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A new species of *Dixonius* (Squamata: Gekkonidae) from Phu Quy Island, Vietnam

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Abstract

We describe a new species of *Dixonius* on the basis of five specimens from Phu Quy Island, Binh Thuan Province, in southern Vietnam. The new species can be distinguished from congeners based on molecular and morphological differences. Diagnostic features are: small size (SVL up to 44 mm); 7 or 8 supralabials; 11 or 12 rows of keeled tubercles on dorsum; 21–23 ventral scale rows; 5 or 6 precloacal pores in males; a canthal stripe running from rostrum through the eye and terminating behind the head; second pair of postmentals about one third to one half size of first pair; ground color of dorsum brown, with one or two rows of light yellow or orange spots in one or two rows along flanks, and irregular bands or a reticulated network of dark marks on dorsum. This is the fifth species of *Dixonius* known to occur in Vietnam.

Key words: *Dixonius tatoi* sp. nov., coastal forest, molecular phylogeny, taxonomy, Binh Thuan Province, southern Vietnam, South China Sea

Introduction

The distribution of the gekkonid genus *Dixonius* extends from Myanmar and Thailand to Laos, Cambodia and Vietnam (Bobrov 1992; Pauwels *et al.* 2000, 2003; Ota *et al.* 2001; Nguyen *et al.* 2006; Bourret 2009; Nguyen *et al.* 2009). Five species of *Dixonius* are currently recognized: *D. aaronbaueri* Ngo & Ziegler 2009, *D. hangseesom* Bauer, Sumontha, Grossmann, Pauwels & Vogel 2004, *D. melanostictus* (Taylor 1962), *D. siamensis* (Boulenger, 1898) and *D. vietnamensis* Das 2004 (Uetz and Hošek, 2014). Four of these are known to occur in Vietnam and *D. vietnamensis* as well as *D. aaronbaueri* were only described in the past decade (Das 2004; Ngo & Ziegler 2009). During surveys in southern Vietnam in 2013 an island gecko population was discovered, that showed the typical features characteristic for the genus *Dixonius*: single pair of enlarged terminal scansors, tuberculate and keeled dorsal scalation, a rostral scale with a median cleft, first supralabials in contact with the nostril, enlarged chinshields and lateral gulars, precloacal pores present, a median row of transversely enlarged caudal scales, the absence of terminal scansorial pad on tail, the presence of cloacal spurs, the reduction in length of the second phalanx of digit IV of the manus, and the proximally bifurcate hypoischium (Bauer *et al.* 1997). However, this *Dixonius* population was neither morphologically nor genetically assignable to any known species and thus is here described as new.

Material and methods

After anaesthetization with ethyl acetate specimens were fixed in 90% ethanol and subsequently transferred into 70% ethanol for permanent storage. Specimens were deposited in the collections of the California Academy of Sciences, San Francisco, USA (CAS 257300), the Institute of Ecology and Biological Resources, Hanoi, Vietnam (IEBR A.2014.26, A.2014.27), the Vietnam National Museum of Nature, Hanoi (VNMN A.2014.4) and the Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK 96680).

For morphological characters we followed Bauer *et al.* (2004) and Ngo and Ziegler (2009). Sex was determined based on the presence or absence of precloacal pores and hemipenial swellings. All measurements were taken on the right side of the body with a digital caliper to the nearest 0.1 mm. For measurements and abbreviations see Table 1.

TABLE 1. List of abbreviations and explanations of morphological and morphometric characters.

SVL	Distance from tip of snout to vent
TL	Distance from vent to tip of tail
TW	Tail width, measured at base of tail
BW	Body width, greatest width of torso, taken at level of midbody
HL	Head length, distance between posterior edge of last supralabial and snout-tip
HW	Head width, measured at angle of jaws
HD	Head depth, maximum height of head, from occiput to throat
EL	Ear length, length along the major axis of ear
FA	Forearm length, distance between palm and elbow
ED	Eye diameter, greatest diameter of orbit
EN	Eye nostril distance, distance between anteriormost point of eyes and nostrils
ES	Eye snout distance, distance between anteriormost point of eyes and nostrils
EE	Eye ear distance, distance from anterior edge of ear opening to posterior corner of eyes
IN	Internarial distance, distance between nares
IO	Interorbital distance, distance between orbits
V	Ventral scale rows, number of longitudinal ventral scale rows counted midway between axilla and groin
PV'tub	Paravertebral scales, number of scales in a paravertebral row from first scale posterior to parietal scale to last scale at the level of vent opening
PV'tub	Paravertebral scales in a row between limb insertions
T4	Lamellae under fourth toe, number of scales from the distal one containing claw to basal one that broadly contacts adjacent fragmented scale
IOS	Interorbital scales, scales at narrowest point between orbits
ICS	Interciliary scales, scales between supraciliaries at midpoint of orbit
SPL	Supralabials
IFL	Infralabials
MO	Number of supralabial at midorbital position
PP	Precloacal pores

