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Research article

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A new species of *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from the Truong Son Mountain Range, Vietnam

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Abstract. We describe a new species of the genus *Acanthosaura* Gray, 1831 from the Truong Son Mountain Range, southern Vietnam, based on integrative taxonomic analyses. *Acanthosaura cuongi* sp. nov. differs from its congeners by a combination of the following diagnostic characteristics: size moderate (snout-vent length: 79.4–104.61 mm); the absence of a diastema between the short nuchal

and dorsal crest spines; vertebral crests composed of two rows of enlarged, keeled, pointed scales, arranged in a zipper line; various body coloration with light-green, orange-yellow, and light or purple-gray; black eye patch extending posteriorly to the anterior edge of tympanum. Maximum likelihood (ML) and Bayesian inference (BI) analyses using two mitochondrial genes (COI and Cytb) support the monophyly of *Acanthosaura cuongi*. Furthermore, the new species differs from the closest lineage consisting of *A. coronata* Günther, 1861 by having pairwise genetic distances of 11.58–12.11%, and have a maximal intraspecific distance of 0.35%. This is the 21st species of *Acanthosaura* and the tenth species of the genus reported from Vietnam.

Keywords. Agamid lizards, morphology, phylogeny, taxonomy.

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Introduction

The genus *Acanthosaura* Gray, 1831 of the family Agamidae Gray, 1827 currently comprises 20 species, which are distributed among all countries of the Indo-Burma region, from northeastern India, southern China, Vietnam, Laos, Myanmar, Cambodia and Thailand, southward to the Malay Peninsula and Sumatra in Indonesia (Gray 1831; Taylor 1963; Manthey 2008; Grismer 2011; Nguyen *et al.* 2018, 2019; Liu & Rao 2019; Ananjeva *et al.* 2020; Liu *et al.* 2022; Trivalairat *et al.* 2022; Uetz *et al.* 2024). Extensive surveys discovered more agamid species and added to a better understanding of the taxonomic status of *Acanthosaura* morphotypes and genetic lineages. Based on integrative taxonomic analyses, viz. combining morphological characters and molecular phylogeny, the number of *Acanthosaura* species has significantly increased, half of them having been discovered in the last ten years (Nguyen *et al.* 2019; Ananjeva *et al.* 2020; Liu *et al.* 2022; Uetz *et al.* 2024).

To date, nine species of *Acanthosaura* are reported from Vietnam, viz. *A. brachypoda* Ananjeva *et al.*, 2011; *A. capra* Günther, 1861; *A. cardamomensis* Wood *et al.*, 2010; *A. coronata* Günther, 1861; *A. lepidogaster* Cuvier, 1829; *A. murphyi* Nguyen *et al.*, 2018; *A. nataliae* Orlov *et al.*, 2006; *A. phongdienensis* Nguyen *et al.*, 2019; and *A. prasina* Ananjeva *et al.*, 2020. Some of them even occur in sympatry, such as *A. capra* and *A. coronata* which are both known from the border area between Vietnam and Cambodia (Nguyen *et al.* 2018; Uetz *et al.* 2024). *Acanthosaura phongdienensis* and *A. prasina* are allopatrically distributed, but can be found in sympatry with the widely distributed *A. nataliae* (Orlov *et al.* 2006; Nguyen *et al.* 2019; Ananjeva *et al.* 2020). It is hypothesized that there is still potential of further cryptic, sympatrically occurring species of *Acanthosaura* to be discovered in Vietnam.

During recent fieldwork in Phu Yen and Khanh Hoa provinces, south-central Vietnam, covering the southeastern forest of the Truong Son Mountain Range, we found individuals of *A. murphyi* at its type locality, but also those of another *Acanthosaura* representative with a distinct morphotype (Nguyen *et al.* 2018). Based on morphological and molecular analyses, these individuals were identified as an unnamed taxon belonging to the genus *Acanthosaura*. We herein describe this newly discovered population as a new species.

Material and methods

Sampling

Two field surveys were conducted in south central Vietnam: Phu Yen Province (Tay Hoa District) and Khanh Hoa Province (Van Ninh District). Lizards were collected by hand and photographed live.

Animals were anaesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate (Simmons 2002), fixed in 85% ethanol and subsequently stored in 70% ethanol. Tissue samples were preserved separately in absolute ethanol. Voucher specimens used for the description were deposited at the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam.

Molecular analyses

Extraction of genomic DNA from three samples (Table 1) was carried out using DNeasy Blood & Tissue Kits (QIAGEN, Germany), the protocol followed the manufacturers' instructions. Total DNA was amplified using an Eppendorf PCR machine. Polymerase chain reaction (PCR) total volume for each reaction was 20 µl, consisting of 10 µl of Mastermix, 7 µl of Nuclease-Free Water, 0.5 µl (10 pmol/µl) of each primer and 2 µl of template DNA. Primers of 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) were used to amplify a fragment of Cytochrome c oxidase subunit I (COI) gene, and other primers of mt A-new L-14995 (5'-TCC CAG CCC CAT CCA ACA TCT CAG CAT GAT GAA ACT TCG-3') and mt F-new H-16060 (5'-AGG GTG GAG TCT TCA GTT TTT GGT TTA CAA GAC CAA TG-3') used for a fragment of Cytochrome b (Cytb) gene (Milto *et al.* 2001). PCR protocols for the amplification included an initial denaturation at 94°C for 5 minutes, 35 cycles at 94°C for 45 s, 52°C for 45 s, 72°C for 45 s and 72°C for 7 minutes for COI, and 94°C for 5 minutes, 35 cycles at 94°C for 45 s, 47°C for 45 s, 72°C for 75 s and 72°C for 7 minutes for Cytb. PCR products were sent to the 1st Base company in Malaysia for sequencing. The obtained sequences were deposited in GenBank under the accession numbers as stated in Tables 1–2.

Two samples of *Calotes versicolor* (Daudin, 1802) ([MK695211](#) and [MK695212](#)) and one of *Pseudocalotes brevipes* (Werner, 1904) ([AY572869](#)) were selected as outgroups (Table 1). The nucleotide sequences were aligned using the MUSCLE algorithm integrated in MEGA 11 (Edgar 2004; Tamura *et al.* 2021) with default settings. Locality information and GenBank accession numbers for all sequences used in this research can be found in Tables 1–2. Pairwise comparisons of uncorrected sequence divergences (p-distance) were calculated with MEGA 11 where the outgroup was excluded. Variance was estimated using bootstrap method with 1000 replicates using nucleotide substitution while gap/missing data were treated via the pairwise deletion option.

Prior to Maximum-likelihood (ML) and Bayesian inference (BI) analyses, the optimum substitution models were chosen using ModelFinder (Kalyaanamoorthy *et al.* 2017) and the chosen best-fit model for ML analysis was the TIM+F+I+G4 model of DNA evolution based on the Bayesian information criterion (BIC) while the closest model chosen for BI analysis was GTR+I+G4. For BI analysis, MrBayes ver. 3.2.7a (Ronquist *et al.* 2012) was used where the Metropolis-coupled Markov chain Monte Carlo analyses were run for 10 million generations and sampled every 100 generations. Two independent runs of four Markov chains were performed, and the first 25% of trees were discarded as burn-in. We checked parameter estimates and convergence using TRACER ver. 1.6 (Rambaut *et al.* 2017). Regarding the ML tree inference, IQ-TREE ver. 1.6.12 (Nguyen *et al.* 2015) was used with 10,000 ultrafast bootstrap (UFB) replications (Hoang *et al.* 2018). We considered Bayesian posterior probability (BPP) and UFB support values of greater than or equal to 0.95 to indicate strong support (Felsenstein 1985; Hoang *et al.* 2018).

Morphological analysis

Measurements of collected specimens were taken to the nearest 0.1 mm with digital calipers. Meristic characters were taken on the right side. All data of currently recognized *Acanthosaura* species were taken from Günther (1861), Boulenger (1885), Ananjeva *et al.* (2008, 2011, 2020, 2021), Wood *et al.* (2009, 2010), Pauwels *et al.* (2015), Nguyen *et al.* (2018, 2019), Trivalairat *et al.* (2020) and Liu *et al.* (2020, 2022).

