



Goniurosaurus gezhi sp. nov., a new gecko species from Guangxi, China (Squamata: Eublepharidae)

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Abstract

Five species of geckos in the genus *Goniurosaurus* had been recorded from Guangxi, China. Here we describe a new species, *Goniurosaurus gezhi* sp. nov. Zhu, He & Li. The new species is similar to those found in Guangxi and Guizhou provinces of China and Northern Vietnam, but unique in a combination of the following characters: (1) three body bands between limb insertions; (2) precloacal pores 18–20; (3) body small (SVL=70.6–83.8 mm); (4) body color orange to yellow. We evaluated the phylogenetic position of this new species based on the *16S* mitochondrial gene. Molecular phylogenies validate this new species as distinct to currently described lineages within *Goniurosaurus*. The type specimens are deposited in the Museum of Biology, East China Normal University (ECNU).

Key words: Gekkota, taxonomy, *16S*, karst, Guangxi, China

Introduction

The genus *Goniurosaurus* includes 20 described species (Mocquard 1897; Orlov & Darevsky 1999; Grismer *et al.* 1999, 2002; Orlov *et al.* 2008; Ziegler *et al.* 2008; Wang *et al.* 2010, 2013, 2014; Honda *et al.* 2014; Yang & Chan 2015; Honda & Ota 2017; Zhou *et al.* 2018; Zhu *et al.* 2020). A recent phylogenetic study recovered four major lineages of *Goniurosaurus* that were closely associated with their geographical distributions (Liang *et al.* 2018). One of the largest clades within *Goniurosaurus* is the *G. luii* group. Species of this group are distributed in Guangxi and Guizhou provinces of China and Northern Vietnam (Liang *et al.* 2018). In Guangxi, five species have been recorded and are mainly associated with karst landscapes (*G. araneus* Grismer, Viets & Boyle, *G. luii* Grismer, Viets & Boyle, *G. kadoorieorum* Yang & Chan, *G. kwangsiensis* Yang & Chan, and *G. lichtenfelderi* Mocquard) (Grismer *et al.* 1999; Yang & Chan 2015). In 2018–2019, we conducted multiple surveys in karst areas in Guangxi. These surveys led to the collection of a previously unknown *Goniurosaurus* species. Here, we describe this taxon as a new species, and validate this species using molecular phylogenies.

Material and methods

Sampling. We collected specimens of the new species by conducting visual survey encounters in karst areas along the ground at night. We collected five males of the new species and used Personalbio Saliva/Swab Genomic DNA Kit to collect DNA (Shanghai Personalbio Biotechnology Co., Ltd.). We followed the protocol by swabbing the

mouth of each individual for about 10 seconds. The saliva DNA samples were preserved at room temperature. We preserved three vouchers in 75% alcohol (ECNU-V0038, 0040, 0042) used in the description, and released the other two individuals. The general collection locality for each specimen is provided in Table 1. All specimens were deposited in the Museum of Biology, East China Normal University (ECNU).

DNA extraction and gene amplification. To validate this new species and understand the evolutionary history of the *G. luyi* group, we sequenced the *16S* ribosomal RNA gene from five individuals of the new species, three *G. kadoorieorum* samples, and one *G. araneus* individual (Table 1). Total genomic DNA was extracted from the saliva DNA samples according to the manufacturer's instructions. We amplified one mitochondrial gene: *16S* rRNA gene (482–503 bp). Primers for PCR amplification are r16S-5L GGTTMMYGCCTGCCAGTG and 16Sbr-H CCG-GTCTGAACTCAGATCACGT (Jonniaux & Kumazawa 2008). Amplification conditions for PCR were as follows: pre-denaturation for 2 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72 °C, then a final extension at 72 °C for 7 min. All PCR products were sequenced directly with the same primers. GenBank accession numbers are provided in Table 1.

TABLE 1. GenBank accession number for each sequence used in this study, * = type specimen.