Molecular sequences were generated or obtained from Genbank for all five named species of *Dixonius* (Tables 2–3). Representatives of two species of *Heteronotia* and seven of *Nactus* were chosen as outgroups, based on previous work suggesting these two genera represent the closest relatives of *Dixonius* (Jackman *et al.* 2008; Gamble *et al.* 2012). The data set consisted of multiple mitochondrial and nuclear genes: the complete sequence of the mitochondrial NADH dehydrogenase subunit 2 (ND2; 1,035–1,041 bp), along with partial or complete sequences of 5 adjacent tRNAs (372–387 bp), plus partial exon sequences of the nuclear genes recombination activating gene 1 (RAG-1; 1,038 bp) and phosphocin (PDC; 395 bp).

TABLE 2. *Dixonius* representatives of which molecular sequences were compared. Collection abbreviations: CAS — California Academy of Sciences, San Francisco, USA; FMNH—Field Museum of Natural History, Chicago, USA; IEBR—Institute of Ecology and Biological Resources, Hanoi, Vietnam; LLG—L. Lee Grismer (La Sierra University, Riverside, USA); VU—Villanova University, Villanova, USA; ZFMK—Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

Species	Collection ID	Country	Locality	GenBank Accession No. ND2
<i>Dixonius aaronbaueri</i>	ZFMK 87274	Vietnam	Nui Chua NP, Ninh Thuan Province	HM997152
<i>Dixonius melanostictus</i>	VU 022	Thailand	Captive	HM997153
<i>Dixonius siamensis</i>	LLG 7378	Cambodia	Phnom Aural, Pursat Province	KP979732
<i>Dixonius siamensis</i>	LLG 7328	Cambodia	Phnom Aural, Pursat Province	EU054299
<i>Dixonius taoi</i> sp. nov.	CAS 257300	Vietnam	Phu Quy Island	KP979734
<i>Dixonius taoi</i> sp. nov.	ZFMK 96680	Vietnam	Phu Quy Island	KP979733
<i>Dixonius taoi</i> sp. nov.	IEBR A 2014-26	Vietnam	Phu Quy Island	KP979735
<i>Dixonius taoi</i> sp. nov.	IEBR A 2014-27	Vietnam	Phu Quy Island	KP979736
<i>Dixonius vietnamensis</i>	FMNH 263003	Cambodia	Keo Seima District, Mondolkiri Province	EU054298

TABLE 3. Uncorrected pairwise p-distances between samples of *Dixonius*.

Taxon	Spec. number	A				
<i>Dixonius aaronbaueri</i>	ZFMK 87274	A	—	B		
<i>Dixonius melanostictus</i>	VU 022	B	0.16635	—	C	
<i>Dixonius vietnamensis</i>	FMNH 263003	C	0.23269	0.20000	—	D
<i>Dixonius siamensis</i>	LLG 7378	D	0.18269	0.13462	0.18846	—
<i>Dixonius siamensis</i>	LLG 7328	E	0.18365	0.13462	0.18846	0.00288
<i>Dixonius taoi</i> sp. nov.	ZFMK 96680	F	0.17613	0.12596	0.19038	0.12500
<i>Dixonius taoi</i> sp. nov.	CAS 257300	G	0.17517	0.12500	0.19135	0.12404
<i>Dixonius taoi</i> sp. nov.	IEBRA 2014-26	H	0.18094	0.13077	0.19519	0.13077
<i>Dixonius taoi</i> sp. nov.	IEBRA 2014-27	I	0.20884	0.16867	0.12851	0.18474

continued.

