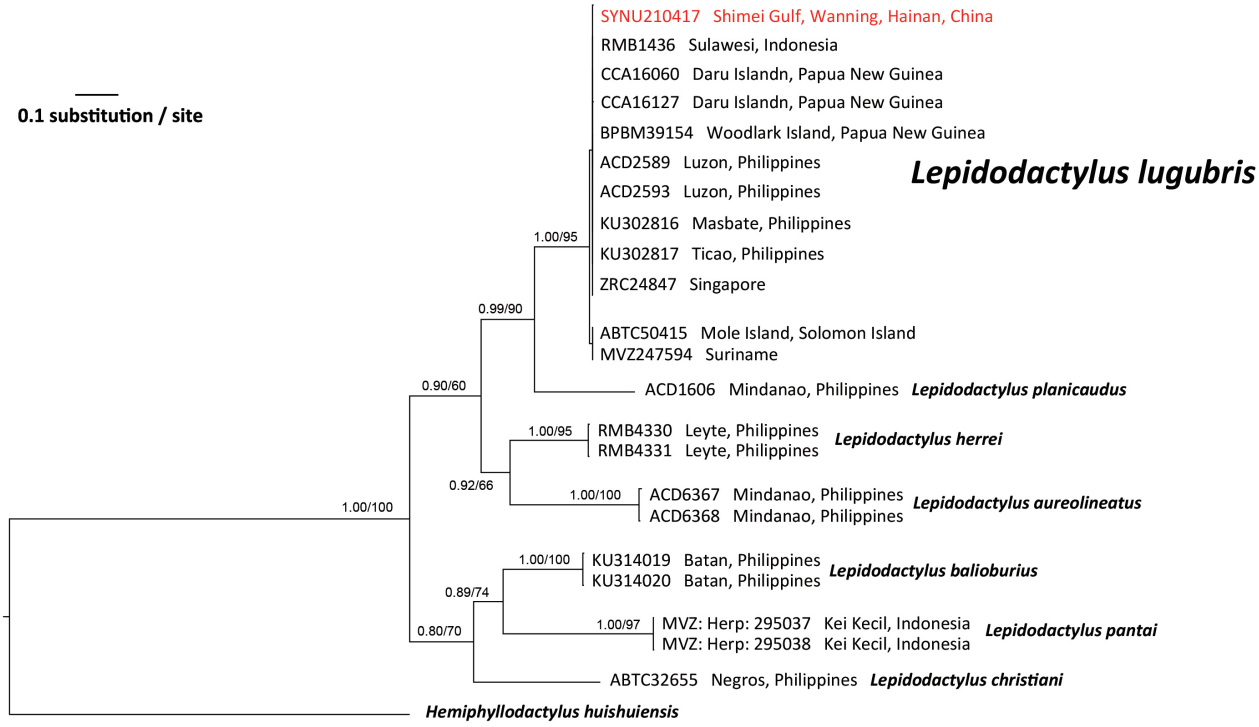

Figure 1. Bayesian inference tree of seven species of *Lepidodactylus*, based on the partial DNA sequences of the mitochondrial ND2 gene. Numbers before slash indicate Bayesian posterior probabilities (BPP) and numbers after slash are bootstrap support for ML (1000 replicates) analyses.

A mature female of medium size (SVL 41.3 mm, TrL 19.9 mm); head depressed, longer than wide (HL/HW 1.17), distinct from neck; snout tapered and rounded at tip, relatively long (SN/HL 0.54), approximately twice of eye diameter (SN/EY 2.2); rostral quadrangular, nearly twice ae wide as high (RW/RH 2.02) and wider than mental (RW/MW 1.53), touching first supralabial and supranasal on each side; nostrils oval, rounded by rostral, first supralabial, supranasal, and two nasals posteriorly; posterior nasal region concave; internasals 2; preorbitals 14/14, preorbital region concave ;eye large (EY/HL 0.25, EY/EN 0.63), pupil vertical, elliptic; ear opening small (Ear/HL 0.053), oval, horizontal, smaller than eye (Ear/EY 0.21); distance between ear and eye slightly larger than eye diameter (EE/EY 1.20);

Table 2. Uncorrected *P*-distance of ND2 gene among seven *Lepidodactylus* species used in this study.