Species	Specimen ID	Locality	<i>16S</i>	References
<i>G. gezhi</i> sp. nov.	ECNU-V0038*	China, Guangxi, Southwest	MT533260	this study
<i>G. gezhi</i> sp. nov.	ECNU-V0040*	China, Guangxi, Southwest	MT533261	this study
<i>G. gezhi</i> sp. nov.	ECNU-V0042*	China, Guangxi, Southwest	MT533262	this study
<i>G. gezhi</i> sp. nov.	ECNU-V0046	China, Guangxi, Southwest	MT533263	this study
<i>G. gezhi</i> sp. nov.	ECNU-V0047	China, Guangxi, Southwest	MT533264	this study
<i>G. araneus</i>	ECNU-V0008	China, Guangxi, Southwest	MT533259	this study
<i>G. araneus</i>	-	Vietnam	AB308460	Jonniaux and Kumazawa (2008)
<i>G. catbaensis</i>	MHNG 2699.49	Vietnam	EU499389	Ziegler <i>et al.</i> (2008)
<i>G. luyi</i>	ECNU-V0012	China, Guangxi, Chongzuo	MK782787	Zhu <i>et al.</i> (2020)
<i>G. luyi</i>	SYSr000255	China, Guangxi, Jingxi	KC765083	Wang <i>et al.</i> (2013)
<i>G. luyi</i>	SYSr000256	China, Guangxi, Jingxi	KC765084	Wang <i>et al.</i> (2013)
<i>G. luyi</i>	GL511	China, Guangxi	KM455054	Liu & Qin (2017)
<i>G. kadoorieorum</i>	ECNU-V0060	Guangxi, Southwest	MT533265	this study
<i>G. kadoorieorum</i>	ECNU-V0061	Guangxi, Southwest	MT533266	this study
<i>G. kadoorieorum</i>	ECNU-V0058	Guangxi, Southwest	MT533258	this study
<i>G. kwangsiensis</i>	ECNU-V0009	Guangxi, Nanning	MK782786	Zhu <i>et al.</i> (2020)
<i>G. liboensis</i>	SYS r000217	China, Guizhou, Libo	KC900230	Wang <i>et al.</i> (2013)
<i>G. lichtenfelderi</i>	ECNU-V0007	Guangxi, Chongzuo	MK782785	Zhu <i>et al.</i> (2020)
<i>G. lichtenfelderi</i>	IEBR 3692	Vietnam	JF799756	Schneider <i>et al.</i> (2011)
<i>G. huuliensis</i>	-	Vietnam	AB853453	Honda <i>et al.</i> (2014)
<i>G. hainanensis</i>	SYS r000349	China, Hainan	KC765080	Wang <i>et al.</i> (2013)
<i>G. hainanensis</i>	BL-RBZ-041	China, Hainan	MH247194	Liang <i>et al.</i> (2018)
<i>G. hainanensis</i>	BL-RBZ-042	China, Hainan	MH247195	Liang <i>et al.</i> (2018)
<i>G. kwanghua</i>	ECNU-V0003	China, Hainan	MK782788	Zhu <i>et al.</i> (2020)
<i>G. kwanghua</i>	ECNU-V0004	China, Hainan	MK782789	Zhu <i>et al.</i> (2020)
<i>G. kwanghua</i>	ECNU-V0005	China, Hainan	MK782790	Zhu <i>et al.</i> (2020)
<i>G. bawanglingensis</i>	BL-RBZ-021	China, Hainan	MH247190	Liang <i>et al.</i> (2018)
<i>G. bawanglingensis</i>	BL-RBZ-022	China, Hainan	MH247191	Liang <i>et al.</i> (2018)
<i>G. bawanglingensis</i>	BL-RBZ-023	China, Hainan	MH247192	Liang <i>et al.</i> (2018)

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TABLE 1. (Continued)

Species	Specimen ID	Locality	16S	References
<i>G. bawanglingensis</i>	BL-RBZ-024	China, Hainan	MH247193	Liang <i>et al.</i> (2018)
<i>G. zhoui</i>	BL-RBZ-001	China, Hainan	MH247196	Liang <i>et al.</i> (2018)
<i>G. zhoui</i>	BL-RBZ-004	China, Hainan	MH247197	Liang <i>et al.</i> (2018)
<i>G. zhoui</i>	BL-RBZ-006	China, Hainan	MH247198	Liang <i>et al.</i> (2018)
<i>G. zhoui</i>	BL-RBZ-007	China, Hainan	MH247199	Liang <i>et al.</i> (2018)
<i>G. zhoui</i>	BL-RBZ-008	China, Hainan	MH247200	Liang <i>et al.</i> (2018)
<i>G. zhelongi</i>	SYS r000816	China, Guangdong, Yingde	KJ423105	Wang <i>et al.</i> (2014)
<i>G. yingdeensis</i>	SYS r000549	China, Guangdong, Yingde	KC765082	Wang <i>et al.</i> (2013)
<i>G. yingdeensis</i>	SYS r000550	China, Guangdong, Yingde	KC900231	Wang <i>et al.</i> (2013)
<i>G. splendens</i>	-	Japan, Ryukyu	AB853449	Honda <i>et al.</i> (2014)
<i>G. splendens</i>	-	Japan, Ryukyu	AB853450	Honda <i>et al.</i> (2014)
<i>G. splendens</i>	-	Japan, Ryukyu	AB853451	Honda <i>et al.</i> (2014)
<i>G. toyamai</i>	-	Japan, Ryukyu	AB853447	Honda <i>et al.</i> (2014)
<i>G. yamashinae</i>	-	Japan, Ryukyu	AB853441	Honda <i>et al.</i> (2014)
<i>G. yamashinae</i>	-	Japan, Ryukyu	AB853442	Honda <i>et al.</i> (2014)
<i>G. kuroiwae</i>	-	Japan, Ryukyu	AB853445	Honda <i>et al.</i> (2014)
<i>G. kuroiwae</i>	-	Japan, Ryukyu	AB853446	Honda <i>et al.</i> (2014)
<i>Coleonyx mitratus</i>	KUZ R47331	Japan, Ryukyu	AB853455	Honda <i>et al.</i> (2014)
<i>Gekko chinensis</i>	SYS r000464	China, Guangdong	KC765079	Wang <i>et al.</i> (2013)