Taxon	Spec. number						
<i>Dixonius aaronbaueri</i>	ZFMK 87274	A					
<i>Dixonius melanostictus</i>	VU 022	B					
<i>Dixonius vietnamensis</i>	FMNH 263003	C					
<i>Dixonius siamensis</i>	LLG 7378	D E					
<i>Dixonius siamensis</i>	LLG 7328	E — F					
<i>Dixonius taoi</i> sp. nov.	ZFMK 96680	F 0.12500 — G					
<i>Dixonius taoi</i> sp. nov.	CAS 257300	G 0.12404 0.00385 — H					
<i>Dixonius taoi</i> sp. nov.	IEBRA 2014-26	H 0.13077 0.00866 0.00674 —					
<i>Dixonius taoi</i> sp. nov.	IEBRA 2014-27	I 0.18474 0.03213 0.03213 0.03614					

For generation of new sequences, genomic DNA was isolated from ethanol-preserved tissue samples using a Qiagen DNeasy tissue and blood kit under manufacturers' protocols. PCR was performed in 25 µL reactions, with primer sequences retrieved from the literature (Jackman *et al.* 2008). Standard reaction conditions included an initial denaturation at 95 °C (2 min), followed by 32–34 cycles of denaturation at 95 °C (35 s), annealing at 50 °C

(35 s), and extension at 72°C (initially 150 s, with 4 s added per cycle). Annealing temperature was lowered for poor-yielding samples. Purification of amplified PCR products was via AMPure magnetic bead system (Agencourt Bioscience). The sequencing reaction employed dye-terminator chemistry, with cycle sequencing followed by purification via the CleanSeq magnetic bead system (Agencourt Bioscience). Capillary electrophoresis and analysis were performed using an ABI 3700 sequencer. Resulting chromatograms were inspected by eye and compared against their reverse complements in BioEdit (Hall 1999) to detect call errors, followed by translation to check for premature stop codons. Sequences were then aligned using Clustal (Thompson *et al.* 1994), and manually corrected to take into account codon deletions and tRNA secondary structure. The separate gene sequences were then concatenated into a single data set for analyses.

Bayesian and maximum likelihood methods were employed for phylogenetic analyses. PhyML (Guindon & Gascuel 2003) and jModelTest 0.91 (Posada 2008) were used to estimate parameters for all standard models of evolution, with the Akaike Information Criterion (AIC) used to identify the best-fitting model of evolution. Based on the AIC, the GTR + I + G model was chosen for the Bayesian and likelihood. For all analyses, alignment gaps were treated as missing data, and pairwise deletion of sites was employed.

The Bayesian analysis was run in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Two parallel runs with three heated and one cold chain were run for 1,000,000 generations, with a sampling frequency of 100 generations per sample. The data were divided into four partitions (ND2, tRNA, RAG-1, PDC), with all parameters unlinked across the partitions. A burnin of 2,000 samples (200,000 generations) was used to discard the region of increasing likelihood values. The average standard deviation of split frequencies (finished at 0.005) and potential scale reduction factors (no value greater than 1.004) were consulted to ensure that the chains ran for an adequate length of time. Branch support was assessed using posterior probabilities. The likelihood analysis was performed using RAxML 7.2.6 (Stamatakis 2006). The same partitions employed in the Bayesian analysis were used in the likelihood analysis. Ten independent searches were employed to find the most likely tree, and branch support was assessed with 1,000 nonparametric bootstrap replicates.

Results

The molecular analyses revealed the *Dixonius* specimens from Phu Quy Island to be a sister taxon of *Dixonius melanostictus* (Fig. 1). However, the *Dixonius* from Phu Quy showed a genetic divergence of 11.3–11.8% (ND2 gene) from other species justifying it to be a distinct taxon.

