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A new species of *Acanthosaura* Gray 1831 (Reptilia: Agamidae) from central Vietnam

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Abstract

Herein, we describe the new agamid species *Acanthosaura phongdienensis* **sp. nov.** from central Vietnam based on morphological and genetic data. Males of the new medium-sized species have a snout-vent length of up to 77.4 mm and females up to 64.7 mm. In both genders, the tail is longer than the snout-vent length. A spine occurs on each side of the neck, the rostral scute is entire, and the nuchal and dorsal crests are continuous. A postorbital spine is about half as long as the diameter of the eye-ball, and small lateral scales intermix with large, keeled scales whose tips point backwards and upwards. The new species differs genetically from the closely related species *A. brachypoda*, *A. coronata*, and *A. lepidogaster* by uncorrected *p*-distances of 13.6%, 21.7%, and 12.8%, respectively. This is the 13th species of *Acanthosaura* and the eighth species in the genus from Vietnam.

Key words: Agamid, *Acanthosaura lepidogaster*, Phong Dien, *COI*

Introduction

The agamid genus *Acanthosaura* Gray, 1831 occurs from northeastern India, southern China, through Vietnam, Laos, Myanmar, Thailand, Cambodia, Malay Peninsula and adjacent archipelagos, to Sumatra, and the Anambas and Natunas archipelagos (Smith 1935; Wood *et al*. 2010). The genus contains 12 species (Nguyen *et al*. 2018; Uetz *et al*. 2019) of which half were described in the last decade. Ananjeva *et al*. (2008) confirmed the occurrence of *A. capra* Günther, 1861, *A. coronata* Günther, 1861, *A. lepidogaster* (Cuvier, 1829), and *A. nataliae* Orlov, Nguyen & Nguyen, 2006 in Vietnam based on molecular (Kalyabina-Hauf *et al*. 2004) and morphological analyses. Ananjeva *et al.* (2011) described *A. brachypoda* Ananjeva, Orlov, Nguyen & Ryabov, 2011 from northern Vietnam. Subsequently, Bobrov (2013) recorded the occurrence of *A. cardamomensis* Wood, Grismer, Grismer, Neang, Chav & Holden in southern Vietnam. Nguyen *et al.* (2018) recently described an additional species, *Acanthosaura murphyi* Nguyen, Do, Hoang, Nguyen, McCormack, Nguyen, Orlov, Nguyen & Nguyen, 2018, from central Vietnam. Hence, seven species of *Acanthosaura* have been recorded from Vietnam so far.

Acanthosaura capra, *A. murphyi*, and *A. nataliae* are characterized morphologically by the absence of a spine between the tympanum and nuchal crests. The last species occurs in central Vietnam, southern Laos (Orlov *et al*. 2006; Nguyen *et al*. 2009) and eastern Cambodia (Stuart *et al*. 2006, 2010). *Acanthosaura capra* occurs in Mondolkiri Province, eastern Cambodia, and central and southern Vietnam at Khanh Hoa, Lam Dong, Binh Phuoc, and Trang Bom (Orlov *et al*. 2006; Stuart 2006; Nguyen *et al*. 2018) and *A. murphyi* occurs in central Vietnam at Khanh Hoa and Phu Yen (Nguyen *et al*. 2018). Occurring in Cambodia (Stuart *et al*. 2006, 2010) and Vietnam (Gia Lai, Dak Lak, Lam Dong, Dong Nai; Ananjeva *et al.* 2008; Kalyabina-Hauf *et al*. 2004; Nguyen *et al*. 2009), *A. coronata* has spines between the tympanum and nuchal crests and the nuchal and dorsal crests are continuous. *Acanthosaura*

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lepidogaster ranges in southern China, Thailand, Laos, Vietnam, and Myanmar (Ananjeva *et al.* 2008). It also has a spine between the tympanum and nuchal crests, but the nuchal and dorsal crests are not continuous. Closely related to *A. lepidogaster*, *A. brachypoda* occurs in northwestern Vietnam. *Acanthosaura cardamomensis*, which has long nuchal and dorsal crests and a long spine between the tympanum and nuchal crests, was recorded from Phu Quoc Islands in southern Vietnam (Bobrov 2013).

Our fieldwork in central Vietnam in 2010 obtained a series of *Acanthosaura* that differs morphologically from its congeners. Genetic analyses also detect differences. Thus, below we describe a new species.

Materials and methods

The genetic dataset consisted of 29 ingroup samples including four samples of *A. brachypoda*, five *A. coronata*, 11 *A. lepidogaster*, three *A. nataliae*, and six tissue samples of the new species from Phong Dien Nature Reserve. Detailed localities for the species were given in Table 1 and Fig. 1. Two samples of *Calotes versicolor* were used as the outgroup in the phylogenetic analyses based on Kalyabina-Hauf *et al*. (2004) and Ananjeva *et al*. (2008).