Phylogenetic analyses. We compiled molecular data for 20 *Goniurosaurus* species, including five *G. gezhi* **sp. nov.** samples, and two outgroup species (*Gekko chinensis* Gray and *Coleonyx mitratus* Peters). Database assembly, sequence alignment, and curation were conducted within SuperCRUNCH (Portik and Wiens 2020). First, we confirmed gene orthology in our database using DC-MEGABLAST (McGinnis and Madden 2004). Next, we adjusted the direction of all sequences and performed multiple sequence alignment using MAFFT (Katoh *et al.* 2009). Finally, we trimmed the sequences using TrimAl (Capella-Gutiérrez *et al.* 2009) under default parameters. The resulting alignment (512 bp) included 46 sequences from 22 species.

We implemented the stochastic algorithms in IQ-TREE (Nguyen *et al.* 2015) to infer the phylogeny of *Goniurosaurus* under maximum likelihood. We selected the best-fitting model in our molecular dataset using ModelFinder (Kalyaanamoorthy *et al.* 2017), also implemented in IQ-TREE. We assessed branch support with 1,000 bootstrap. Alternatively, we used MrBayes (Huelsenbeck and Ronquist, 2001) to infer the phylogenetic relationships under a Bayesian Inference framework. For this, we selected a GTR+I+G model for the full dataset (based on ModelFinder) and analyzed two independent MCMC runs, each with a total of 30 million generations. The maximum clade credibility tree under MrBayes was inferred based on the 90% of posterior samples (i.e. 10% of burn-in). We confirmed convergence under MrBayes in Tracer by assessing whether ESS were at least 200 for the likelihood. IQ-TREE analyses were run from the W-IQ-TREE webserver (Trifinopoulos *et al.* 2016) and MrBayes was run in CIPRES (Miller *et al.* 2010).

Morphology. All specimens were preserved in 75% alcohol and deposited in the Museum of Biology, East China Normal University (ECNU). The following measurements were recorded using digital calipers (± 0.1 mm) following Zhou *et al.* (2018): snout vent length (SVL) from tip of snout to vent; tail length (TaL) from vent to tip of tail; distance between axilla and groin (AG) from posterior edge of forelimb insertion to anterior edge of hindlimb insertion; snout to eye distance (SE) measured from tip of snout to anterior-most point of eye; eye to ear distance (EE) from posterior margin of eye to posterior margin of ear; maximum head width (HW); head length (HL) from tip of snout to posterior margin of ear. Scalation characters recorded are as follows: supralabials (SPL); sublabials/infralabials (SBL); nasal scales surrounding nare (N); internasals (IN); gular scales bordering the internasals (PostIN); postmentals (PM); gular scales bordering the postmentals (GP); eyelid fringe scales or ciliaria (CIL); preorbital scales (PO); granular scales surrounding dorsal tubercles (GST); dorsal tubercle rows at midbody (DTR); paravertebral tubercles between limb insertions (TL); scales around midbody (MB); subdigital lamellae under the

first finger (LD1) and the fourth finger (LD4); subdigital lamellae under the first toe (LT1) and the fourth toe (LT4); precloacal pores (PP); postcloacal tubercles (PAT). Bilateral scale counts are given as left/right.

Results

Phylogenetic relationships. Phylogenetic inference under Maximum Likelihood and Bayesian Inference recovered congruent evolutionary relationships among the 20 *Goniurosaurus* species analyzed in this study (Fig. 2). Our phylogenetic analyses inferred four major lineages within *Goniurosaurus*. These major groups have also been recovered in previous studies of the *Goniurosaurus* phylogeny (e.g., Liang *et al.* 2018). Samples assigned to *G. gezhi* **sp. nov.** based on morphology formed a strongly supported monophyletic group in our phylogenies (bootstrap=99%, posterior probability=1). Finally, our analyses suggest that *G. gezhi* **sp. nov.** belongs to the *G. luii* group (bootstrap=80%, posterior probability=0.86), with *G. araneus* being recovered as its sister species, although without support (bootstrap=53%, posterior probability=0.52) (Fig. 2).

Systematics

Goniurosaurus gezhi Zhu, He & Li **sp. nov.**

(Figs. 1A–B, 3, 4)

Holotype. ECNU-V0038, adult male, from southwestern Guangxi Province, China, 100–200 m in altitude; exact locality withheld because of conservation concerns, available to qualified researchers upon request. Collected during April, 2019 by Zhu Xiao-Yu & He Zhu-Qing.

Paratypes. Two adult males ECNU-V0040 and ECNU-V0042, same data as holotype.