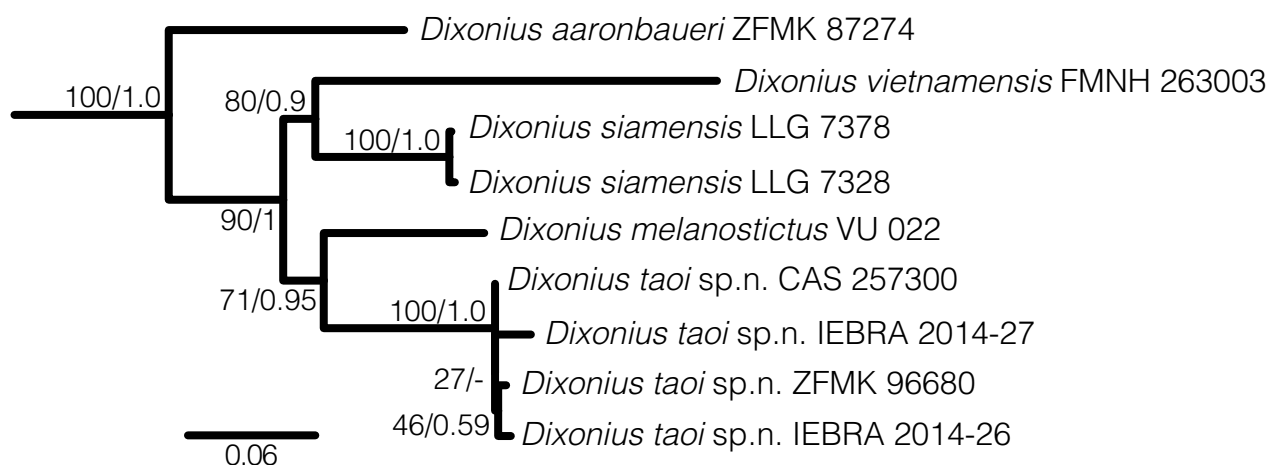


FIGURE 1. Maximum likelihood tree illustrating the relationship of *Dixonius taoi* sp. n. to its congeners. Support values are maximum likelihood bootstraps, followed by Bayesian posterior probabilities. Outgroup taxa (see Materials and Methods) not shown.

Further morphological comparison also revealed the difference between specimens from Phu Quy (Figs. 2-3) and other species of *Dixonius*, based on examination of specimens and data obtained from the literature (Boulenger

1898; Smith 1935; Taylor 1962, 1963; Bauer *et al.* 2004; Das 2004; Stuart *et al.* 2006; Grismer *et al.* 2007; Ngo & Ziegler 2009).

Based on our molecular and morphological findings we describe the *Dixonius* population from Phu Quy Island as:

***Dixonius taoi* sp. nov.**

Holotype: IEBR A.2014.26 collected on 2nd of June 2013 by Trung My Phung, Phu Quy Island, Phu Quy District, Binh Thuan Province, Vietnam.

Paratypes: CAS 257300, IEBR A.2014.27, VNMN A.2014.4, ZFMK 96680, the same data as the holotype.

Diagnosis. A small gecko with up to 44 mm SVL; 7 or 8 supralabials; 11 or 12 rows of keeled tubercles on dorsum; 21–23 ventral scale rows; 5 or 6 precloacal pores in males; a canthal stripe running from rostrum through the eye and terminating at back of head; lateral second pair of postmentals about one third to one half size of first pair; brown dorsal ground color with light yellowish/orange spots in one or two rows along flanks, and irregular bands or a reticulated network of dark marks on dorsum.

Description of holotype. Adult male, snout-vent length 41.5 mm with dorsolaterally flattened body, total tail length 46.4 mm with regenerated tip (10 mm), trunk length 17.5 mm. Head longer than wide, depressed, distinct from slender neck. Head length 7.5 mm, head width 6.6 mm, eye of moderate size 2.8 mm, ear opening oblique (EL 0.9 mm), nare-eye length 3.5 mm, snout-eye length 4.8 mm, internarial distance 1.5 mm.



FIGURE 2. Type series of *Dixonius taoi* sp. n. from Phu Quy, Binh Thuan Province, Vietnam. From left to right CAS 257300, IEBR A.2014.26 (holotype), VNMN A.2014.4, ZFMK 96680, IEBR A.2014.27. Photo: Andreas Botov.

Rostral very large, wider (1.3 mm) than high (1.8 mm), with distinct suture; supralabials 7 (fifth in midorbital position), smaller in subocular rictus; nostril in contact with rostral, first supralabial, supranasal, and two nasals posteriorly on each side; supranasals in broad contact and without internasals; snout flat, covered with granular scales; pupil vertical; ear opening oblique, oval, approximately one third of the eye diameter, without bordering enlarged scales; mental triangular, as wide as long; infralabials 7/6, decreasing gradually in size; mental triangular, wider (2.2 mm) than high (1.6 mm); two pairs of enlarged postmentals, first pair very large and in contact with each other, second pair about one third to one half size of first, in contact with first and second infralabials and separated from each other by four gular scales; dorsal scale rows at midbody 12, keeled and separated from one another by one or two smaller scales which are keeled or at least conical shaped; three to four rows of small scales along vertebrae; ventral scales larger than dorsal scales, in 21 rows at midbody, with distinct serration posteriorly; dorsal surface of fore- and hind limbs covered with shallowly-keeled scales, area around limb insertions covered with small granular scales; lamellae on fourth toe 12/12; femoral pores absent; precloacal pores six, in an angular series; pore bearing scales not enlarged; precloacal depression absent; tail covered with keeled scales, in different sizes; subcaudals 38, enlarged and undivided.

