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A new species of the genus *Calotes* Cuvier, 1817 (Squamata: Agamidae) from southern Vietnam

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Abstract

We describe a new species of the agamid genus *Calotes* Cuvier, 1817 from southern Vietnam, which is most similar to *Calotes mystaceus* Duméril & Bibron, 1837, but can be distinguished from the latter and its other congeners by genetic and morphological differences. We discuss the current distribution of the new species and its sister species *C. mystaceus* in Mainland Southeast Asia.

Key words: Squamata, Agamidae, *Calotes* **sp. nov.**, *Calotes mystaceus*, taxonomy, Vietnam, COI, multivariate morphometric analysis

Introduction

The genus *Calotes* Cuvier, 1817 currently contains 23 species (Vindum *et al.* 2003; Zug *et al.* 2006; Krishnan 2008). Besides the widespread *Calotes versicolor* (Daudin, 1802), which natively inhabits an enormous area from East Iran throughout Asia to Indonesia (Sumatra), the majority of species in the genus *Calotes* are restricted to relatively small geographical regions in India, Sri Lanka and Myanmar (Hallermann 2000). In mainland Southeast Asia, a recently increased number of seven species is known to occur (*C. chincollium* Vindum, 2003; *C. htunwini* Zug & Vindum, 2006; *C. irawadi* Zug, Brown, Schulte & Vindum, 2006; *C. jerdoni* Günther, 1870), of which only three species are known from Thailand and Indochina (*C. emma* Gray, 1845; *C. mystaceus* Duméril & Bibron, 1837; *C. versicolor* (Daudin, 1802)) (Zug *et al.* 2006).

Early faunal publications (Morice 1875; Tirant 1885; Bourret 1927) listed two more species of *Calotes* for Cochinchina (today: southern Vietnam): *Calotes rouxii* Duméril & Bibron, 1837 and *Calotes ophiomachus* Duméril & Bibron, 1837 (currently recognized as a junior synonym of *Calotes calotes* (Linnaeus, 1758)). However, these records were obviously based on misidentifications, as both species are nowadays restricted to the Indian subcontinent including Sri Lanka (Manthey 2008; Uetz *et al.* 2012).

During recent field work in southern Vietnam specimens of the genus *Calotes* were collected and based on their general morphology we preliminary assigned them to *Calotes mystaceus* originally described by Duméril and Bibron (1837) from Myanmar [“Pays de Birmans”]. According to recent reviews on the lizard fauna of Vietnam (Bobrov & Semenov 2008; Nguyen *et al.* 2009), *C. mystaceus* is indicated as a widespread species found mostly in the southern parts of the country (from Kien Giang Province in the south to Thua Thien-Hue in central Vietnam) and several isolated records known from the north (Lang Son, Son La and Nghe An Provinces).

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However, when morphologically compared with specimens of *C. mystaceus* from western Cambodia and Thailand our Vietnamese specimens showed a significantly different color pattern, for example in lacking three characteristic dark brown blotches on the back. This geographic variance was already recognized by Smith (1921) and Bourret (2009), who characterized two forms, one on each side of the Mekong River, however without making any taxonomic decisions. Our extensive geographical sampling of comparative specimens has made it possible to distinguish between two genetically distinct units within the specimens currently referred to *C. mystaceus*. A preliminary analysis of mtDNA sequences variation (655 bp of Cytochrome oxidase I, COI) among the studied samples has shown that these morphotypes appear to be genetically highly divergent with uncorrected pairwise distances (*p*-distances) of 14.33 to 14.67 (%). These genetically distinct units are also geographically separated (Fig. 1). Based on the identified diagnostic features between the two genetically distinct lineages and taking into consideration that the type locality of *C. mystaceus* sensu stricto is in Myanmar, we herein formally describe the new species of *Calotes* from southern Vietnam and discuss its distribution on the eastern side of the Lower Mekong River.

FIGURE 1. Map of Southeast Asia, showing localities from which specimens were used in our analysis. Black: *Calotes* **sp. nov.**; White: *Calotes mystaceus*. Diamond: used in genetic and morphological analyses; triangle: used in genetic analysis only; upside down triangle: used in morphological analysis only.

Material and methods

Sampling: During recent field work from March until June 2009, specimens were collected in Cat Tien National Park, Dong Nai Province, Vietnam by PG. Further specimens and referred material were collected by NAP, EAG and A.B. Vassilieva in Cat Tien National Park, Dong Nai Province, Vietnam, from March until June 2009, from May to July in 2010 and 2011; in Cat Loc area of Cat Tien National Park, Lam Dong Province, Vietnam, from 17 to

27 of June 2011 (by NAP, EAG and A.B. Vassilieva); in Bu Gia Map National Park, Binh Phuoc Province, Vietnam, from 13 to 21 of April 2009 and from 18 to 30 of May 2011 (by NAP and A.B. Vassilieva); in Dong Nai Nature Reserve (Ma Da Forestry, former Vinh Cuu Reserve), Dong Nai Province, Vietnam, from 12 to 16 of June 2011 (by NAP and A.B. Vassilieva); in Yok Don National Park, Dak Lak Province, Vietnam, from 13 to 25 of April 2011 (by A.B. Vassilieva) and from 12 to 24 of December 2011 (by EAG and A.B. Vassilieva); in Binh Chau Xuyen Moc National Park, Ba Ria – Vung Tau Province, Vietnam, from 07 to 18 of November 2010 (by A.B. Vassilieva) and from 07 to 18 November 2011 (by EAG and A.B. Vassilieva); in Loc Bac Forest, Lam Dong Province, Vietnam, from 17 of April to 01 of May 2012 (by NAP and A.B. Vassilieva); in Lo Go-Xa Mat National Park, Tay Ninh Province, Vietnam, from 12 to 21 of May, 2012 (by NAP and A.B. Vassilieva).

Specimens were euthanized, fixed in 90% ethanol, later transferred to 70% ethanol and subsequently deposited at the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam, Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany and the Zoological Museum, Moscow State University (ZMMU), Moscow, Russia.

DNA extraction, PCR and sequencing: Tissue samples were processed at the Canadian Centre for DNA Barcoding (CCDB) and obtained sequences were submitted to the Barcoding of Life Database (BOLD; www.boldsystems.org). Total genomic DNA was extracted from ethanol-preserved muscle or liver tissues of the above mentioned and from recently collected comparative *Calotes* specimens (see Table 1) using a glass-fiber automatic DNA isolation protocol following Ivanova *et al.* (2006) or using standard phenol–chloroform extraction procedures (Hillis *et al.* 1996) followed with isopropanol precipitation. We amplified 655 bp of Cytochrome oxidase I (COI), a mitochondrial gene proved to be useful for species identification in reptiles and widely used as a barcoding marker for vertebrates (Hebert & Gregory 2005; Smith *et al.* 2008). Recently this gene was successfully applied for registration of cryptic diversity in lizards, including agamids (Solovyeva *et al.* 2011; Nazarov *et al.* 2012). Primers used both for PCR and sequencing were the VF1-d (5'-TTCTCAACCAACCACAARGAYATYGG-3') and the VR1-d (5'-TAGACTTCTGGGTGGCCRAARAAYCA-3') (Ivanova *et al.* 2006). The obtained fragments were sequenced in both directions for each sample, and a consensus sequence was generated. PCRs and sequencing were performed after the protocol of Nazarov *et al*. (2012). Obtained sequences are accessible at BOLD systems website (www.boldsystems.org) and are deposited in GenBank (Accession numbers: HM425545, KC016060-067; KC016069-070).

