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First molecular verification of *Dixonius vietnamensis* Das, 2004 (Squamata: Gekkonidae) with the description of a new species from Vinh Cuu Nature Reserve, Dong Nai Province, Vietnam

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Abstract

Based on near-topotypic specimens of *Dixonius vietnamensis* from Khanh Hoa Province in southern Vietnam genetic analyses showed that the recently described *D. taoi* is sister to *D. vietnamensis* and several separate forms exist which previously have been misidentified as *D. vietnamensis* and *D. siamensis*. The *Dixonius* population from Vinh Cuu Nature Reserve, Dong Nai Province, Vietnam, represents an undescribed species. *Dixonius minhlei* **sp. nov.** can be distinguished from its congeners based on the following diagnostic characters: small size (up to 47.5 mm SVL); 7–9 supralabials; 14–15 rows of keeled tubercles on dorsum; 20–23 ventral scale rows; 7 or 8 precloacal pores in males; a canthal stripe running from rostrum through the eye and terminating at back of head; lateral second pair of postmentals maximum one quarter the size of first pair; dorsum olive gray with more or less discernible brownish olive blotches. This is the sixth species of *Dixonius* known to occur in Vietnam.

Key words: *Dixonius vietnamensis*, *Dixonius minhlei* **sp. nov.**, Vietnam, morphology, molecular phylogeny, taxonomy

Introduction

The gekkonid genus *Dixonius* was erected by Bauer *et al.* (1997) and originally contained only two species. Within the past decade, several new species have been discovered so that the genus currently contains six species: *D. aaronbaueri* Ngo & Ziegler, 2004, *D. hangseesom* Bauer, Sumontha, Grossmann, Pauwels & Vogel, 2004, *D. melanostictus* (Taylor, 1962), *D. siamensis* (Boulenger, 1898), *D. taoi* Botov, Phung, Nguyen, Bauer, Brennan & Ziegler, 2015, and *D. vietnamensis* Das, 2004 (Botov *et al.* 2015; Uetz & Hosek 2015). During a survey in southern Vietnam in 2007 a population of *Dixonius* was discovered in Vinh Cuu Nature Reserve, Dong Nai Province that showed the diagnostic characters of the genus (see Bauer *et al.* 1997), but differed from the known species in morphology. In addition, some specimens of *D. vietnamensis* were collected in Nha Trang, Khanh Hoa Province near the type locality of this species and thus serve as the first molecular vouchers of near-topotypic *D. vietnamensis*. Based on this new morphological and molecular dataset we update the knowledge about the phylogeny of *Dixonius* and describe the population from Vinh Cuu as a new species.

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Material and methods

After anaesthetization with ethylacetate, collected specimens were fixed in 90% ethanol and subsequently transferred into 70% ethanol for permanent storage. Specimens were deposited in the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam National Museum of Nature (VNMN), Hanoi, and the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn: *Dixonius vietnamensis* (Nha Trang, Khanh Hoa Province, coll. May 2015): IEBR R.2016.1 (field number KH-NT 2015.2), IEBR R. 2016.2 (field number KH-NT 2015.4), IEBR R. 2016.3 (field number KH-NT 2015.6), VNMN R.2016.3 (field number KH-NT 2015.7), VNMN R.2016.4 (field number KH-NT 2015.8), ZFMK 97747 (field number KH-NT 2015.1), ZFMK 97748 (field number KH-NT 2015.3), and ZFMK 97749 (field number KH-NT 2015.5); *Dixonius* **sp. nov.** (Vinh Cuu, Dong Nai Province, coll. December 2007): IEBR A.0801, IEBR A.0802, VNMN R.2016.1 (formerly IEBR A.0805), VNMN R.2016.2 (formerly IEBR A.0806), ZFMK 97745 (formerly IEBR A.0803), and ZFMK 97746 (formerly IEBR A.0804). Morphological methods followed Bauer *et al.* (2004) and Ngo & Ziegler (2009). Morphological comparisons were 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). 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 abbreviations of taken morphological measurements and scalation data see Table 1.

TABLE 1. Morphological measurements and scalation with abbreviations.