Liver tissues were stored in 95% ethanol in the field and then maintained at –80°C in the laboratory. The mitochondrial DNA gene encoding cytochrome *c* oxidase subunit I (*COI*) was sequenced. DNA extraction from tissue samples was done by using proteinase K and the standard three-step phenol/chloroform procedure (Sambrook *et al*. 1989). PCR was performed using primers Chmf4, 5'-TYT CWA CWA AYC AYA AAG AYA TCG G-3' and Chmr4, 5'-ACY TCR GGR TGR CCR AAR AAT CA-3' (Che *et al*. 2012) in a 25 µl volume with an initial step of 95°C for 4 min followed by 35 steps of 94°C for 1 min, 46°C for 1 min, and 72°C for 1 min. A final extension involved 72°C for 10 min. We used a ratio of 0.55 H_20 : 0.30 ExoI : 0.15 SAP to clean the PCR product (Hanke & Wink 1994). Amplified *COI* fragments were sequenced in both directions using an ABI PRISM 3730 Automated DNA Sequencer (Applied Biosystems) following the manufacturer's protocol.

The *COI* sequences were aligned using ClustalW (Thompson *et al*. 1994) integrated in MEGA 4.1 (Tamura *et al*. 2007) with default parameters. All sequences were translated into amino acids to confirm the absence of premature stop codons in the sequences. We also used MEGA to calculate uncorrected and Kimura 2-parameter (K2P; Kimura 1980) genetic distances between and within species.

Three kinds of character-based tree-building methods were used to hypothesize the matrilineal relationships of the species. Partitioned Bayesian inference (BI) was performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) based on best-fit models of sequence evolution selected by MrModeltest 2.3 (Nylander 2004) under the Akaike information criterion (K80+Γ for the first codon position, and GTR+I for the second and third codon positions). Bayesian posterior probabilities (BPP) were estimated using a Markov chain Monte Carlo sampling approach after one million generations and once the average standard deviations reached 0.006. A 50% majority consensus tree was generated (Fig. 1) after discarding 25% of initial samples as burn-in. Partitioned maximum likelihood (ML) analyses were performed using RAxML (Stamatakis *et al*. 2008) with the gamma model of rate heterogeneity option. Maximum parsimony (MP) analyses were done using TNT (Goloboff *et al.* 2008) with a heuristic search, random addition sequences, TBR branch-swapping, and 1000 pseudoreplicates for bootstrap values. Bootstrap values ≥70% for ML and MP, and BPP of ≥95% were considered *a priori* as being indicators of strongly supported nodes (Felsenstein 2004; Hillis & Bull 1993).

Morphological characters for *Acanthosaura*, some of which were slightly modified from Wood *et al*. (2010), were as follows: SVL: snout–vent length, measured from the tip of the snout to the tip of the vent; TL: tail length, measured from the posterior margin of the vent to the tip of the tail; HL: head length, measured from posterior edge of the rectis of the jaw to the tip of the snout; HW: maximum head width, the width at the level of the tympanum; HH: maximum head height, measured across the parietal region; MW: mental width; MH: mental height; RW: rostral width; RH: rostral height; ED: eye diameter, measured from the posterior to the anterior edge of the eye-opening; TD: tympanum diameter, measured horizontally from the anterior to the posterior border of the tympanum; PS: postorbital spine length; NS: length of the nuchal spine, measured from the base to the tip; NC: length of the longest nuchal crest; DC: length of the longest dorsal crest; SL: number of supralabials; IL: number of infralabials; F4: number of subdigital lamellae on the fourth finger, measured from the site where the fourth separating from the third finger to tip; T4: number of subdigital lamellae on the fourth toe, measured from where the fourth toe separates from the third to tip; and CS: number of canthus-rostralis-supraciliary scales, counted from the nasal scale to the posterior end of the ridge at the posterior margin of the orbit. Institutional abbreviations were ITBCZ (Institute of Tropical Biology Collection of Zoology, Ho Chi Minh City, Vietnam), KIZ, (Kunming Institute of Zoology, China), and ROM (Royal Ontario Museum, Toronto, Canada).

Comparative morphological data were taken from original descriptions and subsequent studies (Ananjeva *et al*. 2008, 2011; Boulenger 1885; Günther 1861; Nguyen *et al.* 2018; Orlov *et al.* 2006; Pauwels *et al*. 2015; Smith 1935; Taylor 1963; Wood *et al*. 2009, 2010). For species concepts, we applied both the morphological species concept (*sensu* Wiley & Lieberman 2011) and the evolutionary species concept (Simpson 1961; Wiley & Lieberman 2011). Genealogical relationships were used to guide species delimitation.

