Vietnam Journal of Biotechnology. DOI: 10.15625/vjbt-23273

GENETIC VARIATION IN THE COMPLEX *Acanthosaura coronata* SPECIES GROUP AND RECORD OF SEXUAL DIMORPHISM

Ngoc Hai Ngo[1,✉], Tu Hoang Linh Le[1], Thien Tao Nguyen[1], Thi Nhu Quynh Nguyen[2], Hoang Van Nguyen[3] and Thi Truc Thanh Nguyen[4,5]

[1]*Institute of Biology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam*

[2]*School of Chemistry and Life Sciences, Hanoi University of Science and Technology, 1 Dai Co Viet, Bach Mai, Hanoi, Vietnam*

[3]*University of Science, Vietnam National University, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam*

[4]*Quy Nhon University, 170 An Duong Vuong, Quy Nhon Nam, Gia Lai, Vietnam*

[5]*Ngo Gia Tu High School, 4 Xo Viet Nghe Tinh, Phu Yen, Dak Lak, Vietnam*

✉To whom correspondence should be addressed. Email: ngohai2709@gmail.com

Received: 14 August 2025 / Accepted: 04 December 2025 / Published online: 19 January 2026

ABSTRACT

The two recently described species, *Acanthosaura cuongi* and *A. grismeri* in southern Vietnam, highlight the potential existence of additional populations or even undescribed species in this region, rather than supporting the previous assumption that *Acanthosaura coronata* is broadly distributed across southern Vietnam. Phylogenetic analysis of COI gene sequences confirmed the monophyly of the *A. coronata* group, comprising *A. coronata*, *A. cuongi*, and *A. grismeri*. In southern Vietnam, two new populations from Gia Lai Province were assigned to *A. cuongi*, while one new population from Tay Ninh Province was identified as *A. coronata*. Within the group, interspecific p-distances ranged from 7.82–8.77% between *A. coronata* and *A. grismeri,* to 13.5–14.92% between *A. coronata* and *A. cuongi*, and 13.84–15.19% between *A. cuongi* and *A. grismeri*. Intraspecific genetic divergences were up to 3.22% in *A. cuongi* and 2.66% in *A. coronata*, suggesting potential cryptic diversity and emphasizing the need for further taxonomic assessment using additional molecular data. Based on morphological data from 66 individuals, this study provided a comprehensive redescription of *A. coronata* species and documented distinct sexual dimorphism. Males exhibit light green to yellow-green dorsal coloration and an orange-red tail likely functioning in sexual signaling, whereas females display dull brown-gray coloration enhancing camouflage. Morphometric analyses revealed female-biased traits, including greater snout–vent length and abdominal width, potentially improving fecundity and egg survival. Conversely, males possess longer heads, limbs, and broader tail bases, which may facilitate greater agility and provide advantages in hunting, territorial defense, and reproductive success. These findings contribute to a better understanding of morphological variation, sexual dimorphism, and taxonomic relationships within the complex *A. coronata* group.

Keywords: COI gene, dimorphism, female-biased pattern, morphology, phylogenetic analysis.

INTRODUCTION

Recent studies on morphology and phylogeny confirmed that *Acanthosaura lepidogaster sensu lato* is composed of at least five narrowly distributed species, rather than being widespread across Vietnam and southern China (Ananjeva *et al.,* 2011; Liu *et al.,* 2022; Liu *et al.,* 2023). It is possible that the wide distribution reported for *A. coronata* in southern Vietnam was likewise overestimated, suggesting a need for consideration. Subsequently, extensive surveys in different provinces of southern Vietnam indeed have discovered two new species, namely *A. cuongi* in Phu Yen (now Dak Lak) and Khanh Hoa Provinces, and *A. grismeri* in Dak Lak Province (Ngo *et al.,* 2025b; Le *et al.,* 2025). These findings raise the possibility that additional populations of the two new species, or even new species within the *A. coronata* group, may still be awaiting discovery (Le *et al.,* 2025). Therefore, in this study, we carried out surveys in some new areas of Gia Lai, Dak Lak and Tay Ninh Provinces, in which individuals of *Acanthosaura* species with morphological characteristics similar to members of the *A. coronata* group were recorded. We performed COI gene sequence analyses and re-evaluated the phylogenetic relationships within the complex *A. coronata* group. In addition, morphological data of *A. coronata* was further collected to provide a sufficient diagnosis and assess the sexual dimorphism.

MATERIALS AND METHODS

Sampling

Field surveys were conducted in Kon Ka Kinh National Park (NP) and Ia Ly Forest – Gia Lai Province, Cat Tien NP – Dong Nai Province, Ba Den Mountain – Tay Ninh Province, Khanh Hoa Province and Phu Yen Province (now Dak Lak) from 2023 to 2024 (Figure 1). Agamid lizards were collected by hand and photographed in life. Some animals were placed in a closed vessel containing a piece of cotton wool dipped with ethyl acetate for anesthesia and euthanasia (Simmons, 2002), then fixed in 85% ethanol and later transferred to 70% ethanol for storage. Liver and muscle tissue samples were preserved separately in absolute ethanol for genetic analysis. The specimens were deposited at the repository of the Institute of Biology, Vietnam Academy of Science and Technology, Hanoi, Vietnam. The specific coordinate data can be shared by the authors upon request.

Molecular analyses

Extraction of genomic DNA from samples (namely, BD.TN.2023.13, BD.TN.2023.25, BD.TN.2023.30, BD.TN.2023.37, Ialy.GL.2023.16, Ialy.GL.2023.20, KKK.2023.148, KKK.2023.150, Table 1) was conducted with the DNeasy Blood and Tissue Kits (Qiagen, Germany) following the protocol provided by the manufacturers. The extracted total DNA was then amplified via a polymerase chain reaction (PCR) using an Eppendorf PCR machine. The total PCR 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. Forward primer Chmf4 (5'-TYT CWA CWA AYC AYA AAG AYA TCG G-3') and reverse primer Chmr4 (5'-ACY TCR GGR TGR CCR AAR AAT CA-3') were used to amplify a fragment of the Cytochrome c oxidase subunit I (COI) gene (Che *et al.,* 2012). PCR protocols were followed Ngo *et al.* (2025b). PCR products were sent to the company 1st Base in Malaysia for sequencing.