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	*L. lugubris*	SYNU210417																					
2	*L. lugubris*	RMB1436	0.13																				
3	*L. lugubris*	CCA16060	0.13	0.00																			
4	*L. lugubris*	CCA16127	0.13	0.00	0.00																		
5	*L. lugubris*	BPBM39154	0.13	0.00	0.00	0.00																	
6	*L. lugubris*	ACD2589	0.13	0.00	0.00	0.00	0.00																
7	*L. lugubris*	ACD2593	0.13	0.00	0.00	0.00	0.00	0.00															
8	*L. lugubris*	KU302816	0.13	0.00	0.00	0.00	0.00	0.00	0.00														
9	*L. lugubris*	KU302817	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00													
10	*L. lugubris*	ZRC24847	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00												
11	*L. lugubris*	MVZ247594	0.76	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63											
12	*L. lugubris*	ABTC50415	0.76	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.00										
13	*L. aureolineatus*	ACD6367	26.03	25.82	25.82	25.82	25.82	25.82	25.82	25.82	25.82	25.82	25.64	25.64									
14	*L. aureolineatus*	ACD6368	25.64	25.44	25.44	25.44	25.44	25.44	25.44	25.44	25.44	25.44	25.26	25.26	0.50								
15	*L. balioburius*	KU314019	33.42	33.37	33.37	33.37	33.37	33.37	33.37	33.37	33.37	33.37	32.40	32.40	29.92	30.34							
16	*L. balioburius*	KU314020	33.42	33.37	33.37	33.37	33.37	33.37	33.37	33.37	33.37	33.37	32.40	32.40	29.72	30.15	0.25						
17	*L. christiani*	ABTC32655	31.68	31.63	31.63	31.63	31.63	31.63	31.63	31.63	31.63	31.63	31.25	31.25	34.66	34.81	25.10	25.10					
18	*L. herrei*	RMB4330	21.06	20.87	20.87	20.87	20.87	20.87	20.87	20.87	20.87	20.87	20.37	20.37	23.68	23.65	31.38	31.38	30.96				
19	*L. herrei*	RMB4331	21.03	20.84	20.84	20.84	20.84	20.84	20.84	20.84	20.84	20.84	20.35	20.35	23.83	23.80	31.33	31.33	31.00	0.38			
20	*L. pantai*	MVZ: Herp: 295037	35.16	35.11	35.11	35.11	35.11	35.11	35.11	35.11	35.11	35.11	34.90	34.90	35.41	35.15	25.35	25.35	29.93	34.93	34.89		
21	*L. pantai*	MVZ: Herp: 295038	35.36	35.31	35.31	35.31	35.31	35.31	35.31	35.31	35.31	35.31	35.10	35.10	35.62	35.35	25.52	25.52	30.12	34.73	34.69	0.13	
22	*L. planicaudus*	ACD1606	18.39	18.21	18.21	18.21	18.21	18.21	18.21	18.21	18.21	18.21	18.36	18.36	28.10	27.34	32.07	32.07	33.62	25.49	25.46	34.33	34.53

mental pentagonal, wider than long (MW/ML 1.51); mental bordered posteriorly by three rows of enlarged gulars that are followed by smaller granular chin scales, distinct chin shields absent; supralabials 12/12; infralabials 9/9.

Body elongated (TrL/SVL 0.48); dorsal scales on head, body, limbs, and throat smooth, granular and juxtaposed in row; tubercles absent; ventrals distinctly larger than dorsal scales, flat, smooth, imbricate, gradually becoming granular from the middle to the sides ; ventral scale rows at midbody 41; scale rows around midbody 119; ventral scales in a row between mental and cloacal 167; precloacal scales enlarged, but no enlarged scales on thighs; six rows tiny scales between enlarged precloacal scales and vent; scales on palms and soles smooth; precloacal or femoral pores absent.

Fore- and hindlimbs relatively small but well-developed (FA/SVL 0.12, CS/SVL 0.14); digits well-developed, moderately dilated (T4W/T4L 0.32); all fingers and toes with claws, except the first; claws laterally depressed, extending slightly beyond terminal lamellae; subdigital lamellae narrow and smooth, II-V fingers and toes with 4 divided terminal lamellae, I fingers and toes with undivided terminal lamellae; lamellae extend for more than half length of each toe (T4lamellaeL/T4L 0.63); lamellae under first finger 9/9, under fourth finger 15/15, under first toe 12/12, under fourth toe 12/12; relative lengths of digits on manus and pes I < II < V< III < IV; web present on fingers and toes, toes only one-fifth webbed (T3T4webL/T4L 0.21).