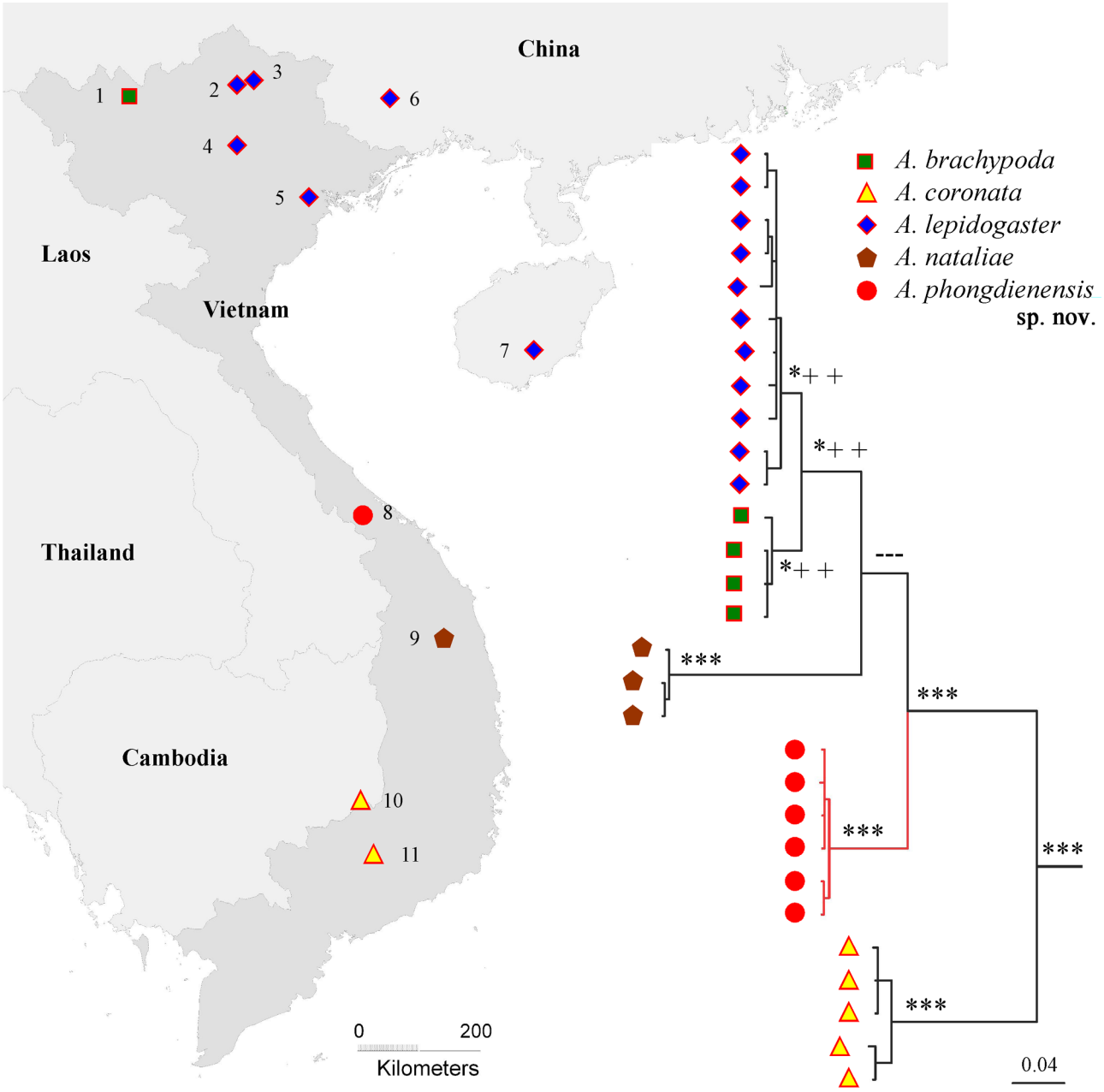

FIGURE 1. Sampling locations of the *Acanthosaura* in Vietnam and southern China (left) and Bayesian inference (BI) tree for the *Acanthosaura* (right). Site numbers refer to Table 1. Symbols at the internal nodes are BI posterior probabilities and bootstrap proportions from maximum likelihood (ML) and maximum parsimony (MP), respectively. Asterisk: supported 100% by BI, ML, and MP; plus sign: supported 95–99% by BI and 70–99% by ML and MP; hyphen: unsupported. Outgroup (*Calotes versicolor*) was not shown on the tree.

