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On the millipede genus *Antheromorpha* Jeekel, 1968 (Diplopoda: Polydesmida: Paradoxosomatidae) from Vietnam, with a description of a new species

Anh D. Nguyen[1,2*], Son G. Nguyen[2], Son X. Le[3]

Abstract. The genus *Antheromorpha* Jeekel, 1968 is reviewed for the Vietnam fauna. A total of four species have been recorded in Vietnam, including a new species, *A. pumatensis*, new species from Nghe An Province. Two other species, *A. paviei* (Brölemann, 1896) and *A. festiva* (Brölemann, 1896) are recorded for the first time in Vietnam. The phylogenetic relationship between *Antheromorpha* species and several orthomorphinine members is analysed based on fragments of the Cytochrome c oxidase subunit I (COI) and 16S rRNA mitochondrial genes. *Antheromorpha* is closely related to the two genera *Orthomorpha* and *Nesorthomorpha*. An identification key to species is also provided.

Key words. Diplopoda, millipede, Paradoxosomatidae, *Antheromorpha*, new species, molecular phylogeny, Vietnam

INTRODUCTION

The name *Antheromorpha* was proposed to replace *Brachytropis* Silvestri, 1896 by Jeekel (1968). The genus consisted of 12−13 species (Jeekel, 1968; Nguyen & Sierwald, 2013). Recently, the genus was revised to comprise only 11 species distributed widely in mainland Southeast Asia, from southern China down to Malaysia, and from Myanmar to Vietnam (Likhitrakarn et al., 2016). The genus is large in body size and has remarkably bright colour patterns. It can also be recognised by the following combination of characters: paraterga well developed; gonopod femorite long and slender, laterally demarcated from postfemoral region by a distinct sulcus; solenophore sheathing almost half of flagelliform solenomere; tip of gonopod strongly divided into two long lobes.

In Vietnam, only one species, *Antheromorpha harpaga* (Attems, 1937), was described from the Hon Ba Mountains in southcentral Vietnam. Continuing our research on millipedes of Vietnam, this paper reviews the genus *Antheromorpha* Jeekel, 1968 from Vietnam, with a description of a new species.

[1]Duy Tan University, 254, Nguyen Van Linh, Da Nang city, Vietnam; Email: ducanh410@yahoo.com; ndanh@iebr.vast.vn (*corresponding author)

[2]Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18, Hoangquocviet Rd., Caugiay, Hanoi, Vietnam

[3]Institute of Tropical Ecology, Vietnamese-Russian Tropical Center, 2, Nguyen Van Huyen, Cau Giay, Hanoi, Vietnam

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MATERIALS AND METHODS

Material was collected from various parts of Vietnam. Specimens were examined under a Olympus SZX10 microscope. Line drawings were made with a drawing tube attached to the microscope. SEM images were taken with an ABT 32 (Topcon Technohouse Co. Ltd) or Hitachi T3300. Colour images were taken using a DinoLite Microscope Camera, and assembled using Adobe Photoshop CS6. The map was created using the software DivaGis ver.7.5.

All material is kept in the millipede collection at the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam. All terminology follows Likhitrakarn et al. (2016).

Total genomic DNA was extracted from head and leg tissue using the DNAeasy Blood & Tissue Kit (Qiagen TM). Primer sets 16S-1F19 (5′- CCG GTT TGA ACT CAG ATCA-3′), 16S-1R20 (5′-TGA CTG TTT AGC AAA GAC AT-3′), COI-1F20 (5′-ACT CTA CTA ATC ATA AGG AT-3′), and COI-1R19 (5′-TAA ACC TCC GGG TGA CCAA-3′) were used to amplify a 520 bp fragment of the mitochondrial 16S rRNA gene and 680 bp fragment of the Cytochrome c oxidase subunit I (COI) gene, respectively. PCR (Polymerase Chain Reaction) protocols for amplification follow Nguyen et al. (2017). PCR products were screened for potentially successful amplification of 16S rRNA or COI fragments through electrophoresis in 1% Agarose - TBE 1X. After the electrophoresis process, successful PCR products were purified using an ExosapIT or QIAquick PCR Purification Kit (Qiagen Inc.), then sent for sequencing at Solgen, Inc (Korea) on an Applied Biosystems automatic sequencer (ABI3130 XL) using the same primer sets used for the initial PCR.

Phylogenetic trees were constructed using both maximum likelihood (ML) and Bayesian inference (BI) models for each

Table 1. Species vouchers and accession numbers deposited in Genbank.

No.	Species	Locality	Voucher	Accession number	
				16S rRNA	COI
1	*Antheromorpha festiva* (Brölemann, 1916)	Yon Don National Park (= NP), Dak Lak Prov.	IEBR-519	KX755577	MG669361
2	*Oxidus gigas* (Attems, 1953)	Sapa, Lao Cai Prov.	IEBR-133	KX755581	MG669363
3	*Oxidus riukiaria* (Verhoeff, 1940)	Okinawa Isls., Japan	IEBR-500J	KX755583	MG669365
4	*Tylopus hilaroides* Golovatch, 1984	Cuc Phuong NP, Ninh Binh Prov.	IEBR-198	KX755588	MG669368
5	*Tylopus roseiparaterga* Nguyen, 2012	Tam Dao NP, Vinh Phuc Prov.	IEBR-185A	KX755590	MG669362
6	*Orthomorpha scabra* Jeekel, 1964	Bi Duop NP, Lam Dong Prov.	IEBR-Myr 432	MG564334	MG669360
7	*Orthomorpha glandulosa* (Attems, 1937)	Quang Nam, Phuoc My	IEBR-Myr 237	MG564333	MG669359
8	*Orthomorpha arboricola* (Attems, 1937)	Ngoc Linh Mts., Kon Tum Prov.	IEBR-Myr 455	MG564332	–
9	*Orthomorphoides setosus* (Attems, 1937)	Bi Duop NP, Lam Dong Prov.	IEBR-Myr 523	MG564335	MG669367
10	*Nesorthomorpha* sp.	Ngoc Linh Mts., Kon Tum Prov.	IEBR-Myr 453	MG564338	–
11	*Nesorthomorpha* sp.	Chu Mon Ray NP, Dak Lak Prov.	IEBR-Myr 561	MG564337	–
12	*Nesorthomorpha* sp.	Chu Mon Ray NP, Dak Lak Prov.	IEBR-Myr 596	MG564339	–
13	*Desmoxytes cervaria* (Attems, 1953)	Sa Pa, Lao Cai Prov.	IEBR-Myr 32	MG564329	MG669370
14	*Desmoxytes proxima* Nguyen et al., 2005	Sa Pa, Lao Cai Prov.	IEBR-Myr 243	MG564331	MG669371
15	*Desmoxytes enghoffi* Nguyen et al., 2005	Phong Nha NP, Quang Binh Prov.	IEBR-IPE 5	MG564330	MG669369
16	*Piccola odontopyga* (Attems, 1953)	Bi Duop NP, Lam Dong Prov.	IEBR-Myr 463	MG564336	MG669366
17	*Antheromorpha pumatensis,* new species	Pu Mat NP, Nghe An Prov.	IEBR-IPE 3	MG669559	MG669372

gene. Maximum likelihood bootstrap analysis was conducted using IQTREE ver. 1.5.5 for Windows (Nguyen et al., 2015) with 1000 replicates. A Bayesian Inference (BI) tree was created using MrBayes ver 3.1.2 (Huelsenbeck & Ronquist, 2001) with 10 million generations, with a heating parameter of 0.06, and sampling every 1,000 generations. All nucleotide sequences were deposited in GenBank. Collection localities, specimen vouchers, and GenBank accession numbers are summarised in Table 1.

TAXONOMY

Class Diplopoda

Order Polydesmida

Family Paradoxosomatidae Daday, 1889

Genus *Antheromorpha* Jeekel, 1968

Brachytropis Silvestri, 1896 (preoccupied): 198; Attems, 1937: 58, treated as a junior synonym of *Orthomorpha*.
Antheromorpha Jeekel, 1968: 57; Likhitrakarn et al., 2016: 23.

Type species. *Orthomorpha miranda* Pocock, 1895, by original designation.

Antheromorpha harpaga (Attems, 1937)
(Figs. 1, 10)

Orthomorpha harpaga Attems, 1937: 77−78, fig. 100; Attems, 1938: 211, figs 32−33; Jeekel, 1963: 269.
Antheromorpha harpaga − Jeekel, 1968: 57; Golovatch, 1983: 181; Enghoff et al., 2004: 33; Likhitrakarn et al., 2016: 49, figs 19−21.