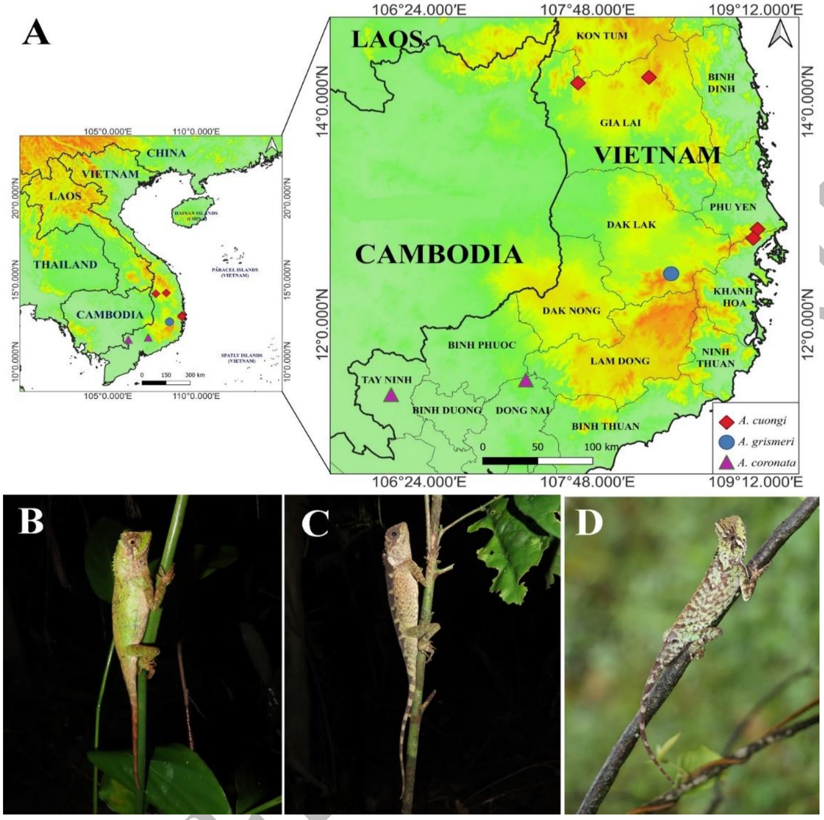

Figure 1. A. Records of species in the *Acanthosaura coronata* group; B. *A. coronata;* C. *A. cuongi*; D. *A. grismeri*.

Table 1. List of samples used for COI genetic analysis in this study.

Genus	Locality	Voucher No.	GenBank Accession No.	Reference
Ingroup				
A. capra	Bu Gia Map NP, Dong Nai Province	BGM 01	MK239022	Nguyen *et al.,* 2018
A. capra	Ba Den Mountain, Tay Ninh Province	BD.TN.2023.25	XXXXXXXX	This study
A. capra	Ba Den Mountain, Tay Ninh Province	BD.TN.2023.37	XXXXXXXX	This study
A. coronata	Bu Gia Map NP, Dong Nai Province	KIZ47	MK695186	Nguyen *et al.,* 2019

A. coronata	Bu Gia Map NP, Dong Nai Province	KIZ48	MK695187	Nguyen *et al.,* 2019
A. coronata	Bu Gia Map NP, Dong Nai Province	KIZ97	MK695188	Nguyen *et al.,* 2019
A. coronata	Cat Tien NP, Dong Nai Province	ROM 42240	MK695189	Nguyen *et al.,* 2019
A. coronata	Cat Tien NP, Dong Nai Province	ROM 42241	MK695190	Nguyen *et al.,* 2019
A. coronata	Ba Den Mountain, Tay Ninh Province	BD.TN.2023.13	XXXXXXXX	This study
A. coronata	Ba Den Mountain, Tay Ninh Province	BD.TN.2023.14	XXXXXXXX	This study
A. cuongi	Deo Ca Mountain (Phu Yen)	IEBR R.5250	PP669986	Ngo *et al.,* 2025b
A. cuongi	Deo Ca Mountain (Phu Yen)	IEBR R.5257	PP669987	Ngo *et al.,* 2025b
A. cuongi	Van Ninh, Khanh Hoa Province	IEBR R.5251	PP669985	Ngo *et al.,* 2025b
A. cuongi	Ialy, Gia Lai Province	Ialy.GL.2023.16	XXXXXXXX	This study
A. cuongi	Ialy, Gia Lai Province	Ialy.GL.2023.20	XXXXXXXX	This study
A. cuongi	Kon Ka Kinh NP, Gia Lai Province	KKK.2023.148	XXXXXXXX	This study
A. cuongi	Kon Ka Kinh NP, Gia Lai Province	KKK.2023.150	XXXXXXXX	This study
A. grismeri	Krong Pong forest, Dak Lak Province	IEBR R.6353	PV646694	Le *et al.,* 2025
A. grismeri	Krong Pong forest, Dak Lak Province	IEBR R.6354	PV646695	Le *et al.,* 2025
A. grismeri	Krong Pong forest, Dak Lak Province	IEBR R.6357	PV646696	Le *et al.,* 2025
A. lepidogaster	Na Hang NR, Tuyen Quang Province	ROM 30505	MK695191	Nguyen *et al.,* 2019
A. lepidogaster	Na Hang NR, Tuyen Quang Province	ROM 30507	MK695192	Nguyen *et al.,* 2019
A. lepidogaster	Cao Bang Province	ROM 30677	MK695193	Nguyen *et al.,* 2019

A. lepidogaster	Cao Bang Province	ROM 30680	MK695194	Nguyen *et al.*, 2019
A. lepidogaster	Tam Dao NP, Phu Tho Province	ROM 30712	MK695195	Nguyen *et al.*, 2019
A. murphyi	Deo Ca Mountain (Phu Yen)	ITBCZ 4603	MK239025	Nguyen *et al.*, 2018
A. murphyi	Hon Ba NR, Khanh Hoa	ITBCZ 3533	MK239026	Nguyen *et al.*, 2018
A. murphyi	Deo Ca Mountain (Phu Yen)	PYU 147	MK239027	Nguyen *et al.*, 2018
A. murphyi	Van Ninh, Khanh Hoa Province	KH.2023.94	XXXXXXXX	This study
A. murphyi	Deo Ca Mountain (Phu Yen)	PY.2023.54	XXXXXXXX	This study
Outgroup				
Calotes versicolor	Ta Kou NR, Binh Thuan Province	KIZ1120	MK695211	Nguyen *et al.*, 2019
C. versicolor	Ta Kou NR, Binh Thuan Province	ITBCZ1034	MK695212	Nguyen *et al.*, 2019

Two samples of *Calotes versicolor* (MK695211 and MK695212) were selected as outgroups (Table 1). Nucleotide sequences were aligned in MEGA 11 using the MUSCLE algorithm with default parameters (Edgar, 2004; Tamura *et al.*, 2021). Locality data and GenBank accession numbers for all sequences are provided in Table 1. Pairwise uncorrected sequence divergences (p-distances) were calculated in MEGA 11, excluding the outgroup. Variance was estimated via the bootstrap method (1,000 replicates) based on nucleotide substitutions, with gaps and missing data handled by pairwise deletion.