Diagnosis. *Goniurosaurus gezhi* **sp. nov.** differs from other congeners by a combination of the following characters: small size (SVL 70.58–83.78 mm in adults); one nuchal loop, three body bands, and one postsacral band; number of precloacal pores 18–20; body color orange or yellow in life.

Description. Holotype. ECNU-V0038, adult male; SVL: 75.86 mm; TaL (regenerated): 39.56 mm; AG: 46.78 mm; SE 11.83 mm; EE: 9.76 mm; HW: 19.48 mm; HL: 27.30 mm; SVL: AG 1.62; SVL: HL: 2.78; HL: HW 1.40; SE: EE: 1.21; head triangular, wider than neck, covered with uniform granular scales interspersed with tubercles on top of head; conspicuous row of enlarged supraorbital tubercles; rostral wider than high, middorsal portion partially sutured dorsomedially, bordered laterally by first supralabial and prenasal, dorsolaterally by supranasal; external nares bordered by 6/7 nasals, anteriorly by prenasal, dorsally by supranasal and two granular scales, posteriorly by three smaller granular scales; prenasals with long recurved ventral portion; supranasals in contact at the midline; supralabials 9/9, rectangular, grading into longer scales posteriorly; eyes relatively large, pupils vertical; eyelid fringe scales 51/50; a fold of skin originating in suborbital region extends posteroventrally across angle of jaw; external auditory meatus elliptical with long axis directed dorsoventrally; tympanum deeply recessed bordered anteriorly by two spinose scales; mental triangular, bordered laterally by first infralabial and posteriorly by three postmentals; postmentals bordered by seven gular scales; infralabials rectangular 9/8. Neck narrower than body, covered with uniform granular scales interspersed with conical tubercles on nape; dorsal body tubercles surrounded by 11–12 granular scales; 21 longitudinal rows of dorsal tubercle at midbody; 32 paravertebral tubercles between limb insertions, distinct vertebral row of scales absent. Body relatively thin, covered with granular scales grading ventrally into larger flattened subimbricate ventral scales; 142–151 scales around midbody; larger ventral scales grade abruptly into smaller granular scales immediately anterior to vent; 20 pore-bearing precloacal scales in a continuous transverse series extending onto proximal regions of thighs; two enlarged postcloacal tubercles laterally on each side at level of vent. Limbs covered dorsally with granular scales interspersed with closely spaced tubercles and ventrally with flat, juxtaposed to subimbricate scales; hind limbs larger and longer than forelimbs; deep axillary pockets present; subdigital lamellae wide, 9/11 on first finger, 20/20 on fourth finger, 12/9 on first toe, 21/23 on fourth toe. Claws sheathed by four scales, two lateral scales long and curved, dorsal one shorter than lateral ones, but much longer than ventral one. Tail regenerated and gradually narrowed to the tip, scales varied in size. In general, dorsal caudal scales small and juxtaposed, subcaudal scales large (2–3 times larger than dorsal caudals), and imbricate.