Table 1 (continued on next two pages). Samples used for molecular analysis (COI fragment) in this study (VN: Vietnam).

Genus	Locality	Voucher no.	GenBank accession no.	Reference
Ingroup – COI				
<i>Acanthosaura cuongi</i> sp. nov.	Phu Yen Province, VN	IEBR R.5250	PP669986	This study
<i>Acanthosaura cuongi</i> sp. nov.	Phu Yen Province, VN	IEBR R.5257	PP669987	This study
<i>Acanthosaura cuongi</i> sp. nov.	Khanh Hoa Province, VN	IEBR R.5251	PP669985	This study
<i>Acanthosaura brachypoda</i>	Sa Pa, Lao Cai Province, VN	ROM 38118	MK695182	Nguyen <i>et al.</i> 2019
<i>Acanthosaura brachypoda</i>	Sa Pa, Lao Cai Province, VN	ROM 38119	MK695183	Nguyen <i>et al.</i> 2019
<i>Acanthosaura brachypoda</i>	Sa Pa, Lao Cai Province, VN	ROM 38120	MK695184	Nguyen <i>et al.</i> 2019
<i>Acanthosaura brachypoda</i>	Sa Pa, Lao Cai Province, VN	ROM 38123	MK695185	Nguyen <i>et al.</i> 2019
<i>Acanthosaura capra</i>	Bu Gia Map, Binh Phuoc Province, VN	BGM 01	MK239022	Nguyen <i>et al.</i> 2018
<i>Acanthosaura coronata</i>	Bu Gia Map, Binh Phuoc Province, VN	KIZ47	MK695186	Nguyen <i>et al.</i> 2019
<i>Acanthosaura coronata</i>	Bu Gia Map, Binh Phuoc Province, VN	KIZ48	MK695187	Nguyen <i>et al.</i> 2019
<i>Acanthosaura coronata</i>	Bu Gia Map, Binh Phuoc Province, VN	KIZ97	MK695188	Nguyen <i>et al.</i> 2019
<i>Acanthosaura coronata</i>	Cat Tien, Dong Nai Province, VN	ROM 42240	MK695189	Nguyen <i>et al.</i> 2019
<i>Acanthosaura coronata</i>	Cat Tien, Dong Nai Province, VN	ROM 42241	MK695190	Nguyen <i>et al.</i> 2019
<i>Acanthosaura crucigera</i>	Tanintharyi, Ma Noe Lone, Lenya, Myanmar	USNM587488	MT607980	Mulcahy <i>et al.</i> 2018
<i>Acanthosaura crucigera</i>	Tanintharyi, Ma Noe Lone, Lenya, Myanmar	USNM587489	MT607981	Mulcahy <i>et al.</i> 2018
<i>Acanthosaura crucigera</i>	Tanintharyi, Ma Noe Lone, Lenya, Myanmar	USNM587487	MT607982	Mulcahy <i>et al.</i> 2018
<i>Acanthosaura lepidogaster</i>	Na Hang, Tuyen Quang Province, VN	ROM 30505	MK695191	Nguyen <i>et al.</i> 2019
<i>Acanthosaura lepidogaster</i>	Na Hang, Tuyen Quang Province, VN	ROM 30507	MK695192	Nguyen <i>et al.</i> 2019
<i>Acanthosaura lepidogaster</i>	Quang Thanh, Cao Bang Province, VN	ROM 30677	MK695193	Nguyen <i>et al.</i> 2019
<i>Acanthosaura lepidogaster</i>	Quang Thanh, Cao Bang Province, VN	ROM 30680	MK695194	Nguyen <i>et al.</i> 2019
<i>Acanthosaura lepidogaster</i>	Tam Dao, Vinh Phuc Province, VN	ROM 30712	MK695195	Nguyen <i>et al.</i> 2019

Table 1. Continued.

Genus	Locality	Voucher no.	GenBank accession no.	Reference
<i>Acanthosaura liui</i>	Jianshui, Honghe, Yunnan Province, China	KIZL2020003	MT769763	Liu <i>et al.</i> 2020
<i>Acanthosaura liui</i>	Jianshui, Honghe, Yunnan Province, China	KIZL2020004	MT769764	Liu <i>et al.</i> 2020
<i>Acanthosaura liui</i>	Jianshui, Honghe, Yunnan Province, China	KIZL2020005	MT769765	Liu <i>et al.</i> 2020
<i>Acanthosaura liui</i>	Jianshui, Honghe, Yunnan Province, China	KIZL2020006	MT769766	Liu <i>et al.</i> 2020
<i>Acanthosaura liui</i>	Jianshui, Honghe, Yunnan Province, China	KIZL2020007	MT769767	Liu <i>et al.</i> 2020
<i>Acanthosaura longicaudata</i>	Jiangcheng, Yunnan Province, China	KIZ_L0051	ON207528	Liu <i>et al.</i> 2022
<i>Acanthosaura longicaudata</i>	Jiangcheng, Yunnan Province, China	KIZ_L0132	ON207529	Liu <i>et al.</i> 2022
<i>Acanthosaura longicaudata</i>	Jiangcheng, Yunnan Province, China	KIZ_L0133	ON207530	Liu <i>et al.</i> 2022
<i>Acanthosaura longicaudata</i>	Jiangcheng, Yunnan Province, China	KIZ_L0150	ON207531	Liu <i>et al.</i> 2022
<i>Acanthosaura murphyi</i>	Deo Ca, Phu Yen Province, VN	ITBCZ 4603	MK239025	Nguyen <i>et al.</i> 2018
<i>Acanthosaura murphyi</i>	Hon Ba, Khanh Hoa Province, VN	ITBCZ 3533	MK239026	Nguyen <i>et al.</i> 2018
<i>Acanthosaura murphyi</i>	Deo Ca, Phu Yen Province, VN	PYU 147	MK239027	Nguyen <i>et al.</i> 2018
<i>Acanthosaura nataliae</i>	Tram Lap, Gia Lai Province, VN	ROM30629	MK695202	Nguyen <i>et al.</i> 2019
<i>Acanthosaura nataliae</i>	Tram Lap, Gia Lai Province, VN	ROM30631	MK695203	Nguyen <i>et al.</i> 2019
<i>Acanthosaura nataliae</i>	Tram Lap, Gia Lai Province, VN	ROM30632	MK695204	Nguyen <i>et al.</i> 2019
<i>Acanthosaura phongdienensis</i>	Phong Dien, Thua Thien Hue Province, VN	ITBCZ6831	MK695205	Nguyen <i>et al.</i> 2019
<i>Acanthosaura phongdienensis</i>	Phong Dien, Thua Thien Hue Province, VN	ROM48013	MK695206	Nguyen <i>et al.</i> 2019
<i>Acanthosaura phongdienensis</i>	Phong Dien, Thua Thien Hue Province, VN	KIZ10657	MK695207	Nguyen <i>et al.</i> 2019

Table 1. Continued.

