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Description of the first Oriental species of the ant genus *Xymmer* (Hymenoptera: Formicidae: Amblyoponinae)

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Abstract

The monotypic ant genus *Xymmer* Santschi, 1914 was established for *X. muticus* (Santschi, 1914) which has so far been known only from the West African subregion. The genus is easily distinguishable from the other amblyoponine genera by the anteromedian part of clypeus which is produced as a short rectangular lobe. Additional undescribed species had been found exclusively from Madagascar and Africa until recently. However, in March, 2015, a *Xymmer* colony was found in northern Central Vietnam. In the present paper *Xymmer phungi* sp. nov. is described as the first Oriental species of the genus. Although the worker of *X. phungi* is morphologically quite similar to that of *X. muticus*, the former is distinguishable from the latter by the following two characteristics of the worker: clypeal lobe 1/6 times as long as broad (vs. 1/3 times as long as broad in *X. muticus*); anterior margin of the lobe weakly concave (vs. almost straight in *X. muticus*). *Xymmer phungi* is well distinguished from Ethiopian *Xymmer* spp. for which 28S sequences are available from GenBank. Our observations suggest that the species feeds on geophilids (Chilopoda: Geophilomorpha). An updated key to Vietnamese genera of the subfamily Amblyoponinae is also provided.

Key words: taxonomy, new species

Introduction

Xymmer was established by Santschi (1914) as a subgenus of *Stigmatomma* Roger, 1859 (type species: *Stigmatomma* (*Xymmer*) *muticum* Santschi, 1914, by monotypy), raised to genus by Wheeler (1922), and then treated as a subgenus of *Amblyopone* Erichson, 1842 by Clark (1934). Later it was synonymized with *Stigmatomma* by Brown (1949), and then with *Amblyopone* by Brown (1960). The treatment by Brown (1960) had been widely accepted. However, Yoshimura & Fisher (2012) resurrected *Xymmer* from synonymy with *Amblyopone* and provided a generic diagnosis based on the male. This view was reconfirmed by a comprehensive molecular phylogenetic analysis of the subfamily (Ward & Fisher 2016).

There is currently only a single named species, *Xymmer muticus*, from the West African subregion (Cameroon, Central African Republic, Côte d'Ivoire, Gabon, Ghana, Nigeria). Additional undescribed morphospecies are known from Madagascar and Africa (Antweb v.5.28.1, https://www.antweb.org/). Additionally, DNA barcoding and morphological observations suggest that at least two species are present in the Oriental region (Thailand); all of the Thai specimens are males (Fisher, unpublished data).

In 2007 Bui T. V. (coauthor of the present paper) found a unique amblyoponine worker from Nui Chua

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National Park, southern Central Vietnam. Unfortunately, when picking up the worker its body was partly destroyed. However, the anterior clypeus had a plate-like projection, like *X. muticus*, instead of the series of dents that is typical for *Stigmatomma* (Eguchi *et al.* 2014). Nevertheless, Eguchi *et al.* (2014) tentatively treated it as "*Stigmatomma* sp. eg-4" until the colony-based material of the species became available. Finally, in March 2015, Eguchi (corresponding author of the present paper) was successful in collecting a colony of the species in Vu Quang National Park, northern Central Vietnam. Thus, we here describe the first Oriental species of *Xymmer* based on workers and the queen, and we provide bionomical observations.

Materials and methods

Abbreviations of the specimen depositories. ACEG, Ant Collection of Katsuyuki Eguchi (see the contact address of the corresponding author); IEBR, Institute of Ecology and Biological Resources, 18 Hoang Quoc Viet Road, Cau Giay, Hanoi, Vietnam; MCZC, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA; MHNG, Muséum d'Histoire Naturelle, Geneva, Switzerland; VNMN, Vietnam National Museum of Nature, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. The images of the type materials provided by AntWeb (http://www.antweb.org/) were also examined.

2D Imaging. Multi-focused, montage images were produced using Helicon Focus Pro from a series of source images taken by a Canon Eos Kiss X5, Olympus Pen Lite E-PL7 or Panasonic Lumix DMC-GX8 digital camera attached to a Nikon AZ100 microscope. Fine hairs and other features not recognized automatically were copied from the focused parts in source images to the montage image using the retouching function of Helicon Focus. Artifacts (ghost images) and unnecessary parts (unfocused appendages, etc.) surrounding or covering target objects were erased and cleaned up using the retouching function of Helicon Focus. Finally, the background was cleaned up, and the color balance, contrast and sharpness were adjusted using Adobe Photoshop CS6.