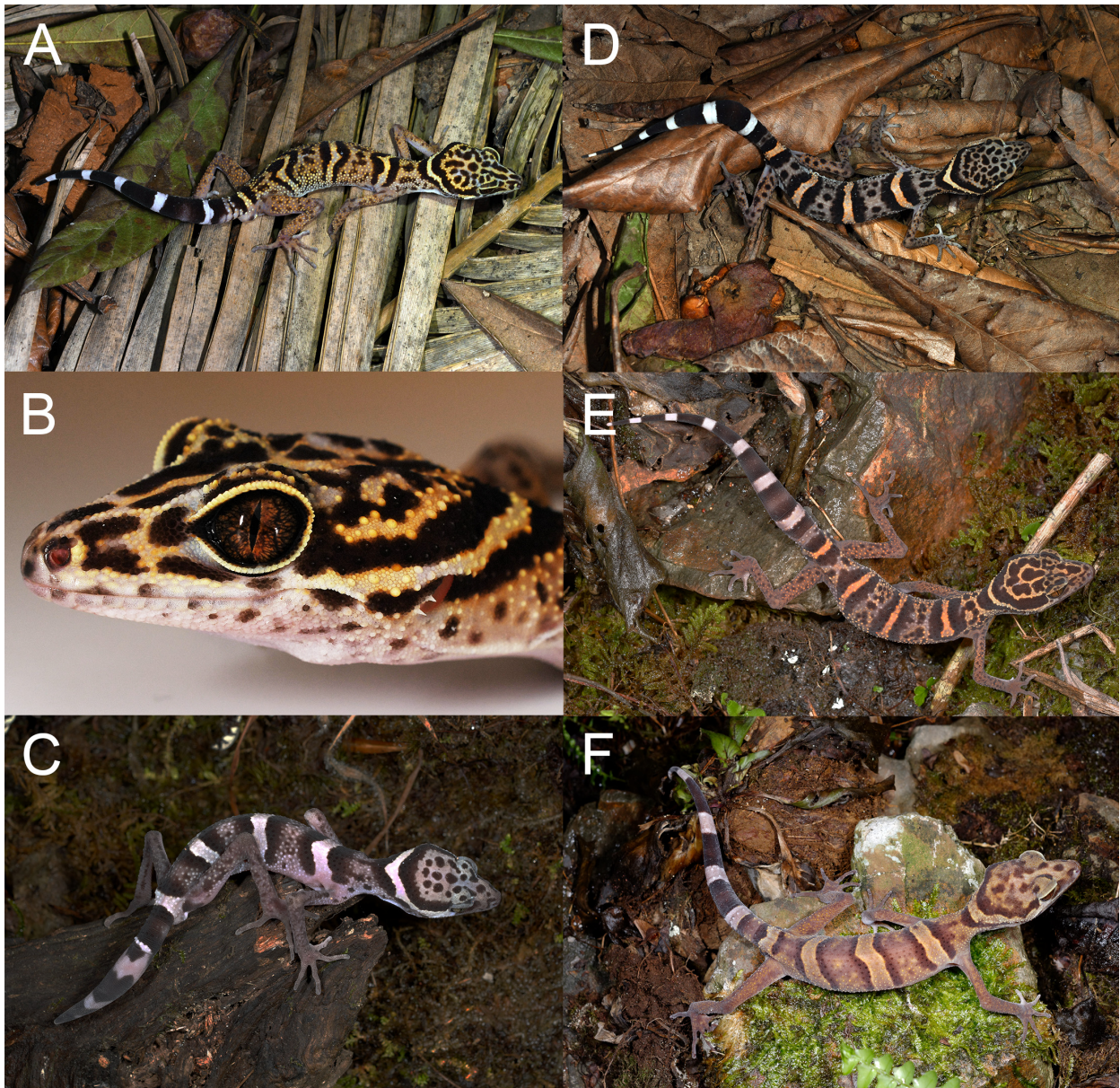


FIGURE 1. *Goniurosaurus* spp. from Guangxi, China. A-B. *G. gezhi* sp. nov., adult males; C. *G. kadoorieorum*; D. *G. luyi*; E. *G. kwangsiensis*; F. *G. araneus*. (photos by Zhu Xiao-Yu)

Coloration. Dorsal ground color of head, body and limbs grey, bearing irregularly shaped small black blotches, black blotches on head and near closely spaced; iris brown; nuchal loop pale yellow, posterior margin rounded, not pointed; three body bands between limb insertions, one postsacral band on tail base; these bands are pale yellow; ventral surfaces of head, body and limbs dull white; ground color of tail black with irregular white markings.

Variation. Measurements and scalation data of the type series are provided in Table 2. Paratypes largely match the overall scalation and coloration characters of the holotype. Paratype ECNU-V0042 has one internasal and the number of precloacal pores is 18. The PostIN and PM of paratype ECNU-V0040 is 5.

Comparisons. *Goniurosaurus gezhi* sp. nov. differs from *G. lichtenfelderi* by having three body bands (*versus* two) It differs from *G. luyi*, *G. huuliensis*, *G. kadoorieorum*, *G. kwangsiensis*, and *G. liboensis* by having 18–20 precloacal pores as opposed to 23–32 collectively. The new species is smaller in body size (SVL 70.6–83.8 mm) than *G. araneus* (SVL 115–124 mm) and *G. catbaensis* (SVL 84.7–111.5 mm) (Table 3). Moreover, the body color of *G. gezhi* is orange or yellow, while it is brown in *G. catbaensis*. The new species has several spots on the body, while there are few spots on the body of *G. araneus*.

TABLE 2. Comparisons of morphometric characters and scale counts for eight *Goniurosaurus* species within the *luiti* group. Character abbreviations are explained in material and methods (data for *G. gezhi* sp. nov. come from this study; *G. kadoorieorum* and *G. kwangsiensis* from Yang & Chan 2015, *G. araneus* and *G. luiti* from Grismer et al. 1999, *G. liboensis* from Wang et al. 2013, *G. huiliensis* from Orlov et al. 2008; *G. catbaensis* from Orlov & Darevsky 1999).