Genus	Locality	Voucher no.	GenBank accession no.	Reference
<i>Acanthosaura phongdienensis</i>	Phong Dien, Thua Thien Hue Province, VN	ITBCZ6830	MK695208	Nguyen <i>et al.</i> 2019
<i>Acanthosaura phongdienensis</i>	Phong Dien, Thua Thien Hue Province, VN	KIZ10695	MK695209	Nguyen <i>et al.</i> 2019
<i>Acanthosaura phongdienensis</i>	Phong Dien, Thua Thien Hue Province, VN	KIZ10697	MK695210	Nguyen <i>et al.</i> 2019
<i>Acanthosaura rubrilabris</i>	Menghai, Yunnan Province, China	KIZ_L0057	ON207534	Liu <i>et al.</i> 2022
<i>Acanthosaura rubrilabris</i>	Menghai, Yunnan Province, China	KIZ_L0010	ON207535	Liu <i>et al.</i> 2022
<i>Acanthosaura rubrilabris</i>	Jinghong, Yunnan Province, China	KIZ_L0006	ON207536	Liu <i>et al.</i> 2022
<i>Acanthosaura rubrilabris</i>	Jinghong, Yunnan Province, China	KIZ_L0040	ON207537	Liu <i>et al.</i> 2022
<i>Acanthosaura rubrilabris</i>	Lancang, Yunnan Province, China	KIZ_L0137	ON207543	Liu <i>et al.</i> 2022
<i>Acanthosaura rubrilabris</i>	Lancang, Yunnan Province, China	KIZ_L0140	ON207544	Liu <i>et al.</i> 2022
Outgroup				
<i>Calotes versicolor</i>	Ta Kou, Binh Thuan Province, VN	KIZ1120	MK695211	Nguyen <i>et al.</i> 2019
<i>Calotes versicolor</i>	Ta Kou, Binh Thuan Province, VN	ITBCZ1034	MK695212	Nguyen <i>et al.</i> 2019

Table 2 (continued on next three pages). Samples used for molecular analysis (Cytb fragment) in this study (VN: Vietnam).

Taxon	Locality	Voucher no.	Genbank accession no.	Reference
Ingroup – Cytb				
<i>Acanthosaura cuongi</i> sp. nov.	Phu Yen Province, VN	IEBR R.5250	PQ119778	This study
<i>Acanthosaura cuongi</i> sp. nov.	Phu Yen Province, VN	IEBR R.5257	PQ119779	This study
<i>Acanthosaura cuongi</i> sp. nov.	Khanh Hoa Province, VN	IEBR R.5251	PQ119777	This study
<i>Acanthosaura cuongi</i> sp. nov. cf.	Krong Pa, Gia Lai Province, VN	ROM 31985	AY572896	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura coronata</i>	Cat Tien, Dong Nai Province, VN	ROM 24840	AY572897	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura coronata</i>	Cat Tien, Dong Nai Province, VN	ROM 27204	AY572898	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura coronata</i>	Cat Tien, Dong Nai Province, VN	ROM 42241	AY572899	Kalyabina-Hauf <i>et al.</i> 2004

Table 2. Continued.

Taxon	Locality	Voucher no.	Genbank accession no.	Reference
<i>Acanthosaura coronata</i>	Ba Ra, Binh Phuoc Province, VN	ZISP F5613	MT376190	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura coronata</i>	Ba Ra, Binh Phuoc Province, VN	ZISP F5618	MT376191	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura tongbiguanensis</i>	Tongbiguan, Dehong, Yunnan Province, China	KIZL201801	MN604012	Liu & Rao 2019
<i>Acanthosaura tongbiguanensis</i>	Tongbiguan, Dehong, Yunnan Province, China	KIZL201802	MN604013	Liu & Rao 2019
<i>Acanthosaura tongbiguanensis</i>	Tongbiguan, Dehong, Yunnan Province, China	KIZL201803	MN604014	Liu & Rao 2019
<i>Acanthosaura crucigera</i>	Bago Division Bago Yoma, Myanmar	CAS 206626	AY572888	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura crucigera</i>	Bago Division Bago Yoma, Myanmar	CAS 208426	AY572895	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phuketensis</i>	Kao Yoi, Phetchaburi, Thailand	No data	AY572887	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phuketensis</i>	Khao Lak Takua Pa Phang Nga, Thailand	PCUM	AY572890	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phuketensis</i>	Khao Lak Takua Pa Phang Nga, Thailand	PCUM	AY572891	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phuketensis</i>	Khao Lak Takua Pa Phang Nga, Thailand	PCUM	AY572892	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phuketensis</i>	Malaysia	No data	AY572893	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phuketensis</i>	Malaysia	No data	AY572894	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura armata</i>	Pulau Pinang, Pinang, Malaysia	PCUM	AY572871	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura armata</i>	Pulau Pinang, Pinang, Malaysia	PCUM	AY572872	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura prasina</i>	Ngoc Linh, Kon Tum Province, VN	ROM 27483	AY572928	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura prasina</i>	Kon Chu Rang, Gia Lai Province, VN	ZISP 30745	MT376205	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura prasina</i>	Kon Chu Rang, Gia Lai Province, VN	ZISP 30746	MT376206	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura prasina</i>	Konplong District, Kon Tum Province, VN	ZISP 28857	MT376212	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura nataliae</i>	Krong Pa, Gia Lai Province, VN	ROM 31983	AY572873	Liu & Rao 2019

Table 2. Continued.

Taxon	Locality	Voucher no.	Genbank accession no.	Reference
<i>Acanthosaura nataliae</i>	Tram Lap, Gia Lai Province, VN	ROM 30627	AY572880	Liu & Rao 2019
<i>Acanthosaura nataliae</i>	Krong Pa, Gia Lai Province, VN	ROM 32161	AY572882	Liu & Rao 2019
<i>Acanthosaura nataliae</i>	Konplong, Kon Tum Province, VN	ZISP F32922	MT376213	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura phongdienensis</i>	Khe Moi, Nghe An Province, VN	ROM 26328	AY572900	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Vu Quang, Ha Tinh Province, VN	ZISP 20753-1	AY572904	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Vu Quang, Ha Tinh Province, VN	ZISP 20753-2	AY572905	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Con Cuong, Nghe An Province, VN	FMNH 255581	AY572917	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Tuong Duong, Nghe An Province, VN	FMNH 255584	AY572918	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Nakai, Khammouane Province, Laos	FMNH 255488	AY572920	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Nakai, Khammouane Province, Laos	FMNH 255487	AY572921	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Vieng Tong, Huaphan Province, Laos	FMNH 255489	AY572922	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Khao Yoi, Thailand	PCUM	AY572923	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura phongdienensis</i>	Kon Chu Rang, Gia Lai Province, VN	ZISP 30747	MT376202	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura phongdienensis</i>	Kon Chu Rang, Gia Lai Province, VN	ZISP 30748	MT376203	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura phongdienensis</i>	Kon Chu Rang, Gia Lai Province, VN	ZISP 30749	MT376204	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura phongdienensis</i>	Pu Mat, Nghe An Province, VN	ZISP FR2017	MT376214	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura phongdienensis</i>	Pu Mat, Nghe An Province, VN	ZISP F52	MT376216	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Chi Linh, Hai Duong Province, VN	ROM 31954	AY572901	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura lepidogaster</i>	Tam Dao, Vinh Phuc Province, VN	ROM 30503	AY572906	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura lepidogaster</i>	Quang Thanh, Cao Bang Province, VN	ROM 36074	AY572909	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura lepidogaster</i>	Chi Linh, Hai Duong Province, VN	ROM 31960	AY572911	Kalyabina-Hauf <i>et al.</i> 2004
<i>Acanthosaura lepidogaster</i>	Thaphabat Bolikhamsay, Laos	FMNH 255491	AY572919	Kalyabina-Hauf <i>et al.</i> 2004

Table 2. Continued.