3D Imaging. Two paratype workers preserved in 80% ethanol (vouchers IMG20151021-1 and IMG20151021-5) were dehydrated in 100%. Each specimen was mounted with 100% ethanol inside a 20-µL polypropylene pipette tip on which the apex was heat-sealed. Basal openings of the pipette tips were then sealed with plasticine caps and parafilm to prevent the specimens from drying out during scanning.

The mounted samples were scanned with ScanXmate-D160TSS105/1100 (Comscantecno Co., Ltd) at voltage of 40 kV and current of 166 µA, and scans were performed over 360 degrees with 1500 projections. The resolution of radiographic images was 0.9 µm/pixel. The tomographic images were reconstructed with conCTexpress v.1.76 (White Rabbit Co., Ltd)

Because each 3D image was reconstructed from a combined data set of two stack tomographic image sets with MolcerPlus v. 1.35 (White Rabbit Co., Ltd), a weak unconformity line appeared. Movies showing the 3D images were uploaded to YouTube (URL: https://youtu.be/gwHggpJWlGM; https://youtu.be/csImvZPebrk). Original images are available from the corresponding author.

Detailed information on imaging protocols will be separately provided as a technical paper by Osamu Sasaki.

Measurements. Photographs for measuring were taken by an Olympus Pen Lite E-PL7 digital camera attached to a Nikon AZ100 microscope under suitable magnification. The following body parts were measured using ImageJ 1.49m (National Institute of Mental Health, USA, available at http://imagej.nih.gov/ij/) and then the indices were calculated: HL, maximum length of head in full-face view from the level crossing anterolateral ends of anteromedian clypeal lobe to the level crossing posterolateral ends of head; HW, maximum width of head in full-face view (excluding eyes in queens); MBL, with head in full-face view, maximum length of mandible from outer side of mandibular insertion to mandibular apex; SL, maximum measurable length of scape, from the proximal point of scape shaft, not including the condyle, to the distal end of scape; ML, mesosomal length in dorsal view measured from the midpoint of anterior margin of promesonotal dome to the midpoint of a transverse line spanning the posteriormost points of mesosoma; PNW, maximum width of pronotum in dorsal view; HFL, length of hind femur; PL, maximum diagonal length of petiole (abdominal segment II) in lateral view measured from the anteroventral corner of petiolar peduncle to the posterodorsal corner of the petiole; PH, maximum height of petiole in lateral view measured from the apex of the ventral broadly-triangular prominence to the top of the petiolar node; PW, maximum width of the petiolar node in dorsal view; A3W, maximum width of abdominal tergite III (gastral tergite I) in dorsal view; A4W, maximum width of abdominal tergite IV (gastral tergite II) in dorsal view; CI=HW/

HLx100; MBI=MBL/HWx100; SI=SL/HWx100; MI=ML/PNWx100; HFI=FL/HWx100; A3I=A3W/PWx100; A4I=A4W/A3Wx100.

DNA Barcoding. DNA was extracted from three workers of the colony Eg20iii15-01, to which the type series belongs (voucher ID: Ext20151110-1, -9, -15). The Chelex-TE-ProK method was used (Eguchi *et al.* 2016). Then the CO1 gene of mitochondrial DNA and 28S ribosomal DNA of Ext20151110-1 and Ext20151110-9 were amplified and sequenced using the following primer sets (Saux *et al.* 2004; Eguchi *et al.* 2016): LCO-EG (TTTCAACAAATCACAAAGAYATYGG) and HCO-EG (TAAACTTCAGGRTGACCRAAAAATCA) for CO1; D2B (GTCGGGTTGCTTGAGAGTGC) and D3Ar (TCCGTGTTTCAAGACGGGTC) for 28S. The PCR thermal regime consisted of one cycle of 2 min at 94 °C; five cycles of 10 sec at 98 °C, 30 sec at 45 °C, and 45 sec at 68 °C; 40 cycles of 10 sec at 98 °C, 30 sec at 48.5 °C (CO1) or 51 °C (28S), and 45 sec at 68 °C; and a final cycle of 7 min at 68 °C. The sequences obtained were submitted to the DNA Data Bank of Japan (accession no: LC154069–LC154072).