Material examined. 1 male, 1 female (IEBR-Myr 229) Khanh Hoa Prov., Hon Ba Mts. (12°01′45″−12°12′00″N; 108°54′04″–109°05′00″N), bamboo forest, near stream, 800 m a.s.l., 20 April 2003, coll. Anh D. Nguyen.

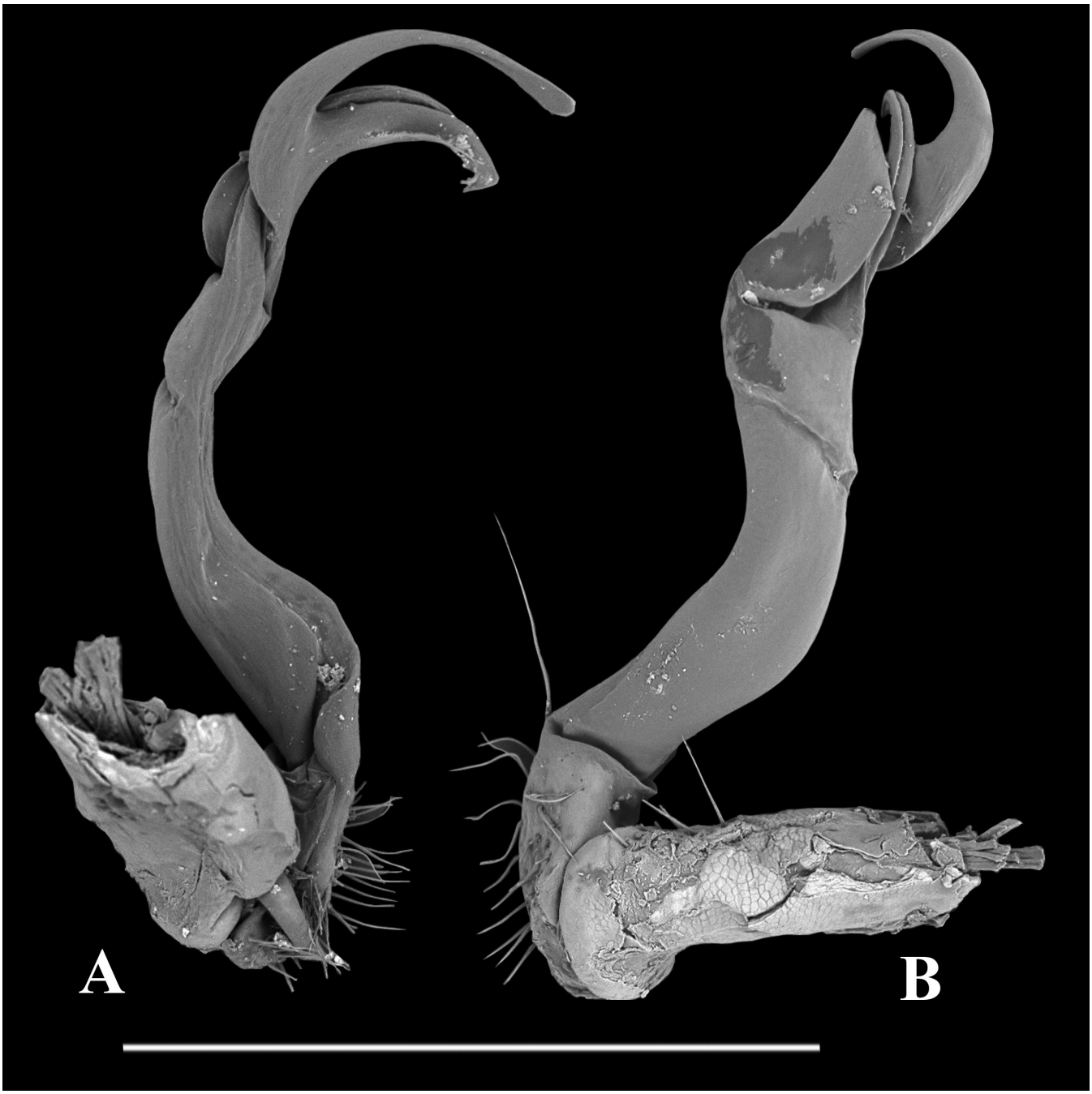

Fig. 1. *Antheromorpha harpaga* (Attems, 1937) from Hon Ba Mts. Right gonopod, subdorsal view (A); lateral view (B). Scale bar = 1 mm.

Diagnosis. This species can be distinguished from its congeners by the following features: metaterga delicately rugose, with two rows of setae (3+3 presulcus and 3+3 postsulcus setae); sternum 5th with a highly elevated, setiferous, bifid trapeziform process between coxae 4; epiproct with two lateroapical, remarkably curved caudoventrally, claw-shaped tubercles; gonopod tip deeply bifid including a long, slender and pointed process (D); the other process with two tiny denticles.

Remarks. This species has only been known from its type locality (Suoi Dau, Khanh Hoa Prov.) (Attems, 1937). No other records have been reported since its description. Recently, Likhitrakarn et al. (2016) re-described this species based on two males preserved in Natural History Museum of Wien (Austria). However, there are no additional specimens except the two specimens referenced in this work. The new record is close to its type locality, about 20 km from Suoi Dau. Since Likhitrakarn et al. (2016: p.19, fig. 19) already provided excellent illustrations, it is not necessary to include additional images for this species.

Antheromorpha festiva (Brölemann, 1896) (Figs. 2, 3, 10)

Orthomorpha festiva Brölemann, 1896: 1; Attems, 1937: 60.
Antheromorpha festiva – Jeekel, 1968: 57; 1980: 85; Golovatch, 1983: 181; Enghoff et al., 2004: 37; Enghoff, 2005: 95; Nguyen & Sierwald, 2013: 1234; Likhitrakarn et al., 2016: 45, figs 16–18, 21.