TABLE 1. Samples used in the molecular analysis.

Species	ID	Voucher	Locality	GenBank accession no.
Calotes **sp. nov.**	1	ZFMK 88935 (HOLOTYPE)	Cat Tien NP, Dong Nai Prov., Vietnam	KC016061
Calotes **sp. nov.**	2	IEBR A.2012.23 (PARATYPE)	Cat Tien NP, Dong Nai Prov., Vietnam	KC016062
Calotes **sp. nov.**	3	ZMMU NAP-00301	Bu Gia Map NP, Binh Phuoc Prov., Vietnam	HM425545
Calotes mystaceus	4	ZFMK 90413	Kulen Promtep WS, Preah Vihear Prov., Cambodia	KC016060
Calotes mystaceus	5	ZFMK 88341	Phnom Kulen NP, Siem Reap Prov., Cambodia	KC016063
Calotes versicolor	6	ZFMK 90414	Kulen Promtep WS, Preah Vihear Prov., Cambodia	KC016066
Calotes versicolor	7	ZMMU NP-VR-08-11-1	Cat Tien NP, Dong Nai Prov., Vietnam	KC016064
Calotes versicolor	8	ZFMK 88882	Mui Ne, Binh Thuan Prov., Vietnam	KC016065
Calotes versicolor	9	ZMMU NP-VR-08-30-1	Cat Tien NP, Dong Nai Prov., Vietnam	KC016067
Draco maculatus	10	ZFMK 90304	Phnom Kulen NP, Siem Reap Prov., Cambodia	KC016069
Eutropis multifasciata	11	ZFMK 88946	Cat Loc, Lam Dong Prov., Vietnam	KC016070

Phylogenetic analysis: The final alignment used for our phylogenetic analysis contained 600 bp of COI gene for six specimens of the *Calotes mystaceus* complex, five specimens of *Calotes versicolor* (to compare the interspecific distance to the *C. mystaceus* complex) and two specimens representing the outgroups, one being as well an agamid lizard (*Draco maculatus*) and one a more distantly related scincid lizard (*Eutropis multifasciata*) (summarized in Tab. 1). Sequences were aligned by eye using BioEdit Sequence Alignment Editor 5.0.9 (Hall 1999). Phylogenetic analyses were conducted in PAUP version 4.0b4a (Swofford 1998) and MEGA5 (Tamura *et al.* 2011) software. Uncorrected genetic distances (*p*-distance) between sequences were calculated in MEGA5 (see Table 2) (Tamura *et al.* 2011). Optimal Neighbour-Joining (NJ) trees were constructed in MEGA5 basing on evolutionary distances obtained using the Maximum Composite Likelihood method (Tamura *et al.* 2004) with transitions, transversions and all three codon-positions included into analysis. We partitioned the data by codon and selected the best-fit model of DNA sequence evolution for each data partition using the jModelTest (Posada 2008). The proportion of invariable sites, I = 0.55, for among-site rate variation followed a gamma distribution, with the shape parameter α = 1.32. The preferred model was (GTR + I + G), as suggested by the Akaike information criterion (AIC). Confidence in the tree topology was tested by non-parametric bootstrap analysis (Felsenstein985) with 1000 replicates for the MP and NJ analyses. We performed a heuristic ML search (Felsenstein 1981) with ten random-addition sequence replicates and TBR branch swapping was performed with the (GTR + I + G) model. Branches with bootstrap values 70% or greater were regarded as sufficiently resolved (Huelsenbeck & Hillis 1993).

TABLE 2. Uncorrected pairwise distances (%) of the mtDNA sequences (COI) within (diagonal) and between (below diagonal) species of *Calotes* included in the molecular analysis.

	Calotes **sp. nov.**	*Calotes mystaceus*	*Calotes versicolor*
Calotes **sp. nov.** (n = 3)	0.00–0.33		
Calotes mystaceus (n = 2)	14.33–14.67	0.67	
Calotes versicolor (n = 4)	20.50–20.83	17.83–18.00	0.00–0.83

Morphological comparison: Examined specimens are listed in the Appendix 1. Measurements were taken to the nearest of 0.1 mm with dial calipers. We obtained mensural and meristic characters in accordance to Zug *et al.* (2006). A list of characters used can be found in Appendix 2. The morphological data of *Calotes* **sp. nov.** and *Calotes mystaceus* was analyzed by using XLSTAT 2011.4.04 (Fahmy 2011). Subadult and juvenile specimens were excluded from the statistical morphological analyses. We performed an analysis of variance (ANOVA) of morphological characters to identify characters showing significant differences between the two OTUs (sexes were analyzed separately). Characters that were not ($p>0.05$) or not nearly ($p>0.10$) significantly different were excluded from the concluding principal component analysis (PCA).

Results

Phylogenetic analysis: Among 600 bp of the COI gene studied 149 characters were found to be variable, of those 148 were potentially parsimony-informative; the transition–transversion bias was estimated as 2.89 (all data given for ingroups only). Nucleotide frequencies were equal (A = 25%, T = 25%, C = 25%, G = 25%).

All analyses resulted in essentially similar topologies. The specimens (ID 1–5) originally assigned to *Calotes mystaceus*, were found to represent two well separated clades of *Calotes*. One clade consists of *Calotes mystaceus* sensu stricto (ID 4–5) and is represented by specimens from northern Cambodia, the other clade contains all samples from southern Vietnam (ID 1–3), which form a clearly distinct monophyletic lineage and represent a so far undescribed *Calotes* species (see Fig. 2).

p-distances between and within species of *Calotes* included in our molecular analysis are given in Table 2. Genetic variation among studied samples within the two lineages of the *Calotes mystaceus* complex is minimal. The evolutionary divergence between COI genes of *Calotes mystaceus* sensu stricto from Cambodia and specimens from southern Vietnam is profound and varies from 14.3 to 14.7 % of substitutions; this is slightly less than the

divergence between the *Calotes mystaceus* complex and a taxonomically distant species *Calotes versicolor* ranging from 17.8 to 20.8 % of substitutions. On the contrary, *p*-distances between Cambodian and Vietnamese samples of *C. versicolor* were found to be very small (0.83%). The values of *p*-distances between Vietnamese and Cambodian lineages of the *Calotes mystaceus* complex correspond to species-level divergence and are comparable or even higher than *p*-distance values in COI previously reported for other lizard groups (Solovyeva *et al.* 2011; Nazarov *et al.* 2012).