The 28S sequences obtained (accession no: LC154069, LC154070) were then aligned with the homologue sequences of "*Amblyopone mutica*" (= *Xymmer muticus*, AY325926, EF012960), "*Amblyopone* sp. CS-2003-9" (= *Xymmer* sp., AY325922) and "*Amblyopone silvestri*" (= *Stigmatomma silvestrii* Wheeler, AY325918, AY325919, as outgroups) using MUSCLE (Edgar, 2004) built in MEGA 7 (Kumar *et al.*, 2016). Then pairwise divergences were calculated using p-distance (obtained by dividing the number of nucleotide differences by the total number of nucleotides compared) and the K2P distance model (Kimura, 1980).

Taxonomy

Xymmer phungi sp. nov.

Stigmatomma sp. eg-4: Eguchi *et al.*, 2014, 26.

Holotype. worker [voucher: IMG20160228-1, colony: Eg20iii15-01, IEBR], Vietnam: Ha Tinh: Vu Quang National Park: TK 189, N18˚16'36–38", E105˚22'06–13", K. Eguchi leg., 20/III/2015.

Paratypes. 15 workers, 1 dealate queens from the same colony as holotype [IEBR, VNMN, MCZC & ACEG].

Non-type material examined. Vietnam: Ninh Thuan: Nui Chua National Park: Cau Gay, 1 worker, Bui T. V. leg., 11/V/2007.

Worker. Monomorphic. Head in full-face view subrectangular, with posterior margin weakly concave, in lateral view relatively flattened dorsoventrally; vertex without median furrow; anterolateral corner of head beside each mandibular insertion not forming a tooth; preoccipital carina weakly present dorsally and laterally; frontal lobe narrowly separated by posteromedian portion of clypeus; frontal carina and antennal scrobe absent; anteromedian part of clypeus produced as a short rectangular lobe (hereafter referred to as "clypeal lobe") which is 1/6 as long as broad and has a weakly concave anterior margin; the margin without a series of dents or tubercles; mandible elongate and linear; apical and preapical teeth triangular, followed by five more or less bifid teeth and then a minute denticle; both maxillary and labial palps 3-segmented (one worker dissected); antennal socket in full-face view almost concealed by frontal lobe; antenna 12-segmented; scape relatively flattened dorsoventrally; funiculus incrassate apically but not forming a distinct club; antennal segment II longer than broad; segment III to XI broader than long, terete, not flattened; apical segment (segment XII) much longer than broad, terete, not flattened, bluntly pointed apically; eye absent. Mesosoma in lateral view very weakly convex dorsally; promesonotal suture present and flexible; mesonotum in dorsal view weakly constricted; mesopleuron not subdivided into anepisternum and katepisternum by suture or furrow; metanotal groove almost entirely absent, but weakly present on the ventrolateral part of mesosoma (in front of metapleuron); metapleural gland bulla, when seen through metapleuron, bean-shaped, located at the posterolateral corner of metapleuron; the anterior margin of bulla close to or reaching the level of propodeal spiracle; junction of dorsal and posterior faces of propodeum round, without any transverse carina; posterior face of propodeum laterally without spines/carinae; orifice of propodeal spiracle round, situated around midheight of the side of mesosoma; propodeal lobe very low, in lateral view with a roundly convex posterior outline. Mid tibia without spurs; hind tibia with a reduced barbulate anterior spur and a well-developed pectinate posterior spur; pretarsal claws simple, without teeth. Petiole with a distinct anterior