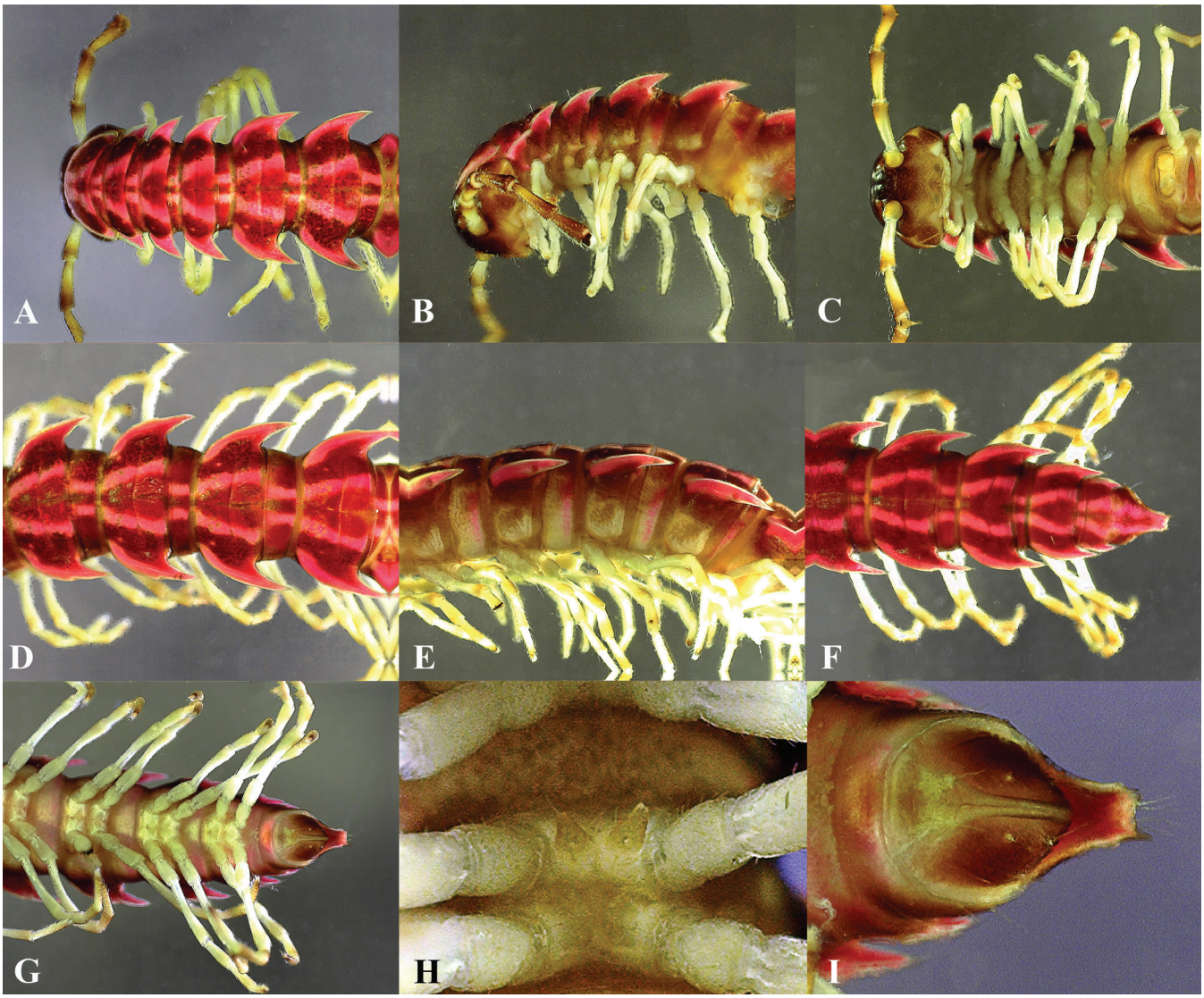

Fig. 2. *Antheromorpha festiva* (Brölemann, 1896) from Lo Go - Xa Mat National Park. Anterior body part, dorsal view (A); lateral view (B); ventral view (C); Midbody part, dorsal view (D); lateral view (E); posterior body part, dorsal view (F); ventral view (G); sternum 5th, ventral view (H); epiproct and hypoproct, ventral view (I). No scale.

Material examined. 4 males, 1 female (IEBR-Myr 25) Kien Giang Prov., U Minh Thuong National Park (9°31–9°39′N; 105°03′–105°07′E); forests, Nov. 2007, coll. Hoang Ngoc Khac; 2 males, 3 females (IEBR-Myr 519) Dak Lak Prov., Yok Don National Park (12°45′–13°10′N; 107°29′30″–107°48′30″E); deciduous forests, on the way to the station No.6, 9 June 2013, coll. Phung T.H.L.; 1 male, 4 females (IEBR-Myr 522) Dak Lak Prov., Yok Don National Park (12°45′–13°10′N; 107°29′30″–107°48′30″E); deciduous forests, on the way to the station No.9, 8 June 2013, coll. Phung T.H.L.; 1 male (IEBR-Myr 661) Tay Ninh Prov., Tan Bien District, Lo Go Xa Mat National Park (11°02′–11°47′N; 105°57′–106°04′E); March 2014, forest, coll. Dang Van An.

Diagnosis. This species is differentiated by its red or red-brown colour, two paramedian red longitudinal stripes on the metaterga; sternum 5th with two independent, sparsely setose, highly elevated tubercles between coxae 4; gonopod tip deeply bifid including a long, slender and pointed process d.

Remarks. Likhitrakarn et al. (2016) stated that the species seems to be widely distributed across the southern half of the Malay Peninsula (southern Thailand and western Malaysia). This species was found at elevations of less than 60 m (Likhitrakarn et al., 2016). However, it seems likely that the species is distributed throughout Indochina, because it is recorded in several localities in southern Vietnam, even at elevations of 800–900 m.

Antheromorpha paviei (Brölemann, 1896) (Figs. 4, 5, 10)

Orthomorpha Paviei Brölemann, 1896: 1.
Pratinus Paviei – Attems, 1937: 122.
Orthomorpha paviei – Jeekel, 1968: 56; Enghoff et al., 2004: 34; Enghoff, 2005: 97; Likhitrakarn et al., 2011: 52; 2014: 2.
Antheromorpha paviei – Likhitrakarn et al., 2016: 30, figs 4–6, 21.

Material examined. 2 males (IEBR-451) Da Nang, Ba Na National Park (15°59′44.4″N; 107°59′39.13″E); forest, 1,200 m a.s.l., July 2009, coll. Truong Xuan Lam.

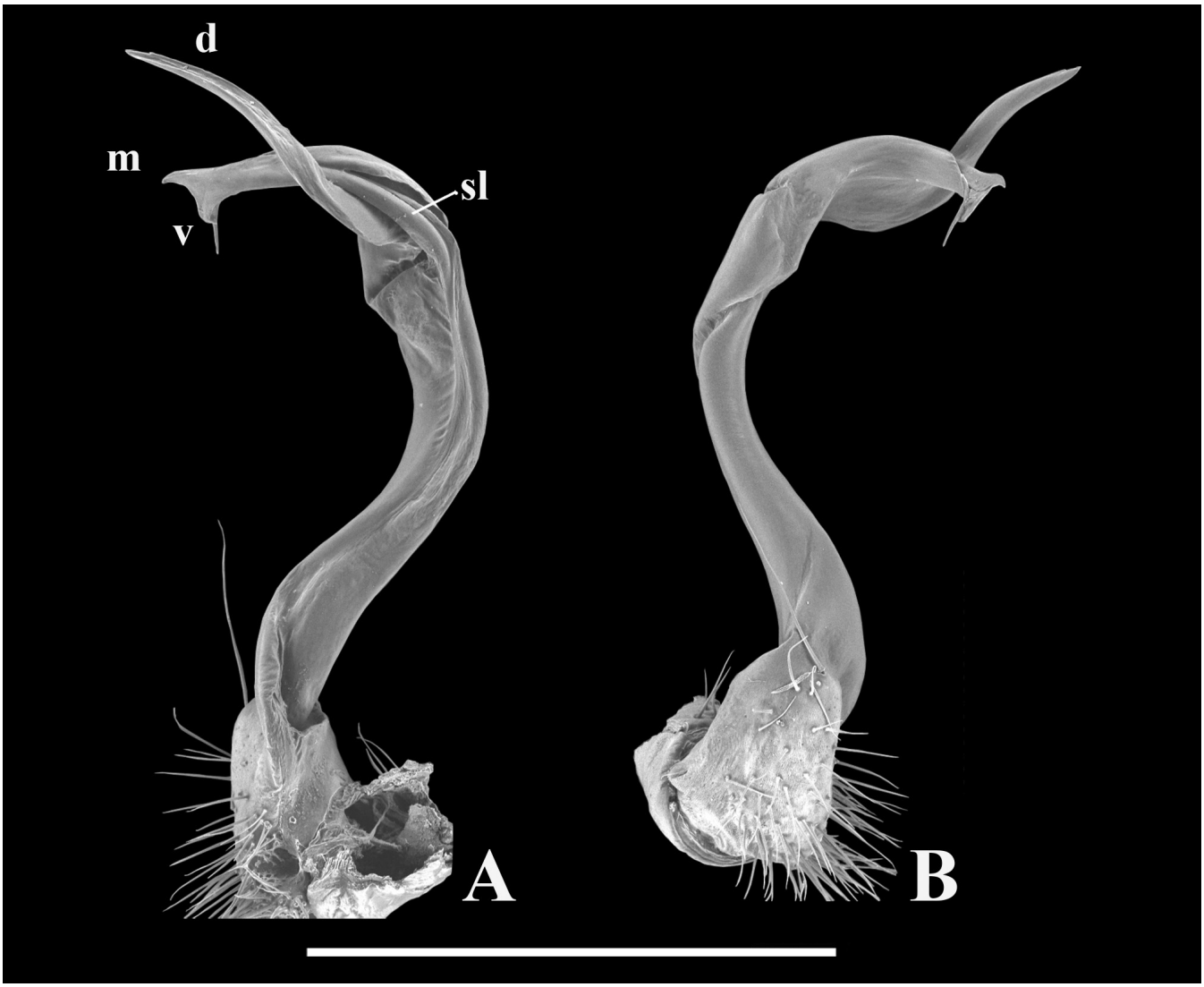

Fig. 3. *Antheromorpha festiva* (Brölemann, 1896) from Lo Go - Xa Mat National Park. Right gonopod, mesal view (A); ventral view (B). Scale bar = 1 mm

Diagnosis. This species can be distinguished from its congener by: metaterga faintly rugose, with two rows of 2+2 and 4+4 setae borne on minute knobs in front of and behind transverse sulcus, respectively; paraterga well developed, set higher than metatergal surface, with long protruding caudal corner. Tip of gonopod strongly divided into two lanceolate lobules.