	SVL	SPL	SBL	PO	CIL	PM	GST	TL	MB	LT4	PP
<i>G. gezhi</i>	mean	9.33	9.00	17.17	48.33	4.00	11.33	34.67	136.83	23.50	19.33
	SD	0.52	0.63	1.47	3.26	1.41	0.82	3.78	10.03	1.52	1.15
	Range	71–84	8–10	15–19	44–52	3–5	10–12	32–39	123–151	21–25	18–20
<i>G. araneus</i>	mean	8.80	8.70	16.70	64.00	5.30	9.80	35.80	135.70	23.50	19.80
	SD	0.98	0.52	1.86	2.19	0.82	4.33	2.71	7.26	0.55	1.71
	Range	115–124	8–9	13–18	61–67	4–6	10–14	32–38	129–147	23–24	18–22
<i>G. luiti</i>	mean	9.50	10.00	15.80	59.50	3.00	12.20	33.80	134.50	23.50	26.00
	SD	0.55	0.63	1.17	1.87	0.89	1.34	0.75	12.00	1.38	2.58
	Range	107–116	9–11	14–17	57–61	2–4	9–14	33–34	119–144	21–24	23–29
<i>G. kadoorieorum</i>	mean	10.33	9.00	16.17	50.83	5.00	12.00	32.00	128.67	22.00	26.75
	SD	0.52	0	0.75	2.77	0	1.10	2.00	4.16	0.89	0.96
	Range	112.5–118	9	15–17	47–54	5	11–13	30–34	124–132	21–23	26–28
<i>G. huiliensis</i>	mean	10.40	9.80	N/A	43.50	2.40	12.20	34.60	123.60	17.40	26.75
	SD	0.52	0.63	N/A	0.95	0.55	0.44	0.89	3.91	0.70	1.26
	Range	108.78–117.34	9–11	N/A	41–44	2–3	12–13	34–36	118–129	17–19	25–28
<i>G. kwangsiensis</i>	mean	9.38	8.38	17.00	54.50	4.00	11.50	30.25	125.25	24.12	32.00
	SD	0.84	0.82	1.63	1.17	1.41	1.21	2.36	2.75	0.82	1.41
	Range	97.6–109.1	7–9	15–19	52–58	3–6	10–13	27–32	122–128	22–27	31–33
<i>G. catbaensis</i>	mean	8.80	7.70	N/A	53.70	2.70	9.70	33.30	119.30	23.30	18.70
	SD	0.40	0.80	N/A	1.00	0.60	1.50	0.60	7.50	0.80	2.50
	Range	84.7–111.5	6–8	N/A	52–55	2–3	8–11	33–34	112–127	22–24	16–21
<i>G. liboensis</i>	mean	10.38	10.38	16.83	55.83	4	12.00	27.67	128	24.33	23
	SD	0.92	0.92	0.75	2.64	1.15	1.12	0.58	1.15	1.21	0
	Range	103.2–110.3	9–12	16–18	52–59	3–5	10–13	27–28	127–129	23–26	23

TABLE 3. Morphological measurements (in mm) and scalation characters of the type specimens of *Goniurosaurus gezhi* sp. nov. Abbreviations defined in the morphology study part. * = regenerated tail.

	HOLOTYPE ECNU-V0038	PARATYPE ECNU-V0040	PARATYPE ECNU-V0042
sex	male	male	male
SVL	75.86	70.58	83.78
TaL	39.56*	36.45*	44.68*
AG	46.78	44.13	52.12
HL	27.3	26.44	29.32
HW	19.48	17.91	20.8
SE	11.83	11.11	12.19
EE	9.76	9.22	11.17
SVL:HL	2.78	2.67	2.86
SVL:AG	1.62	1.60	1.61
HL:HW	1.40	1.48	1.41
SE:EE	1.21	1.20	1.09
SPL	9/9	9/10	9/10
SBL	9/8	9/9	9/10
N	7/6	6/7	7/7
IN	0	0	1
PostIN	3	5	3
PM	3	5	3
GP	7	8	7
PO	15/16	17/18	19/18
CIL	51/50	44/45	48/52
MB	142—151	123—128	138—139
GST	11—12	11—12	10—12
TL	32	33	39
DTR	21	21	20
LD1	9/11	11/10	9/10
LD4	20/20	20/21	20/21
LT1	12/9	12/12	11/12
LT4	21/23	24/25	23/25
PP	20	20	18
PAT	2	2	2

Distribution and Life History. *Goniurosaurus gezhi* sp. nov. is only known from southwestern Guangxi, China. It is found at 100–200 m elevation. Some individuals were found on limestone within crevices at night, while others were collected on the road near farmland. This species co-occurred with Moellendorff's Rat Snake (*Elaphe moellendorffi* Boettger) and the Tokay Gecko (*Gekko gekko* L.) in the limestone area. Our surveys suggest they are most active from April to October.

Etymology. The specific epithet *gezhi* is for the Chinese phonetic alphabet 格致, which was noted in ancient Chinese literature *Daxue* around 2000 years ago. It means researching something carefully and then summarizing the truth behind it. The epithet is a noun in apposition. For the common name, we suggest “Gezhi Cave Gecko.”