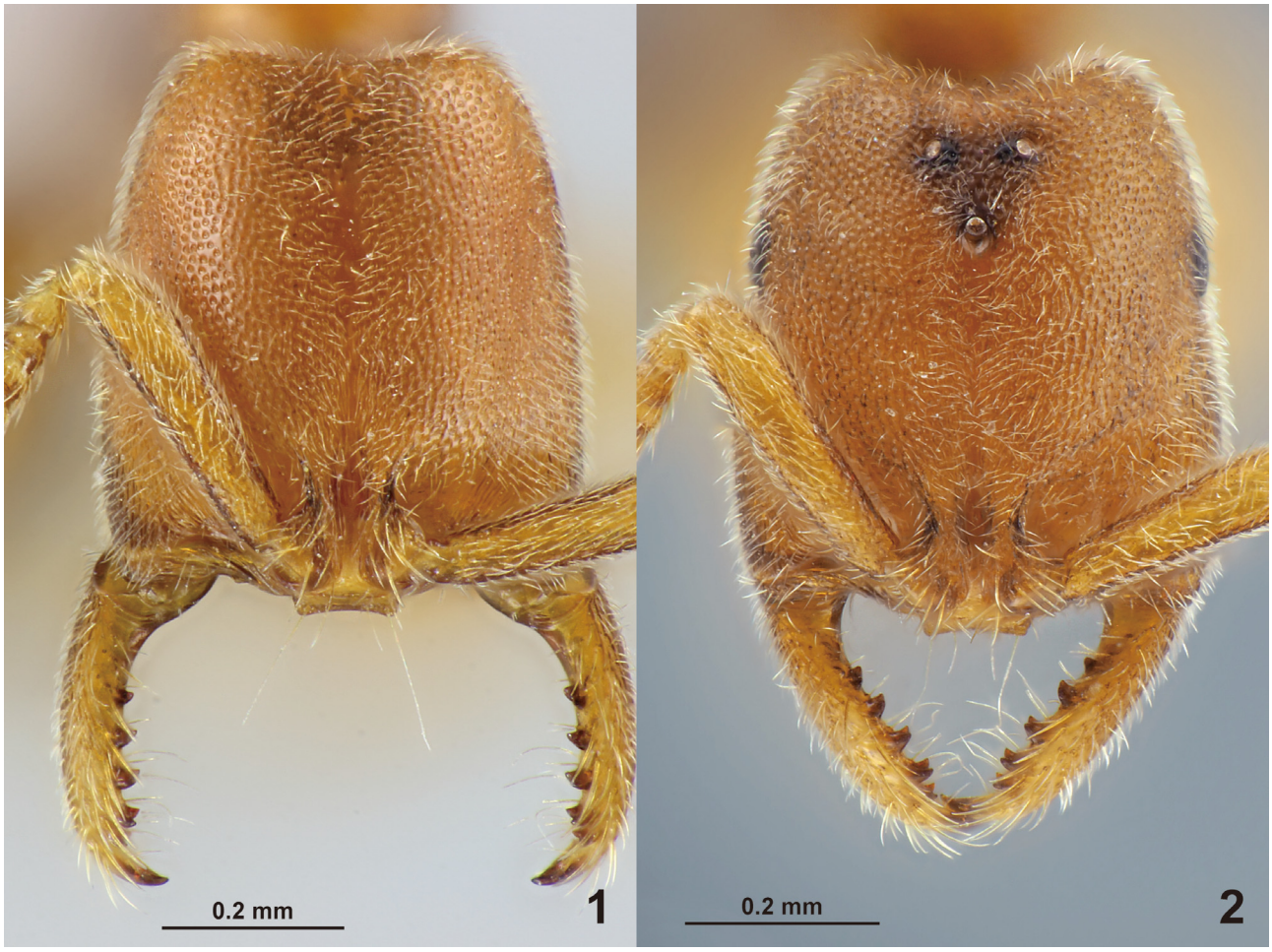
peduncle which has an anteroventral corner; ventrum of the petiole in lateral view with a broadly triangular prominence; petiolar node in lateral view with very weakly concave anterior and very weakly convex dorsal outlines; petiolar spiracle in lateral view located close to the junction of dorsal face of petiolar peduncle and anterior face of petiolar node; posteriormost part of petiole broadly attached to abdominal segment III, in lateral view without a free posterior face; petiole in ventral view with inverted U-shaped sulcus; petiolar sternite in ventral view recognized as a ligulate sclerite of which the anteriormost part comes into contact with the inverted U-shaped sulcus; abdominal segment III above the helcium without a free anterior face; anteroventral outline of abdominal segment III below helcium gently curved; girdling constriction between abdominal segment III and IV distinct; pygidium large, convex across; pygidium and hypopygium laterally and posteriorly without spines/tubercles; sting well developed.

Dorsum of head densely and distinctly sculptured with hair pits, but with a smooth and hairless longitudinal band on vertex and posterior part of frons; lateral and ventral faces of head, pronotum, mesonotum, and dorsum of propodeum sparsely and weakly sculptured with hair pits; mesopleuron and metapleuron finely rugose; lateral and posterior faces of propodeum almost smooth and shining; petiolar node and gaster densely and weakly sculptured with hair pits.