Remarks. The species *Antheromorpha paviei* (Brölemann, 1896) was described as *Orthomorpha paviei*. It was questionably relocated to the genus *Prionopeltis*, then to the genus *Pratinus* (Attems, 1914, 1937). Jeekel (1963) assigned this species back to the genus *Orthomorpha*, however, he still questioned its generic status. Recently, this species was transferred to the genus *Antheromorpha* due to the deeply split tip of its gonopod.

This species has been reported from Thailand and Laos (Enghoff, 2005; Likhitrakarn et al., 2014, 2016). This is the first time the species is recorded for Vietnam.

Antheromorpha pumatensis, new species (Figs. 6−9, 10)

Material examined. Holotype: 1 male (IEBR-461H) Nghe An Provinve, Pu Mat National Park, Khe Thoi (19°04′47.7″N; 104°38′13.7″E); forest, 4−10 April 2011, coll. Anh. D. Nguyen.

Paratypes: 2 males, 4 females (IEBR-461P) same data as sample IEBR-461H; 1 male, 2 females (IEBR-462), same locality, but Thac Chem (18°58′17.4″N; 104°48′02.9″E); forest, 4−10 April 2011, coll. Anh. D. Nguyen.

Diagnosis. This species differs from its congeners by having: metaterga with a median yellowish brown stripe, paraterga pink, tip of gonopod strongly divided into two processes: one being spiniform, the other being a serrated lobe.

Etymology. Named after the locality where the type specimens were collected.

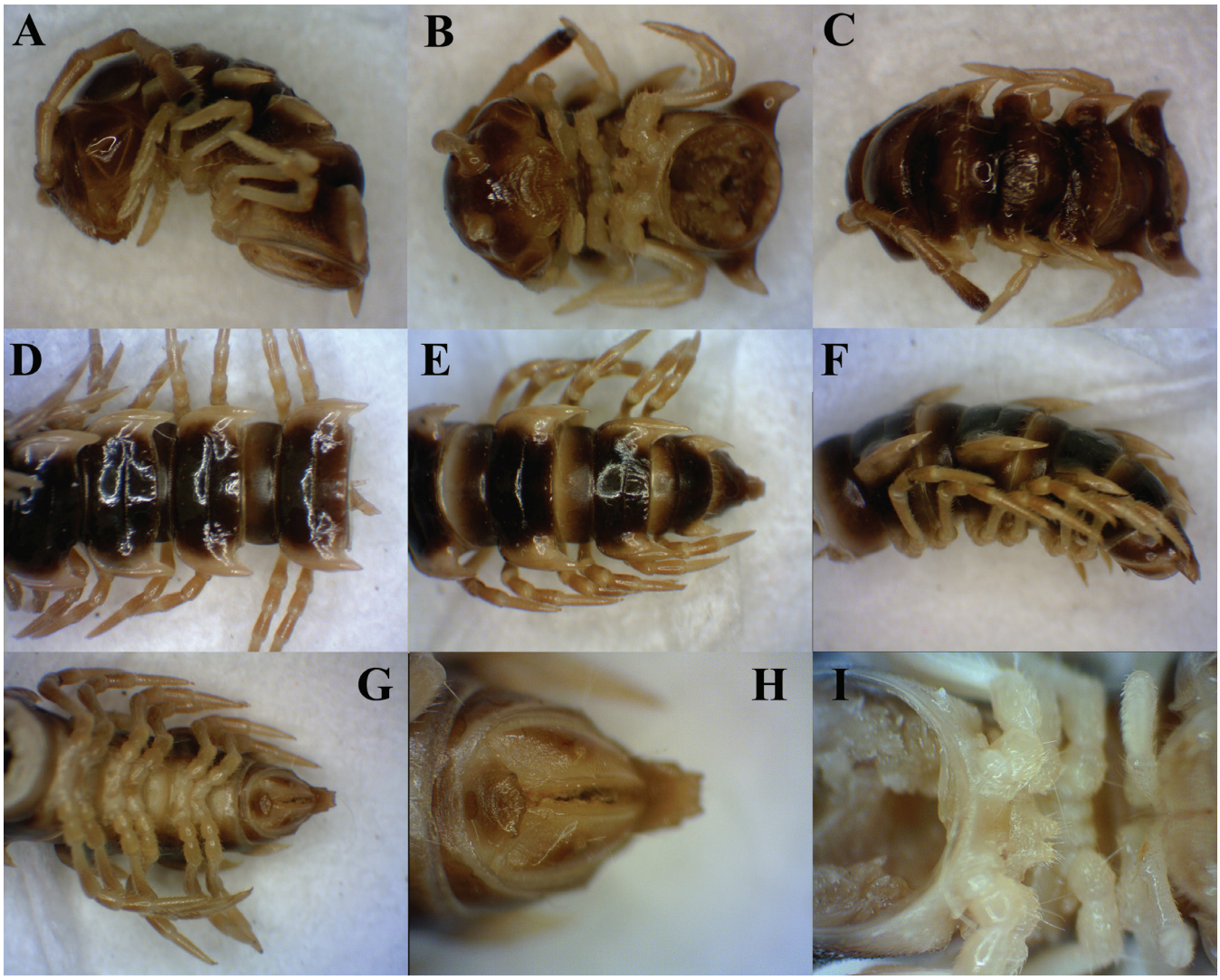

Fig. 4. *Antheromorpha paviei* (Brölemann, 1896) from Da Nang. Anterior part of body, lateral (A); ventral (B) and dorsal view (C); segments 8−10, dorsal view (D); posterior part of body, dorsal (E); lateral (F) and ventral view (G); Hypoproct and Epiproct (H); Sternum 5th, subventral view (I). No scale bar.

Description. Body length about 28.1−37.4 mm (male); 28−36.5 mm (female); width of midbody pro- and metazona 2.3−2.9 mm (male); 2.9−3.4 mm (female) and 3.5−4.1 mm (male); 3.7−4.5 mm (female); respectively. Holotype length ca. 28.1 mm, width of midbody pro- and metazona 2.3 mm and 3.5 mm, respectively.

Prozona and metaterga with a median broad pink stripe and two paramedian castaneous/blackish brown regions (Figs. 6, 7). Paraterga pink or red (both dorsal and ventral sides). Pleura, head, distal part of antennomere 6 and whole antennomere 7 blackish brown. Antennae, sterna, and legs light brown.

Head (Fig. 7A−C) slightly broader than collum; clypeolabral region sparsely setose. Frons weakly convex, divided into 2 parts by a distinct epicranial suture. Antennae (Fig. 7B, C) claviform, reaching metatergum 3 if stretched dorsally. Most antennomeres subequal in length except for the shortest antennomeres 1 & 7.

Collum (Fig. 7A) slightly narrower than body ring 2. Surface smooth and shining. Paratergum modestly developed, ear−shaped or subtriangular with broadly rounded anterior corner. Lateral side without setiferous incision.

Body ring (Fig. 7A−F) 3<4<2=5−16 in width, thereafter gradually tapering towards telson. Prozona shining and smooth. Metaterga shining, almost smooth except lateroposterior area faintly rugose. Transverse sulcus incompletely present on metatergum 4; well present, thin, distinct on metaterga 5−19, but not reaching base of paraterga. Metaterga with traces of rows of 2+2 and 5+5 setae in front of and behind transverse sulcus, respectively. The second row located near posterior margin. Axial line thin, brown, present on all prozona and metaterga.

Pleura considerably rugose, with dense microgranules. Pleurosternal carinae present as a full crest with a small caudal tooth on body rings 2−4; reduced to a small caudal denticle on body rings 5−14 (male); on body rings 5−11 (female); and absent on subsequent body rings.