Before constructing phylogenetic trees using Maximum Likelihood (ML) and Bayesian Inference (BI), the optimal nucleotide substitution model was determined with ModelFinder (Kalyaanamoorthy *et al.*, 2017) integrated in IQ-TREE v2.3.5 (Nguyen *et al.*, 2015). The best-fit model selected for ML analysis was TIM+F+I+G4, based on the Bayesian Information Criterion (BIC), which was translated to the closest equivalent model for BI analysis: GTR+I+G4. The BI analyses were conducted in MrBayes v3.2.7a (Ronquist *et al.*, 2012) with Metropolis-coupled Markov chain Monte Carlo (MCMC) running 10 million generations and sampling every 100 generations. Two independent runs of four Markov chains each were performed, and the first 25% of trees were discarded as burn-in. Parameter estimates and convergence were assessed using the built-in sump command in MrBayes. ML tree inference was performed in IQ-TREE v2.3.5 with 10,000 ultrafast bootstrap replicates (UFB) (Hoang *et al.*, 2018). Node support was considered strong when Bayesian posterior probabilities

(BPP) and UFB values were greater than or equal to 0.95/95 (Felsenstein, 1985; Hoang *et al.,* 2018).

Morphological data

Measurements of collected specimens were taken to the nearest 0.1 mm with digital calipers. Meristic characters were taken on the right side. The morphometric and meristic data obtained in this study followed the subsequent studies (Günther, 1861; Boulenger, 1885; Orlov *et al.,* 2006; Ananjeva *et al.,* 2008; Ananjeva *et al.,* 2011; Ananjeva *et al.,* 2020; Wood *et al.,* 2009; Wood *et al.,* 2010; Pauwels *et al.,* 2015; Nguyen *et al.,* 2018; Nguyen *et al.,* 2019; Liu and Rao, 2019; Liu *et al.,* 2020; Liu *et al.,* 2022; Trivalairat *et al.,* 2020; Ngo *et al.,* 2025a; Ngo *et al.,* 2025b): snout-vent length (SVL), measured from the tip of the snout to the vent; tail length (TAL), measured from the posterior margin of the vent to the tip of the tail; tail base width (TBW); axilla to groin distance (AG), measured from the posterior base of the forelimb at its emergence from the body to the anterior base of the hindlimb at its emergence from the body; body width (BW); head length (HL), measured from the posterior edge of the lower jaw to the tip of the snout; head width (HW), maximum head width measured at the level of the tympanum; maximum head height (HH), snout length (SL), measured from the tip of the snout to the medial canthus; orbit diameter (ORBIT), measured from the posterior to the anterior edge of the bony orbit; eye to ear distance (EE), measured from posterior margin of eye to posterior margin of ear opening; tympanum diameter (TD), measured horizontally from the anterior to the posterior border of the tympanum; mouth length (MoL); mouth width (MW); forelimb length (FOREL), measured from the axilla to the tip of the fourth finger; hindlimb length (HIND), measured from the groin to the tip of the fourth toe; number of spikes on the nuchal crest (NC); number of scales surrounding the occipital spine (NSSOS); number of supralabials (SUPRAL); number of infralabials (INFRAL); number of canthus rostralis - supraciliary scales (CS), counted from the nasal scale to the posterior end of the ridge at the posterior margin of the orbit; number of internasal scales (NS), counted between the nasals; number of scales surrounding the rostral scale (RS); number of scales surrounding the mental scale (PM); number of ventral scales (VENT), counted along the midline from the anterior edge of the shoulders to the anterior edge of the vent; number of subdigital lamellae on the fourth finger (FI); number of subdigital lamellae on the fourth toe (TO); number of scales between the nasals and the rostral (NR); light-colored knee patch (LKP).

The dimorphism in coloration was assessed by taking photos from four directions (e.g., both lateral, ventral and dorsal sides). The sex of each individual was determined by the presence of a large, swollen cloaca in males, and the absence in females.

Statistical analysis

The sexual dimorphism index (SDI) was calculated to identify the pattern of sexual size dimorphism (SSD) by the revised formula that was first suggested by Lovich and Gibbons (1992), in which SDI = (mean size of males / mean size of females) – 1. Accordingly, SDI is a positive value when males are the larger sex (SDI > 0) as a male-biased pattern, a negative value when females are the larger sex (SDI < 0) as a female-biased pattern and zero when the sexes are equal in size (SDI = 0) as an unbiased pattern. The Shapiro-Wilk's test was used to test the assumption of normality. A Wilcoxon test was performed to determine the difference in the SVL between males and females. All statistical analyses were performed by using the software environment R.3.8.1 (R Core Team, 2024). Due to high collinearity, the raw differences in other morphological characteristics may be intrinsically affected by the differences in overall body size. To independently assess the differences and limit the correlative effects, a normalization equation following an allometric growth model first suggested by Thorpe (1975) was applied to adjust raw data of morphology through the allom () function in the R package GroupStruct (Chan and Grismer, 2021). Subsequently, we used the t-test approach to examine the inter-sexual differences in these adjusted variables. For all of these tests, we applied a significance level of P-value = 0.05.

A Principal Component Analysis (PCA) was conducted on all adjusted variables, excluding SVL, to evaluate overall intersexual morphological variation. Morphological space between sexes was visualized by plotting and clustering individuals as color-coded points along the PC1 and PC2 axes, allowing assessment of potential overlap. PC1 and PC2 scores were extracted separately, and intersexual differences were tested using t-tests. PCA was performed with the packages "factoextra" (Kassambara and Mundt, 2020) and "FactoMinerR" (Le *et al.,* 2008).