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
PVtub	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

For molecular analyses, we extracted genomic DNA from tissue of eight *Dixonius* samples (Table 2). Genomic DNA was isolated from ethanol-preserved tissues via Qiagen DNeasy blood and tissue kit. A single large mitochondrial segment including the locus ND2 and succeeding tRNAs (NADH dehydrogenase subunit 2, Trp, Ala, Asx, Cys, Tyr: 1445 bp) were amplified in 25 mL reactions with published primer pairs for ND2 (Table 3). Polymerase chain reaction followed published conditions: denaturation at 95ºC for two minutes, followed by 34 cycles of: denaturation at 95ºC for 35 s, annealing at 48ºC for 35 s, and 1.5 m extension at 72ºC. Amplified products were visualized on 1.5% agarose gels, and successful amplifications were purified using AMPure magnetic bead system (Agencourt Bioscience). Sequencing reactions used ABI Prism BigDyeTerminator (Applied Biosystems), and primer pairs for ND2 (METF1, ND2F17, TRPR3, CO1R1, and product was purified using Agencourt CleanSeq magnetic bead system (Agencourt Bioscience). Sequencing was carried out on an automated ABI 3730 for electrophoresis, and electropherograms were imported into Geneious 7.0 for careful consideration by eye, and identification of heterozygous sites. Sequences were manually aligned in TextWrangler. Final alignments were uploaded to the CIPRES Science Gateway (Miller *et al.*, 2012) for Maximum Likelihood analysis using RAxML 8.0 (Stamatakis, 2006), and run for 1000 bootstrap replicates using a general time reversible (GTR) model with gamma distributed rate variation among sites (Γ).

TABLE 2. *Dixonius* samples used in the molecular analyses, including collection numbers, locality information and GenBank Accession numbers. *CAS—California Academy of Sciences, San Francisco, USA; FMNH—Field Museum of Natural History, Chicago, USA; IEBR—Institute of Ecology and Biological Resources, Vietnam; LSUHC—La Sierra University Herpetology Collections, USA; VNMN—Vietnam National Museum of Nature; VU—Villanoa University, USA; WAM—Western Australian Museum, Australia; ZFMK—Zoological Research Museum Alexander Koenig, Bonn, Germany.

Species	Collection ID*	Country	Locality	GenBank Accession No. ND2
Dixonius aaronbaueri	ZFMK 87274	Vietnam	Nui Chua NP, Ninh Thuan Province	HM997152
Dixonius minhlei **sp. nov.**	ZFMK 97745	Vietnam	Vinh Cuu, Dong Nai Province	KX379194
Dixonius cf. *siamensis*	VU 023	Thailand	Captive	KX379195
Dixonius melanostictus	VU 022	Thailand	Captive	HM997153
Dixonius siamensis	LSUHC 7378	Cambodia	Phnom Aural, Purset Province	KP979732
Dixonius siamensis	LSUHC 7328	Cambodia	Phnom Aural, Purset Province	EU054299
Dixonius sp.	FMNH 263003	Cambodia	Keo Seima District, Mondolkiri Province	EU054298
Dixonius sp.	LSUHC 9466	Thailand	Sai Yok, Kanchanaburi Province	KX379196
Dixonius taoi	CAS 257300	Vietnam	Phú Qúy Island, Binh, Thuan Province	KP979734
Dixonius taoi	ZFMK 96680	Vietnam	Phú Qúy Island, Binh, Thuan Province	KP979733
Dixonius taoi	IEBR A 2014-26	Vietnam	Phú Qúy Island, Binh, Thuan Province	KP979735
Dixonius taoi	IEBR A 2014-27	Vietnam	Phú Qúy Island, Binh, Thuan Province	KP979736
Dixonius vietnamensis	ZFMK 97747	Vietnam	Nha Trang, Khánh Hòa Province	KX379197
Dixonius vietnamensis	IEBR R.20163	Vietnam	Nha Trang, Khánh Hòa Province	KX379198
Dixonius vietnamensis	VNMN R.2016.3	Vietnam	Nha Trang, Khánh Hòa Province	KX379199
Dixonius vietnamensis	VNMN R.2016.4	Vietnam	Nha Trang, Khánh Hòa Province	KX379200
Dixonius cf. *vietnamensis*	ZFMK 87273	Vietnam	Nui Chua, Ninh Thuan Province	KX379201
Heteronotia spelea	WAM R157719	Australia	Packsaddle Range, Western Australia	HQ840102

TABLE 3. Primers used for PCR amplification and sequencing.

Gene	Primer name	Sequence	Primer reference
ND2	MetF1 L4437	5'- AAGCTTTCGGGCCCATACC -3'	Macey *et al.*, 1997
	ND2F17	5'-TGACAAAAAATTGCNCC-3'	Macey *et al.*, 2000
	TRPR3 H5540	5'-TTTAGGGCTTTGAAGGC-3'	Macey *et al.*, 1997
	CO1R1	5'-AGRGTGCCAATGTCTTTGTGRTT-3'	Macey *et al.*, 1997