Taxon	Locality	Voucher no.	Genbank accession no.	Reference
<i>Acanthosaura lepidogaster</i>	Na Hang, Tuyen Quang Province, VN	ZISP F11775	MT376192	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Na Hang, Tuyen Quang Province, VN	ZISP F11777	MT376193	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Che Tao, Mu Cang Chai, Yen Bai Province, VN	ZISP F11846	MT376200	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Che Tao, Mu Cang Chai, Yen Bai Province, VN	ZISP F11881	MT376201	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Tam Dao, Vinh Phuc Province, VN	ZISP F20977	MT376207	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Tam Dao, Vinh Phuc Province, VN	ZISP F20978	MT376208	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Wuchi, Central Hainan, China	ZISP F27794	MT376209	Ananjeva <i>et al.</i> 2020
<i>Acanthosaura lepidogaster</i>	Wuchi, Central Hainan, China	ZISP F27795	MT376210	Ananjeva <i>et al.</i> 2020
Outgroup				
<i>Pseudocalotes brevipes</i>	Na Hang, Tuyen Quang Province, Vietnam	ROM 30515	AY572869	Kalyabina-hauf <i>et al.</i> 2004

In this study, the following morphometric and meristic data were collected:

- AG = axilla to groin distance, measured from posterior base of forelimb at its emergence from body to anterior base of hindlimb at its emergence from body
 BEP = presence (1) or absence (0) of a black eye patch
 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
 DC = number of undivided scales in the vertebral column
 DIAS = length of the diastema, measured from the posterior end of the nuchal crest to the anterior end of the dorsal crest
 DSN = total number of scales and pairs of scale on vertebral scale column
 EYE = eye diameter, measured from the posterior to the anterior edge of the eye
 FI = number of subdigital lamellae on the fourth finger
 FOREL = forelimb length, measured from axilla to the proximal edge of the palmar region
 GP = size of gular pouch, scored as absent (0), small (1), medium (2), large (3), or very large (4)
 HH = maximum head height, measured across the parietal region
 HINDL = hindlimb length, measured from groin to the proximal edge of the plantar region
 HL = head length, measured from posterior edge of the jaw to the tip of the snout
 HW = head width, maximum head width, the width at the level of the tympanum
 INFRAL = number of infralabials
 LKP = presence (1) or absence (0) of light knee patch

MH	=	mental height
MW	=	mental width
NC	=	number of spines on nuchal crest big and small
NR	=	number of scales between the nasals and the rostrals
NS	=	number of scales between nasals
NSSOS	=	number of scales surrounding the occipital spine
OF	=	oblique fold anterior to the fore limb insertions
Orbit	=	orbit diameter, measured from the posterior to the anterior edge of the orbit
OS	=	occipital spine length
PM	=	number of scales surrounding the mental
PS	=	postorbital spine length
RH	=	rostral height
RS	=	number of scales surrounding the rostral
RW	=	rostral width
SDL	=	maximum length of the vertebral scale
SDW	=	maximum width of the vertebral scale
SL	=	snout length, measured from the tip of the snout to the medial canthus
SNL	=	maximum length of the largest spine in the nuchal crest measured from the base to the tip
SNW	=	maximum width of the largest spine in the nuchal crest
SUPRAL	=	number of supralabials
SVL	=	snout-vent length, measured from the tip of the snout to the vent
TaL	=	tail length, measured from the posterior margin of the vent to the tip of the tail
TBW	=	tail base width
TD	=	tympanum diameter, measured horizontally from the anterior to the posterior border of the tympanum
TN	=	scales absent on tympanum
TO	=	number of subdigital lamellae on the fourth toe
VENT	=	number of ventral scales, counted at the midline from the anterior edge of the shoulders to the edge of the vent

Comparative morphological data were taken from original descriptions and subsequent studies (Günther 1861; Boulenger 1885; Smith 1935; Taylor 1963; Orlov *et al.* 2006; Ananjeva *et al.* 2008, 2011, 2020; Wood *et al.* 2009, 2010; Pauwels *et al.* 2015; Nguyen *et al.* 2018, 2019; Trivalairat *et al.* 2020; Liu *et al.* 2020, 2022).

Repositories

IEBR	=	Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam
ITBCZ	=	Institute of Tropical Biology Collection of Zoology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam
KIZ	=	Kunming Natural History Museum of Zoology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China
ROM	=	Royal Ontario Museum, Toronto, Canada
USNM	=	The National Collection of Amphibians and Reptiles, National Museum of Natural History, Washington D.C., United States

Results

The sequence alignments contained 600 bp in length of Cytochrome Oxidase Subunit 1 (COI) and 1000 bp in length of Cytochrome b (Cytb). The Bayesian inference and Maximum Likelihood analyses of each sequenced gene yielded similar topologies. All new sequences were deposited in GenBank with the accession numbers of PP669985–PP669987 for COI sequences and PQ119777–PQ119779 for Cytb sequences (Tables 1–2). The COI analyses among selected specimens of twelve *Acanthosaura* species estimated interspecific differences with uncorrected p-distances from 3.91% (between *A. brachypoda* and *A. lepidogaster*) to 25.75% (between *A. nataliae* and *A. capra*). The new species differed from investigated congeners with p-distances from 14.21% (*A. coronata*) to 25.62% (*A. nataliae*) (Table 3). Regarding Cytb analyses, *Acanthosaura* species had uncorrected p-distances ranging from 11.58% (between the new species and *A. coronata*) to 24.34% (*A. prasina* and *A. coronata*). The p-distances between the new species and its congeners were from 11.58–12.11% (*A. coronata*) to 21.5–23.97% (*A. lepidogaster*) (Table 4). Intraspecific differences in terms of both genes were only 0–0.35% among investigated specimens of the new species (Tables 3–4).

The phylogenetic trees of the BI and ML analyses recovered a strong nodal support (except for the ML analysis of COI with a value of 87 < 95%) for the monophyly of the new species with *A. coronata* being its sister lineage thus warranting recognition as a distinct taxon (Figs 1–2).

Taxonomy

Class Reptilia Laurenti, 1768
Order Squamata Oppel, 1811
Suborder Iguania Cope, 1864
Family Agamidae Gray, 1827
Subfamily Draconinae Fitzinger, 1826
Genus *Acanthosaura* Gray, 1831

Acanthosaura cuongi sp. nov.

urn:lsid:zoobank.org:act:FDB2D34D-A091-4BB2-8456-A5E43E28BCAA

Figs 1–8; Tables 3–4; Supp. file 1

Diagnosis

Moderately-sized agamid lizard (SVL 79.40–87.06 mm in males, 85.69–104.61 mm in females); head triangular, relatively long (HL/SVL 0.27–0.31, HW/SVL 0.18–0.21), tail relatively long (TaL/SVL 1.44–1.51 in males and 1.09–1.38 in females), postorbital and occipital spines present; gular pouch undeveloped; 8–10 scales between nasals; four scales bordering mental scale; 10–13 supralabials; 10–13 infralabials; 5–7 scales bordering rostral scale; nuchal crest composed of 7–9 spines relatively high and one spine short; dorsal crest present, undeveloped; double rows of vertebral scales followed the dorsal crest; nuchal and dorsal crests continuous, filled with small spine scales; lateral scales small, intermixed with large, keeled scales, keels directed backward and back-upward; number of subdigital lamellae 14–17 on fourth finger and 19–23 on fourth toe (Table 3); black nuchal collar present; dorsum with black bands; a black patch extending from eye posteriorly to the anterior edge of tympanum.

Etymology

The specific epithet is dedicated to the Vietnamese herpetologist Dr Cuong The Pham, Institute of Ecology and Biological Resources (IEBR), Hanoi, in recognition of his support for field surveys in Khanh Hoa and Phu Yen provinces and his great contributions to herpetological research in Vietnam.