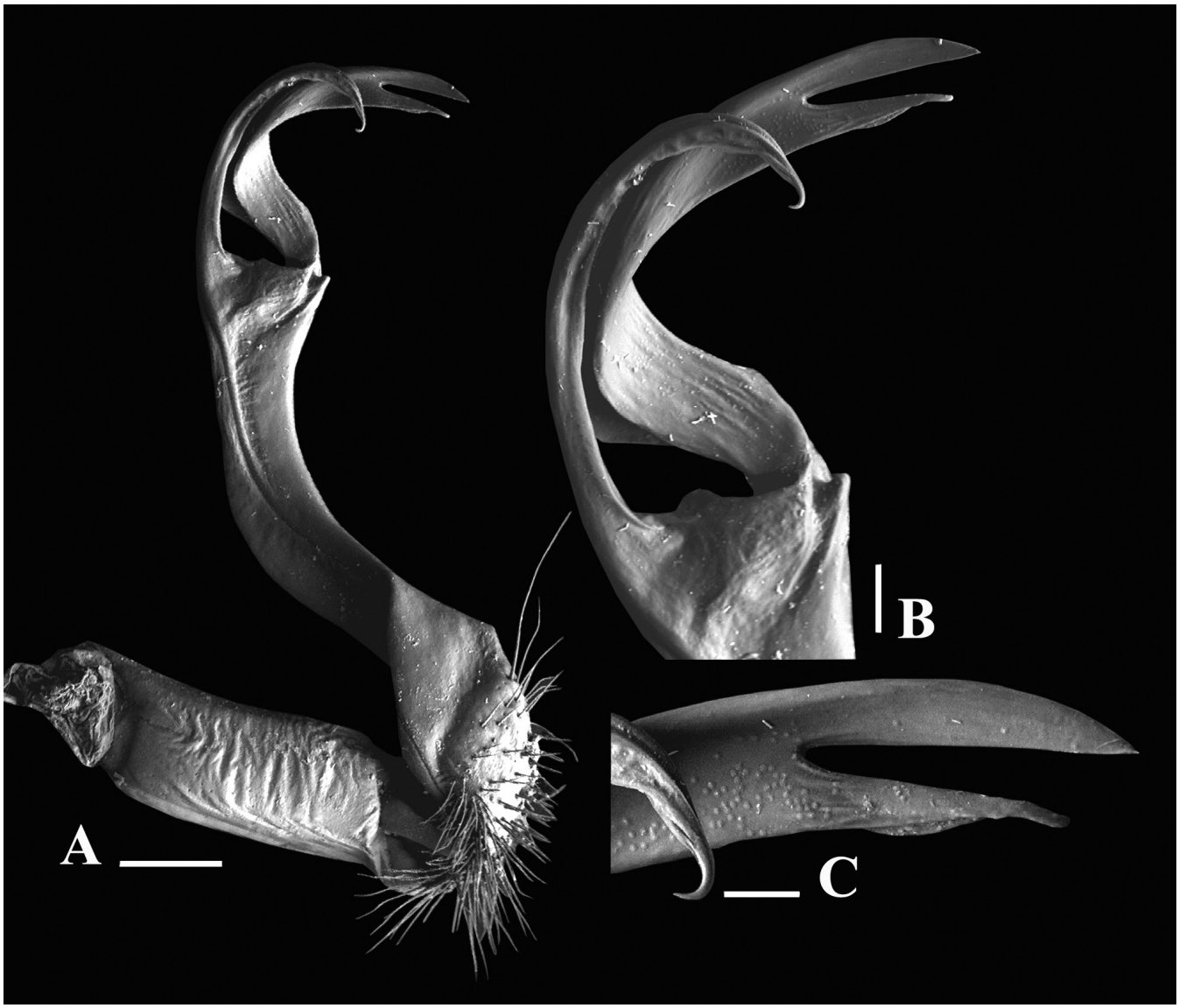

Fig. 5. *Antheromorpha paviei* (Brölemann, 1896) from Da Nang. Right gonopod, mesal view (A); distal part of gonopod, mesal view (B); tip of gonopod, mesal view (C). Scale bar = 200 μm (A); 66.7 μm (B) and 40 μm (C).

Paraterga (Fig. 7A, B, D−G) well developed, sub-horizontal, set lower than metatergal surface; thin, like blunt blades, but thicker on pore−bearing body rings from lateral view. Paratergum 2 subsector-shaped, with anterior and posterior corners rounded. Paraterga 3−4 shorter than others; caudal corners weakly acute, but not pointed. Caudal corners of paraterga 5−15 strongly acute, pointed, but more protruding on paraterga 15−19. Paraterga 16−19 with a small hook at caudal corners. Calluses small, narrow in both poreless and pore-bearing paraterga, with two setiferous incisions at 1/3 and ½ length of paraterga laterally. Paraterga exceeding posterior contour on body rings 16−19 (female) and on body rings 13−19 (male). Ozopores located on a small depression at 2/3 of paratergal length laterally.

Legs (Fig. 7E, G) long and slender, about 1.1−1.3 (females) 1.3−1.6 (males) times as long as midbody height. Tarsal brushes present on legs 1−11, thinner on legs 12−15 (male); thereafter missing on subsequent legs. Prefemora not swollen. Femora without modifications.

Sterna sparsely setose, without modifications except for the fifth sternum with a median pair of separately, but contiguously elevated setiferous conical processes between coxae 4 (Fig. 7L). Epiproct (Fig. 7K) long, broadly truncated, dorsoventrally flattened, with two small lateroapical tubercles. Tip with four spinnerets. Hypoproct (Fig. 7J) subtriangular with two distolateral, well separated, setiferous knobs.

Gonopod (Figs. 8, 9) simple. Coxite cylindrical, shorter than femorite; distoventral part modestly setose. Prefemorite densely setose, about ½ times as long as femorite, set off from femorite by a transverse sulcus laterally. Femorite long, slender, not expanded distally, suberect, demarcated from postfemoral region by an oblique sulcus laterally, with evidence of weak torsion basally. Flagelliform solenomere almost sheathed by solenophore. Tip of gonopod strongly divided into two processes, one being lanceolate process (D); the other with serrated lobe (m) and a distal minute process (v).

Fig. 6. *Antheromorpha pumatensis*, new species, from Pu Mat National Park, habitus. No scale.