Results

Based on the morphological examination, the *Dixonius* series from Nha Trang, Khanh Hoa Province could be identified as *D. vietnamensis* (Fig. 1). The population from Nha Trang differed only slightly in a higher number of precloacal pores (5–7 versus 5–6 in the original description, see Das 2004), 13–17 keeled dorsal tubercle rows (versus 16 in Das 2004) and 12–15 lamellae on fourth toe (versus 13 in Das 2004). As the original description comprised only four individuals, these slightly deviating characters in an extended series from near the type locality can be considered being within normal intraspecific variation. Thus, the extended diagnosis for *D. vietnamensis*, based on the data provided in the original description by Das (2004) and the newly collected series from Nha Trang is as follows: A medium-sized species of *Dixonius* (SVL to 42.4 mm), diagnosable from congeneric species in showing the following combination of characters: head wider than long in adults; two supranasals in contact narrowly; dark canthal stripe present, but terminating at back of head; lips unbarred; and dorsal surface of body and tail brownish olive, with irregular dark blotches or reticulation; blotches tend to form dark transversal bands on the occiput. 13–17 keeled dorsal tubercle rows, 15–21 ventral scale rows, 5–7 precloacal pores in males and 12–15 lamellae on fourth toe (for measurements and scalation, see Table 4). A female individual from Nui Chua, Ninh Thuan Province, mentioned as *Dixonius* cf. *vietnamensis* (ZFMK 87273) in Ngo & Ziegler (2009) was not considered for the extended diagnosis, as it showed somewhat deviating scalation characters (see Table 4, and discussion).

Based on the new *Dixonius* collection from Nha Trang, we were able for the first time to include near-topotypic, "true" *D. vietnamensis* in a phylogenetic tree, as well as the afore mentioned conspecific, ZFMK 87273 from Ninh Thuan Province (see Ngo & Ziegler 2009). Molecular divergence as estimated by pairwise distances between these localities is estimated at 5% (see Fig. 2). Based on that together with the somewhat deviating scalation of the individual from Nui Chua, we followed Ngo & Ziegler (2009) and continue to name it as *D.* cf. *vietnamensis* in Fig. 2. The first verified, near-topotypic molecular sample of *D. vietnamensis* also revealed the recently described *D. taoi* not to represent the sister taxon of *D. melanostictus*, as was published by Botov *et al.* (2015), but instead forming a clade with *D. vietnamensis*. The new molecular dataset also includes five of six currently recognized *Dixonius* species and revealed the existence of at least three unnamed *Dixonius* (Fig. 2, Tab. 5). Molecular data suggest a divergence of >12% between the sample from Vinh Cuu and its closest congener FMNH 263003 from Keo Seima, Cambodia. Based on this molecular result we describe the *Dixonius* population from Vinh Cuu as a new species in the following:

Dixonius minhlei sp. nov.

Holotype (Figs. 3–5): IEBR A.0802 (male) collected between 28th and 29th of December 2007 by Tao Thien Nguyen and Cuc Thu Ho in Vinh Cuu Nature Reserve, Dong Nai Province, Southern Vietnam (11°22'40"N, 107°03'37"E, 70 m a.s.l. elevation).

Paratypes (Figs. 5–6): IEBR A.0801 (female), VNMN R.2016.1 (female), VNMN R.2016.2 (female), ZFMK 97745 (female), and ZFMK 97746 (male), same data as for the holotype.

Diagnosis. A small gecko with up to 47.5 mm SVL; 7–9 supralabials; 14–15 rows of keeled tubercles on dorsum; 20–23 ventral scale rows; 7 or 8 precloacal pores in males; a canthal stripe running from rostrum through the eye and terminating at back of head; lateral second pair of postmentals maximum one quarter the size of first pair; dorsum olive gray with more or less discernible brownish olive blotches.

TABLE 4. Measurements (mm) and scalation of near-topotypic *Dixonius vietnamensis* from Nha Trang, opposite to the *D.* cf. *vietnamensis* female ZFMK 87273 from Nui Chua (Ngo & Ziegler 2004); minimum and maximum measurements do only refer to adult individuals.