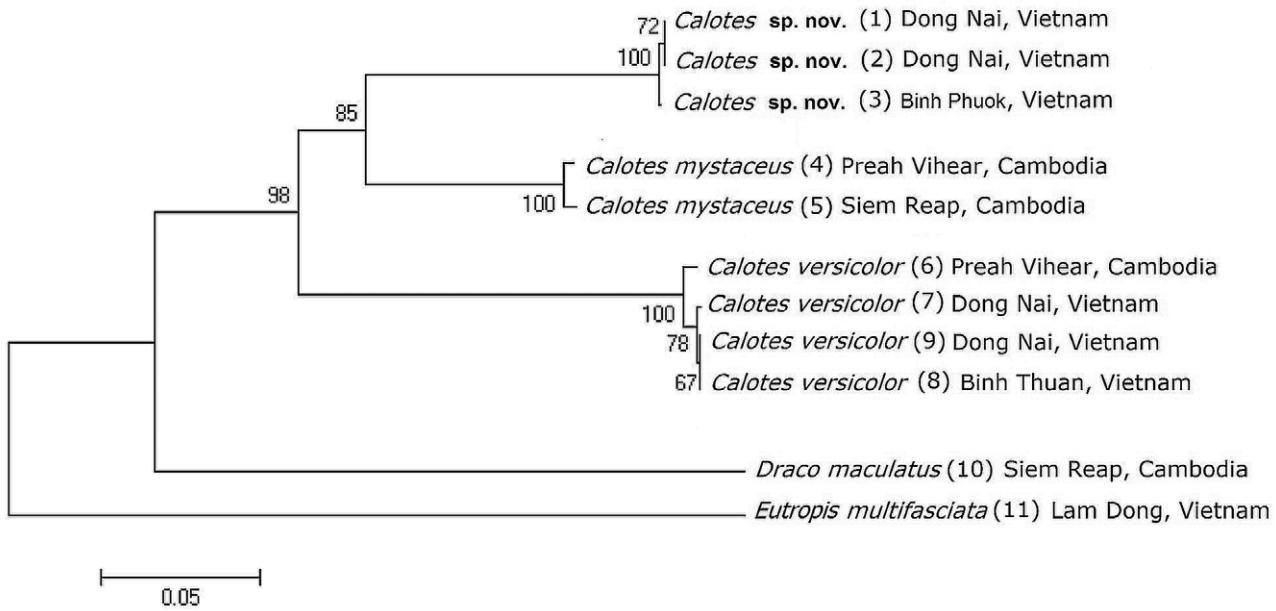

FIGURE 2. Fifty percent majority-rule consensus trees inferred by using the Maximum Likelihood method based on the GTR model. Numbers at nodes are the bootstrap supports (1000 replicates). *Eutropis multifasciata* (ZFMK 88946) and *Draco maculatus* (ZFMK 90304) were used as outgroups. Numbers in parentheses refer to ID in Table 1.

Morphological comparison: Specimens of the new species and *C. mystaceus* are morphologically obviously different to other congeners (see comparison). Hence, other taxa of *Calotes* were not included in the PCA. Total samples of 22 males (3 *Calotes* **sp. nov.**; 19 *Calotes mystaceus*) and 28 females (3 *Calotes* **sp. nov.**; 25 *C. mystaceus*) were included in the PCA. The morphological characters used in the PCA are listed in Table 3 and are also marked (*) in Table 4. In males, F1 had an eigenvalue of 2.703 (explaining 33.78 % of variability) and F2 1.653 (20.67 %). In females, the eigenvalue of F1 was 3.219 (35.77 %) and F2 1.974 (21.93 %).

TABLE 3. Showing the contribution of morphological characters to the variables created by the PCA (in %).

	Males		Females	
	F1	F2	F1	F2
4FingL/SVL	11.83	12.36	-	-
4ToeL/SVL	-	-	13.07	17.94
HindFL/SVL	-	-	13.81	24.23
TailH/SVL	15.48	17.95	9.93	6.47
TailL/SVL	-	-	16.65	0.13
TailW/SVL	6.22	4.39	7.76	24.12
TrunkL/SVL	3.74	22.30	7.23	6.19
UpArmL/SVL	17.33	4.55	-	-
UpLegL/SVL	16.65	0.03	20.43	2.09
VertS	13.39	21.38	3.87	14.10
MidbS	15.36	17.04	7.25	4.73

TABLE 4. Mean values and standard deviation of morphometric and meristic characters of *Calotes* **sp. nov.** and *C. mystaceus*. All morphometric characters are given as percentage of SVL. Character abbreviations are explained in Appendix 2. * = characters that in combination can be used to distinguish *Calotes* **sp. nov.** from *C. mystaceus* ($p < 0.10$).

Character	Male			Female		
	Calotes **sp. nov.** (n = 3)		*C. mystaceus* (n = 19)	*Calotes* **sp. nov.** (n = 3)		*C. mystaceus* (n = 26)
Mensural indices						
EyeEar/SVL	6.36±0.87		6.77±0.51	5.68±0.45		5.87±0.37
HeadH/SVL	17.35±1.77		18.90±1.77	16.38±0.10		17.44±1.35
HeadL/SVL	24.60±1.03		24.25±1.03	24.16±1.22		23.30±1.00
HeadW/SVL	18.70±1.67		20.11±1.67	18.06±1.05		17.30±0.99
Interorb/SVL	11.23±0.84		11.04±0.84	10.70±0.24		10.99±0.70
JawW/SVL	16.79±1.12		17.12±1.12	16.98±1.10		16.08±0.88
NarEye/SVL	6.73±0.40		6.82±0.40	6.30±1.11		6.74±0.64
SnEye/SVL	11.47±0.71		11.54±0.71	11.03±0.89		11.30±0.69
SnW/SVL	6.41±2.46		6.63±2.46	6.09±0.65		6.25±0.46
4FingL/SVL	12.24±0.50	*	11.49±0.50	12.32±0.36		12.01±0.89
4ToeL/SVL	15.99±1.23		14.89±1.23	16.07±0.86	*	14.55±1.17
CrusL/SVL	20.37±1.19		20.75±1.19	20.79±0.97		20.11±1.31
ForefL/SVL	15.78±1.33		15.43±1.33	15.97±0.96		15.41±1.38
HindfL/SVL	28.73±1.65		27.08±1.65	28.67±1.04	*	26.67±1.73
LoArmL/SVL	17.62±1.20		17.63±1.20	17.32±1.19		17.14±1.51
SnForeL/SVL	34.67±3.11		34.1±3.11	35.83±1.94		34.63±3.22
TailH/SVL	10.24±1.19	*	12.51±1.19	7.52±0.52	*	9.60±1.01
TailL/SVL	225.62±13.49 [1]		196.32±13.49 [2]	228.62±14.39	*	189.55±22.41 [3]
TailW/SVL	9.84±1.08	*	11.1±1.08	7.71±0.58	*	10.47±1.03
TrunkL/SVL	43.70±2.96	*	47.08±2.96	45.73±0.33	*	48.47±2.13
UpArmL/SVL	16.82±0.79	*	15.41±0.79	15.42±0.61		15.20±0.76
UpLegL/SVL	21.68±1.07	*	19.52±1.07	21.98±1.82	*	19.36±1.08
Meristics						
CanthR	8.7±0.6		8.7±0.8	9.0±1.0		8.4±0.8
Eyelid	13.3±0.6		13.1±0.9	13.3±0.6		13.4±1.1
HeadSLn	15.3±1.5		16.4±2.6	15.3±2.3		16.2±1.4
HeadSTr	16.0±1.0		17.3±1.4	16.7±1.5		17.4±1.6
Inflab	10.3±0.6		10.6±1.0	10.3±0.6		10.6±0.8
SnS	9.0±1.0		8.4±1.2	8.3±0.6		8.7±1.2
Suplab	10.0±0		10.7±1.1	10.3±0.6		10.7±0.9
4FingLm	20.3±0.6		20.4±1.3	20.0±2.0		20.1±0.9
4ToeLm	23.3±0.6		23.8±1.8	24.0±1.7		23.3±1.6
VertS	39.7±4.0	*	46.4±3.2	44.3±1.5	*	48.4±2.9 [4]
MidbS	45.7±2.1	*	52.4±2.5	46.6±1.2	*	51.9±2.7