Results

Sequence variation, genealogy, and genetic distances

The aligned *COI* nucleotide sequences contained 563 positions with 220 potentially parsimony-informative charac-

ters. No gaps, missing data, and premature stop codons occurred. All unique sequences were deposited in GenBank with the accession numbers MK695182–MK695212 (Table 1).

Matrilineal genealogies from BI, ML, and MP well-resolved all four described species plus a lineage from Phong Dien Nature Reserve (site 8) with high nodal support values. The trees resolved the same topology for all taxa and, thus, only the 50% major consensus tree from BI analysis was shown in Fig. 1, but with bootstrap values from ML and MP.

Uncorrected *p*-distances (Table 2) between species ranged from 4.2% (between *A. brachypoda* and *A. lepidogaster*) to 25.0% (between *A. coronata* and *A. nataliae*), averaging 17.8%. The *Acanthosaura* from Phong Dien differed from *A. brachypoda*, *A. coronata*, *A. lepidogaster*, and *A. nataliae* in 15.4%, 26.0%, 14.3%, and 22.9% in K2P and 13.6%, 21.7%, 12.8%, and 19.3% in *p*-distances, respectively. In contrast, the *p*-distances within species were low, ranging from 0.1% (*A. nataliae*) to 1.3% (*A. coronata*).

The morphological data (below) and genetic distances indicated that the population from Phong Dien Nature Reserve comprised a new species. It is described as follows:

TABLE 1. Specimens, localities, and *COI* GenBank accession numbers of Vietnamese agamids used in this study. Location numbers were mapped in Fig. 1.

Taxon	Voucher	Site no. and locality	**GenBank Accession No.**
Acanthosaura brachypoda	ROM38118	1. Sa Pa, Lao Cai, Vietnam	MK695182
A. brachypoda	ROM38119	1. Sa Pa, Lao Cai, Vietnam	MK695183
A. brachypoda	ROM38120	1. Sa Pa, Lao Cai, Vietnam	MK695184
A. brachypoda	ROM38123	1. Sa Pa, Lao Cai, Vietnam	MK695185
A. coronata	KIZ47	10. Bu Gia Map, Binh Phuoc, Vietnam	MK695186
A. coronata	KIZ48	10. Bu Gia Map, Binh Phuoc, Vietnam	MK695187
A. coronata	KIZ97	10. Bu Gia Map, Binh Phuoc, Vietnam	MK695188
A. coronata	ROM42240	11. Cat Tien, Dong Nai, Vietnam	MK695189
A. coronata	ROM42241	11. Cat Tien, Dong Nai, Vietnam	MK695190
A. lepidogaster	ROM30505	2. Na Hang, Tuyen Quang, Vietnam	MK695191
A. lepidogaster	ROM30507	2. Na Hang, Tuyen Quang, Vietnam	MK695192
A. lepidogaster	ROM30677	3. Quang Thanh, Cao Bang, Vietnam	MK695193
A. lepidogaster	ROM30680	3. Quang Thanh, Cao Bang, Vietnam	MK695194
A. lepidogaster	ROM30712	4. Tam Dao, Vinh Phuc, Vietnam	MK695195
A. lepidogaster	ROM30715	4. Tam Dao, Vinh Phuc, Vietnam	MK695196
A. lepidogaster	ROM30720	4. Tam Dao, Vinh Phuc, Vietnam	MK695197
A. lepidogaster	ROM34679	4. Tam Dao, Vinh Phuc, Vietnam	MK695198
A. lepidogaster	ROM31954	5. Chi Linh, Hai Duong, Vietnam	MK695199
A. lepidogaster	KIZ12720	6. Fulong, Guangxi, China	MK695200
A. lepidogaster	YPX19177	7. Diaoluoshan, Hainan, China	MK69501
A. nataliae	ROM30629	9. Tram Lap, Gia Lai, Vietnam	MK695202
A. nataliae	ROM30631	9. Tram Lap, Gia Lai, Vietnam	MK695203
A. nataliae	ROM30632	9. Tram Lap, Gia Lai, Vietnam	MK695204
A. phongdienensis **sp. nov.**	ITBCZ6831	8. Phong Dien, Thua Thien-Hue, Vietnam	MK695205
A. phongdienensis **sp. nov.**	ROM48013	8. Phong Dien, Thua Thien-Hue, Vietnam	MK695206
A. phongdienensis **sp. nov.**	KIZ10657	8. Phong Dien, Thua Thien-Hue, Vietnam	MK695207
A. phongdienensis **sp. nov.**	ITBCZ6830	8. Phong Dien, Thua Thien-Hue, Vietnam	MK695208
A. phongdienensis **sp. nov.**	KIZ10695	8. Phong Dien, Thua Thien-Hue, Vietnam	MK695209
A. phongdienensis **sp. nov.**	KIZ10697	8. Phong Dien, Thua Thien-Hue, Vietnam	MK695210
Calotes versicolor	KIZ1120	Ta Kou, Binh Thuan, Vietnam	MK695211
Calotes versicolor	ITBCZ1034	Ta Kou, Binh Thuan, Vietnam	MK695212

TABLE 2. Uncorrected *p*-distances (upper-right) and Kimura 2-parameter distances (lower-left) between species and *p*-distance within species (in boldface) of *Acanthosaura* used in this study.