	IEBR R.2016.3	VNMN R.2016.3	ZFMK 97747	IEBR R.2016.1	VNMN R.2016.4	ZFMK 97748	IEBR R.2016.4	ZFMK 97749	Min	Max	ZFMK 87273
Sex	male	male	male juvenile	female	female	female	female juvenile	female juvenile			female
SVL	39.0	39.6	34.1	43.5	43.7	45.2	31.2	29.2	39.0	43.7	42
TL	partly regenerated	48.1	43.9	-	broken	-	38.5	30.7		48.1	49
TW	2.2	3.8	3.4	-	-	-	2.6	1.9	1.9	3.8	3.4
BW	6.5	7.8	4.7	7.6	8.6	10.3	5.2	4.8	4.7	10.3	8.3
HL	6.9	7.2	6.2	7.6	7.7	8.5	5.7	5.2	6.2	8.5	6.9
HW	6.8	7.0	5.7	6.9	7.7	8.2	5.8	4.8	5.7	8.2	6.5
HD	4.2	4.7	4.1	4.7	4.7	5.7	3.7	3.1	4.1	5.7	4.4
EL	1.1	0.8	0.9	1.0	1.1	1.3	0.9	0.9	0.8	1.3	1.2
ED	2.8	2.5	2.3	2.7	2.8	2.9	2.4	2.5	2.3	2.9	2.9
EN	2.9	3.4	2.7	3.1	3.4	3.8	2.4	2.1	2.7	3.8	3.2
ES	3.9	4.6	3.6	4.5	4.7	5.4	3.4	2.9	3.6	5.4	4.0
EE	3.1	3.3	2.5	2.7	3.6	4.3	2.4	2.3	2.5	4.3	3.0
IN	1.1	1.3	1.2	1.3	1.3	1.5	1.0	1.0	1.1	1.3	1.2
IO	1.6	1.7	1.3	1.6	1.8	2.7	1.2	1.2	1.3	2.7	1.5
FA r	4.7	5.2	4.2	5.0	5.5	5.5	3.1	3.1	4.7	5.5	4.7
TBL r	6.5	6.5	5.9	6.2	6.3	6.6	4.8	4.9	6.2	6.6	5.6
AG r	14.8	16.6	12.3	19.2	18.2	19.2	11.9	11.1	14.8	19.2	17.2
EL/ED	0.39	0.32	0.39	0.37	0.39	0.44	0.38	0.36	0.32	0.44	0.35
SPL r/l	8/8	7/8	8/7	7/7	8/7	8/7	8/8	7/7	7	8	7
IFL r/l	6/6	6/6	6/6	6/6	7/7	6/6	7/7	6/7	6	7	6
MO	6/5	5/6	6/5	6/5	6/6	6/6	6/6	6/5	5	6	5/6
IOS	10	9	10	8	9	8	7	8	7	10	7
ICS	25	23	24	22	25	23	25	26	22	26	29
V	19	19	15	18	20	20	21	19	15	21	20
DTR	15	15	13	16	15	17	16	17	13	17	14
T4 r/l	12/15	14/13	14/13	14/13	13/13	14/14	13/12	13/14	12	15	17/16
PP	6	5	7	-	-	-	-	-	5	7	-
canthal stripe	+	+	+	+	+	+	+	+			+

FIGURE 1. Newly collected specimens of *Dixonius vietnamensis* from Nha Trang, Khanh Hoa Province, Vietnam: VNMN R.2016.4 (top), and VNMN R.2016.3 (bottom). Photos: D. T. Do.

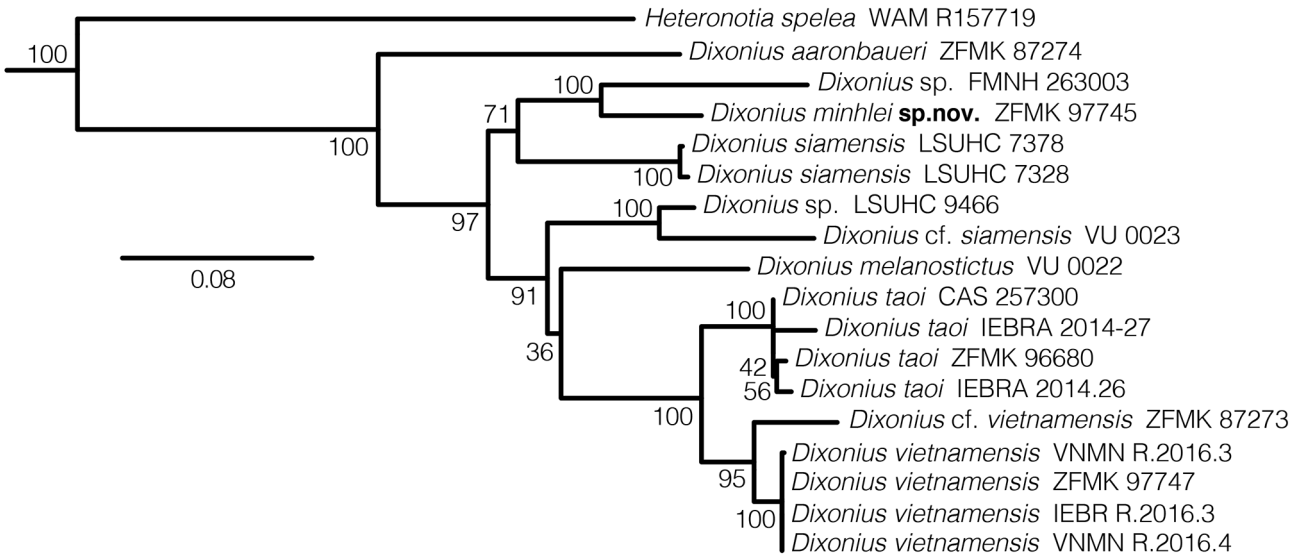

FIGURE 2. Maximum likelihood tree illustrating the relationship of *Dixonius minhlei* **sp. nov.**, and the first verified near-topotypic samples of *D. vietnamensis*, to their congeners. Values at nodes indicate ML boostrap support. Phylogenetic tree also includes the recently described *D. taoi* (Botov *et al.*, 2015), and three additional, currently undescribed taxa.