[1] (n = 2); [2] (n = 15); [3] (n = 22), [4] (n = 24)

The performed PCA discriminated well between the two species in both sexes (see Fig. 3). Our molecular and morphological analyses (see below) clearly provide evidence that the southern Vietnamese population represents a distinct new species of *Calotes*, which we describe herein as:

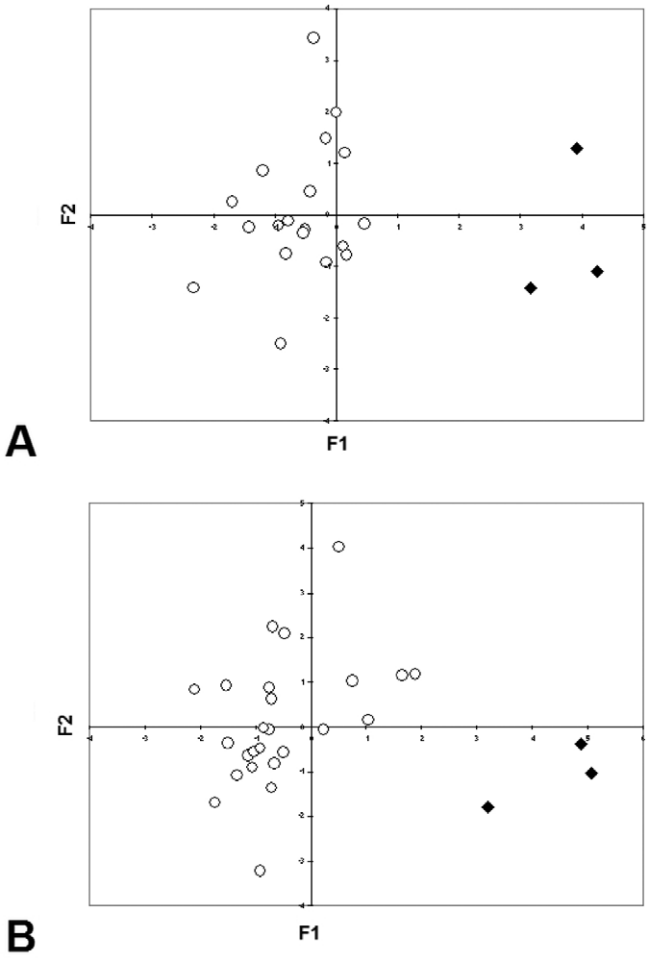

FIGURE 3. Principal component plot showing the success of multivariate morphometrics and meristics in discriminating the new species (black diamonds) from *Calotes mystaceus* (white circles). A. Males, B. Females. The statistically significantly different characters used are marked (*) in Table 4, their contribution to the variables F1 and F2 is given in Table 3.

***Calotes bachae* sp. nov.**
Fig. 4; Tables 4–5

Holotype: ZFMK 88935, an adult male (Fig. 4A) from Vietnam, Dong Nai Province, Cat Tien National Park (11°38'04" N 107°27'24" E), 104 m elevation, collected 10 May 2009 by PG.

Paratypes: An adult female (IEBR A.2012.23; Fig. 4B) and a juvenile (ZFMK 88937; Fig. 4E), same data as holotype (except collection dates: 20 April 2009 (IEBR A.2012.23) and 14 June 2009 (ZFMK 88937)) by PG. Two adult males (ZMMU NAP-02910-911; Fig. 4D) from Phuoc Hoa environs, Bu Gia Map National Park, Dak Ka River valley, Bu Gia Map commune, Bu Gia Map District, Binh Phuoc Province, southern Vietnam (12°19.6' N, 107°25.2' E; ca. 400 m above sea level), collected on 26 May 2011 by NAP. Two adult females (ZMMU NAP-01509, 01512) from Ma Da forestry, Dong Nai Nature Reserve (former Vinh Cuu Nature Reserve), Dong Nai Province, southern Vietnam, 80 m elevation, collected 12.06.2011 by NAP.

Diagnosis: A medium-sized *Calotes* with a maximum SVL of 97 mm, distinguished from all congeners by the combination of the following characters: 1) head and body robust; 2) body scales relatively small, homogeneous, feebly keeled, arranged in regular rows; 3) upper dorsolateral scales pointing backwards and upwards; 4) 44–50 midbody scale rows; 5) two short and well separated spines, surrounded by three to four scales on either side of the

upper head above tympanum; 6) nuchal and dorsal crest continuous, composed of erect compressed scales, directed posteriorly; 7) vertebral spines and scales 35–42 in males and 43–46 in females; 8) oblique fold of skin in front of forelimb insertion dinstict, covered with small granular dark scales; 9) extremities and tail relatively long and slender (see comparison with *C. mystaceus* for details); 10) bluish to turquoise head and anterior body part, this coloration not well exceeding front limb insertion; 11) yellowish light stripe at upper lip reaching from below anterior corner of eye to posterior end of head; 12) very faint or no dorsal brownish blotches, medially extending across the vertebral crest.