RESULTS

Genetic variation

Sequence alignments consisted of 600 bp in length of Cytochrome Oxidase Subunit 1 (COI) gene. Within the complex *A. coronata* group, the interspecific p-distances among species are from 7.82–8.77 % (between *A. coronata* and *A. grismeri*) to 13.5–14.92% (*A. coronata* and *A. cuongi*) and 13.84–15.19% (*A. cuongi* and *A. grismeri*) (Table 2). The difference was also noted with the p-distances more than 13.46% compared to sympatric *Acanthosaura* species (i.e., *A. capra* and *A. murphyi*). It is noteworthy that the intraspecific differences among distinct geographical populations of *A. coronata* group were up to 2.66 % (between Ba Den Mountain and the type population in Cat Tien NP of *A. coronata*) and up to 3.23% (between Kon Ka Kinh NP and the type locality of *A. cuongi*). The BI and ML analyses yielded congruent topologies (Figure 2). Accordingly, a new population of Ba Den Mountain is placed into the monophyletic *A. coronata* (BPP/UFB = 0.99/91) and new populations in Kon Ka Kinh NP and Ia Ly forest, Gia Lai Province, into the strongly supported clade (BPP/UFB = 1/99) of the recently discovered species of *A. cuongi*.

Table 2. Genetic distances (uncorrected p-distances) among samples and between *Acanthosaura* species in Vietnam, based on partial COI gene sequences.

	Species	1	2	3	4	5	6
1	*A. coronata*	0.0-2.66					
2	*A. grismeri*	7.82-8.77	0.0-0.0				
3	*A. cuongi*	13.5-14.92	13.84-15.19	0.0-3.23			

4	*A. capra*	14.44-17.33	16.32-17.35	13.46-15.7	0.0-0.78		
5	*A. murphyi*	16.11-18.33	17.87-18.66	15.01-16.3	5.67-7.25	0.0-0.96	
6	*A. lepidogaster*	21.67-22.91	22.48-22.74	21.68-23.1	20.83-21.7	20.6-21.8	0.0-1.6

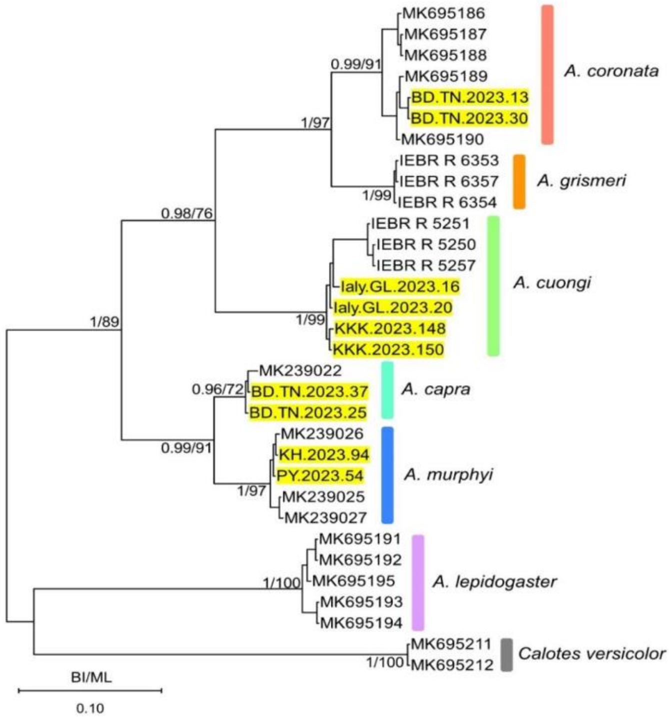

Figure 2. Bayesian - Maximum Likelihood tree based on partial COI gene sequences of specimens used in this study. Values at each node indicate Bayesian Posterior Probabilities (BPP) and Ultrafast Bootstrap Values (UFB) from Maximum Likelihood analysis, respectively (BI/ML).

Morphology diagnosis

Morphometric data from 66 individuals of *A. coronata* species (39 males, 27 females) and meristic data from 39 individuals (23 male, 16 females) were presented in detail in Table 3, for taxonomic identification: Moderately-sized agamid lizard (SVL 55.6–100.3 mm); head triangular in dorsal view, relatively short, 14.0–27.4 mm in length (average HL/SVL 0.30), 10.3–18.7 mm in width (HW/HL 0.61), 8.9–15.2 mm in height (HH/HL 0.47); tail relatively long (TAL/SVL 1.73); postorbital and occipital spines present; gular pouch relatively undeveloped; 5–10 internasals; 8–10 scales between nasals; 2–3 scales bordering mental scale; 10–14 supralabials; 9–14 infralabials; 2–6 scales bordering rostral scale; nuchal crest comprises 6–11 enlarged, triangular spines relatively high; dorsal crest present, underdeveloped; single row of vertebral scales followed the dorsal crest; nuchal crests continue into the dorsal crests forming a prominent mid-dorsal ridge; lateral scales (40–63) small, intermixed with large, keeled scales, keels directed backward and back-upward; number of subdigital lamellae 11–

17 on the fourth finger and 15–22 on the fourth toe; a light spot on the knee; no dark stripe around the eye (Figure 3 and Table 3).

Sexual dimorphism

A phenotypic characteristic of coloration is distinctly different between males and females of *A. coronata*. Females are predominantly light brown or gray on the dorsal surfaces of the head, body, and tail. In contrast, males exhibit a light green or yellow-green dorsal coloration, often with brownish-black bands or longitudinal stripes. Notably, males display an orange-red coloration on the tail, whereas females have a light brown tail (Figure 3).

Figure 3. *Acanthosaura coronata*: male (A) and female (B).

Table 3. Comparison of morphological characteristics between male and female individuals of *Acanthosaura coronata*.

Traits	Males	Females	Total	Tests	Adjusted test
Morphometric characteristics (Males = 39; Females = 27)					
SVL	65.9 ± 7.4 (55.6–79.9)	77.3 ± 11.7 (58.4–100.3)	55.6–100.29	8.88897E-05	2.62436E-08
TAL	89.1 ± 10.3 (67.1–119.9)	84.3 ± 11.5 (43.2–99.5)	43.23–99.48	0.122235366	0.432205126
TBW	8.3 ± 1.6 (5.48–11.33)	8.02 ± 1.73 (5.3–11.5)	5.3–11.5	0.547663859	0.000169908
AG	33.3 ± 4.7 (24.78–41.41)	42.4 ± 8.7 (25.39–57.12)	24.78–57.12	3.66271E-05	0.020187258
BW	15.4 ± 2.9 (11.06–21.24)	25.03 ± 4.4 (16.3–33.59)	11.6–33.59	3.43195E-08	3.93149E-06
HL	18.8 ± 2.4 (14–23.91)	21.5 ± 3.4 (15.1–27.42)	14–27.42	0.000429132	0.600578508
HW	13.4 ± 1.6 (11–17.25)	15.1 ± 2.2 (10.34–18.65)	10.34–18.65	0.000425917	0.145982462