TABLE 5. Pairwise Distance Matrix.

Taxon		A																
Dixonius aaronbaueri ZFMK 87274	A	0	B															
Dixonius cf. *siamensis* VU 0023	B	0.1588	0.0000	C														
Dixonius cf. *vietnamensis* ZFMK 87273	C	0.1719	0.1331	0.0000	D													
Dixonius melanostictus VU 0022	D	0.1583	0.1066	0.1291	0.0000	E												
Dixonius siamensis LLG 7328	E	0.1617	0.1271	0.1306	0.1274	0.0000	F											
Dixonius siamensis LLG 7378	F	0.1603	0.1263	0.1306	0.1274	0.0042	0.0000	G										
Dixonius vietnamensis ZFMK 97747	G	0.1712	0.1304	0.0521	0.1237	0.1203	0.1194	0.0000	H									
Dixonius vietnamensis IEBR R.2016.3	H	0.1713	0.1305	0.0522	0.1238	0.1204	0.1195	0.0000	0.0000	I								
Dixonius vietnamensis VNMN R.2016.3	I	0.1722	0.1296	0.0530	0.1247	0.1212	0.1204	0.0008	0.0008	0.0000	J							
Dixonius vietnamensis VNMN R.2016.4	J	0.1713	0.1305	0.0522	0.1238	0.1204	0.1195	0.0000	0.0000	0.0008	0.0000	K						
Dixonius taoi CAS 257300	K	0.1616	0.1240	0.0797	0.1184	0.1215	0.1208	0.0546	0.0547	0.0555	0.0547	0.0000	L					
Dixonius taoi IEBRA 2014 26	L	0.1664	0.1281	0.0867	0.1222	0.1267	0.1260	0.0622	0.0622	0.0631	0.0622	0.0065	0.0000	M				
Dixonius taoi IEBRA 2014 27	M	0.1592	0.1393	0.1039	0.1201	0.1433	0.1417	0.0573	0.0574	0.0574	0.0574	0.0145	0.0162	0.0000	N			
Dixonius taoi ZFMK 96680	N	0.1637	0.1280	0.0829	0.1200	0.1230	0.1223	0.0588	0.0589	0.0597	0.0589	0.0050	0.0093	0.0145	0.0000	O		
Dixonius sp. FMNH 263003	O	0.2064	0.1737	0.1912	0.1754	0.1583	0.1583	0.1785	0.1786	0.1794	0.1786	0.1712	0.1759	0.1223	0.1725	0.0000	P	
Dixonius minhlei **sp. nov.** ZFMK 97745	P	0.1601	0.1205	0.1538	0.1288	0.1078	0.1078	0.1371	0.1372	0.1380	0.1372	0.1323	0.1374	0.1254	0.1345	0.1284	0.0000	Q
Dixonius sp. LSUHC 9466	Q	0.1244	0.0446	0.0994	0.0801	0.0884	0.0877	0.0816	0.0816	0.0808	0.0816	0.0825	0.0850	0.1142	0.0843	0.1316	0.0826	0

Description of the holotype. Adult male, snout-vent length 43.9 mm, body dorsolaterally flattened, tail length 53.2 mm (tail regenerated), trunk length 18.7 mm. Head almost as long as wide, depressed, distinct from slender neck. Head length 7.3 mm, head width 7.7 mm, eye of moderate size 2.7 mm, ear opening oblique (EL 1.5 mm), naris-eye length 3.7 mm, snout-eye length 5.0 mm, internarial distance 1.6 mm.

Rostral very large, wider (1.8 mm) than high (1.2 mm), with a distinct suture; supralabials 8 (sixth in midorbital position), smaller in subocular rictus; nostril in contact with rostral, first supralabial, supranasal, and two nasals posteriorly on each side; supranasals in contact anteriorly; internasals absent; snout flat, covered with granular scales; pupil vertical; ear opening oblique, oval, approximately one half of the eye diameter, without bordering enlarged scales; mental triangular, wider (2.2 mm) than high (1.7 mm); infralabials 6 on both sides, decreasing gradually in size; mental triangular, wider (2.2 mm) than high (1.7 mm); two pairs of enlarged postmentals, first pair very large and in narrow contact, second pair about less than one quarter the size of first, in contact with first and second infralabials and separated from each other by four gular scales; 12 keeled dorsal scale rows at midbody 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 22 rows at midbody, with distinct posterior serration; dorsal surface of fore- and hindlimbs covered with shallowly-keeled scales, area around limb insertions covered with small granular scales; 14 lamellae on fourth toe; no femoral pores; 7 precloacal pores in an angular series; pore bearing scales not enlarged; no precloacal depression; caudal scales keeled and covered with scales of different sizes, like on dorsum; 61 enlarged and undivided subcaudals on regenerated tail.