0.04 substitutions/site

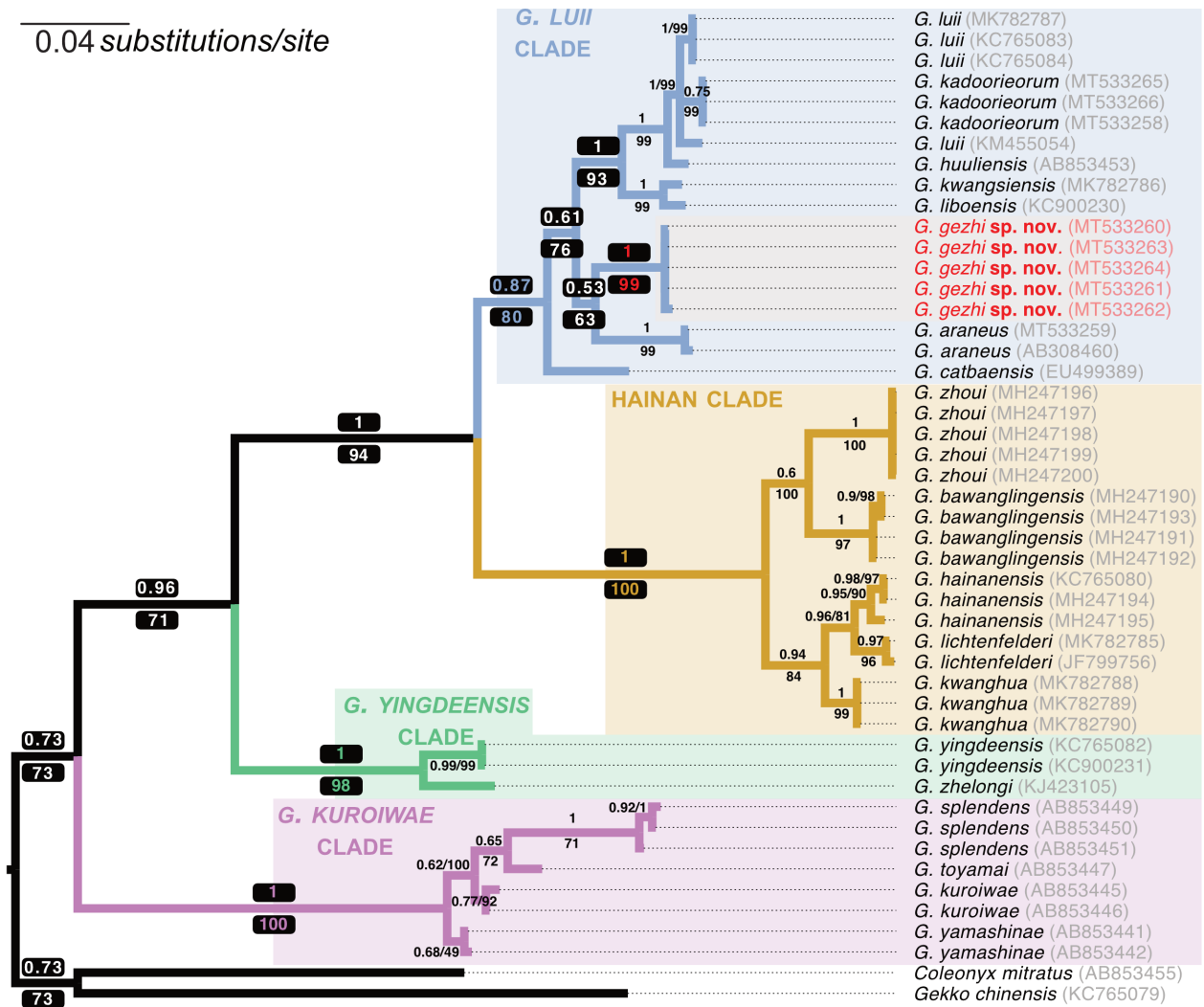


FIGURE 2. Phylogenetic relationships among 20 *Goniurosaurus* species. Support values for the major lineages within *Goniurosaurus* based on Bayesian Inference under MrBayes (posterior probabilities) and maximum likelihood under IQ-TREE (bootstrap values) are shown above and below branches, respectively. In some cases, posterior probabilities are shown before bootstrap support values. The tree is a Bayesian topology. The position of *G. gezhi sp. nov.* is highlighted in the tree.