HH	11.2 ± 1.4 (8.8–14.19)	12.4 ± 1.64 (8.99–15.19)	8.8–15.19	0.002370456	0.046742473
MW	12.30 ± 1.72 (9.6–16.19)	13.96 ± 2.1 (9.59–17.31)	9.59–17.31	0.000687526	0.875911081
MoL	13.2 ± 1.8 (10.55–17.07)	14.8 ± 1.99 (11.3–19.19)	10.55–19.19	0.001538462	0.021607771
ID	4.70 ± 0.76 (3.3–6.55)	5.35 ± 0.86 (3.4–6.49)	3.3–6.55	0.001898614	0.358036488
IO1	9.00 ± 1.11 (7.3–11.25)	9.52 ± 1.24 (7.6–11.91)	7.3–11.91	0.120632229	0.002566366
IO2	11.5 ± 1.18 (9.85–13.51)	11.9 ± 1.2 (10.02–14.46)	9.85–14.46	0.237746734	0.02083338
SL	7.25 ± 1.21 (5.2–9.94)	8.11 ± 1.15 (5.95–10.9)	5.2–10.9	0.005231142	0.120151867
ORBIT	5.67 ± 1.18 (3–8.19)	5.60 ± 1.41 (3.3–8.77)	3–8.77	0.823042881	0.493244893
EE	4.64 ± 1.52 (2.62–7.88)	5.63 ± 1.64 (3.32–9.03)	2.62–9.03	0.018890787	0.432205126
TD	2.67 ± 0.56 (1.46–4.24)	3.04 ± 0.52 (1.8–4.06)	1.46–4.24	0.007712517	0.627943653
FOREL	35.5 ± 4.05 (27.68–41.4)	38.75 ± 4.8 (28.5–44.81)	27.68–44.81	0.003868971	0.086225226
HIND	53.9 ± 5.8 (43.95–63.76)	56.2 ± 7.1 (42.58–67.96)	42.58–67.96	0.156959042	3.66485E-05
Meristic characteristics (Males = 23; Females = 16)					
NC	6–11	7–10	6–11		
NSSOS	4–6	4–8	4–8		
CS	8–13	7–11	7–13		
SUPRAL	10–13	10–14	10–14		
INFRAL	9–14	10–13	9–14		
NS	5–9	7–10	5–10		
RS	2–6	3–5	2–6		
PM	2–3	2–3	2–3		
VENT	46–63	40–57	40–63		
FI	11–17	12–16	11–17		
TO	15–22	16–22	15–22		
LKP	presence	presence	presence		

The mean SVL of females is on average larger than that of males (77.30 mm vs. 65.93 mm; P-value < 0.05). In addition, the SDI value is negative ($-0.147 < 0$) confirming that *A. coronata* displays the sexual dimorphism in size of female-biased pattern. Other body indices, including AG, BW, and head-related measurements such as HL, HW, HH, MoL, MW, IO1, IO2, ID, SE and TD are all significantly larger in females than in males (Table 3; P-values < 0.05). Based on the adjusted morphological data, females also exhibit significantly larger abdominal size indices, including AG and BW (Figure 4; P-values < 0.05). In contrast, males show significantly greater measurements in HH, MoL, IO1, IO2, HIND, and TBW (Figure 4; P-values < 0.05).

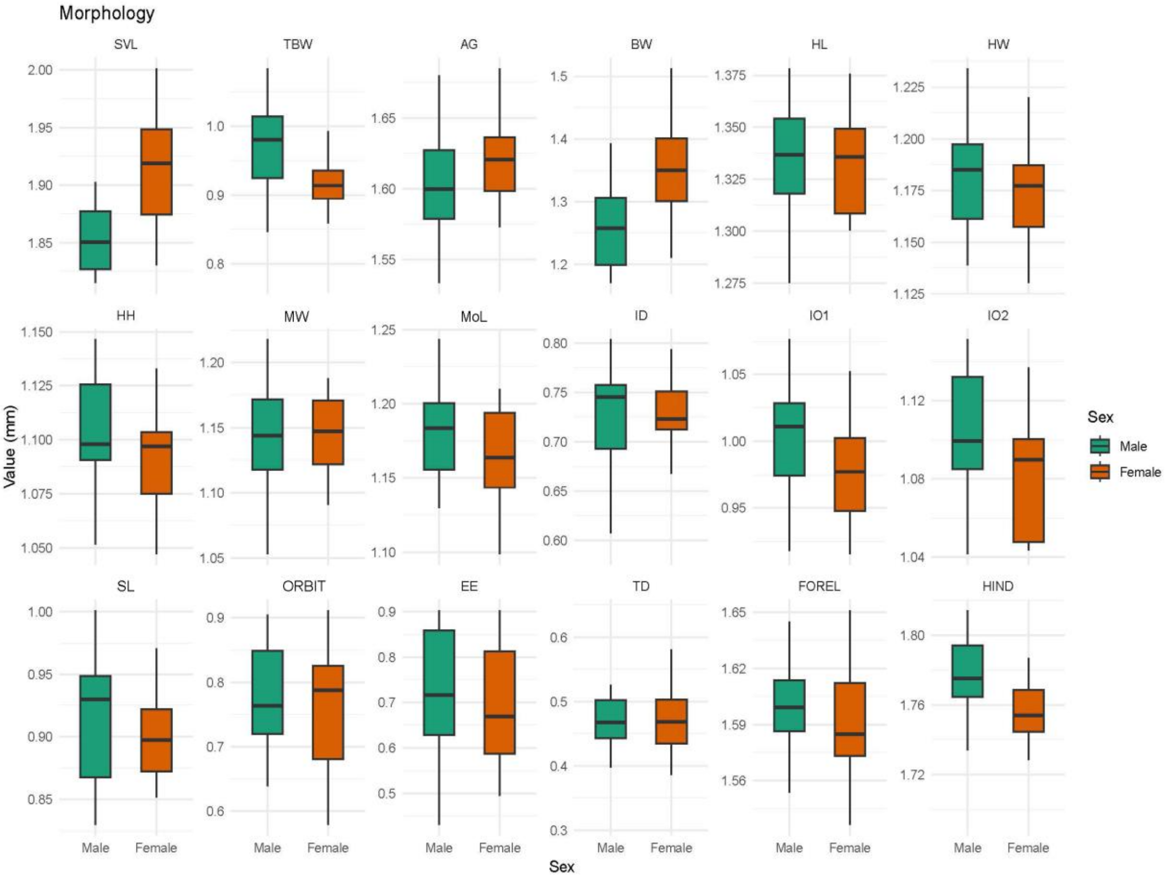

Figure 4. Boxplots comparing adjusted morphological characteristics between the two sexes of *Acanthosaura coronata*

PCA revealed that PC1 and PC2 account for 34.34% and 16.00% of the morphological variance in *A. coronata*, respectively. Although there is a considerable overlap between sexes in the morphospace (Figure 5), PC1 values differed significantly between males and females ($t = -3.3694$, df = 69.183, P-value = $0.0012 < 0.05$). In contrast, PC2 values did not differ significantly between sexes ($t = -0.66$, df = 61.1, P-value = $0.5 > 0.05$).