Dorsum olive gray in life, with round brownish olive blotches. A stripe of the same colour running from rostrum through eyes and ending at back of head. Dorsum of head with blotches of same colour. Dorsum and dorsal part of original tail covered with brownish olive blotches. Two specimens with a very pale, almost indiscernible

pattern. Venter cream and regenerated tail without blotches. In preservative, dorsum light brown with dark grey-brown blotches on head and body; colouration of tail and on dorsal surface of fore- and hindlimbs similar, but with less contrast.

FIGURE 3. Holotype of *Dixonius minhlei* **sp. nov.** (IEBR A.0802) from Vinh Cuu, Dong Nai Province in preservative. Photo: T. Ziegler.

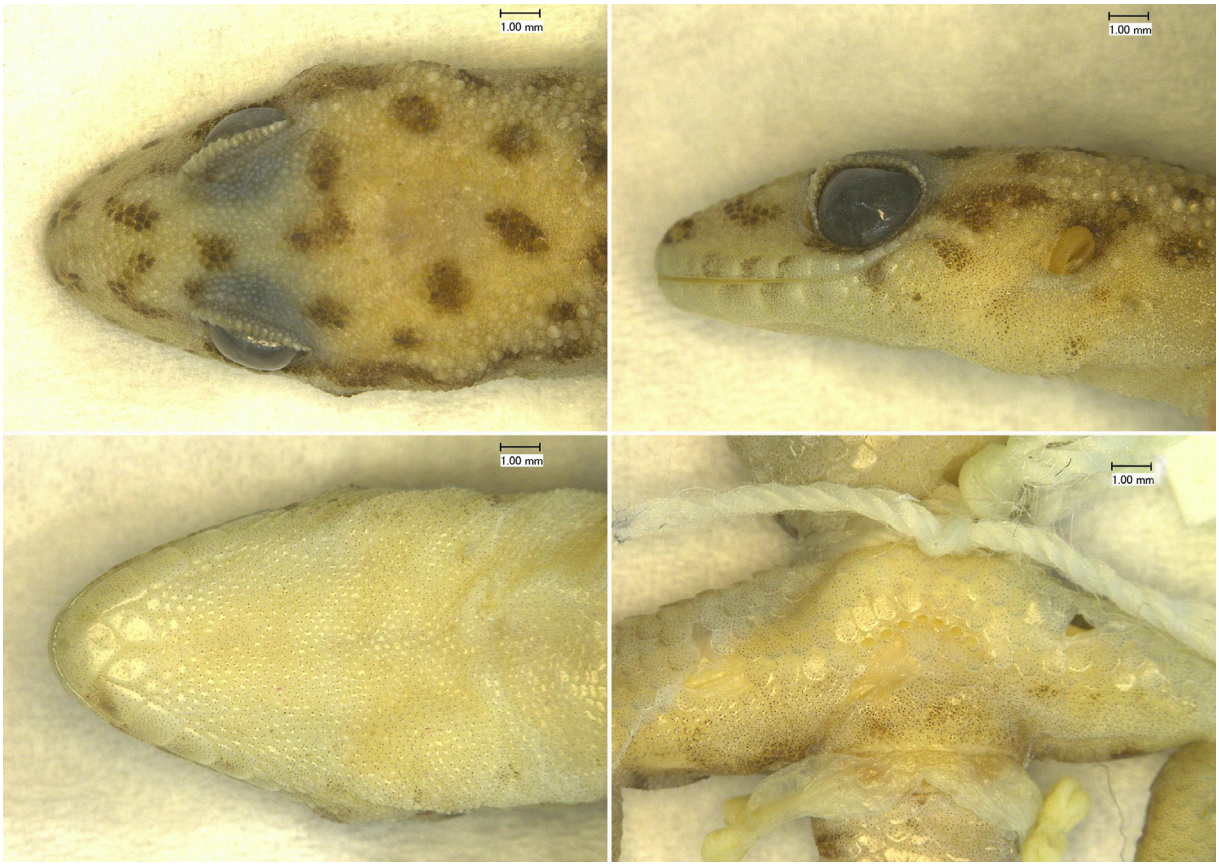

FIGURE 4. Different head views and cloacal region of the holotype of *Dixonius minhlei* **sp. nov.** (IEBR A.0802) from Vinh Cuu, Dong Nai Province in preservative. Photos: A. Botov.