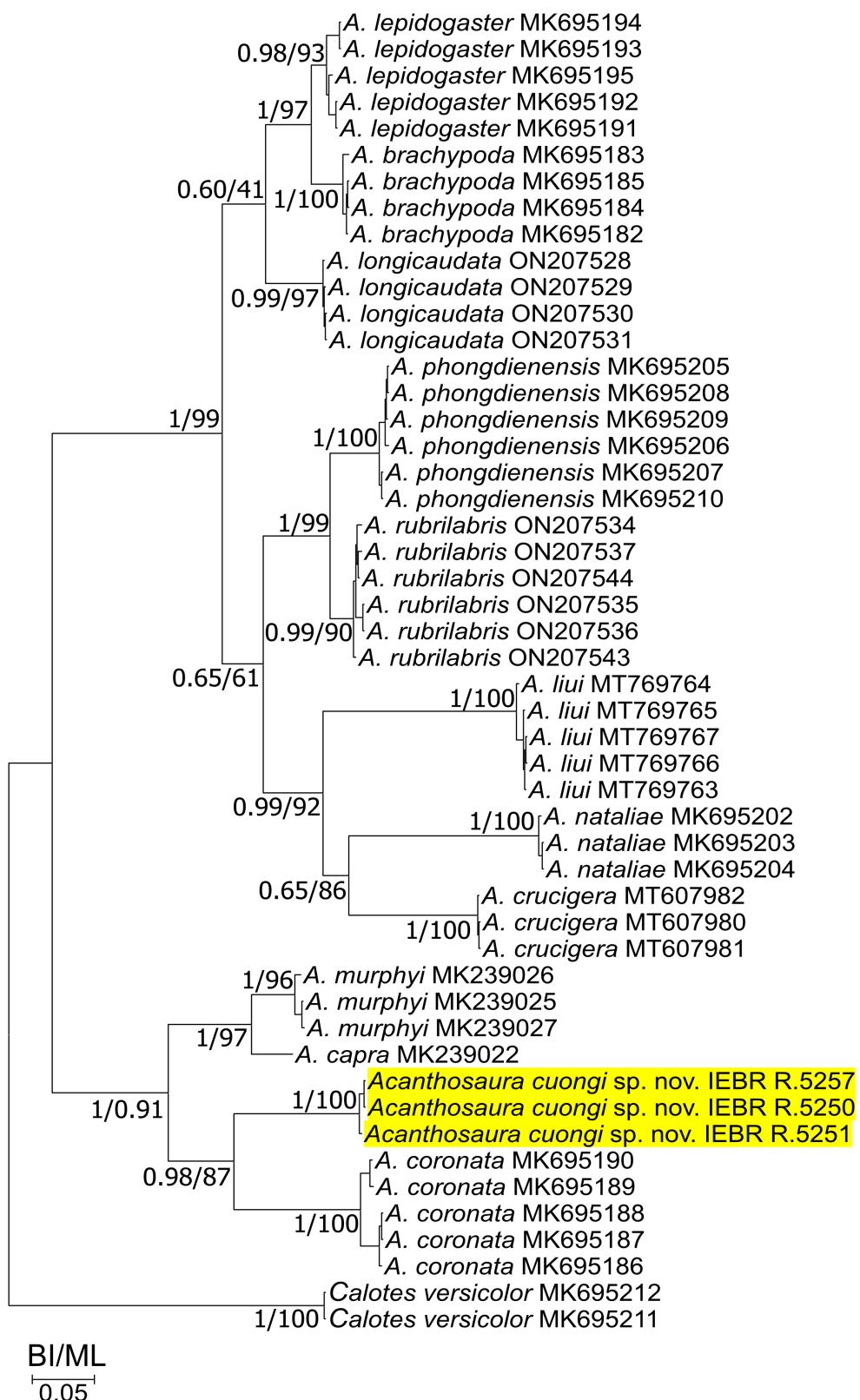


Fig. 1. Bayesian – Maximum Likelihood tree based on partial COI gene sequences of specimens used in this study. Values at each node indicate Bayesian Posterior Probabilities and Ultrafast Bootstrap Values from Maximum Likelihood analysis respectively (BI/ML).

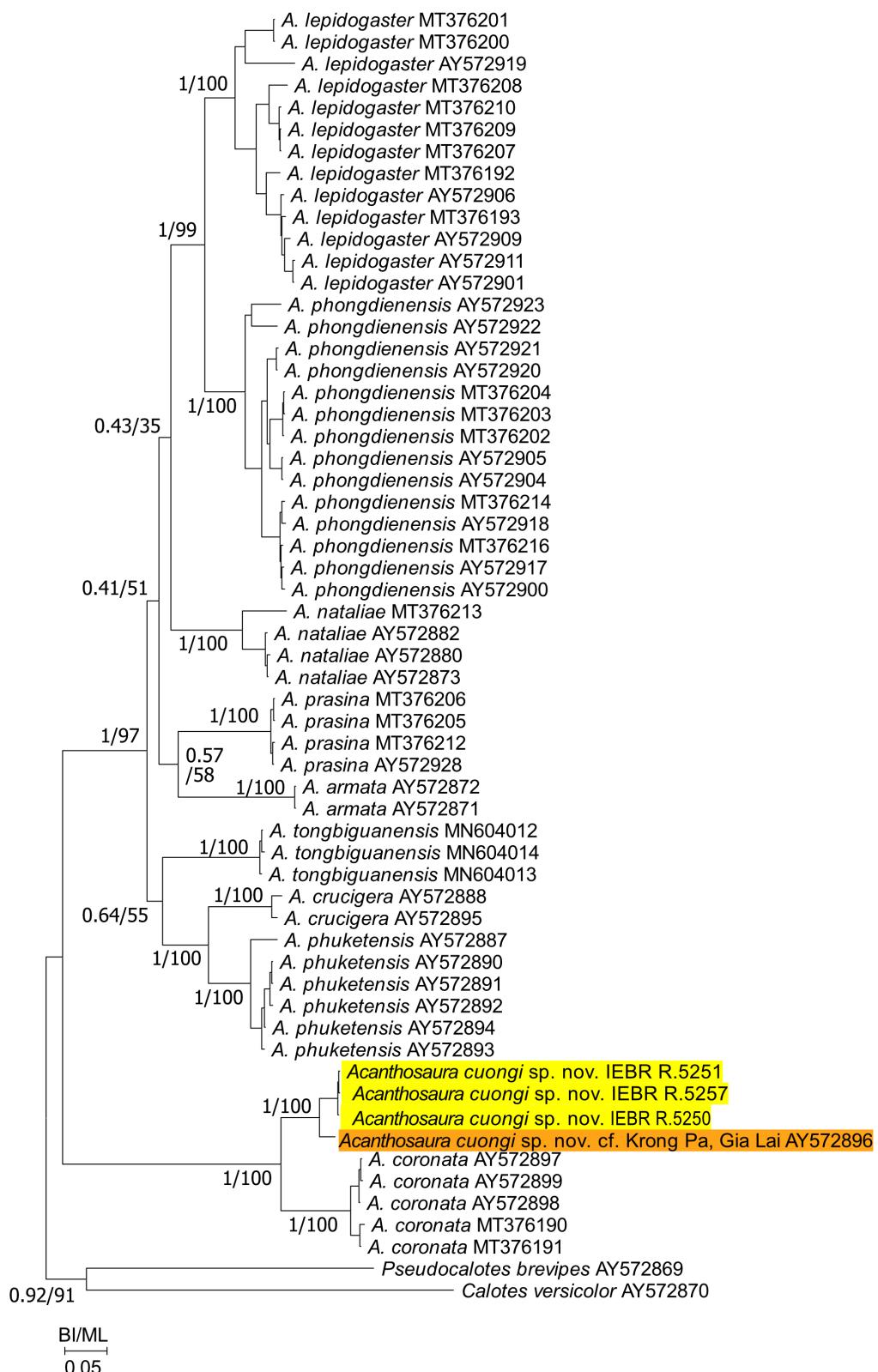


Fig. 2. Bayesian – Maximum Likelihood tree based on Cytb gene sequences of specimens used in this study. Values at each node indicate Bayesian Posterior Probabilities and Ultrafast Bootstrap Values from Maximum Likelihood analysis respectively (BI/ML).

digits on manus; subdigital scales keeled, subdigital lamellae 15/15 on the fourth finger; five digits on pes; subdigital scales keeled, subdigital lamellae 20/20 on the fourth finger; tail covered by small keeled scales, directed posteriorly.

Coloration of live holotype

Dorsal surface of head brown-green with a short transverse band light-green between orbital regions; occipital surface light-green; a black patch extending from behind the eye backward and slightly upward to anterior margin of tympanum; upper and lower lips green; lateral side of neck grey-white in most parts; nuchal crest spines pastel-orange (third, fifth and sixth spines) and green (remaining spines); iris yellow-brown; tongue blueish gray; dorsal surface of body light-green with seven brownish blackblured stripes, oriented obliquely forward from both sides of the vertebral region and one on shoulder; dorsal surface of limbs light-green, light spots on elbows and knees present; gular region and ventral side yellow-white in most parts; ventral sides of limbs yellow-green in the upper half part and white in the remaining part; black and light-green rings on tail, the rings become indistinct on posterior part of tail (Fig. 3).