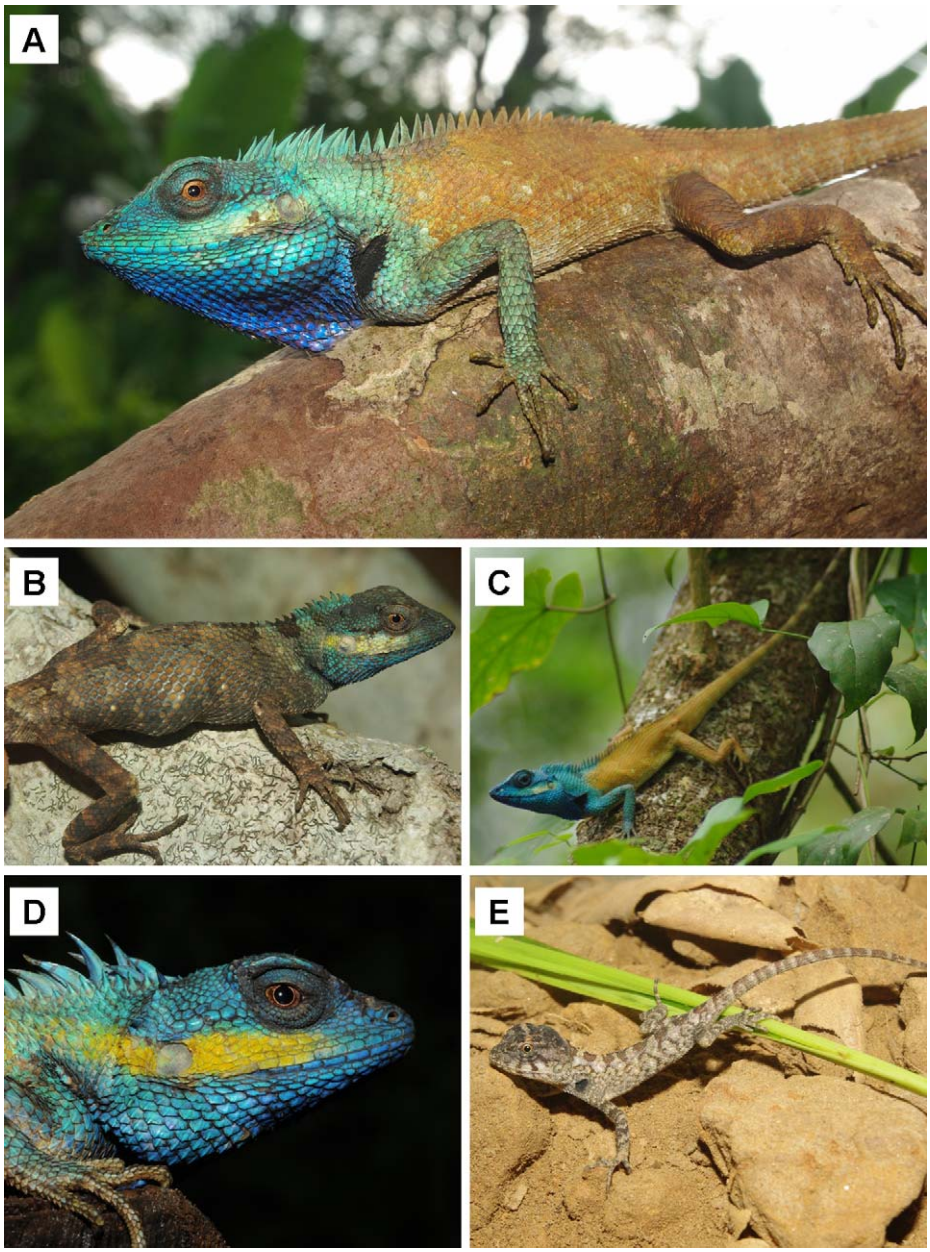

FIGURE 4. *Calotes bachae* **sp. nov.** in life. A) Holotype, adult male (ZFMK 88935), Dong Nai Province, Vietnam. Photograph by PG. B) Paratype, adult female (IEBR A.2012.23), Dong Nai Province, Vietnam. Photograph by PG. C) Uncollected adult male during breeding season, Dong Nai Province. Photograph by PG. D) Close up of paratype, adult male (ZMMU NAP-02910), Binh Phuoc Province, Vietnam. Photograph by NAP. E) Paratype, juvenile (ZFMK 88937), Dong Nai Province, Vietnam. Photograph by PG.

Description of Holotype: Moderately large male (SVL 90 mm). Tail and extremities relatively long and slender (TailL 199 mm). For further measurements see Table 5.

TABLE 5. Measurements and morphological characters of the type series of *Calotes bachae* **sp. nov.**; measurements in mm. *= tail broken.

	ZFMK 88935 (Holotype)	IEBR A.2012.23	ZFMK 88937	ZMMU NAP-01509	ZMMU NAP-01512	ZMMU NAP-02910	ZMMU NAP-02911
Gender	♂	♂	juv.	♀	♀	♂	♂
SVL	90	74	27.4	86	85	97	83
EyeEar	5.3	3.91	1.81	4.82	5.24	7.15	4.84
HeadH	14.88	12.19	4.9	14.09	13.83	18.22	13.88
HeadL	21.8	16.92	9.24	20.91	21.5	25.72	19.15
HeadW	17.04	14.12	5.93	14.6	15.4	20.02	13.71
Interorb	10.09	7.85	3.44	9.43	8.94	11.69	8.66
JawW	15	13.45	5.74	13.78	14.23	17.94	12.62
NarEye	6.16	3.75	1.88	6.19	5.65	6.97	5.12
SnEye	10.44	7.58	3.02	9.32	10.2	11.54	9.07
SnW	5.45	4.06	1.89	5.83	5.1	6.56	5.32
4FingL	11.79	8.88	3.69	10.93	10.42	10.94	10.24
4ToeL	15.08	11.83	5.37	14.59	12.96	14.17	13.78
CrusL	19.21	14.58	5.78	18.15	18.33	19.83	16.03
ForefL	13.74	12.11	4.97	12.79	14.17	15.59	13.28
HindfL	26.14	20.34	7.68	25.3	24.74	26.97	24.35
LoArmL	15.59	11.95	5.49	14.87	15.75	18.64	13.55
SnForeL	30	25	9.7	31	32	37	27
TailH	8.71	5.12	1.38	6.76	6.62	10.35	8.62
TailL	199	157	50.2	202	203	*	191
TailW	8.44	5.27	1.58	7.12	6.56	9.57	8.54
TrunkL	42	34	11.5	39	39	41	35
UpArmL	14.73	11.77	23.9	12.68	13.28	15.78	14.79
UpLegL	20.24	14.85	24.8	19.08	20.14	21.83	16.65
CanthR	9	8	9	9	10	8	9
Eyelid	14	14	13	13	13	13	13
HeadSLn	17	18	14	14	14	14	15
HeadSTr	16	18	14	15	17	17	15
Inflab	11	10	10	10	11	10	10
SnS	9	8	6	8	9	10	8
Suplab	10	10	10	10	11	10	10
4FingLm	21	18	20	22	20	20	20
4ToeLm	23	22	24	25	25	23	24
VertS	42	46	43	43	44	42	35
MidbS	48	48	48	46	46	44	45

Head distinct from neck; area posterior of jaw-angle swollen, snout-tip blunt; rostral small; nostril in a single scale, separated from rostral by one scale; a median longitudinal row of four bluntly ridged scales on snout; canthus rostralis sharp and straight; canthal scales continous with supraciliary scales; lateral sides of head flat; supralabials 10/10 (right/left), separated from the orbit by three rows of small scales; five feebly ridged scales from orbit to

above tympanum; tympanum distinct; two spines above tympanum, surrounded by four or five slightly pointed scales; infralabials 11/11; mental small, bordered by two postmentals; separated from each other; only first pair of postmentals touching infralabials; scales on chin and throat strongly keeled; a continuous vertebral crest consisting of elevated spine-like scales from nape to well behind tailbase, spine height gradually decreasing posterior of neck; dorsal and lateral scales keeled, pointing upwards and backwards; caudal scales directed backwards and slightly upwards; scales on venter parallel keeled; subcaudal scales strongly and parallel keeled. Arms and legs relatively slender, fourth finger and toe longest.

Color of Holotype in life: (Fig. 4A). The breeding coloration of the adult male is a brightly colored bluish to turquoise head, bluish coloration continuing posteriorly to behind the forelimb. A relatively faded light stripe at upper lip crossing the tympanum from beneath the eye to end of head. Gular pouch being colored in a darker blue, interscale skin black. Triangular to crescent-shaped patch of small black scales in front of shoulder. Very faint brownish blotches extending middorsally over the vertebral crest, from above forelimb insertion on to tail; posterior to forelimb insertion brownish orange in color on trunk and tail; hind limb in a slightly darker brown; venter cream.

Color of Holotype in preservative: Head and anterior part of body bluish, although artificially faded to whitish grey at top of head and between eyes and tympanum. Coloration behind forelimb insertion brownish to grey at flanks; dorsum and tail light grey; at posterior tail faded darker blotches still discernible; venter cream.