TABLE 6. Measurements (mm) and scalation of the type series of *Dixonius minhlei* **sp. nov.**

Character	IEBR A.0802 (holotype)	ZFMK 97746 (paratype)	IEBR A.0801 (paratype)	ZFMK 97745 (paratype)	VNMN R.2016.1 (paratype)	VNMN R.2016.2 (paratype)	Min	Max
Sex	male	male	female	female	female	female		
SVL	43.9	40.6	45.9	47.5	43.3	46.7	40.6	47.5
TL	53.2	broken	52.4	51.0	regenerated	regenerated	51.0	53.2
TW	4.1	4.2	3.7	4.4	3.8	4.2	3.7	4.4
BW	9.4	8.5	9.7	9.6	9.3	9.2	8.5	9.7
HL	7.3	6.7	7.2	7.6	7.1	7.7	6.7	7.7
HW	7.7	6.0	6.6	6.8	6.5	6.2	6.0	7.7
HD	4.7	4.3	5.2	4.7	4.4	4.6	4.3	5.2
EL	1.5	1.3	1.2	1.5	1.3	1.2	1.2	1.5
ED	2.7	2.2	3.3	3.1	2.5	3.1	2.2	3.3
EN	3.7	3.2	3.4	3.5	3.5	3.8	3.2	3.8
ES	5.0	4.4	4.4	4.9	4.6	5.2	4.4	5.2
EE	3.5	3.6	3.4	3.9	3.8	3.6	3.4	3.9
IN	1.6	1.3	1.5	1.5	1.5	1.5	1.3	1.6
IO	4.0	3.5	3.7	3.7	3.8	3.4	3.4	4.0
FA	6.2	6.7	5.9	6.0	6.1	6.6	5.9	6.7
TBL	7.7	7.0	7.2	7.3	7.5	7.0	7.0	7.7
AG	18.7	18.2	21.2	21.5	20.6	20.3	18.2	21.2
AG/SVL	0.43	0.45	0.46	0.45	0.48	0.43	0.43	0.48
EL/ED	0.56	0.59	0.36	0.48	0.52	0,39	0.36	0.59
SPL	8/8	8/8	8/9	7/8	8/8	8/8	7	9
IFL	6/6	7/6	7/7	6/6	6/6	6/7	6	7
MO	6/6	6/6	6/6	5/6	5/6	6/6	5	6
IOS	10	10	10	10	8	7	7	10
ICS	28	28	26	29	25	27	25	29
V	22	23	22	23	23	20	20	23
DTR	14	14	15	15	14	15	14	15
PVtub	42	44	44	42	44	38	38	44
PV'tub	23	26	26	24	26	23	23	26
T4 r/l	14	15/14	12	13	15	13	13	15
PP	7	8	0	0	0	0	7	8
Canthal stripe	+	+	+	+	+	+		

Variation of paratypes: Blotches on head, dorsum and tail may appear very pale or almost absent in some individuals (Figs. 5–6). Variation in scalation is shown in Table 6.

Comparisons. *Dixonius minhlei* **sp. nov.** differs from all known *Dixonius* as follows: from *D. aaronbaueri* by having more ventral scale rows at midbody (20–23 *versus* 18–19 in *D. aaronbaueri*); more dorsal tubercle rows (14–15 *versus* 11 in *D. aaronbaueri*), more precloacal pores in males (7–8 *versus* 5 in *D. aaronbaueri*) and different color pattern (olive gray ground color on dorsum, with more or less discernible brownish olive blotches *versus* an unpatterned dorsum in *D. aaronbaueri*); from *D. hangseesom* by having fewer ventral scale rows at midbody (20–23 vs 22–26 in *D. hangseesom*); more dorsal tubercle rows (14–15 *versus* 12–14 in *D. hangseesom*) and coloration of tail same as the dorsum (*versus* orange tail in *D. hangseesom*); from *D. melanostictus* by having

more dorsal tubercle rows (14–15 *versus* 10–11 in *D. melanostictus*); fewer precloacal pores (7–8 *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 smaller size (47.5 mm max. SVL *versus* 57 mm in *D. siamensis*), more precloacal pores (7–8 *versus* 6–7 in *D. siamensis*) and in having a distinct canthal stripe (*versus* absence of canthal stripe in *D. siamensis*); from *D. taoi* by having more precloacal pores (7–8 *versus* 5–6 in *D. taoi*) and different dorsal pattern (dorsum olive gray with more or less discernible round brownish olive blotches *versus* presence of one or two irregular rows of yellowish marks running from head along flanks in *D. taoi*); from *D. vietnamensis* by having more precloacal pores (7–8 versus 5–7 in *D. vietnamensis*) and in dorsal head and body pattern (blotches on head and dorsum round, more or less discernible vs. dark transversal bands on the occiput and irregular blotches or reticulation on dorsum in *D. vietnamensis*).