Body densely covered with short decumbent hairs (Figs. 1, 3, 6). Clypeal lobe anterolaterally with a pair of long setae; outer surface of mandible with a single row of setae along the inner margin; posterior dorsum of petiole with several suberect hairs; tip of gaster densely growing long setae.

Whole body light brown, with antennae and legs lighter and yellowish.

Measurements and indices of the holotype: HL 0.634 mm; HW 0.528 mm; MBL 0.380 mm; SL 0.371 mm; ML 0.899 mm; PNW 0.320 mm; HFL 0.400 mm; PL 0.477 mm; PH 0.298 mm; PW 0.246 mm; A3W 0.387 mm; A4W 0.418 mm; CI 83; MBI 72; SI 70; MI 281; HFI 76; A3I 157; A4I 108.



FIGURES 1–2. *Xymmer phungi* **sp. nov.**, head in full-face view. Fig. 1, paratype worker (IMG20151129-1). Fig. 2, paratype queen (IMG20160227-4).

FIGURES 3–5. *Xymmer phungi* **sp. nov.** Fig. 3, body in lateral view, paratype worker (IMG20151129-1). Fig. 4, mesosoma in lateral view, paratype worker (IMG20151129-1). Fig. 5, mesosoma in lateral view, paratype queen (IMG20160227-4).

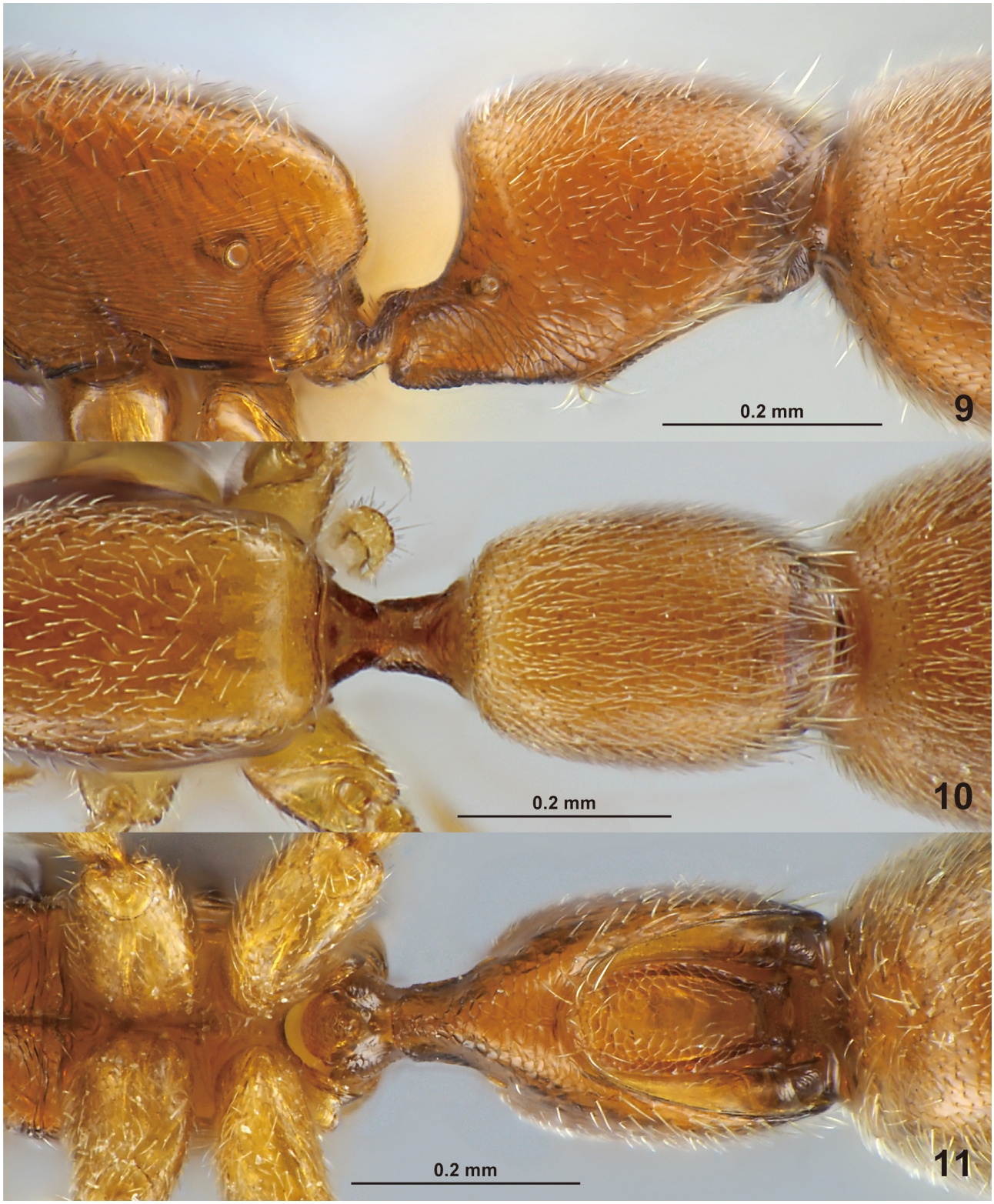
Measurements and indices of the paratypes (n=4): HL 0.616–0.638 mm; HW 0.513–0.532 mm; MBL 0.373–0.389 mm; SL 0.362–0.374 mm; ML 0.878–0.918 mm; PNW 0.317–0.331 mm; HFL 0.391–0.402 mm; PL 0.470–0.487 mm; PH 0.291–0.308 mm; PW 0.241–0.251 mm; A3W 0.377–0.391 mm; A4W 0.410–0.423 mm; CI 83; MBI 72–74; SI 70–71; MI 275–280; HFI 75–77; A3I 156–161; A4I 107–110.