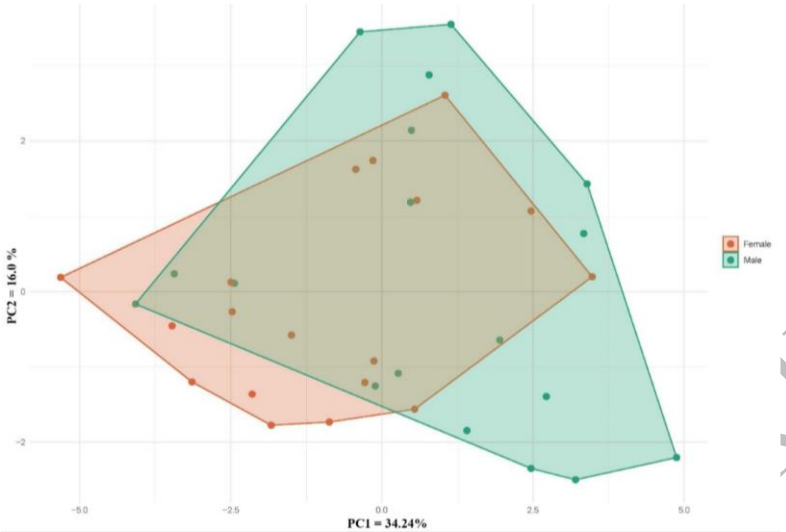

Figure 5. Principal Component Analysis of seven adjusted morphometrics from *Acanthosaura coronata* males and females.

DISCUSSION

Phylogenetic review in the *Acanthosaura coronata* group

Phylogenetic analysis of the COI gene in this study revealed that the *A. coronata* group forms a distinct cluster comprising three species: *A. coronata*, *A. cuongi*, and *A. grismeri* (Figure 2). This complex is clearly divergent from the clade containing *A. capra* and *A. murphyi*, with a substantial genetic difference evidenced by p-distances ranging from 13.46% to 18.66% and strong phylogenetic support at the diverging node (BPP/UFB = 1/89). Such phylogenetic distinctions were highly supported by the morphological differences in the body size and the length of both nuchal and dorsal crests (Ngo *et al.*, 2025b; Le *et al.*, 2025).

Furthermore, two new populations from Kon Ka Kinh NP and Ia Ly Forest, Gia Lai Province were placed into the species of *A. cuongi* and a new population of Ba Den Mountain, Tay Ninh Province was identified as *A. coronata.* However, the intraspecific genetic divergences within the group require careful consideration. Specifically, these divergences between the two populations of Gia Lai Province and the type population of *A. cuongi* is approximately 2.44–3.22%, and up to 2.66% between Ba Den Mountain and type population of Cat Tien NP of *A. coronata*. Recent surveys in Dien Bien and Lai Chau Provinces recorded the species of *A. longicaudata* in Vietnam and its populations showed the genetic divergence (3.25–4.03%) compared to the type population of *A. longicaudata* from China (Ngo *et al.*, 2025a). With the genetic divergence being just over 3.37% (up to 4.6%) of the COI gene fragment, Ananjeva *et al.* (2011) confirmed the interspecific distinction between *A. brachypoda* and *A. lepidogaster* based on an additional analysis of morphology. However, the difference in morphology might simply result from the sexual dimorphism due to the single specimen of female being used as the holotype of *A. brachypoda* for all comparisons. In this study, we consider such genetic differences in new populations to be insignificant and only three species have been known in the complex *A. coronata* group so far. Further examinations in different genetic fragments and morphological comparisons are essential to accurately confirm the valid level of taxonomic species in such cases.

Sexual dimorphism

In this study, we provided the sufficient diagnosis of *A. coronata*, based on morphological data of 66 individuals in Cat Tien NP and Ba Den Mountain, southern Vietnam, approved with the molecular analysis. The sexual dimorphism of *A. coronata* was further documented. In the coloration, we noted males of *A. coronata* having a striking green-yellow or light green coloration and an orange-red tail, while females have a simple coloration with a dominant light brown and gray coloration. The difference in coloration has also been documented in an agamid species of the Water Dragon lizard - *Physignathus cocincinus* in Vietnam (Ngo *et al.*, 2024). Like most diurnal agamids, mate selection in *A. coronata* may be visually driven – females are attracted to colorful males, while simple coloration in females likely provides enhanced camouflage (Andersson, 1994; Stuart-Fox *et al.,* 2004; Kuo *et al.,* 2009).

In this study, females of *A. coronata* exhibited longer SVL and greater abdominal measurements (AG and BW), strongly favoring the female-biased pattern. In contrast, males of *P. cocincinus* exhibited greater SVL, reflecting the male-biased size trend (Ngo *et al*., 2024). The sexual dimorphism in favor of females with larger abdominal and body sizes may help increase the number of eggs, the fecundity rate and the survival of eggs better in females with more horizontal and vertical abdominal space, especially in species of the family Agamidae containing a large number of eggs in gravid females (Vitt and Congdon, 1978; Andersson, 1994; Cox *et al*., 2003; Kuo *et al.,* 2009; Nguyen *et al*., 2018; Nguyen *et al*., 2024).

The study recorded males of *A. coronata* exhibiting longer limbs. The difference in limb length of males may facilitate greater agility and flexibility in hunting strategies, territorial defense, and reproductive success (Malhotra and Thorpe, 1997; Butler and Losos, 2002; Irschick *et al.,* 2005; Schwarzkopf, 2005; Ngo *et al.,* 2024). Accordingly, males can utilize a larger gripping surface, thereby enhancing sprinting ability across broader areas (Losos, 1990; Irschick and Losos, 1998; Beuttel and Losos, 1999; Kuo *et al.,* 2009). During mating, males with longer and more muscular limbs tend to exert better control and grasp females, thus ensuring the successful copulation (Butler and Losos, 2002; Schwarzkopf, 2005; Iraeta *et al.,* 2010).