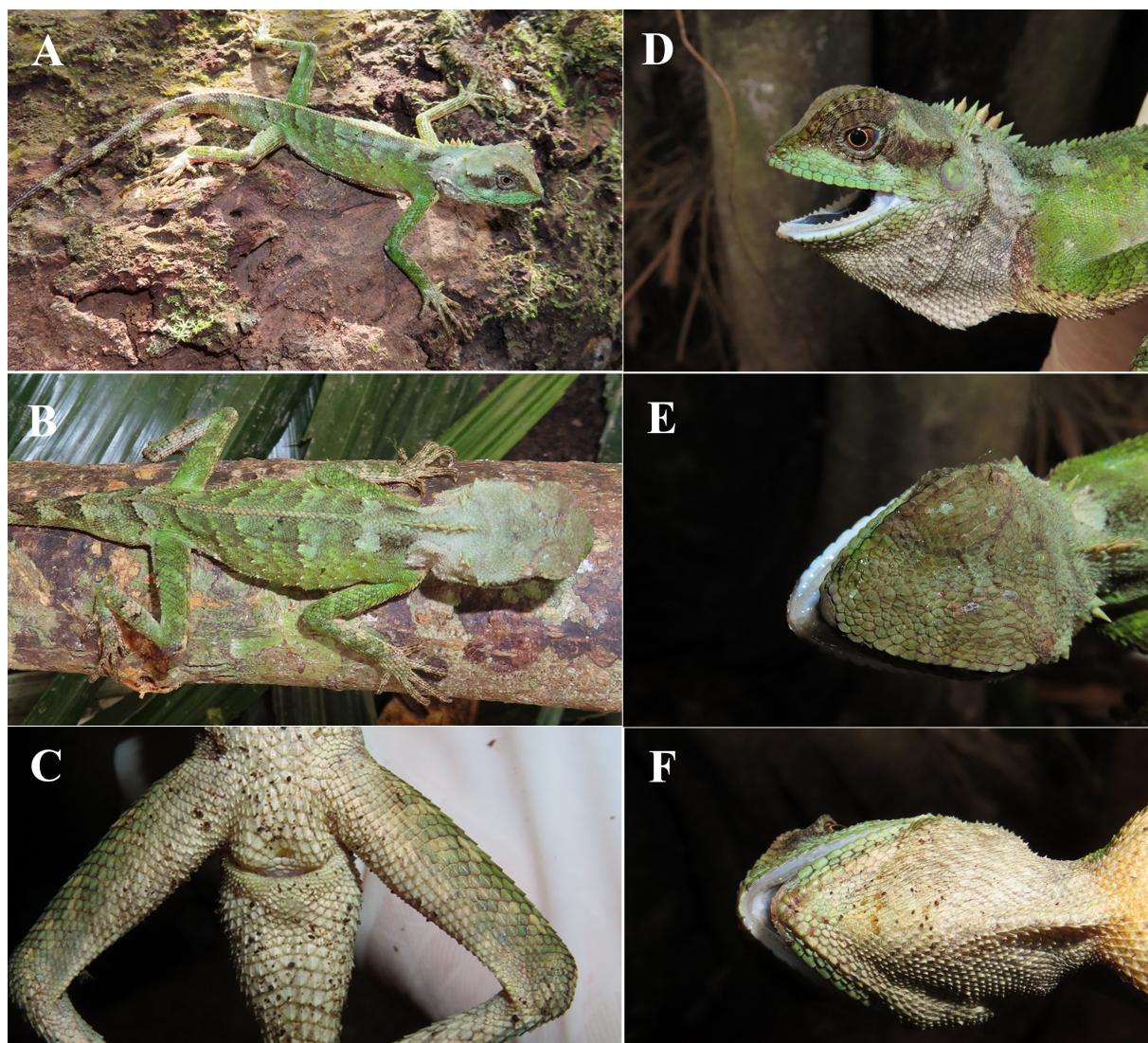


Fig. 3. Live male holotype of *Acanthosaura cuongi* sp. nov. (IEBR R.5250). **A.** Dorsolateral view. **B.** Dorsal view. **C.** Cloacal region. **D.** Head, lateral view. **E.** Head, dorsal view. **F.** Head, ventral view.

Coloration of holotype in preservative

In preservative, the light-green on the dorsal surface of the body, dorsal and ventral surface of limbs and rings on tail faded to dark gray but the pattern remained unchanged (Fig. 4).

Variations

Morphometric and meristic data for the type series are provided in Supp. file 1. The dorsal ground color of the body varies with green, orange-yellow, light to purplish gray, and deep-brown; paratype IEBR R.5258 lacks patches on the back; paratype IEBR R.5256 has brown upper and lower lips and the nuchal crest is grayish black, green and yellow (Figs 5–6).

Sexual size dimorphism

The observed sexual dimorphism in size of *A. cuongi* sp. nov., is that of a female-biased pattern: SVL of females (98.1 ± 6.0 mm, 85.69–104.61 mm, $n = 8$) is significantly longer than that of males (83.19 ± 4.2 mm, 79.4–87.06 mm, $n = 4$) (Supp. file 1).

Distribution

Acanthosaura cuongi sp. nov. is currently known from two sites: Van Ninh District of Khanh Hoa Province and Tay Hoa District of Phu Yen Province, with findings recorded from nearby the coastline, south-central Vietnam (Fig. 7).



Fig. 4. Dorsolateral (above) and ventral (below) views of the male holotype of *Acanthosaura cuongi* sp. nov. (IEBR R.5250) in preservative.

Natural history

Lizards were collected at elevations ranging from 120 to 387 m a.s.l. in evergreen forests, belonging to the southeast Truong Son Mountain Range of Phu Yen and Khanh Hoa provinces (Fig. 8). Lizards were observed during night surveys, clinging to, and sleeping in shrubs, thin trunks of small trees, beneath dense forest canopy. The height of occupied perches ranged from 0.1–1.8 m above the ground. The new species occurs in sympatry with *A. murphyi* in the aforementioned provinces.

Comparisons

Acanthosaura cuongi sp. nov. differs from *A. armata* (Gray, 1827), *A. aurantiacrista* Trivalairat et al., 2020, *A. capra*, *A. cardamomensis*, *A. crucigera* Boulenger, 1885, *A. meridiona* Trivalairat et al., 2022, *A. murphyi*, *A. nataliae*, *A. phuketensis* Pauwels et al., 2015 and *A. tongbiguanensis* Liu & Rao, 2019 by having a shorter body length (max snout-vent length: 104.61 vs 138 mm, 130 mm, 137.9 mm, 149 mm, 127 mm, 118.1 mm, 127.3 mm, 158 mm, 123.5 mm, 115.6 mm, respectively), shorter postorbital spines (0.4–1.49 vs 4.9–12 mm, 5.5–19.1 mm, 5.2–10.2 mm, 3.2–12.7 mm, 1.9–7.8 mm, 3.4–7 mm, 5.6–11.8 mm, 7.7–17.8 mm, 4.6–11.8 mm, 3.6–6.3 mm, respectively), shorter nuchal crest spines (1.63–2.66 mm vs 5.5–11.2 mm, 5.5–21.6 mm, 4.2–14.7 mm, 3.8–17.4 mm, 3.2–8.9 mm, 2.6–6.9 mm, 7–14.9 mm, 8.5–23.8 mm, 4.1–12.2 mm, 4–6.7 mm, respectively), shorter dorsal crest spines (0.6–1.14 vs 4.9–11.3 mm, 2.4–8.7 mm, 3.5–6.8 mm, 2–14.2 mm, 2–5.5 mm, 1.4–3.9 mm, 2.6–10.5 mm, 6–17.7 mm, 2.3–8.3 mm, 2.4–4.2 mm, respectively), and the absence of a diastema between nuchal and dorsal crests (vs presence) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. bintangensis* Wood et al., 2009 and *A. titiwangsaensis* Wood et al., 2009 by having a shorter body length (max snout-vent length: 104.61 vs 142 mm, 118.4 mm,