Taxon	1	2	3	4	5
1. *A. brachypoda*	**0.3**	23.0	4.2	17.7	13.6
2. *A. coronata*	28.0	**1.3**	22.0	25.0	21.7
3. *A. lepidogaster*	4.4	26.4	**1.0**	18.2	12.8
4. *A. nataliae*	20.8	30.9	21.5	**0.1**	19.3
5. *A. phongdienensis* **sp. nov.**	15.4	26.0	14.3	22.9	**0.2**

Species description

Acanthosaura phongdienensis sp. nov.

Holotype (Fig. 2). KIZ 10695, an adult male collected on 22 October 2010, 16° 28' 03.6" N, 107° 16' 40.8" E, at Phong Dien Nature Reserve, Thua Thien-Hue Province, Vietnam (site 8, Fig. 1), about 197 m a.s.l. by Robert W. Murphy, Wei-Wei Zhou, Ba Dinh Vo, Luan Thanh Nguyen, and Sang Ngoc Nguyen.

Paratypes. ROM 48013 and ITBCZ 6830, adult males; KIZ 10657, KIZ 10697, and ITBCZ 6831, adult females; and KIZ 10617, a sub-adult male (Table 3). Other data were the same as for the holotype except for KIZ 10464, which was collected in September 2010 at an elevation of 1019 m a.s.l.

Diagnosis. Medium size, SVL 73–77 mm in males and 59–65 mm in females; TL much longer than SVL; rostral scute entire; nuchal spine at middle of nuchal crest and tympanum; tympanum naked; postorbital spine about one half of ED; nuchal and dorsal crests continuous (Figs. 2–4); lateral scales small, intermixed with large, keeled scales whose keels point backward and back-upward; SL 9–12; IL 10–11.

Description of holotype. Adult male, SVL 74.2 mm. SL 11, IL 10. Head moderately tall (HH/HL = 0.63) and with HH/HW = 0.94. Rostral entire and wide, nearly trapezoidal (RH/RW = 0.5), upper wider than lower, not touching nostril, bordered posteriorly by four smaller smooth scale and laterally by first supralabials. When mouth closed, rostral in contact with mental and first infralabials. Nasals with pits, not touching supralabials. Scales on dorsum of head large and keeled. Canthus-rostralis-supraciliary scales slightly serrated, composed of 11 enlarged scales, separated from postorbital spine by a notch. Postorbital spine length about one half ED. An enlarged scale row below orbit. Interorbital scales keeled. Nuchal spine present midway between nuchal crest and tympanum, surrounded by spine-like scales. Tympanum naked, not round, surrounded by small scales. TD smaller than ED. A row of large and keeled scales from posterior edge of orbit to nuchal spine. Mental nearly pentagonal, posteriorly in contact with two elongate postmentals that contact with each other. Throat with two large scale rows parallel with infralabials. Gular scales strongly keeled except for smooth scales near the mental. Standard counts and measurements provided in Table 3.

Body elongate with dorsal and nuchal crest connected by lower crests (Fig. 2B). Nuchal crests longer than dorsal ones (3.81 mm vs. 1.56 mm) that reduce posteriorly. Dorsal scales heterogeneous, small, imbricate, intermixed with large, strongly keeled scales that have points directed backward or back-upward. An oblique fold in each side of neck, in front of shoulder. Ventrals strongly keeled. Limbs slender. Hind limb stretched forwards reaches nostril. Dorsal surface of limbs with large, strongly keeled scales. Narrow subdigital lamellae under fourth finger and toe 14 and 20, respectively. Tail longer than snout–vent length (TL/SVL = 1.8), covered with uniform strongly keeled scales; postcloacal region of tail strongly swollen.

In life, dorsum of body green with black lateral blotches. Venter whitish. Large black area in front of shoulder. Green to yellow-green wide band expands from snout through labials, tympanum, and neck to lateral of body. Upper side of head and neck black. Upper part of lateral view of head and neck also black. Black colored area becomes narrow at neck and expands at dorsum forming a diamond-shaped band in dorsal view. Tips of nuchal and postorbital spines, and nuchal crests yellow-green. Iris brown with a round pupil. Elbow and knee with white blotches. Original tail dark brown with white-cream or light green alternating rings.