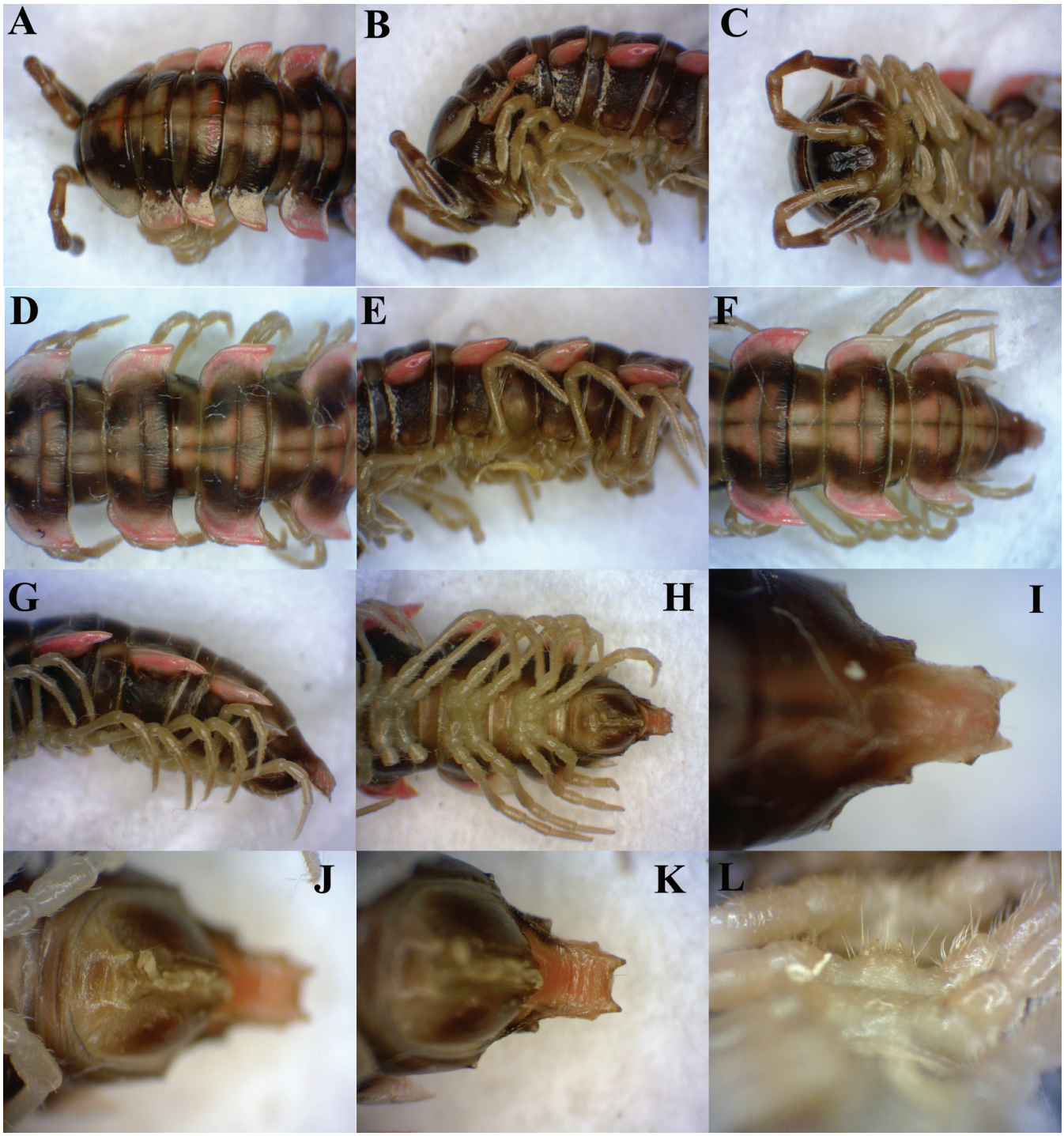

Fig. 7. *Antheromorpha pumatensis*, new species from Pu Mat National Park. Anterior part of body, dorsal (A); lateral (B) and ventral view (C); Midbody part, dorsal (D) and lateral view (E); posterior part of body, dorsa (F); lateral (G) and ventral view (H); Epiproct, dorsal (I) and ventral view (K); hypoproct, ventral view (J); sternum 5th, subventral view (L).

Remarks. Like *Anthromorpha paviei*, the new species is not typical for the genus *Antheromorpha*. It seems to be an intermediate species between *Orthomorpha* and *Antheromorpha*. In comparison to other *Antheromorpha* species, *A. pumatensis*, new species is differentiated by the colour pattern (red or brownish red); large size, and more importantly by gonopod conformation. The gonopod of the new species is long, slender and erect; tip deeply bifid, but the process (d) shorter than in other species, and process (m) serrated apically with short tubercle (v).

The species is only known from its type locality in central Vietnam.

KEY TO *ANTHEROMORPHA* SPECIES FROM VIETNAM

1. Lobules of gonopod tip as long as a half of solenophore length (Figs. 1, 3) 2
– Lobules of gonopod tip shorter than a half of solenophore length (Figs. 5, 8, 9) 3

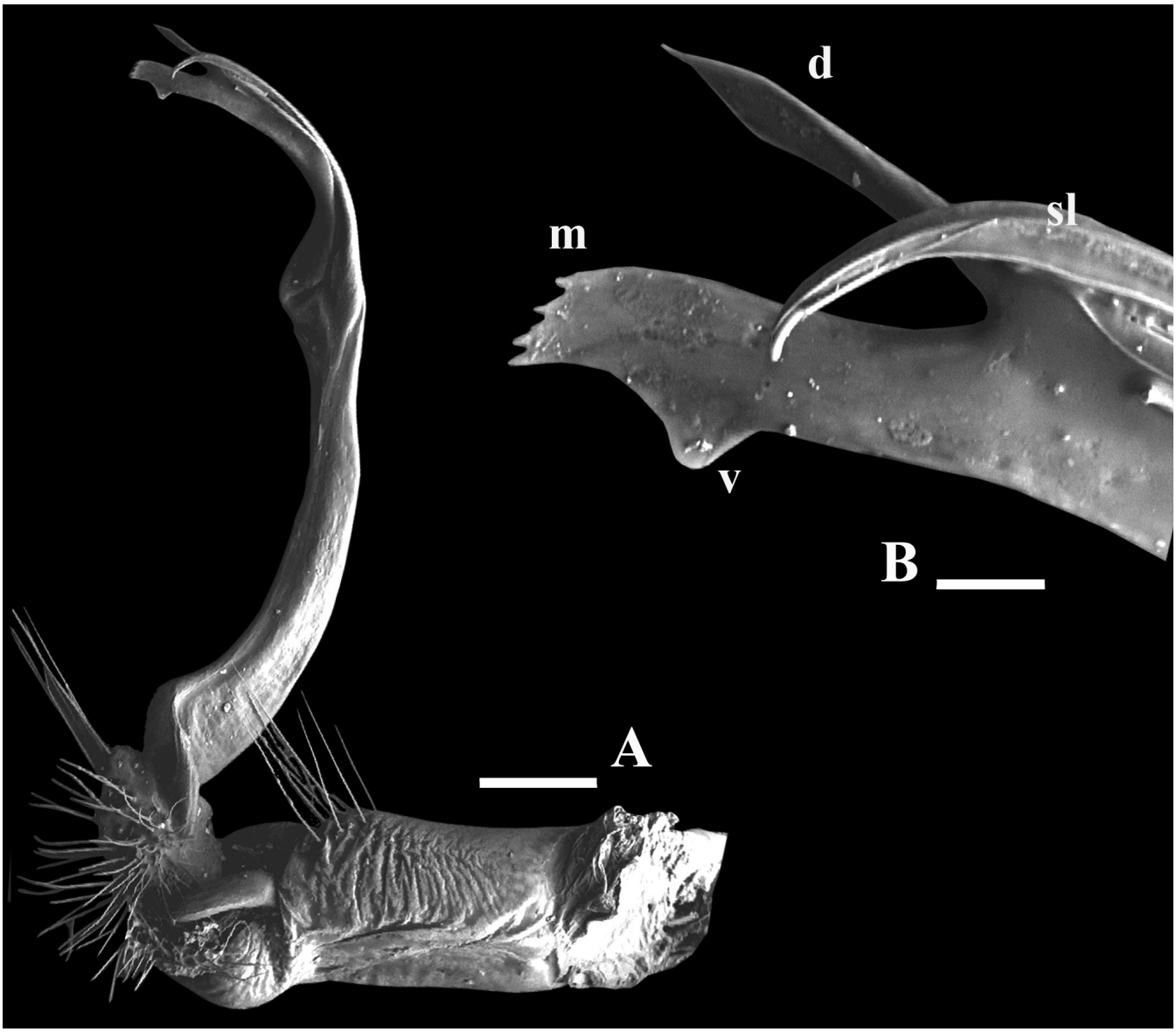

Fig. 8. *Antheromorpha pumatensis*, new species, holotype. Left gonopod, mesal view; tip of gonopod, mesal view (B). Scale bar = 250 µm (A) and 40 µm (B).

2. Body colour red or red-brown, two paramedian red longitudinal stripes on metaterga (Fig. 2); sternum 5th with two independent, sparsely setose, highly elevated tubercles between coxae 4 (Fig. 2H); epiproct with two straight, small, lateroapical tubercles (Fig. 2I).....*A. festiva*
− Body colour not red or red-brown, metaterga delicately rugose, without two paramedian red longitudinal stripes; sternum 5 with a high, medially emarginated, sparsely setose lobe between coxae 4; epiproct with two, claw-shaped, small, lateroapical tubercles (see Likhitrakarn el al., 2016: p.50, fig. 19)..... .....*A. harpaga*

3. Body red or brownish red (Figs. 6, 7). Gonopod tip strongly divided into two processes, one being lanceolate, the other being serrated apically (Figs. 8, 9).....*A. pumatensis*
− Body black or brown-blackish. Gonopod tip strongly split into two subequal, lanceolate processes (Fig. 5).....*A. paviei*

PHYLOGENETIC RELATIONSHIPS

The ML and BI analyses were performed for 15 species belonging to 8 genera, including two out groups in the genera *Tylopus* and *Oxidus* (tribe Sulciferini).

In the phylogenetic tree inferred from a 500 bp fragment of 16S rRNA, both ML and BI trees show a close relationship between the two species, *Antheromorpha festiva* and *A. pumatensis* with strong ML and BI values (93% and 1.00 bpp) (Fig. 11). The genus *Antheromorpha* appears to be a sister clade to the two genera *Orthomorpha* and *Nesorthomorpha*. This relationship is modestly/strongly supported with ML and BI values of 75% and 0.91 bpp, respectively, in the phylogenetic tree inferred from a 500 bp fragment of 16S rRNA.