Tail regenerated behind cloacal sacs, moderately laterally depressed, significantly shorter than body (TL/SVL 0.67), relatively wide (TW/SVL 0.13); tail slightly thickened at base, postcloacal tubercles absent; caudals small, flat, smooth, larger than dorsals, slightly larger ventrally than dorsally; divided terminal (Figs 2, 3).

Measurements. SVL 41.3 (mm), TrL 19.9, TL 27.6 (regeneration), TW 5.2, FA 5.2, CS 6.0, HL9.9, HW 8.4, Ear 0.5, EE 3.0, EY 2.5, SN 5.4, EN 4.0, IN 2.0, RW 1.5, RH 0.7, MW 1.0, ML 0.7, T4L 3.8, T4W 1.2, T4lamellaeL 2.4, T3T4webL 0.9, T4T5webL 1.0.

Coloration. In life, dorsal surface of head reddish brown; a narrow dark brown stripe along the canthus rostralis, crossed the eye, to near shoulder; the major colors of the body, limbs and tail are yellowish sepia, as is the head; a series of indistinct W-shaped markings down the center of the neck and tail, each angular base of the W usually with a small blackish spot, especially the markings at the neck and the base of tail; dark brown stripes all over the dorsal limbs; some light and dark spots on dorsal tail; ventral surface of head milk white, ventral body coverd by lemon yellow band, with brownish spots on each side; ventral tail light brown in forepart and gradual deepening towards the end with dark brown spots (Fig. 2A, B).

In preservative, dorsal surface of specimen grayish white, ventral surface discolored to milky white; the W-shaped patterns indistinct, leaving mostly black bars (Fig. 3A, B).

Distribution and ecological notes. Currently *L. lugubris* is known from almost all over tropical Asia (China, Sri Lanka, India, Myanmar, Malaysia, Vietnam, Japan, Indonesia etc.), Indo-Pacific (Christmas Island)and Oceania (Fiji Islands, Rotuma, New Caledonia, Loyalty Islands, Vanuatu, Tonga etc.), even introduced into the continent of the Americas and the Caribbean (Behm 2018; Urra et al. 2020; Uetz et al. 2022). In China, this species is only known on Hainan Island (Wanning City) and eastern and southern Taiwan Island (including Orchid Island and Green Island; Lee et al. 2019).

The specimen was found at 20:30 on a banyan tree about 1.5m above the ground near a coastal beach (Fig. 2C). Two opal eggs were visible through the skin of the belly.