In preservative (Fig. 2), color faded with unchanged pattern. Upper side of body and limbs with alternating turquoise, white, and black blotches. Labials, tympanum, and lateral view of neck white.

DNA Barcode. Type series with GenBank Accession No. MK695205–MK695210.

TABLE 3. Measurements (in mm) and scale counts for the type series of *Acanthosaura phongdienensis* **sp. nov.** SVL and TL in males and females were calculated separately. The sub-adult male was excluded in range and mean SD calculations. For character abbreviations see Materials and Methods.

	Holotype	Paratypes						Range	mean ± SD
	KIZ 10695	ROM 48013	ITBCZ 6830	KIZ 10697	ITBCZ 6831	KIZ 10657	KIZ 10617		
Sex	Male	Male	Male	Female	Female	Female	Sub-male		
SVL	74.2	77.4	73.2	58.5	64.7	60.9	62.2	m: 73.2–77.4	74.9 ± 2.2
								f: 58.5–64.7	61.4 ± 3.1
TL	132.6	136.2	137.2	97.5	94.6	99.5	104.7	m: 132.6–137.2	135.3 ± 2.4
								f: 94.6–99.5	97.2 ± 2.5
TL/SVL	1.8	1.8	1.9	1.7	1.5	1.6	1.7	1.8–1.9	1.8 ± 0.1
								1.5–1.7	1.6 ± 0.1
HL	22.4	23.8	21.9	18.6	19.6	18.7	17.8	18.6–23.8	20.8 ± 2.2
HW	14.4	15.9	15.3	13.3	13.8	13.1	12.6	13.1–15.9	14.3 ± 1.1
HH	13.5	13.6	13.1	10.7	11	10.4	10.5	10.4–13.6	12.1 ± 1.5
MW	1.35	1.52	1.33	0.87	1.03	1.44	1.3	0.87–1.52	1.3 ± 0.3
MH	1.36	1.60	1.60	1.07	1.04	1.52	1.4	1.04–1.60	1.4 ± 0.3
RW	2.65	2.47	2.46	2.33	2.10	2.07	2.13	2.07–2.65	2.3 ± 0.2
RH	1.32	1.16	1.14	1.08	1.00	1.17	1.23	1.00–1.32	1.1 ± 0.1
ED	3.97	4.17	4.00	3.10	3.42	3.18	3.3	3.10–4.17	3.6 ± 0.5
TD	2.81	2.68	2.38	1.92	2.41	1.78	1.62	1.78–2.81	2.3 ± 0.4
PS	2.07	–	–	1.18	1.28	1.54	–	1.18–2.07	1.5 ± 0.4
NS	2.64	4.15	2.26	1.53	2.19	2.34	2.45	1.53–4.15	2.5 ± 0.9
NC	3.81	4.18	3.77	1.84	2.28	1.24	1.75	1.24–4.18	2.9 ± 1.2
DC	1.56	1.65	1.31	0.65	0.61	0.58	1.55	0.58–1.65	1.1 ± 0.5
SL	11	11	10	11	12	9	10	9–12	10.6 ± 1.0
IL	10	11	11	11	11	10	11	10–11	10.7 ± 0.5
CS	11	13	12	11	9	11	12	9–13	11.3 ± 1.3
F4	14	15	17	15	15	15	15	14–17	15.1 ± 0.9
T4	20	20	23	19	21	20	22	19–23	20.7 ± 1.4

Variation. Males significantly larger than females (SVL 74.9 mm versus 61.4 mm; $p < 0.01$). Relative tail length of males significantly longer than females (TL/SVL 1.8 versus 1.6, $p < 0.01$). Rostral scale in paratype KIZ 10657 half-separated from upper but still forms an entire scale. Spines and crests of females shorter than those of males. Variation in size and squamation provided in Table 3. Color and pattern of males and females differ; males similar to holotype with green ground color, green to yellow at labial, tympanum to lateral of neck, upper side of head and neck black or dark brown. Females with brown color on dorsal and lateral views (Fig. 4). Lateral side of head in female radiatiform from eye through labials. Juvenile color pattern unknown.