Variation: For the variation of paratypes see Table 5. The paratypes largely correspond with the description of the holotype (see Fig. 4B, 4D). The juvenile paratype (ZFMK 88937) differs from the other types in coloration pattern (see Fig. 4E): speckled greyish to beige in ground color; head with two well defined dark brown bands transversely above eyes; dark brown radiating streaks around the eye; well defined dark brown blotches middorsally from neck onto tail, below and between each dark blotch an indifferently light grayish-colored mottle.

Comparisons: Comparisons with all Indochinese congeners are based on examined specimens (see Appendix 1) and data obtained from the literature (Smith 1935, Taylor 1963, Vindum et al. 2003, Zug et al. 2006). *Calotes bachae* **sp. nov.** is distinguished from *C. htunwini*, *C. irawadi* and *C. versicolor* by possessing a crescent to triangular-shaped patch of small granular scales in front of the shoulder. It differs from *C. jerdoni* by the absence of two parallel rows of compressed scales above tympanum. It can be distinguished from *C. emma* by the absence of a large postorbital spine. It differs from *C. chincollium* in having a lower number of midbody scale rows (44-50 vs. 59-74) and a bluish coloration of the head in life (vs. brownish-grey with black mask).

Calotes bachae **sp. nov.** resembles *C. mystaceus* in all the above mentioned characters, but can be distinguished from the latter by a combination of the following characters (for further details see table 4): In males, *C. bachae* **sp. nov.** tends to have relatively longer fourth fingers, upper arms and upper legs than male of *C. mystaceus*. Furthermore, their tails tends to be relatively slimmer and flatter at base than in *C. mystaceus*. In females, *C. bachae* **sp. nov.** tends to have relatively longer forth toes, hind foots and upper legs than in *C. mystaceus*. Compared to *C. mystaceus*, their tails are relatively longer, slimmer and flatter at base. In both sexes, the relative distance between leg insertions was significantly shorter in *C. bachae* **sp. nov.** compared to *C. mystaceus*.

In addition, the number of vertebral spines and scales (M: 35–42, F: 43–46) and the number of midbody scale rows (44–50) tend to be lower compared to *C. mystaceus* (**VertS**: M: 42–51, F: 44–53; **MidbS**: 48–59).

The color pattern of *C. bachae* **sp. nov.** is also different from *C. mystaceus* (see Fig. 4–5). The bluish to turquoise colored anterior body part of the former does not well exceed behind the forelimb insertion, whereas in the latter it often reaches posteriorly up to behind the hind limb. In *C. mystaceus* the eponymous whitish stripe at upper lip extend from between nostril and eye to well behind the head often reaching the end of neck (vs. a yellowish light stripe from below anterior corner of eye to posterior end of head in *C. bachae* **sp. nov.**). *C. bachae* **sp. nov.** bears no or only faint brownish dorsal blotches, which are fused medially across the vertebral crest (vs. well defined dorsolateral dark brown blotches, which are well separated by the vertebral crest).

Etymology: We dedicate this species to Rike Bach (Bonn), in gratitude for supporting the second author (PG) during several fieldtrips in Indochina.

Distribution: *Calotes bachae* **sp. nov.** is currently only known from the territory of Vietnam. The type series consists of specimens from Dong Nai (Cat Tien NP and Dong Nai NR) and Binh Phuoc (Bu Gia Map NP) Provinces. We also recorded the new species from Dak Lak (Yok Don NP), Lam Dong (Cat Tien NP, Loc Bao Forestry), Ba Ria—Vung Tau (Binh Chau—Phuoc Buu NP) and Tay Ninh (Lo Go—Xa Mat NP) Provinces, as well

as from Ho Chi Minh City (Saigon). However, the actual distribution of the *Calotes mystaceus* complex within Indochina is known to be much wider (Bobrov & Semenov 2008; Nguyen *et al.* 2009). Future research has to show which documented populations of the *Calotes mystaceus* complex in Indochina have to be assigned to *Calotes bachae* **sp. nov.**.

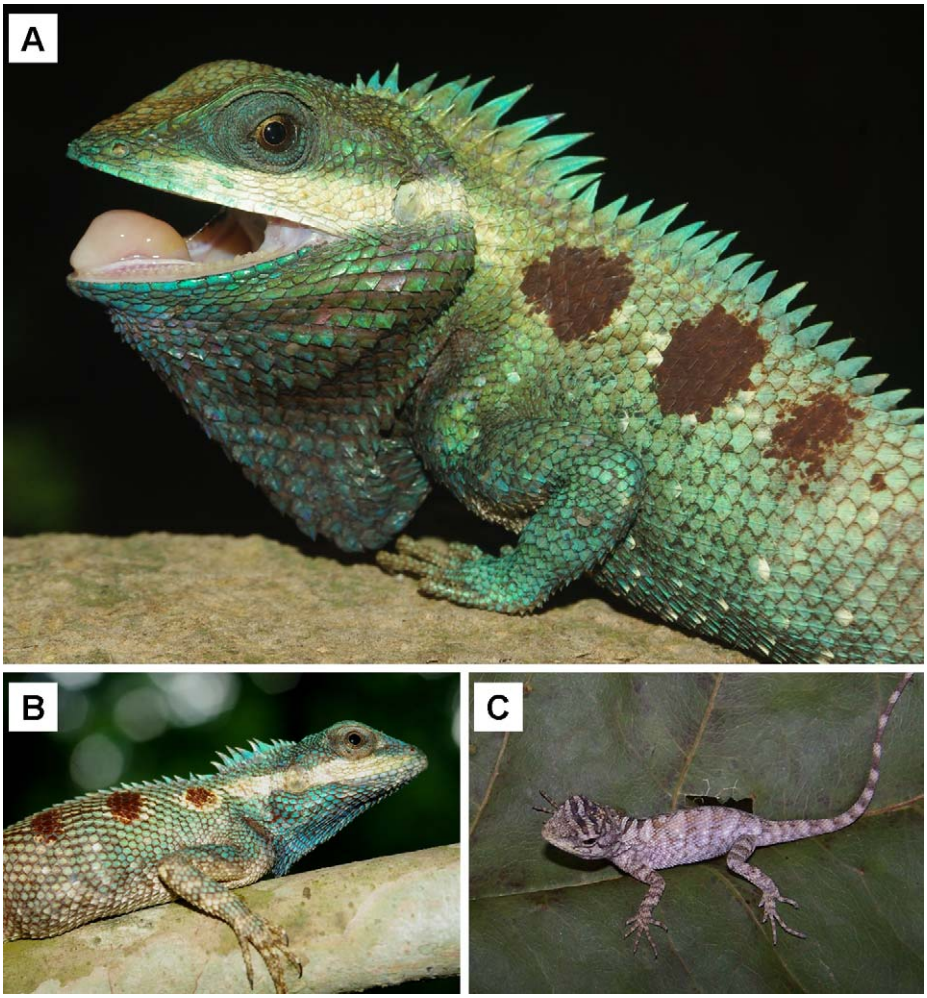

FIGURE 5. *Calotes mystaceus* in life. A) Adult male (ZFMK 92606), Siem Reap Province, Cambodia. Photograph by PG. B) Adult female (ZFMK 92607), Siem Reap Province, Cambodia. Photograph by PG. C) Subadult (ZFMK 90413), Preah Vihear Province, Cambodia. Photograph by TH.