Queen. The queen is similar to the worker except for the following features: compound eye relatively well

developed, situated behind midlength of side of head; ocelli present; median ocellus in full-face view located at the level of the posterior margin of compound eye; distance between median ocellus to lateral ocellus a little longer than that between lateral ocelli; compound eye consisting of ca. 25 ommatidia; mesosoma fully segmented; mesoscutum without parapsidal and notaular lines; axillae separated from the remainder part of mesoscutum by scutal suture; scuto-scutellar suture distinct; mesopleuron separated from metapleuron and lateral face of propodeum by a conspicuous groove; metanotum recognized as a transverse strip; the anteriormost portion of metapleural gland bulla reaching the level of propodeal spiracle. The area defined by three ocelli blackish brown, much darker than the rest of the body (Fig. 2).

Measurements and indices of the paratype: HL 0.595 mm; HW 0.504 mm; MBL 0.332 mm; SL 0.347 mm; ML 0.891 mm; PNW 0.305 mm; HFL 0.383 mm; PL 0.465 mm; PH 0.286 mm; PW 0.254 mm; A3W 0.418 mm; A4W 0.458 mm; CI 85; MBI 66; SI 69; MI 292; HFI 76; A3I 165; A4I 110.

FIGURES 6–8. *Xymmer phungi* **sp. nov.** Fig. 6, body in dorsal view, paratype worker (IMG20151129-2). Fig. 7, mesosoma in dorsal view, paratype worker (IMG20151129-2). Fig. 8, mesosoma in dorsal view, paratype queen (IMG20160227-4).



FIGURES 9–11. *Xymmer phungi* **sp. nov.**, waist. Fig. 9, in lateral view, paratype worker (IMG20160227-2). Fig. 10, in dorsal view, paratype worker (IMG20151129-2). Fig. 11, in ventral view, paratype worker (IMG20160227-1).

Recognition. The worker of *Xymmer phungi* is morphologically quite similar to that of *X. muticus*. However, the former is distinguishable from the latter by the following two characteristics of the worker: clypeal lobe 1/6 times as long as broad (vs. 1/3 times as long as broad in *X. muticus*); anterior margin of the lobe weakly concave (vs. almost straight in *X. muticus*).

DNA barcoding (Table 1)**.** *Xymmer phungi* is well distinguished from Ethiopian *Xymmer* spp. for which 28S

sequences are available from GenBank: "*Amblyopone mutica*" (AY325926, EF012960) and "*Amblyopone* sp. CS-2003-9" (AY325922). These species were re-identified by Fisher (unpublished data) as *Xymmer muticus* and *X.* sp., respectively, prior to the present study (see also Yoshimura & Fisher 2012).

TABLE 1. Pairwise divergences among 28S sequences, calculated using the p-distance (upper right) and the K2P distance model (lower left). The p-distance is percentage.