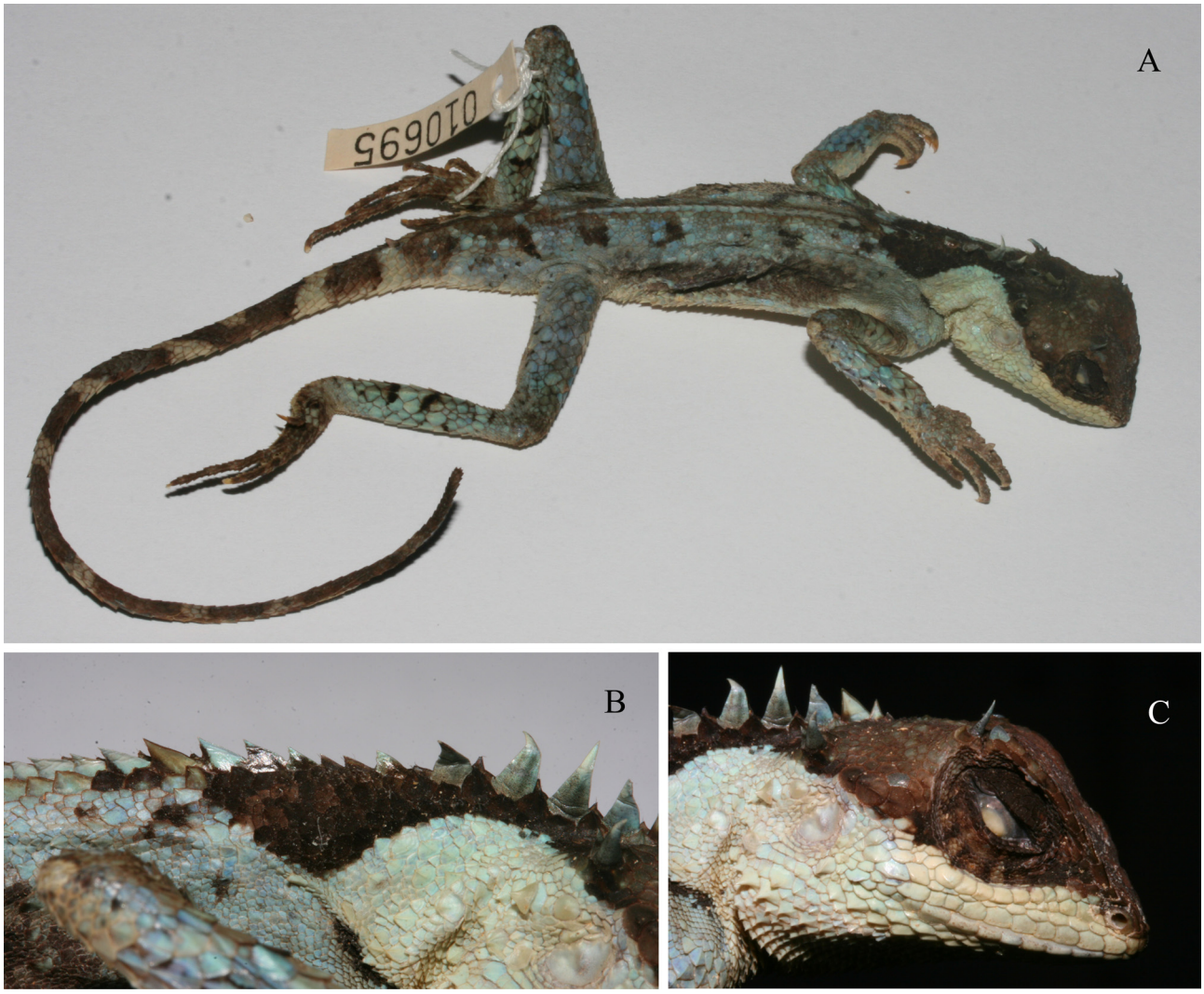

FIGURE 2. Holotype of *Acanthosaura phongdienensis* **sp. nov.** A, dorsal-lateral view; B, Close-up to the connection between nuchal and dorsal crests; C, lateral view of head.

Comparison

Acanthosaura phongdienensis **sp. nov.** differs from *A. capra*, *A. murphyi*, and *A. nataliae* by the absence of a spine on each side of neck. By having continuous dorsal-nuchal crests, the new species differs from *A. armata* (Gray 1827 *in* Hardwicke & Gray 1827), *A. bintangensis* Wood, Grismer, Grismer, Ahmad, Onn & Bauer, 2009, *A. cardamomensis*, *A. crucigera* Boulenger, 1885, *A. phuketensis* Pauwels, Sumontha, Kunya, Nitikul, Samphanthamit, Wood & Grismer, 2015, and *A. titiwangsaensis* Wood, Grismer, Grismer, Ahmad, Onn & Bauer, 2009. The new species is intermediate morphologically between *A. coronata* and *A. lepidogaster* in having continuous nuchal-dorsal crests (like the former), entire rostral scute, and in appearance (like the later). From *A. coronata*, the new species differs by having longer postorbital and nuchal spines (2.5 mm versus 1.1–1.6 mm), longer nuchal and dorsal crests, and having entire rostral scute (versus divided into two or three small scutes; Ananjeva *et al.* 2008). Genetically, the new species differs from *A. coronata* by an uncorrected *p*-distance (*COI*) of 21.7%. *Acanthosaura phongdienensis* **sp.**