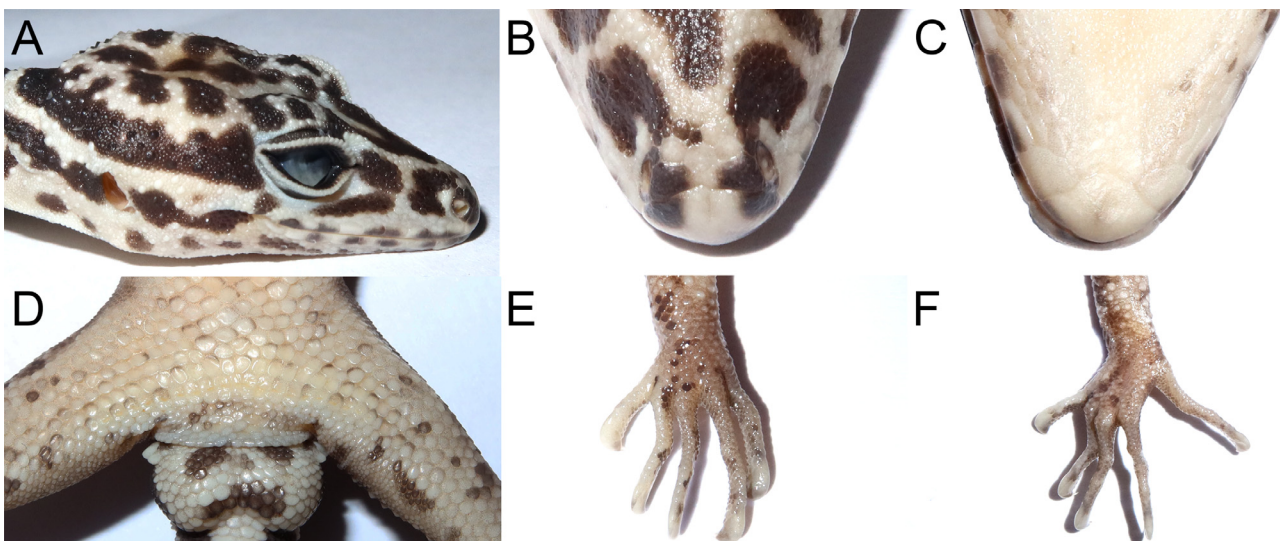


FIGURE 3. *Goniurosaurus gezhi sp. nov.* (ECNU-V0038). A. head in lateral view; B. dorsal view of the snout tip; C. ventral view of the chin; D. preloacal region; E. manus; F. pes.



FIGURE 4. Type series of *Goniurosaurus gezhi* **sp. nov.** left, ECNU-V0038 (Holotype); middle, ECNU-V0040 (Paratype); right, ECNU-V0042 (Paratype).

Discussion

Our study provides the most comprehensive molecular phylogeny for nearly all extant *Goniurosaurus* species based on *16S* ribosomal RNA gene region. In line with previous research, we found strong evidence for the four major geographically partitioned lineages (Liang *et al.* 2018). The *G. luyi* group, containing seven known species, is the largest clade within *Goniurosaurus* (Liang *et al.* 2018). This lineage is only found in southwestern China and Northern Vietnam. Consistent with the geographical distribution, our molecular phylogenetic analyses indicate that *G. gezhi* **sp. nov.** belongs to the *G. luyi* group. Morphologically, *G. gezhi* **sp. nov.** has a different number of precloacal pores compared to other species in this clade except *G. araneus* and *G. catbaensis*. This may not be surprising given that *G. araneus* and *G. gezhi* **sp. nov.** are sister species. The geographical distribution of these two species is also similar, both occurring in Southwest Guangxi. However, *G. gezhi* differs in banding, spotting, and coloration compared to *G. araneus*.

Our study found *G. huuliensis*, *G. kadoorieorum*, and *G. luyi* are closely related. These results are consistent with morphological evidence (Orlov *et al.* 2008, Yang & Chan 2015). The ranges of their precloacal pore number also highly overlap. However, probably as a consequence of incomplete lineage sorting or simply reflecting limited information in our dataset, our analyses did not recover monophyly *G. luyi* as monophyletic. A previous study described *G. kadoorieorum* with no molecular data (Yang & Chan 2015). In this study, we provide *16S* gene sequences from three *G. kadoorieorum* individuals. Combining the recently published molecular data of *G. kwangsiensis* (Zhu *et al.* 2020), our analyses inferred the phylogenetic position of *G. kadoorieorum* and *G. kwangsiensis*. However, we note that published molecular data is still limited for many species in the *G. luyi* group. For example, only *16S* gene sequences are available for *G. liboensis* on GenBank. Future studies should provide better sampling and sequencing efforts for species with limited data, such as *G. catbaensis*, *G. huuliensis*, and *G. liboensis*. Sequencing genes other than *16S* such as *ND2*, *Cytb*, *COI*, *COII*, and nuclear makers will help better resolve the phylogeny of this large clade within the *Goniurosaurus*.

The genus *Goniurosaurus* is generally associated with karst topography. Previous studies in animals and plants have found high levels of species richness and endemism in karst areas across the southwestern China and Northern Vietnam (Luo *et al.* 2016; Wang *et al.* 2017). It has been proposed that allopatric speciation by geographical isolation might be the predominant mode of speciation in these limestone areas (Wang *et al.* 2017). However, the biogeographical patterns underlying speciation in these areas are still poorly understood. *Goniurosaurus* provides a good system to study the process of speciation in the karst areas. Future studies should provide better understanding of the population structure, gene flow, and demographic history among the different species. Our study of *G. gezhi* **sp. nov.** gives another case of speciation in the karst area. Given the small population size and high level of endemism of many *Goniurosaurus* species, our discovery highlights the importance of conservation efforts conducted within these global diversity hotspots.

Acknowledgments

We thank Aaron M. Bauer, L. Lee Grismer, Chris R. Reardon, Connor M. French, and an anonymous reviewer for their helpful comments and suggestions on the manuscript.

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