	Species name	Accession No.	1	2	3	4	5	6	7
1	*Xymmer phungi* **sp. nov.** (paratype worker)	LC154069		0.0	2.65	1.16	0.81	2.11	1.95
2	*Xymmer phungi* **sp. nov.** (paratype worker)	LC154070	0.000		2.65	1.16	0.81	2.11	1.95
3	*Xymmer muticus*	EF012960	0.027	0.027		0.84	1.09	2.29	2.19
4	*Xymmer muticus*	AY325926	0.012	0.012	0.008		0.42	1.61	1.52
5	*Xymmer* sp.	AY325922	0.008	0.008	0.011	0.004		1.34	1.25
6	*Stigmatomma silvestrii*	AY325918	0.021	0.021	0.023	0.016	0.014		0.08
7	*Stigmatomma silvestrii*	AY325919	0.020	0.020	0.022	0.015	0.013	0.001	

Bionomics. The type series (colony Eg20iii15-01) was collected from a lowland evergreen forest at the northern end of the Trung Son Mountains. The colony nested under a wood fragment on the forest floor beside a trail on a steep slope. By sifting the nesting site, 132 workers, a dealate queen, and 46 larvae were obtained. The majority of the adults in the colony were probably captured successfully; on the other hand, many larvae were probably overlooked. Workers ran very fast on and under debris of litter and soil fallen on the bottom of a white pan. The majority (but not all) of the larvae were about the same size, suggesting the development of larvae is synchronized.

We reared a small colony fragment (consisting of 10 workers and 27 larvae) for 7 days in a small plastic case with its bottom covered thinly by soil. A weak vibration triggered workers to start migration. Larvae were transported by workers in a fixed posture: the neck of a larva was grasped by a worker and the slender body of the larva was stretched under the body of the worker along the body axis. By referring to Masuko (1990, 1993), who found *Stigmatomma silvestri* and *Leptanilla japonica* to be specialized predators of geophilid centipedes, we placed weakened small geophilids (ca. 20 mm in length) on the ground near the aggregation site of the colony fragment. The colony moved away from the site. However, after placing the rearing container in a dark place for several hours, we found the workers assembled around the geophilids, and the larvae were placed on or beside the body of the geophilids. We observed the same phenomenon a total of two times, showing that the species can feed on geophilids, but the degree of specialization on geophilids remains unknown.

Etymology. The specific epithet is named after a Vietnamese revolutionary leader, Mr. Phan Dinh Phung, who was born in 1847 in Ha Tinh Province and led rebel armies against French colonial forces.

Updated key to Vietnamese genera of the subfamily Amblyoponinae based on the worker caste

The key to Vietnamese genera of the subfamily Amblyoponinae based on the worker caste is provided in Eguchi *et al.* (2014), and then partly modified by Eguchi *et al.* (2015) because of a discovery of "*Bannapone*" in Vietnam. However, Ward and Fisher (2016) synonymized *Bannapone* with *Stigmatomma*, and place *Opamyrma* in the subfamily Leptanillinae. So, the key should be again modified as below.

1 Mandible elongate-triangular, armed with 3 teeth; in closed position inner margin of mandibles not forming a conspicuous gap against the anterior clypeal margin. . . . . *Prionopelta*

\- Mandible linear, armed with more than 3 teeth; if mandible armed with 3 teeth, the apical teeth forming an elongate and falcate apical blade, and 2nd and 3rd teeth blunt or truncate apically; in closed position inner margin of mandibles separated from anterior clypeal margin by a large gap. 2

2 Apex of mandible round, with minute teeth; body covered with spatulate setae . . . . . *Mystrium*

\- Apex of mandible with distinct apical tooth/teeth; body without spatulate setae. 3

3 A few apical segments of antenna distinctly flattened; outer surface of middle tibia with thickened peglike setae scattered

among normal finer background pilosity. . . . . *Myopopone*

- All of antennal segments terete, not flattened; outer surface of mesotibia without peglike or spiniform setae (the surface of mesotibia often with relatively long standing setae scattered among background pilosity). 4

4 Anteromedian part of clypeus produced as a clypeal lobe; petiole with a distinct anterior peduncle. . . . . *Xymmer*

- Anteromedian part of clypeus never produced as a clypeal lobe; petiole essentially sessile. . . . . *Stigmatomma* (= *Bannapone*).

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