Coloration. In life, dorsum olive gray (Fig. 3) with irregular brownish olive blotches. A brownish olive stripe running from rostrum through eyes, ending at back of head, edged above by a thin yellowish stripe. Dorsal head with brownish olive bands or blotches. Four to seven round yellowish spots, arranging in one or two irregular rows from back of head and continuing along tail. Venter cream. In preservative, dorsum grey with irregular dark greyish brown blotches on forehead and body. Yellowish blotches faded to whitish marks in preservative.



FIGURE 3. Dorsolateral view of *Dixonius taoi* sp. n. from Phu Quy Island, Vietnam. Adult male holotype (A: IEBR A.2014.26) and adult female paratype with partially regenerated tail (B: IEBR A2014.27). Photos: Trung My Phung.

Variation of paratypes: Coloration of all paratypes corresponds with the description of the holotype. Variation in scalation and morphometry is shown in Table 4.

TABLE 4. Selected morphometric measurements and morphological characters of the type series of *Dixonius taoi* sp. nov. For abbreviations see Table 1. Measurements in mm; measurements taken on right side; SPL/IFL/MO given in right/left order; Min = minimum; Max = maximum; + = present.

Character	IEBR A.2014.26 (holotype)	CAS 257300	VNMN A.2014.4	ZFMK 96680	IEBR A.2014.27	Min	Max
Sex	male	male	male	male	female		
SVL	41.5	42.1	42.8	43.9	36.6	36.6	43.9
TL	46.4 (tip regenerated 10 mm)	broken	broken	54.1	regenerated	46.4	54.1
TW	4.3	4.2	4.4	4.3	2.8	2.8	4.4
BW	9.2	8.5	8.1	8.4	8.0	8.0	9.2
HL	7.5	6.7	7.8	7.5	6.6	6.6	7.8
HW	6.6	6.1	6.5	7.3	5.4	5.4	7.3
HD	4.8	4.6	4.9	4.9	3.7	3.7	4.9
EL	0.9	0.8	1.0	1.2	0.9	0.8	1.2
ED	2.8	2.6	2.9	2.7	2.4	2.4	2.9
EN	3.5	3.6	3.8	3.4	3.2	3.2	3.8
ES	4.8	4.7	5.1	4.7	4.1	4.1	5.1
EE	4.0	3.6	3.8	3.6	3.1	3.1	4.0
IN	1.5	1.6	1.7	1.5	1.2	1.2	1.7
IO	4.1	3.8	3.7	3.9	3.1	3.1	4.1
FA	5.4	5.7	6.2	5.3	4.9	4.9	6.2
TBL	6.8	6.4	7.4	7.7	6.0	6.0	7.7
AG	17.5	18.8	17.6	18.3	17.2	17.2	18.8
AG/SVL	0.42	0.45	0.41	0.42	0.47	0.41	0.47
EL/ED	0.32	0.31	0.34	0.44	0.38	0.31	0.44
SPL	7/7	8/8	8/7	7/7	8/8	7	8
IFL	7/6	7/7	6/6	5/6	6/7	5	7
MO	6/5	6/6	6/6	6/6	5/6	5	6
IOS	9	7	10	9	10	7	10
ICS	31	26	26	30	28	26	31
V	21	22	22	21	23	21	23
DTR	12	11	11	11	12	11	12
PVtub	36	42	31	35	33	31	42
PV'tub	18	25	20	20	24	18	25
T4 r/l	12/12	13/12	14/13	14/12	13/12	12	14
PP	6	6	5	6	0	5	6
Canthal stripe	+	+	+	+	+		