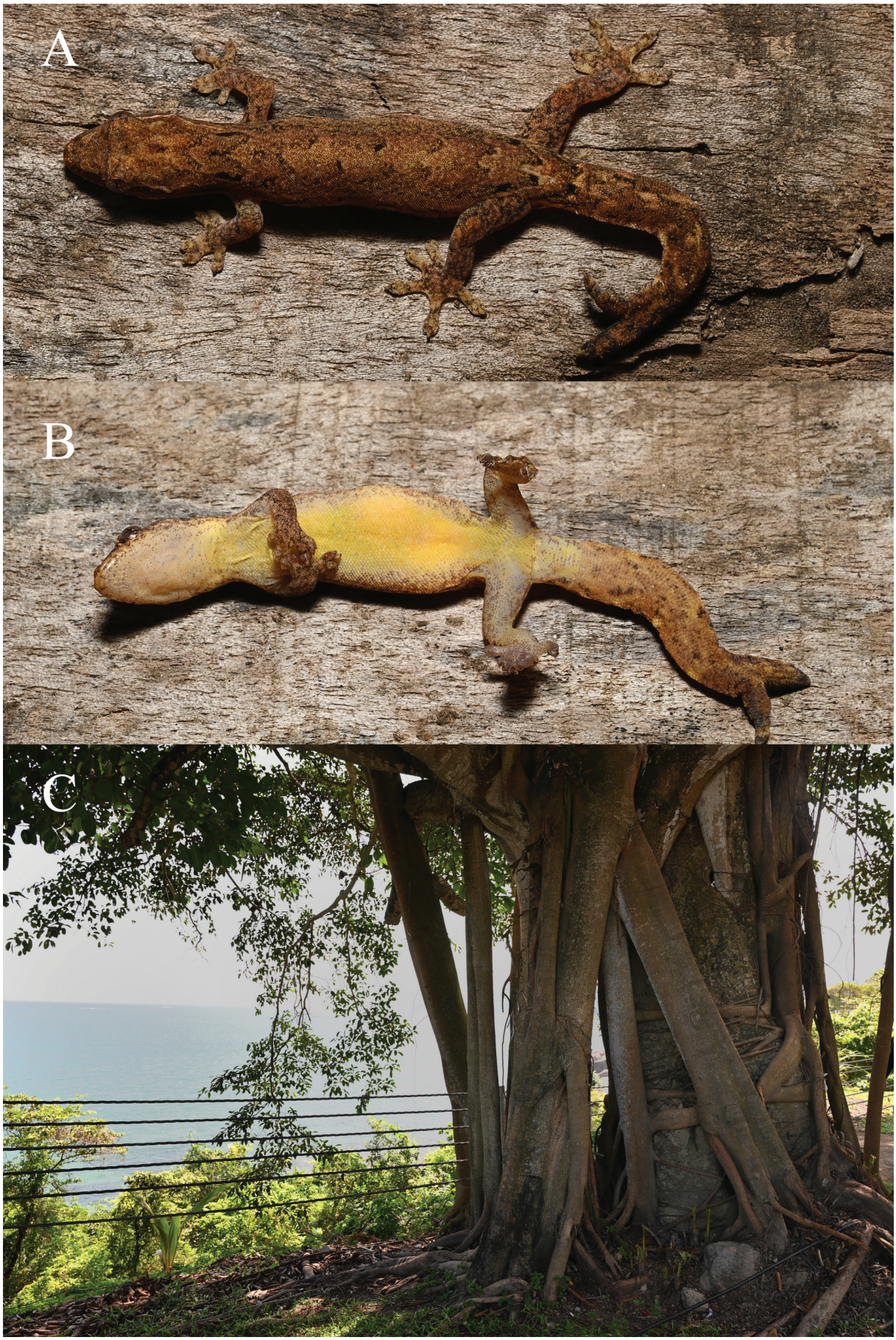

Figure 2. The specimen (SYNU210417) collected from Shimei Gulf, Wanning City, Hainan Province, China in life and its micro habitats. **A.** Dorsal view; **B.** Ventral view; **C.** Micro-habitats.