Ecology: The new species was mainly observed in dipterocarp lowland forests and cultural landscape up to 700 m above sea level (a.s.l.) (maximum altitude recorded in Loc Bao Forestry, Lam Dong Province). During observations in Cat Tien National Park the species appeared to be quite heliophile, preferring more open habitats without closed canopies, but was also observed in dense monsoon tropical forests with closed canopy in Dong Nai Reserve and Bu Gia Map National Park. Within the Cat Tien National Park the species was abundant both in open gallery forests along Dong Nai River as well as in anthropogenic habitats like roadsides within the forest or open park sceneries within the headquarters of the park. The gallery forests were dominated by deciduous *Lagerstroemia* species and *Afzelia xylocarpa*. In Ho Chi Minh City the new species is abundant within the city center in the old park of the Saigon Zoo and Botanical gardens, as well as in the park of the Palace of Reunification and other green areas. In these anthropogenic habitats *C. bachae* **sp. nov.** occurs sympatrically with *Calotes versicolor*. In Cat Tien and Lo Go – Xa Mat National Parks and Dong Nai Reserve these two sympatric species of *Calotes* seem to differ in preferable habitats and *C. versicolor* is found in more open and disturbed habitats and usually is not observed in primary forests, whereas *C. bachae* **sp. nov.** often penetrates to undisturbed primary forest.

C. bachae **sp. nov.** is a diurnal, semiarboreal lizard, easily climbing on tree trunks at a height of 5–10 meters above the ground. Like many other tropical agamid lizards these animals spend night time on thin terminal branches and leaves. According to the content of feaces obtained from the population in the Cat Tien National Park,

Calotes bachae **sp. nov.** forages on numerous insects (Hymenoptera: Formicidae; Coleoptera; Orthoptera) and other arthropods (Myriapoda).

In the end of February to beginning of March, males acquire bright coloration, as described above (see coloration of holotype in life). Four gravid females were found in the middle of April 2012. Oviposition by two females was observed in April and May 2009. First, the female paratype (IEBR A.2012.23) laid four eggs on 20 April 2009. The eggs were incubated in soil substrate at temperatures of 22 to 25° C. After 56 days of incubation four juveniles hatched (SVL 24–31 mm, TailL 56–63 mm). One juvenile (Paratype ZFMK 88937; Fig. 4E) was euthanized and fixed after hatching. Second, on 25 May 2009, an uncollected female from Cat Loc Area, Cat Tien National Park, Lam Dong Province was digging a ca. 4 cm deep hole in which it laid five eggs (see Fig. 6B). After oviposition the hole was covered with soil and leaf litter.

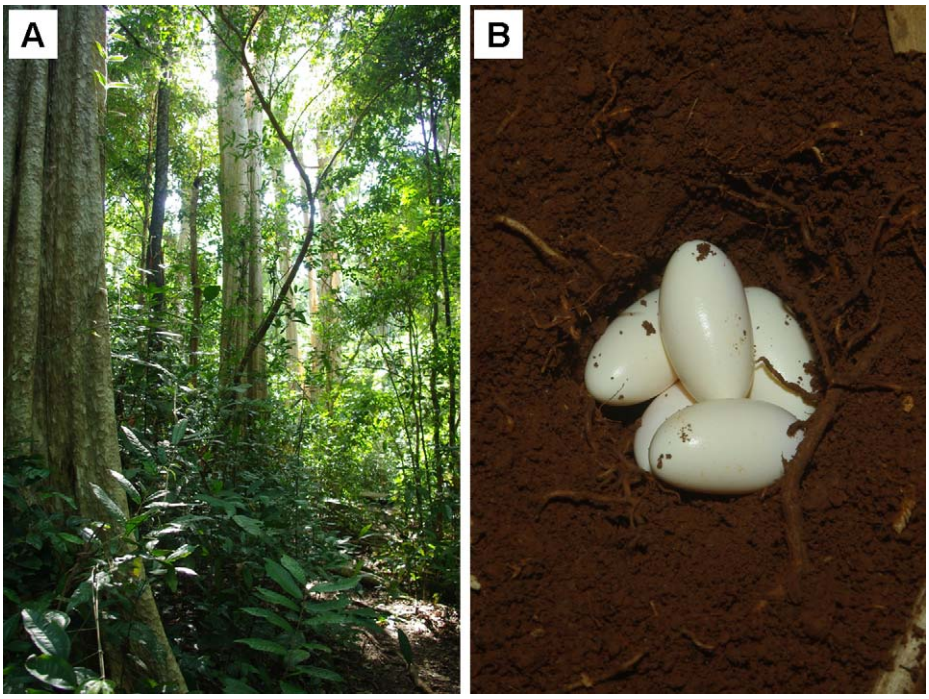

FIGURE 6. A) Habitat of *Calotes bachae* **sp. nov.**, deciduous gallery forest at Dong Nai River, Cat Tien National Park, Dong Nai Province, Southern Vietnam. Photograph by PG. B) Clutch of *C. bachae* **sp. nov.** immediately after oviposition, in a ca. 4 cm deep hole dug into clay soil at Cat Loc Area, Lam Dong Province, southern Vietnam. Photograph by PG.

Discussion

Our description of *Calotes bachae* **sp. nov.** raises the number of known *Calotes* occurring in Mainland Southeast Asia to eight.

So far, we only have provable knowledge of *C. bachae* **sp. nov.** from southern Vietnam. Our field observations, mtDNA sequencing and examination of collected specimens confirm the presence of the new species in Dong Nai, Binh Phuoc, Dak Lak, Lam Dong, Ba Ria – Vung Tau, and Tay Ninh Provinces, as well as in Ho Chi Minh City. Depicted specimens in the literature indicate its occurrence in two other localities in southern Vietnam as well as in adjacent Cambodia and southern Laos. Bobrov and Semenov (2008) depict one male specimen from Ba Ria – Vung Tau Province, further Nguyen *et al.* (2009) imaged one male specimen from Ho Chi Minh City. Teynié and David (2010) depict a specimen from Xepian National Park, Champasak Province, Laos and Bezuijen *et al.* (2009) show a specimen from Koh Khlap Island, Kratie Province, Cambodia. These specimens clearly resemble the distinct coloration pattern of *C. bachae* **sp. nov.**. Assumingly, the **southern** Lower Mekong River (South of the Khone Phapheng Falls) could be the key zoogeographic barrier between the so far allopatric distribution ranges of *C. mystaceus* and *C. bachae* **sp. nov.** as it was already indicated by Smith (1921) and Bourret (2009). Bourret (2009) also provides one watercolor painting of a specimen from “Mao Son, Tonkin”, today: Northern Vietnam, which by its appearance shows more similarity to *C. bachae* **sp. nov.** than to *C. mystaceus*.

Recent reviews of the lizard fauna of Vietnam (Bobrov & Semenov 2008; Nguyen *et al.* 2009, and references therein) indicate the presence of *Calotes mystaceus* in Vietnam from Lang Son Province in the North to Kien Giang Province in the South. Future redeterminations of all populations are needed to clarify which of the populations formerly assigned to *C. mystaceus* belong to the new species and if both species occur in Vietnam. We consider an occurrence of *C. mystaceus* sensu stricto in northern Vietnam to be likely, taken into consideration that specimens of *Calotes mystaceus* sensu stricto are known from east of the **northern** Lower Mekong (Sainyabuli and Vientiane Provinces, northern Laos; ZFMK 49242-246, ZFMK 47080-081). Unfortunately, our sampling along the Mekong is not dense enough to cover the area of possible contact between the two species and we do not know if they are geographically isolated from each other or if there is a contact zone. However, taken in consideration that the two species indeed may be isolated from each other by the **southern** Lower Mekong given the proximity of its known range to the Cambodian-Vietnamese border, it is reasonable to assume that *Calotes bachae* **sp. nov.** also occurs in adjacent parts of Cambodia and possibly in southern Laos as well. The extant of this species in Vietnam, Laos and Cambodia requires further investigations.