Sexual dimorphism was further documented in TBW, with males displaying considerably broader tail bases than females. This characteristic is attributed to the presence of testes in males, which enlarge progressively with sexual maturity and during the breeding season (Chikwendu *et al.,* 2005; Nguyen *et al.,* 2024).

ACKNOWLEDGMENTS

The authors express their gratitude to the local authorities and Forest Protection Departments of Dak Lak, Gia Lai, Khanh Hoa, and Tay Ninh Provinces for their assistance in fieldwork and the issuance of necessary permits. We also thank T. Q.

Nguyen and H. H. Nguyen (Institute of Biology, Hanoi) for their valuable support of the research in Vietnam. This work was funded by the Vietnam Academy of Science and Technology (Grant No. THTEXS.01/23-25 to N. H. Ngo).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Ananjeva N. B., Ermakov O. A., Nguyen S. N., Nguyen T. T., Murphy R. W., Lukonina S. A., *et al*. (2020). A new species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Central Highlands, Vietnam. *Russian Journal of Herpetology, 27*(4), 217-230. https://doi.org/10.30906/1026-2296-2020-27-4-217-230

Ananjeva N. B., Orlov N. L., and Kalyabina-Hauf S. A. (2008). Species of *Acanthosaura* Gray, 1831 (Agamidae: Sauria, Reptilia) of Vietnam: Results of molecular and morphological study. *Biology Bulletin, 35*(2), 178-186. https://doi.org/10.1134/S106235900802012X

Ananjeva N. B., Orlov N. L., Nguyen T. T., and Ryabov S. A. (2011). A new species of *Acanthosaura* (Agamidae, Sauria) from Northwest Vietnam. *Russian Journal of Herpetology, 18*(3), 195-202. https://doi.org/10.30906/1026-2296-2011-18-3-195-202

Andersson M. (1994). *Sexual selection*. Princeton University Press.

Beuttel K., and Losos J. B. (1999). Ecological morphology of Caribbean anoles. *Herpetological Monographs, 13*, 1-28.

Boulenger G. A. (1885). *Catalogue of the lizards in the British Museum (Natural History)* (2nd ed.). Taylor and Francis. https://doi.org/10.5962/bhl.title.53974

Butler M. A., and Losos J. B. (2002). Multivariate sexual dimorphism, sexual selection and adaptation in Greater Antillean *Anolis* lizards. *Ecological Monographs, 72*(4), 541-559. https://doi.org/10.1890/0012-9615(2002)072[0541:MSDSSA]2.0.CO;2

Chan K. O., and Grismer L. L. (2021). A standardized and statistically defensible framework for quantitative morphological analyses in taxonomic studies. *Zootaxa, 5023*(3), 293-300. https://doi.org/10.11646/zootaxa.5023.2.9

Che J., Chen H., Yang J., Jin J., Jiang K., Yuan Z., *et al*. (2012). Universal COI primers for DNA barcoding amphibians. *Molecular Ecology Resources, 12*(2), 247-258. https://doi.org/10.1111/j.1755-0998.2011.03090.x

Chikwendu V., and Adeleke J. (2005). Seasonal testicular histology and reproductive cycle of the rainbow lizard, *Agama agama agama* (Agamidae, Reptilia), in Ile-Ife, southwestern Nigeria. *Animal Research International, 2*(3), 393-398. https://doi.org/10.4314/ari.v2i3.40876

Cox R. M., Skelly S. L., and John-Alder H. B. (2003). A comparative study of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution, 57*(7), 1653-1669. https://doi.org/10.1111/j.0014-3820.2003.tb00371.x

Edgar R. C. (2004). MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics, 5*, 113. https://doi.org/10.1186/1471-2105-5-113

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution, 39*(4), 783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Günther, A. (1861). Second list of Siamese reptiles. *Annals and Magazine of Natural History, 8*, 266-268.

Hoang D. T., Chernomor O., Von Haeseler A., Minh B. Q., and Vinh L. S. (2018). UFBoot2: improving the ultrafast bootstrap approximation.

Molecular Biology and Evolution, 35(2), 518-522. https://doi.org/10.1093/molbev/msx281

Kassambara A., and Mundt F. (2020). *Package "factoextra": Extract and visualize the results of multivariate data analyses* (Version 1.0.7).

Iraeta P., Salvador A., Monasterio C., and Díaz J. (2010). Effects of gravidity on the locomotor performance and escape behaviour of two lizard populations: the importance of habitat structure. *Behaviour, 147*(2), 133-150. https://doi.org/10.1163/000579509X12512773678411

Irschick D. J., and Losos J. B. (1998). A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution, 52*(1), 219-226.

Irschick D. J., Vanhooydonck B., Herrel A., and Meyers J. A. Y. (2005). Intraspecific correlations among morphology, performance, and habitat use within a green anole lizard (*Anolis carolinensis*) population. *Biological Journal of the Linnean Society, 85*(2), 211-221. https://doi.org/10.1111/j.1095-8312.2005.00486.x

Kalyaanamoorthy S., Minh B. Q., Wong T. K. F., Von Haeseler A., and Jermiin L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods, 14*(6), 587-589. https://doi.org/10.1038/nmeth.4285

Kuo C. Y., Lin Y. T., and Lin Y. S. (2009). Sexual size and shape dimorphism in an agamid lizard, *Japalura swinhonis* (Squamata: Lacertilia: Agamidae). *Zoological Studies, 48*(3), 351-361.

Le T. H. L., Ngo N. H., Nguyen T. T., Nguyen Q. T., Ziegler T., and Do T. D. (2025). Unexpected diversity of pricklenape agamas in Vietnam: Another new cryptic species of *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from the Central Highlands. *Zootaxa, 5686*, 373-391. https://doi.org/10.11646/zootaxa.5686.3.3

Le S., Josse J., and Husson F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software, 25*(1), 1-18.