FIGURE 5. Type series of *Dixonius minhlei* **sp. nov.** from Vinh Cuu, Dong Nai Province; from left to right: IEBR A.0801, IEBR A.0802 (holotype), ZFMK 97745, ZFMK 97746, VNMN R.2016.1, and VNMN R.2016.2. Photo: A. Botov.

Etymology. The new species is named after our friend and colleague Dr. Minh D. Le from the Faculty of Environmental Sciences, Hanoi University of Science, Vietnam National University, Hanoi, for his continuous and significant contributions towards a better understanding of the diversity and phylogenetic relationships of species from Vietnam and surrounding countries.

Distribution. *Dixonius minhlei* **sp. nov.** currently is only known from the type locality (Fig. 7).

Natural history. The type series of *Dixonius minhlei* **sp. nov.** was collected at night, between 19:00 and 23:00, on the ground of the evergreen forest (Fig. 8). Further reptile species observed at the type locality were *Acanthosaura lepidogaster, Calotes emma,* and *Cyrtodactylus cattienensis.*

Discussion

In the molecular phylogeny provided in the description of *Dixonius taoi* by Botov *et al.* (2015), the latter species was presented as sister taxon to *D. melanostictus*, and *D. vietnamensis* was sister to *D. siamensis*. The *D. vietnamensis* sample (FMNH 263003) used in the phylogeny by Botov *et al.* (2015) is derived from a population

FIGURE 6. Paratypes of *Dixonius minhlei* **sp. nov.** from Vinh Cuu, Dong Nai Province, in life: ZFMK 97745 (top), and VNMN R.2016.1 (bottom). Photos: T. T. Nguyen.

from Keo Seima, Mondolkiri Province, Cambodia, that was discovered 13 years ago and identified at that time as *D. vietnamensis* (Stuart *et al.* 2006). However, since we now have for the first time molecular reference for *D. vietnamensis* from Nha Trang from near the type locality it becomes obvious that the population from Keo Seima was incorrectly identified as *D. vietnamensis*. The taxon from Keo Seima, the status of which is currently unresolved, is instead revealed to be sister of the species described herein. Our phylogeny further shows that near-topotypic *D. vietnamensis* in fact is sister to the recently described *D. taoi*. These species differ by 5.5–5.9% genetic divergence and by different morphological characters such as number of ventral scale rows at midbody, enlarged dorsal scale rows at midbody, and colour pattern (see Botov *et al.* 2015). As there currently is only one female *Dixonius* specimen from Nui Chua, Ninh Thuan available, that differs from near-topotypic *D. vietnamensis* by deviating scalation characters, such as 17/16 subdigital lamellae below the fourth toe (versus 12–15 in the extended description of *D. vietnamensis*) or 29 interciliary scales (versus 22–26 in the extended description of *D. vietnamensis*), together with 5% molecular divergence, we followed Ngo & Ziegler (2004) in treating it as *D.* cf. *vietnamensis* as a precaution. Only the study of a larger series from Nui Chua, in particular including so far missing male individuals, will help to explain whether the morphological and molecular divergences can be explained by intraspecific variation or whether that points to a separate taxon. Our molecular phylogeny also points to another *Dixonius* population, from Sai Yok, Kanchanaburi Province, Thailand (LSUHC 9466), the status of which is currently unresolved. In addition, *D. siamensis* samples from Cambodia (Phnom Aural, Purset Province, LSUHC 7328, 7378) and Thailand (VU 0023) used herein are also highly divergent from named taxa (>12%) and appear to represent undescribed species. Thus, the recent *Dixonius* species descriptions together with the data presented in this paper and as yet unpublished data of our working group show that *Dixonius*, in particular the *D. siamensis* and *D. vietnamensis* species groups, obviously consist of a complex of cryptic species. Further field work together with an integrative taxonomic approach combining morphological and molecular datasets will be required to resolve the taxonomy and phylogeny of this so far poorly understood gecko genus.

FIGURE 7. Map showing the type locality of *Dixonius minhlei* **sp. nov.** (red star) and further localities mentioned in the text: Nha Trang, Khanh Hoa Province, Vietnam; Nui Chua, Ninh Thuan Province, Vietnam; Phu Quy, Binh Thuan Province, Vietnam; Keo Seima, Mondolkiri Province, Cambodia; Phnom Aural, Purset Province, Cambodia.

FIGURE 8. Habitat of *Dixonius minhlei* **sp. nov.** in the evergreen forest.

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