In the phylogenetic tree inferred from a 576 bp fragment of COI, the new species is also closely related to its congener, *Antheromorpha festiva* with modest ML and BI values of

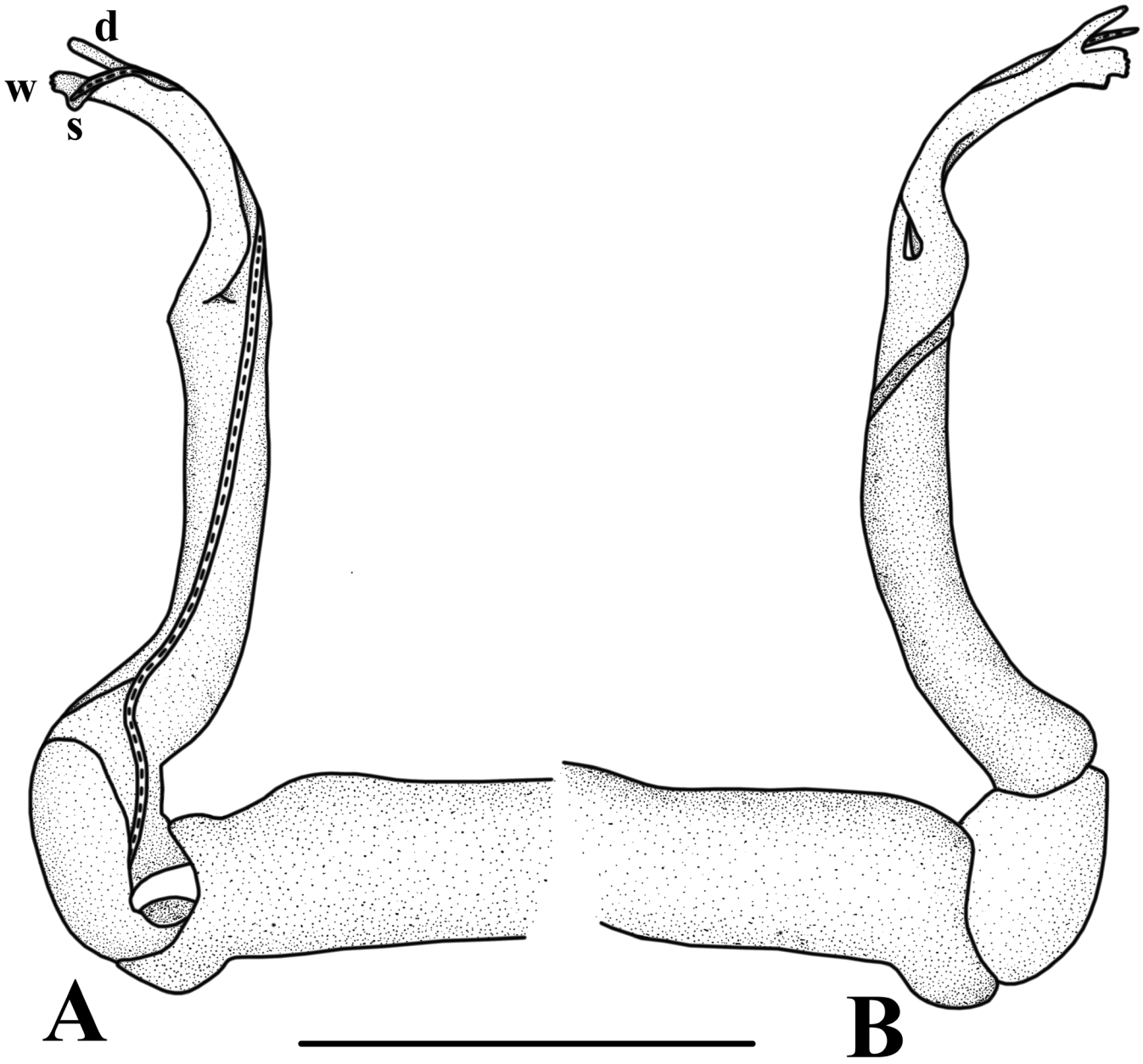

Fig. 9. *Antheromorpha pumatensis*, new species, holotype. Left gonopod, mesal view (A); lateral view (B). Scale bar = 1 mm.

79% and 0.8 bpp, respectively (Fig. 12). Similarly, the genus *Antheromorpha* is more closely related to *Orthomorpha* than to other orthomorphinine genera. This relationship is strongly supported with ML and BI values (95% and 1.00 bpp, respectively).

In both trees, the genus *Desmoxytes* is considered as a sister clade to three genera: *Orthomorpha*, *Nesorthomorpha*, and *Antheromorpha*. This relationship is modestly supported by ML and BI values. Other orthomorphinine genera, *Piccola* and *Orthomorphoides*, seem to be a single clade or grouped together.

CONCLUSION

Presently, a total of four species of the genus *Antheromorpha* have been recorded from Vietnam. Presumably, more unknown *Antheromorpha* species are awaiting discovery. Additionally, more data is needed to clarify the relationship between *Antheromorpha* species and other members of the Orthomorphini.

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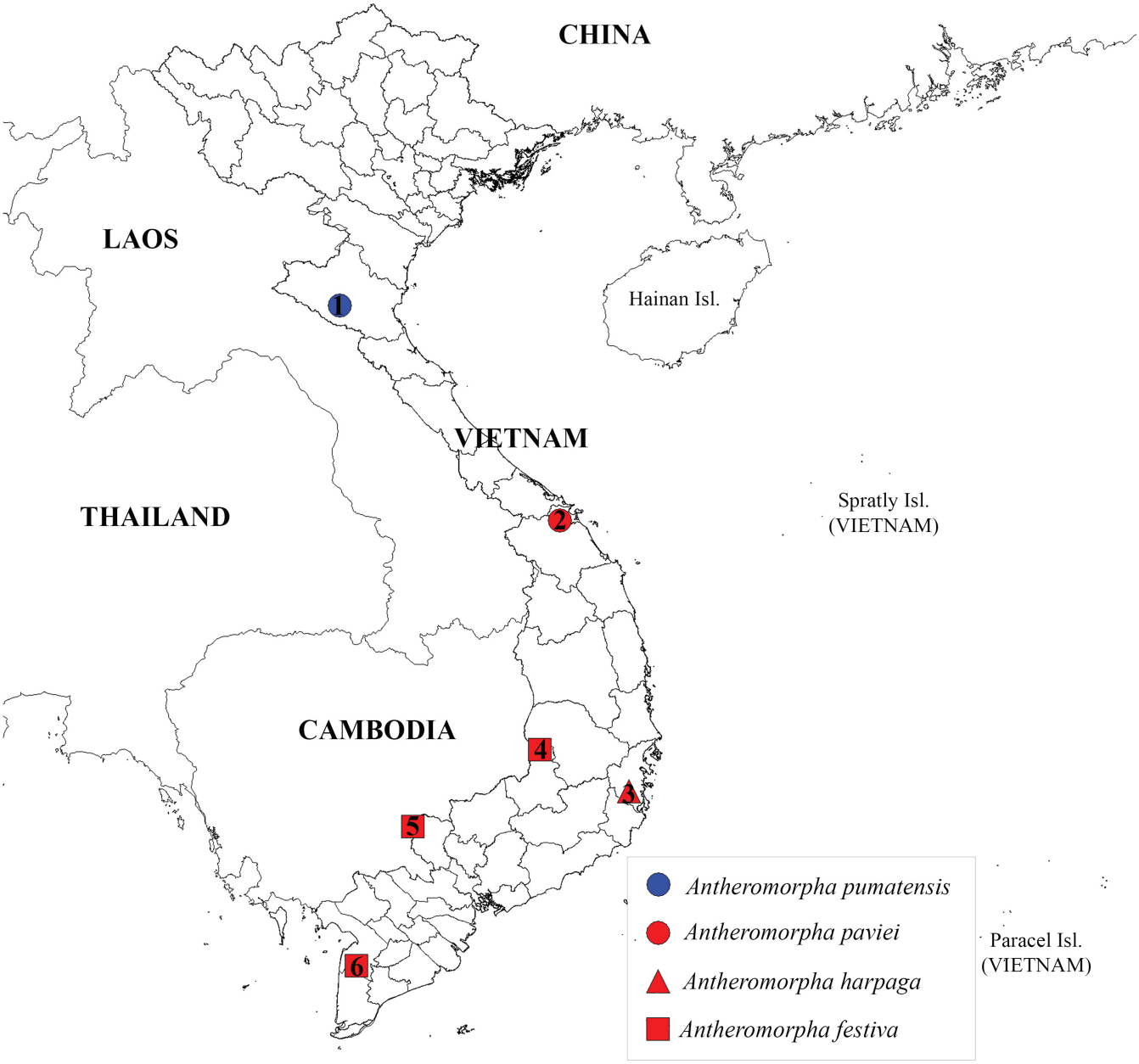

Fig. 10. Distribution map of the genus *Antheromorpha* in Vietnam. 1, Pu Mat NP; 2, Ba Na NP; 3, Hon Ba Mts.; 4, Yok Don NP; 5, Lo Go - Xa Mat NP; 6, U Minh Thuong NP.