Liu S., and Rao D. (2019). A new species of the genus *Acanthosaura* from Yunnan, China (Squamata, Agamidae). *ZooKeys, 888*, 105-132. https://doi.org/10.3897/zookeys.888.38491

Liu S., Hou M., and Rao D. (2020). A new species of the genus *Acanthosaura* (Squamata, Agamidae) from Yunnan, China, with comments on its conservation status. *ZooKeys, 959*, 113-135. https://doi.org/10.3897/zookeys.959.54601

Liu S., Rao D., Hou M., Orlov N. L., Ananjeva N. B., and Zhang D. (2022). Two new species of *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from Yunnan Province, China. *Russian Journal of Herpetology, 29*(2), 93-109. https://doi.org/10.30906/1026-2296-2022-29-2-93-109

Liu S., Zhang D., Hou M., Orlov N. L., Rao D., Ananjeva N. B., *et al.* (2023). Taxonomic assessment of *Acanthosaura lepidogaster* sensu lato (Reptilia: Agamidae) in China through extensive sampling. *Russian Journal of Herpetology, 30*(3), 127-143. https://doi.org/10.30906/1026-2296-2023-30-3-127-143

Losos J. B. (1990). Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecological Monographs, 60*(3), 369-388.

Lovich J. E., and Gibbons J. W. (1992). A review of techniques quantifying sexual size dimorphism. *Growth Development & Aging, 56*(4), 269–281.

Ngo N. H., Phan Q. T., Nguyen Q. T., Nguyen V. H., Do T. D., Gewiss L. R., *et al.* (2024). Distinct sexual dimorphism in a vulnerable lizard, *Physignathus cocincinus*, in Vietnam: an indirect cause for male-biased exploitation? *Salamandra, 60*(3), 254-262.

Ngo N. H., Le H. T. L., Hoang V. C., Ha T. T. L., Nguyen V. H., Nguyen V. T., *et al.* (2025a). Range expansion of *Acanthosaura longicaudata*: First record in Vietnam and prediction of potential distribution. *Russian Journal of Herpetology, 32*(4), 233-245. https://doi.org/10.30906/1026-2296-2025-32-3-233-245

Ngo N. H., Le T. H. L., Nguyen T. N., Nguyen T. T., Nguyen M. T., Phan Q. T., *et al.* (2025b). A new species of *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from the Truong Son mountain range, Vietnam. *European Journal of Taxonomy, 976*, 108-132.
https://doi.org/10.5852/ejt.2025.976.2781

Nguyen L. T., Do D. T., Hoang H. V., Nguyen T. T., McCormack T. E. M., Nguyen T. Q., *et al.* (2018). A new species of the genus *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from Central Vietnam. *Russian Journal of Herpetology, 25*(4), 259-274. https://doi.org/10.30906/1026-2296-2018-25-4-259-274

Nguyen L. T., Schmidt H. A., Von Haeseler A., and Minh B. Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution, 32*(1), 268-274. https://doi.org/10.1093/molbev/msu300

Nguyen S. N., Jin J.-Q., Vo B. D., Nguyen L. T., Zhou W.-W., Che J., *et al.* (2019). A new species of *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from Central Vietnam. *Zootaxa, 4612*(4), 555-565. https://doi.org/10.11646/zootaxa.4612.4.7

Nguyen T. V., Nguyen T. T., Ngo H. N., Ninh H. T., Nguyen H. Q., Pham C. T., *et al.* (2024). Examination of phylogenetic relationship and sexual dimorphism in the Vietnam warty newt (*Paramesotriton deloustali* (Bourret, 1934)) in Vietnam. *Vietnam Journal of Biotechnology, 22*(4), 543-559. https://doi.org/10.15625/vjbt-21656

Malhotra A., and Thorpe R. S. (1997). Size and shape variation in the Lesser Antillean anole, *Anolis oculatus* (Sauria: Iguanidae) in relation to habitat. *Biological Journal of the Linnean Society, 60*(1), 53-72. https://doi.org/10.1006/bijl.1996.0088

Orlov N. L., Truong N. Q., and Sang N. V. (2006). A new *Acanthosaura* allied to *A. capra* Günther, 1861 from Central Vietnam and Southern Laos. *Russian Journal of Herpetology, 13*(1), 61-76. https://doi.org/10.30906/1026-2296-2006-13-1-61-76

Pauwels O. S. G., Sumontha M., Kunya K., Nitikul A., Samphanthamit P., Wood P. L. Jr., *et al.* (2015). *Acanthosaura phuketensis* (Squamata: Agamidae), a new long-horned tree agamid from southwestern Thailand. *Zootaxa, 4020*(3), 473-494. https://doi.org/10.11646/zootaxa.4020.3.4

R Core Team. (2024). *R: A language and environment for statistical computing* (Version 3.8.1). https://www.r-project.org/

Ronquist F., Teslenko M., Van der Mark P., Ayres D. L., Darling A., Höhna S., *et al.* (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology, 61*(3), 539-542. https://doi.org/10.1093/sysbio/sys029

Schwarzkopf L. (2005). Sexual dimorphism in body shape without sexual dimorphism in body size in water skinks (*Eulamprus quoyii*). *Herpetologica, 61*(2), 116-123. https://doi.org/10.1655/04-66

Simmons J. E. (2002). *Herpetological collecting and collections management* (Rev. ed.). Society for the Study of Amphibians and Reptiles.

Stuart-Fox D. M., and Ord T. (2004). Sexual selection, natural selection, and the evolution of dimorphic coloration and ornamentation in agamid lizards. *Proceedings of the Royal Society B: Biological Sciences, 271*(1554), 2249-2255. https://doi.org/10.1098/rspb.2004.2802

Tamura K., Stecher G., and Kumar S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022-3027.
https://doi.org/10.1093/molbev/msab120

Trivalairat P., Kunya K., Chanhome L., Sumontha M., Vasaruchapong T., Chomngam N., *et al.* (2020). *Acanthosaura aurantiacrista* (Squamata: Agamidae), a new long-horned lizard from northern Thailand. *Biodiversity Data Journal, 8*, e48587. https://doi.org/10.3897/BDJ.8.e48587

Vitt L. J., and Congdon J. D. (1978). Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. *The American Naturalist, 112*(987), 595-608.

Wood P. L. Jr., Grismer J. L., Grismer L. L., Ahmad N., Onn C. K., and Bauer A. M. (2009). Two new montane species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Peninsular Malaysia. *Zootaxa, 2012*(1), 28-46. https://doi.org/10.11646/zootaxa.2012.1.2

Wood P. L. Jr., Grismer L. L., Grismer J. L., Neang T., Chav T., and Holden J. (2010). A new cryptic species of *Acanthosaura* Gray, 1831 (Squamata: Agamidae) from Thailand and Cambodia. *Zootaxa, 2488*(1), 22-38. https://doi.org/10.11646/zootaxa.2488.1.2