The ecology of *C. bachae* **sp. nov.** seems to be very similar to the one of *C. mystaceus*. Both species are strictly diurnal, inhabiting evergreen forests at mid-altitudes, lowland forests, as well as anthropogenic parks and gardens (Das 2010). Both species are partly arboreal and feed on large arthropods (Cox *et al.* 1998). The number of eggs per clutch, incubation period and hatchlings measurements are also resembling each other (Smith 1935; Cox *et al.* 1998). Future studies on the autecology of the two species are needed to find out whether there are also ecological traits distinguishing the two species.

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APPENDIX 1. Morphologically examined specimens

***C. bachae* sp. nov.:** VIETNAM: Dong Nai Province: Cat Tien National Park (*Holotype*: ZFMK 88935, *Paratypes*: IEBR A.2012.23, ZFMK 88937); Dong Nai Province: Ma Da (*Paratypes*: ZMMU NAP 01509 & 01512); Binh Phuoc Province: Bu Gia Map (*Paratypes*: ZMMU NAP 02910-911).

***C. mystaceus*:** CAMBODIA: Siem Reap Province: Phnom Kulen National Park (ZFMK 88341 & 92606-607). LAOS: Sainyabuli Province: 30 km east of Muang-Pak-Lay (ZFMK 49242-246); Vientiane Province: Muang Phon-Hong (ZFMK

47080-081). MYANMAR: Kayin State: Kaekanik (ZFMK 45553-554); Mon State: Mudon (ZFMK 45490-502). THAILAND: Kanchanaburi Province: Kanchanaburi (ZFMK 84867); Khon Kaen Province: Ban Phai (ZFMK 16640); Nakhon Ratchasima Province: Khon Buri (ZFMK 44893-894); Nan Province: Nan (ZFMK 55610-612); Petchabun Province: 5 km north of Lom Sak (ZFMK 49219-221); Phetchabun Province: Nam Len (ZFMK 49202); Phetchaburi Province: Cha-am (ZFMK 16641); Phitsanulok Province: Phitsanulok (ZFMK 43906); Saraburi Province: Ditang (ZFMK 49164); Ubon Ratchathani Province: Lam Dom Noi (ZFMK 40561); Uthai Thani Province: Uthai Thani (ZFMK 43930-932).

***C. emma*:** VIETNAM: Ha Tinh Province: Huong Son (ZFMK 92036-92038); Thanh Hoa Province: Ben En National Park (ZFMK 92829).

***C. versicolor*:** CAMBODIA: Preah Vihear Province: Kulen Promtep Wildlife Sanctuary (ZFMK 90414). VIETNAM: Binh Thuan Province: Mui Ne (ZFMK 88882).

APPENDIX 2. Morphological characters used in multivariate morphological analyses to discriminate between *Calotes bachae* **sp. nov.** and *Calotes mystaceus*. Characters after Zug *et al.* (2006).

Mensural Characters

Eye-ear length (**EyeEar**): Distance from anterior edge of tympanum to posterior of orbit (not pupil opening).
Head height (**HeadH**): Dorsoventral distance from top of head to underside of jaw at transverse plane intersecting angle of jaws.
Head length (**HeadL**): Distance from anterior edge of tympanum to tip of snout.
Head width (**HeadW**): Distance from left to right outer edge of temporal or jaw muscles at their widest point without compression of soft tissue.
Interorbital width (**Interorb**): Transverse distance between anterodorsal corners of left and right orbits.
Jaw width (**JawW**): Distance from left to right outer edge of jaw angles; this measurement excludes jaw musculature broadening of head.
Naris-eye length (**NarEye**): Distance from anterior edge of orbit to posterior edge of naris.
Snout-eye length (**SnEye**): Distance from anterior edge of orbit to tip of snout (rostral scale).
Snout width (**SnW**): Internasal or internarial distance of other authors; transverse distance between left and right nares.
4th finger (**4FingLng**): Distance from juncture of 3rd and 4th digits to distalmost extent (outer/distalmost surface of claw) of 4th finger.
4th toe (**4ToeLng**) Distance from juncture of 3rd and 4th digits to distal end of 4th digit on hindfoot.
Crus length (**CrusL**): Length of tibia from knee to heel.
Forefoot length (**ForefL**): Distance from proximal end of forefoot to tip of fourth digit.
Hindfoot length (**HindfL**): Distance from proximal end (heel) of hindfoot to distalmost surface of fourth toe.
Lower arm length (**LoArmL**): Distance from elbow to distal end of wrist, or just before underside of forefoot.
Snout-vent length (**SVL**)
Snout-forelimb length (**SnForeL**): Distance from anterior of forelimb, or shoulder, to tip of snout.
Tail height (**TailH**): Distance from dorsal to ventral surface of tail base measured just posterior to vent.
Tail length (**TailL**): Distance from vent to distal end of tail; noting completeness or regeneration of tail.
Tail width (**TailW**): Distance from left to right side of tail base just posterior to vent.
Trunk length (**TrunkL**): Body length or axilla-groin length of others; distance between posterior edge of forelimb insertion (axilla) to anterior edge of hindlimb insertion (inguen).
Upper arm length (**UpArmL**): Distance from anterior insertion of forelimb, or shoulder, to elbow.
Upper leg length (**UpLegL**): Distance from anterior edge of hindlimb insertion to knee.

Meristic Characters

Canthus rostralis (**CanthR**): number of elongate scales along 'dorsolateral snout ridge' from above posterodorsal corner of nasal scale to and including posteriormost supraciliary scale.
Dorsal eyelid scales (**Eyelid**): Number of scales found along dorsal edge of eyelid.
Dorsal head scales (**HeadSLn**): Number of scales longitudinally on midline between interparietal and rostral scale.
Head scales (**HeadSTr**): Number of scales in transverse line between posteriormost left and right supraciliary scales, just anterior of interparietal.
Infralabials (**Inflab**): posterior end defined by posteriormost enlarged scales that touches with Suplab at rear corner of mouth.
Snout scales (**SnS**): Number of scales on line transversally between left and right nasal scales (single scale surrounding naris).
Supralabials (**Suplab**): posterior end defined by posteriormost enlarged scales that touches Inflab at rear corner of mouth.
Forefoot lamellae (**4FingLm**) Number of 4th digit lamellae; from 1st lamella at digits' cleft that is wider than deep and touches dorsal digital scale (on at least one side) to most distal lamella; fragmented proximal scales are excluded.
Hindfoot lamellae (**4ToeLm**) analogous to **4FingLm** at 4th toe.
Vertebral scales or spines (**VertS**) Number of middorsal scales (spines or not), beginning with first enlarged spine-like scale on nape to above vent.
Midbody scale rows (**MidbS**) Number of scale rows around trunk at midbody.