Fig. 5. Dorsolateral (above), dorsal (middle) and ventral (below) views of male paratype IEBR R.5259 (left) and female paratype IEBR R.5255 (right) of *Acanthosaura cuongi* sp. nov. in preservative.

respectively), fewer subdigital lamellae on the fourth finger (14–17 vs 23, 20–21, respectively) and on the fourth toe (19–23 vs 26–28, 23–27, respectively), fewer canthus rostralis scales (9–11 vs 14–15, 14–15, respectively), and the presence of light knee spot (vs absence) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. brachypoda* by having a relatively longer postorbital spine (0.4–1.9 vs 3.2 mm), shorter nuchal crest spines (1.63–2.66 vs 4.7 mm), fewer ventral scales (51–60 vs 63), fewer subdigital lamellae on the fourth finger (14–17 vs 18) and on the fourth toe (19–23 vs 24), and the absence of a diastema between nuchal and dorsal crests (vs presence) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. coronata* by having a longer body length (max snout-vent length: 104.61 vs 86.1 mm) and head length (23.34–31.1 vs 14.4–16.3 mm), the presence of dorsal crest spines, more subdigital lamellae on the fourth toe, fewer canthus rostralis scales (9–11 vs 12–15), fewer scales bordering the rostral scale (5–7 vs 9), and fewer scales between the nasal and the rostral (1 vs 3–4) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. lepidogaster* by having a shorter orbit diameter (orbit/HL: 0.27–0.38 vs 0.4–0.57), shorter snout length (SL/HL: 0.29–0.39 vs 0.42–0.66), fewer subdigital lamellae on the fourth finger (14–17 vs 17–19), the absence of the diastema between nuchal and dorsal crests (vs presence), black patch extending from behind the eye backward and slightly upward to the anterior edge of tympanum (vs upward to through occipital spine to reach anterior edge of black nuchal collar) (Supp. file 2).

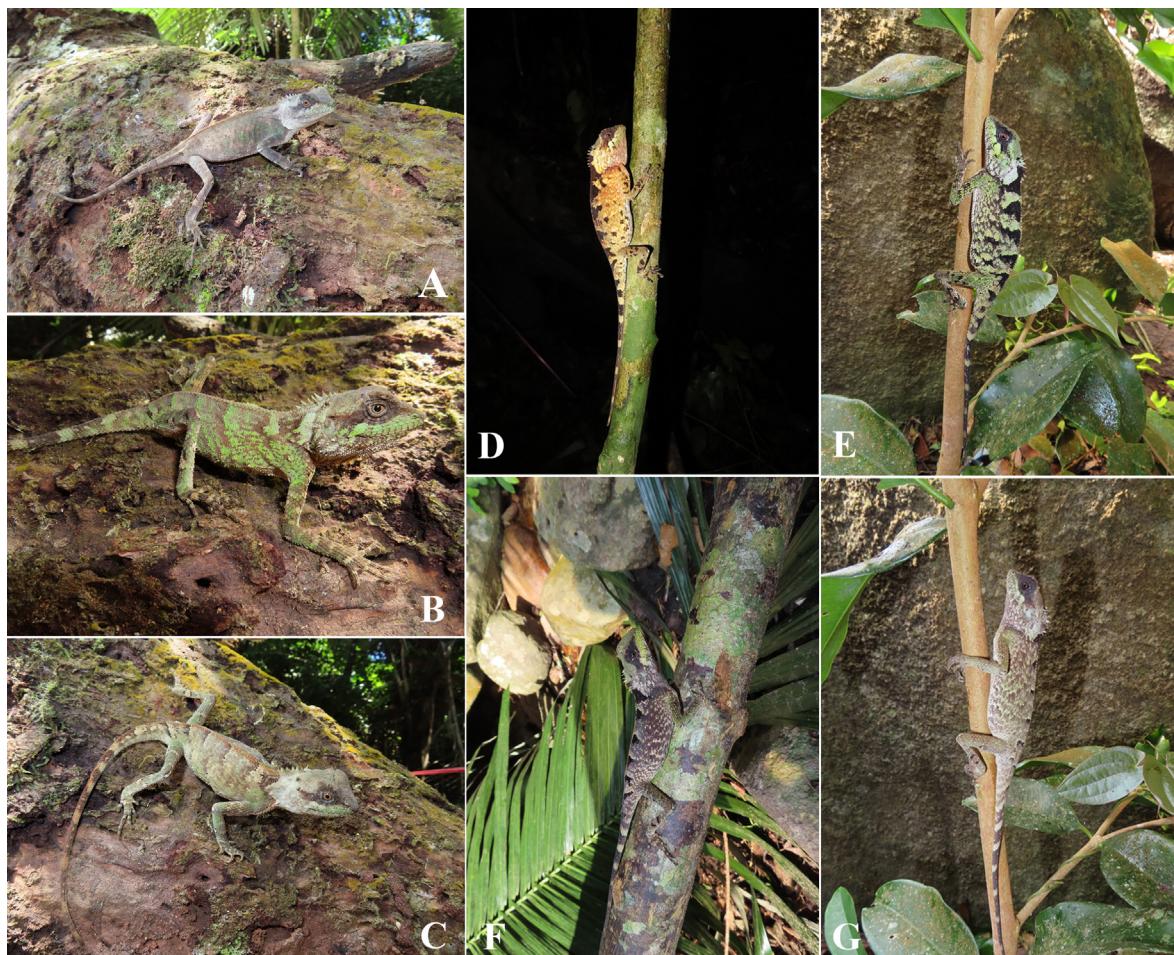


Fig. 6. Color variations of live paratypes of *Acanthosaura cuongi* sp. nov.

Acanthosaura cuongi sp. nov. differs from *A. liui* Liu et al., 2020 by having a shorter nuchal crest spines (1.63–2.66 vs 2.2–7.1 mm), a shorter dorsal crest spines (0.6–1.14 vs 2.7–3.7 mm), more nuchal crest scales (7–9 (1) vs 5–7), shorter occipital spine (1.24–2.54 vs 3.6–4.8 mm), fewer canthus rostralis scales (9–11 vs 12), fewer scales bordering the rostral scale (5–7 vs 8–9), fewer scales bordering the mental (4 vs 5–6), the absence of a diastema between nuchal and dorsal crests (vs presence) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. longicaudata* Liu et al., 2022 by having more nuchal scales (7–9 (1) vs 6–7), fewer subdigital lamellae on the fourth finger (14–17 vs 17–19) and on the fourth toe (19–23 vs 22–25), fewer canthus rostralis scales (9–11 vs 11–13), fewer scales between the nasal and the rostral scales (1 vs 1–3), a black patch extending from behind the eye backward and slightly upward to the anterior edge of tympanum (vs upward to through occipital spine to reach anterior edge of black nuchal collar) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. phongdienensis* by having a longer body length (79.4–104.61 vs 58.5–77.4 mm), a longer head (23.34–31.1 vs 18.6–23.8 mm), a broader head (15.94–19.6 vs 13.1–15.9 mm), a shorter orbit diameter (orbit/HL: 0.27–0.38 vs 0.4–0.57), two rows of slightly low dagger-like vertebral spines (vs single row, black eye patch extending backward and slightly upward to