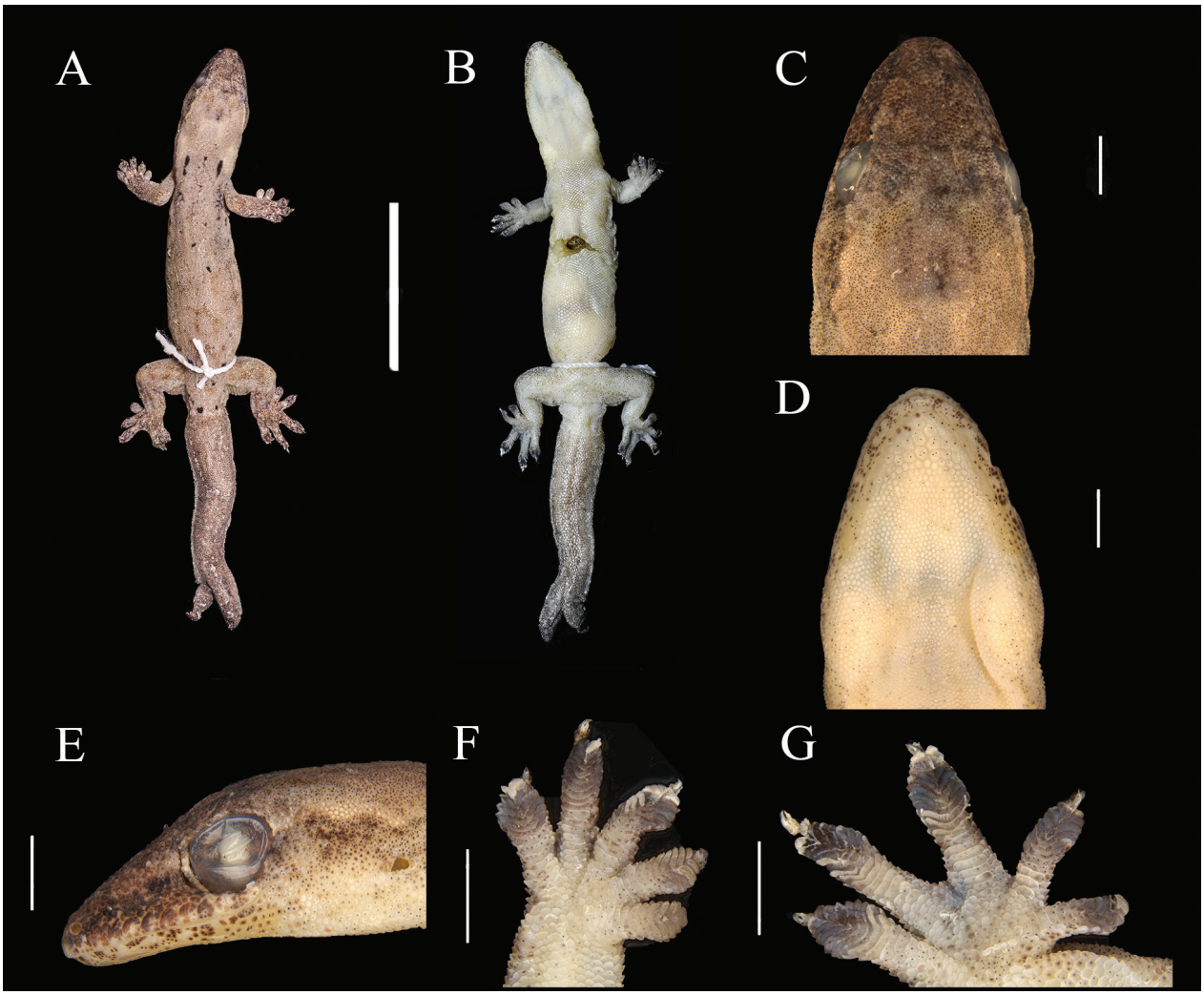

Figure 3. Morphological features of specimen SYNU210417 in preservative. **A.** Dorsal view of entire body; **B.** Ventral view of entire body; **C.** Dorsal view of head; **D.** Ventral view of head; **E.** left head; **F.** right hand; **G.** right foot. Scale bars: 2cm (**A, B**); 2mm (**C–G**).

Discussion

The discovery of the new record brings the total species of Gekkonidae in Hainan Province to six. The five species known from the province are *Gekko (Japonigekko) similignum* Smith, 1923, *Hemidactylus bowringii* (Gray, 1845), *H. garnotii* Duméril & Bibron, 1836, *H. frenatus* Duméril & Bibron, 1836, and *Gehyra mutilata* (Wiegmann, 1834). It is noted that *Lepidodactylus* represents a new genus for Hainan Province.

The species *L. lugubris* actually contains several diploid and triploid clones, in which diploid clone A and triploid clone C are similar and confused on morphology (Ineich 1988, 1999). Based on previous studies, Ineich (2015) summarized the morphological differences between clone C and other clones when studying samples from the Wallis and Futuna Islands. The main morphological differences include the black stripes on the sides of the W-shape on the neck that never disappeared and the ventral surface covered by lemon yellow band. By these two characteristics, we can easily determine that the Hainan specimen belongs to clone C (Fig. 2). This clone is widespread but uncommon (Ineich 2015). Hence, the origin of *L. lugubris* in Hainan Island is awaiting further study. Meanwhile, given that the species has not been found on the island for a long time and that it is a well-known invasive species, we tend to consider it a non-native species.

Nania et al. (2020) reconstructed the geographic expanding history of *L. lugubris* and predicted further expansion of its geographic range over time, in which Hainan Island was considered one of the suitable areas for the species. Although the existence of stable populations in Hainan Island needs to be surveyed once again, our findings tentatively support this hypothesis.

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