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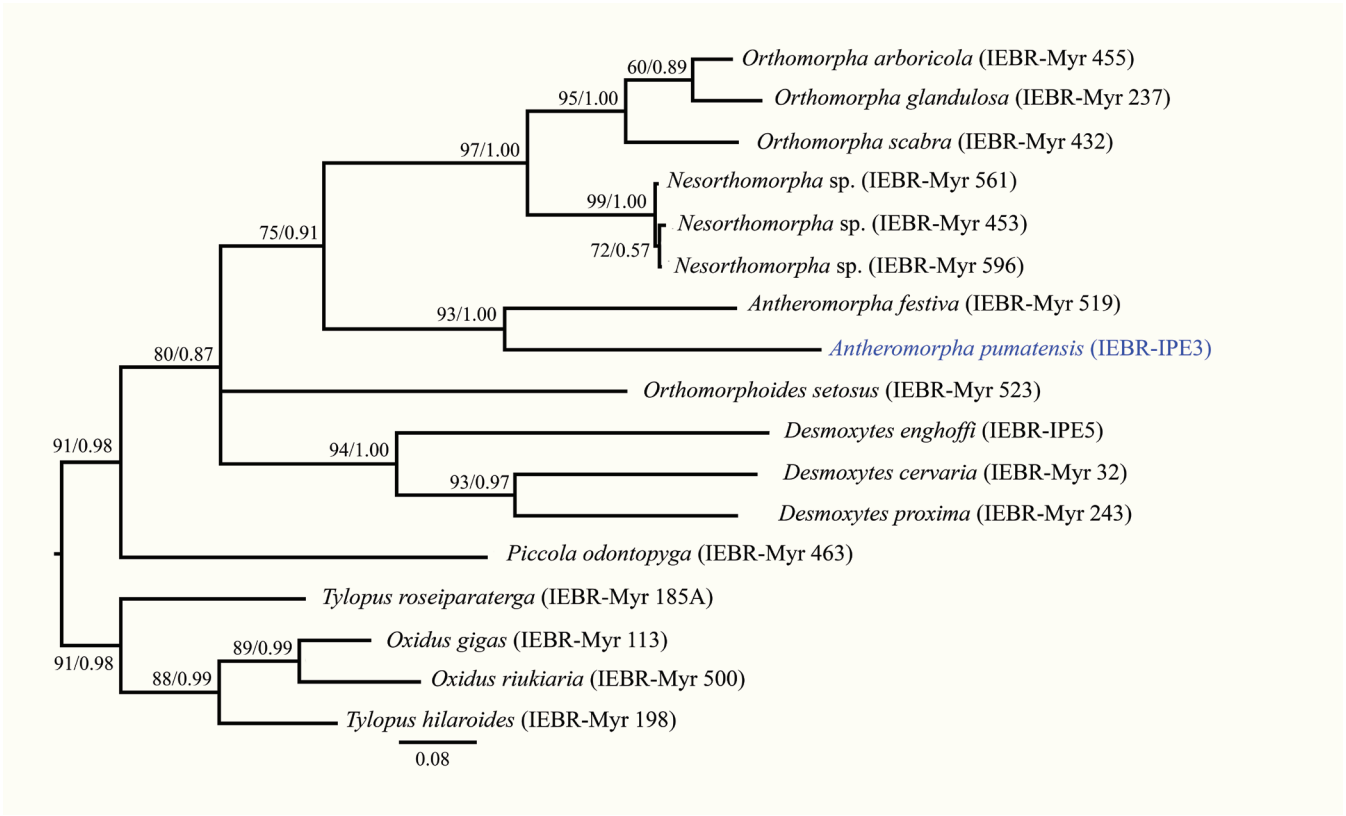

Fig. 11. The phylogenetic tree based on Maximum Likelihood and Bayesian Inference analysis inferring from a 500 bp fragment of the mitochondrial 16S rRNA.

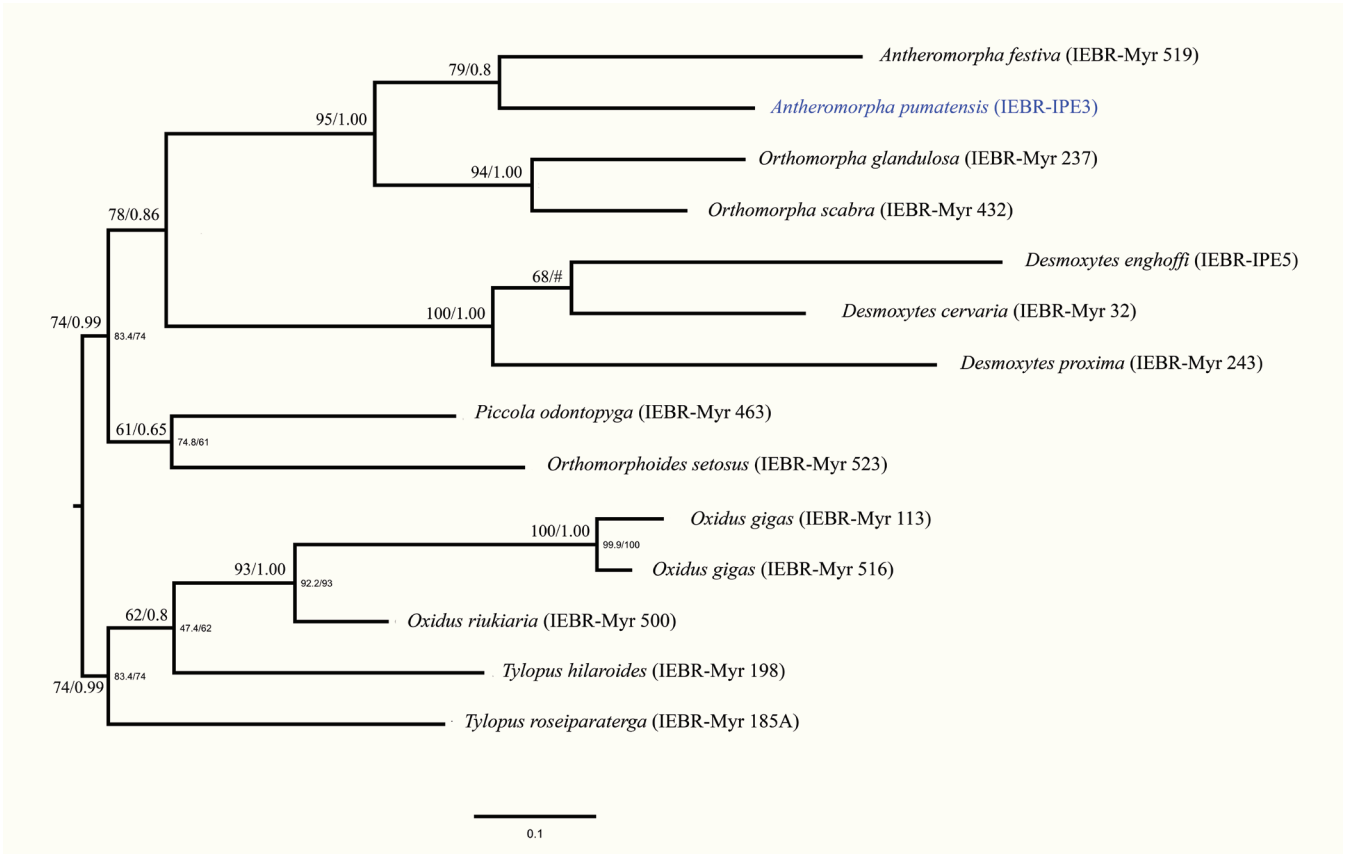

Fig. 12. The phylogenetic tree based on Maximum Likelihood and Bayesian Inference analysis inferring from a 576 bp fragment of the mitochondrial COI.

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