Comparisons. The new species from Phu Quy differs from all known *Dixonius* species as follows: from *D. aaronbaueri* by having more ventral scale rows at midbody (21–23 versus 18–19 in *D. aaronbaueri*) and different color pattern (dorsum brown with light yellowish/orange spots and dark irregular bands versus unpatterned in *D. aaronbaueri*); from *D. hangseesom* by having fewer ventral scale rows at midbody (21–23 versus 22–26 in *D. hangseesom*), fewer precloacal pores in males (5–6 versus 6–8 in *D. hangseesom*), coloration of tail the same as the dorsum (versus orange tail in *D. hangseesom*); from *D. melanostictus* in having a smaller size (max. SVL 44 mm

versus 50 mm in *D. melanostictus*), fewer supralabials (7–8 versus 9 in *D. melanostictus*), fewer precloacal pores in males (5–6 versus 9 in *D. melanostictus*), and canthal stripe continues behind orbit to back of head (versus canthal stripe extending along flanks in *D. melanostictus*); from *D. siamensis* in having a smaller size (max. SVL 44 mm versus 57 mm in *D. siamensis*), the presence of a distinct canthal stripe (absent in *D. siamensis*); from *D. vietnamensis* in having more ventral scale rows at midbody (21–23 versus 20 in *D. vietnamensis*), fewer enlarged dorsal scale rows at midbody (11–12 versus 16 in *D. vietnamensis*), and the presence of one or two irregular rows of yellowish marks running from head along flanks and meeting at base of tail in adults (versus scattered orange tubercles in *D. vietnamensis* in juveniles only) (Tab. 5).

TABLE 5. Comparison of selected morphometrical and morphological characters of *Dixonius taoi* sp. n. and its congeners. For abbreviations see Table 1. Measurements in mm; Min = minimum; Max = maximum; + = present, - = absent; * 6–9 precloacal pores in *D. vietnamensis* are reported from Cambodia (Stuart *et al.*, 2006).

Character	<i>Dixonius taoi</i> sp. nov.	<i>D. aaronbaueri</i>	<i>D. hangseesom</i>	<i>D. melanostictus</i>	<i>D. siamensis</i>	<i>D. vietnamensis</i>
Max SVL	43.9	38.6	42.1	50.0	57.0	42.4
Max TL	54.1	37.1	51.5	56.0	58.0	37.8
SPL	7–8	8–9	8	9	7–8	7
IFL	5–7	6–8	6+2	7	6–7	6–7
MO	5–6	6–7	6	7		5–6
IOS	7–10	8–10	10			8–10
ICS	26–31			29		
V	21–23	18–19	22–26	22	18–25	20
DTR	11–12	11	12–14	10–11	10–14	16
PVtub	31–42	45–50				
PV'tub	18–25	29–32				
T4 r/l	12–14	13–15	13	15	12–16	13
PP in males	5–6	5	6–8	9	6–7	5–6*
Canthal stripe	+	+	+	+	-	+

Etymology. The new species is named in honor of our colleague and friend Dr. Nguyen Thien Tao from the Vietnam National Museum of Nature in Hanoi, in recognition of his numerous and groundbreaking scientific contributions towards a better understanding of the herpetofauna of Vietnam.

Distribution. Phu Quy Island, Binh Thuan Province, Vietnam (Fig. 4).

Natural history. The type series were found in secondary forest just above sea level (Fig. 5). The predominant vegetation, which is interspersed with large volcanic stones, consists of small prickly shrubs and species of the families Pandanaceae, Ebenaceae, and Fabaceae. The geckos were collected during a rainy night, when they had left their shelters between stones and shrubs. A few specimens were found in the small shrubs in agricultural lands. Most of the forests in this area have been destroyed, and only a few small patches are left along the coast.

Discussion

The type locality of the new *Dixonius* species is Phu Quy Island in Binh Thuan Province, ca. 80 km offshore in the East Sea (or South China Sea), where *D. taoi* inhabits disturbed forest in coastal region. The distance from the type locality to the mainland is likely a significant barrier to dispersal and genetic exchange with mainland populations may be assumed to be virtually nil. Bain and Hurley (2011) assumed that these islands have been separated from the mainland for at least 10,000–15,000 years ago, however, the deep divergence between *D. taoi* and its mainland relatives suggests that much older historical events may have promoted cladogenesis. *D. taoi* is the sixth species of *Dixonius* known worldwide, and the fifth species known to occur in Vietnam. In our molecular phylogeny, the new species proved to be sister to *D. melanostictus*. However, we are aware of additional, undescribed species in the

genus and a meaningful interpretation of the historical biogeography of the group must await the documentation of this still under-appreciated diversity.



FIGURE 4. Map showing the type locality of *Dixonius taoi* sp. n. (encircled) in southern Vietnam.



FIGURE 5. Natural habitat of *Dixonius taoi* sp. n. on Phu Quy Island, Vietnam. Photos: Trung My Phung.

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