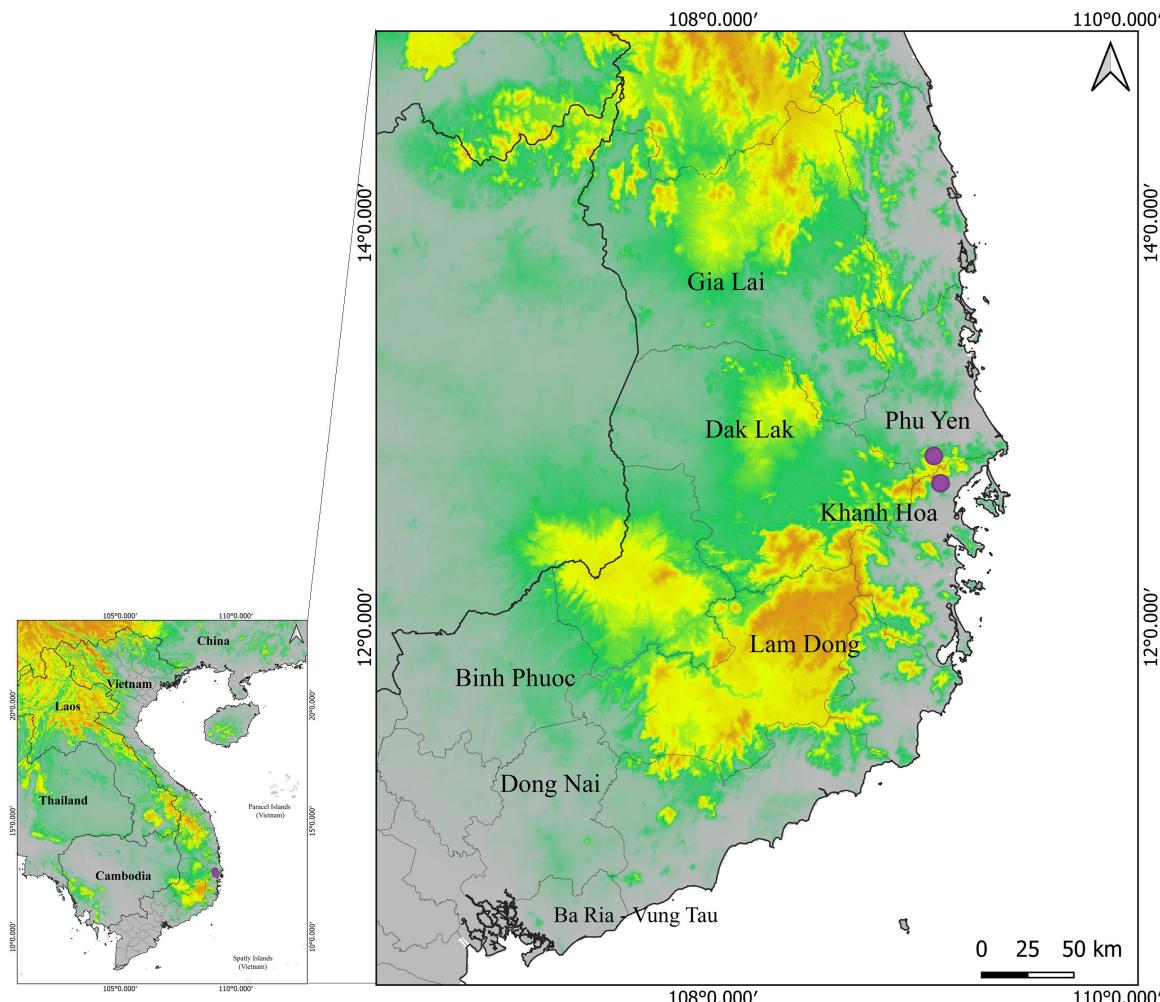


Fig. 7. Distribution of *Acanthosaura cuongi* sp. nov. in Vietnam (violet circles).

the anterior edge of the tympanum vs upward to through occipital spine to reach anterior edge of black nuchal collar) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. prasina* by having a longer body length (79.4–104.61 vs 79.8–88.4 mm), a longer head (23.34–31.1 vs 20.7–22.4 mm), fewer ventral scales (51–60 vs 59–63), fewer subdigital lamellae on the fourth toe (19–23 vs 23–26), more canthus rostralis scales (9–11 vs 5–6), scales of nuchal crest and vertebral scales light orange (vs white), and lips green (vs white) (Supp. file 2).

Acanthosaura cuongi sp. nov. differs from *A. rubrilabris* Liu *et al.*, 2022 by having shorter nuchal crest spines (1.63–2.66 vs 1.9–7.0 mm), more nuchal scales (7–9 (1) vs 3–6), fewer canthus rostralis scales (9–11 vs 11–14), and the absence of a bright red pattern on upper and lower lips (vs presence) (Supp. file 2).

Discussion

The species diversity of the genus *Acanthosaura* has been underestimated for a long time, with most species having been described recently, and new species still being discovered (Liu *et al.* 2020; Trivalairat *et al.* 2022; Uetz *et al.* 2024). In Vietnam, seven species of *Acanthosaura* were documented from the Truong Son Mountain Range, namely *A. lepidogaster*, a widespread species from northern to southern parts (Nguyen *et al.* 2009), *A. capra* and *A. coronata* in the southwest, *A. murphyi* in the southeast (Nguyen *et al.* 2018), *A. nataliae* in the north and center (Orlov *et al.* 2006), *A. phongdiensis* in the north (Nguyen *et al.* 2019), and *A. prasina* in the center (Ananjeva *et al.* 2020). Together with the species described herein, the genus *Acanthosaura* currently comprises a total of 21 species, of which 10 are known from Vietnam, with eight of them endemic to this mountain range (Uetz *et al.* 2024).

The ML and BI analyses based on the COI and Cytb genes recovered the new species being the sister species to *A. coronata*. From the Cytb tree, an individual (available in GenBank with accession number [AY572896](#)) from Krong Pa, Gia Lai Province was previously identified as *A. coronata* (Ananjeva *et al.* 2020), although it has a significant genetic difference compared with *A. coronata* (11.48–11.91%;



Fig. 8. Forest habitat of *Acanthosaura cuongi* sp. nov in Phu Yen Province, Vietnam.

Table 4). The phylogenetic analysis herein revealed that the specimen from Krong Pa clustered with the new species, and the p-distances between them ranged from 3.6–3.8% which is slightly lower than the interspecific difference between *A. lepidogaster* and *A. brachypoda* (3.91–4.62%). Here, we provisionally consider the specimen from Gia Lai Province as a representative of *A. cuongi* sp. nov. In terms of distribution, the finding from Gia Lai is approximately 120 km distant from the type locality of the new species (Fig. 8), whereas the *A. coronata* complex is currently known from a wide distributional range, including high mountains in Dak Lak and Lam Dong provinces, and lowland in Ba Ria–Vung Tau, Binh Phuoc and Dong Nai provinces (Kalyabina-Hauf *et al.* 2004; Nguyen *et al.* 2009; Nguyen *et al.* 2014; Ananjeva *et al.* 2020). Further surveys in Gia Lai Province and the range from where the *A. coronata* complex is known, are recommended in concert with integrative taxonomic analyses to uncover potential further cryptic diversity in the genus.

Acknowledgments

We thank the authorities and forest protection departments in Phu Yen and Khanh Hoa provinces. For the fruitful cooperation within joint research projects, we cordially thank Nguyen V. T. (HUS, Hanoi), Le H. A. (IEBR, Hanoi), Nguyen H. H. (IGR, Hanoi), as well as T. Pagel and C. Landsberg (Cologne Zoo). Thanks to Nguyen Q. H. (IGR, Hanoi) who assisted during field surveys. This research is funded by the Vietnam Academy of Science and Technology (Grant number THTEXS.01/23-25). Field work in southern Vietnam was partially funded by the Rufford Foundation (Grant no. 39056-1) to C. T. Pham.

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Supplementary files

Supp. file 1. Morphometric (in mm) and meristic data for the type series of *Acanthosaura cuongi* sp. nov. For abbreviations see materials and methods. (H) is for holotype, (P) is for paratype, and the number in brackets indicates the number of small fore-spines on nuchal crest.

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Supp. file 2. Morphological comparisons in fifteen characteristics between *Acanthosaura cuongi* sp. nov. and nineteen congeners. <https://doi.org/10.5852/2025.976.2781.12709>