nov. mainly differs from *A. brachypoda* and *A. lepidogaster* by having a continuous nuchal-dorsal crest (Ananjeva *et al*. 2008, Figs. 2B, 3). In addition, the new species differs from *A. brachypoda* and *A. lepidogaster* by uncorrected *p*-distances of 13.6% and 12.8%, respectively. Using the key provided by Ananjeva *et al*. (2008) for identification of Vietnamese *Acanthosaura*, the new species is most similar to *A. coronata*. However, the entire rostral scute readily distinguishes the new species from *A. coronate*, which possesses two or three small rostrals.

Etymology. The specific epithet *phongdienensis* refers to the Vietnamese name Phong Điền Nature Reserve where the new species was discovered.

Distribution and habitat. *Acanthosaura phongdienensis* **sp. nov.** is known only from Phong Dien Nature Reserve, Thua Thien-Hue Province, Vietnam (site 8 in Fig. 1). Lizards were collected on small trees in evergreen forest and along small streams at elevations ranging from 165 to 1019 m a.s.l. All lizards were found at night during the wet season.

FIGURE 3. *Acanthosaura phongdienensis* **sp. nov.** (left) and *A.* cf. *lepidogaster* from northern Vietnam (Phu Tho Prov.) (right). Red arrows show the continuity of nuchal and dorsal crests in the new species, while crests in the later species are clearly separated by a long diastema covered by small scales.

Discussion

The type locality of *Acanthosaura lepidogaster*, which occurs widely in southern China, Thailand, Laos, Vietnam, and Myanmar, is uncertain. It was given as 'Cochinchina' but this locality (region) is vague. Starting in the 16th Century, Cochinchina referred to Vietnam in its entirety. However, from the beginning of the 19th Century Cochinchina was restricted to the southern provinces of Vietnam (Adler 2009; Ananjeva *et al.* 2008). Ananjeva *et al*. (2008) interpreted Cochinchina as including all of modern Vietnam. No data further restrict the type locality for *A. lepidogaster*, although DNA sequence taken from the type specimen might yield further insight. Previous DNA analyses showed that the species occurs in Vietnam from Ha Tinh Province northward (Ananjeva *et al.* 2008) and morphological analyses suggested that *A. lepidogaster* occurs northward from Kon Tum Province (Ananjeva *et al.* 2008). We include five localities in Vietnam from within this range and two additional sites in southern China (Fig. 1). Further, our sequencing uses two samples (ROM 31954 and ROM 30720) identified as *A. lepidogaster* by Kalyabina-Hauf

et al. (2004) and Ananjeva *et al*. (2008) from northern Vietnam. *Acanthosaura phongdienensis* **sp. nov.** appears to occur within the range of *A. lepidogaster* in Vietnam, and, thus, it is possible that the two taxa are conspecific. However, the new species differs from *A. lepidogaster* morphologically. A diastema separates the nuchal and dorsal crests of *A. lepidogaster* as recently confirmed by Ananjeva *et al.* (2008) (2.0–8.6 mm, $n = 25$), Hallermann (2000) (> 7 mm, $n = 12$), and Wood *et al*. (2010) (10–12 mm, $n = 3$). In contract, the new species has a continuous nuchal-dorsal crest. Further, an uncorrected *p*-distance of 12.8% also separates the two species.

The matrilineal genealogy (Fig. 1) resolves well all five Vietnamese species of *Acanthosaura* in this study with high levels of nodal support. However, DNA barcoding does not resolve genealogical relationships between the new species, *A. nataliae*, and *A. brachypoda-lepidogaster* because of a polytomy. Although morphologically intermediate between *A. coronata* and *A. lepidogaster*, the new species is genetically closer to the latter species. The uncorrected *p*-distance between the new species and *A. lepidogaster* is much higher than that between *A. brachypoda* and *A. lepidogaster* (12.8% vs. 4.2%, respectively). Our study resolves little genetic variation in *A. lepidogaster*, whose distribution ranges in northern Vietnam and southern China, including Hainan Island (1% in both *p*-distance and K2P). Our description gives *Acanthosaura* 13 species in total, including eight in Vietnam.

FIGURE 4. *Acanthosaura phongdienensis* **sp. nov.** in life. Female (upper) is brown and male (lower) exhibits green-dark colors.

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