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SHORT COMMUNICATION

A new subterranean *Crematogaster* with one ommatidium from Cambodia, based on morphology and DNA (Hymenoptera: Formicidae)

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Abstract. A subterranean myrmicine ant *Crematogaster monocula* sp. nov. is described based on two worker specimens from Cambodia. This species belongs to the *C. biroi* group of the subgenus *Orthocrema* Santschi, 1918, which is characterized by developed subpetiolar process, developed subpostpetiolar process and sculptured mesosoma. The new species is easily distinguished from other species of *Crematogaster* Lund, 1831 by having only one ommatidium. DNA sequences comprising a 761 bp fragment of the 3' region of the mitochondrial COI gene were amplified and analyzed for *C. monocula* sp. nov. and other closely related species from the same group. The interspecific COI divergence is 17.8% for *C. monocula* sp. nov. and *C. reticulata* Hosoishi, 2009. This species is unique in having the smallest eye in the genus *Crematogaster*.

Key words. Hymenoptera, Formicidae, Myrmicinae, *Crematogaster*, *Orthocrema*, subterranean, ommatidium, compound eye, DNA sequencing, Cambodia, Asia

Zoobank: http://zoobank.org/urn:lsid:zoobank.org:pub:A7B483BE-DDC7-4804-AE39-7BE707F3F85B

Introduction

The ant genus *Crematogaster* Lund, 1831 has a global distribution, and more than 500 species have been described to date (Bolton 2019). The genus is currently divided into two subgenera: *Crematogaster* s. str. and *Orthocrema* Santschi, 1918 (Blaimer 2012). The subgenus *Orthocrema* consists of more than 150 species distributed all around the world, and 27 species are presently known from Asia (Hosoishi & Ogata 2016).

Most members of the genus *Crematogaster* have developed compound eyes consisting of more than 20 ommatidia, while a few subterranean species in the subgenus *Orthocrema* have reduced eyes (Longino 2003, Hosoishi et al. 2010, Hosoishi & Ogata 2016). Hosoishi et al. (2010) reviewed the subterranean species from the Asian *Orthocrema* fauna and showed that *C. masukoi* Hosoishi, Yamane & Ogata, 2010 and *C. myops* Forel, 1911 have reduced compound eyes (approximately 5–6 ommatidia) and depigmented yellowish bodies. In a systematic revision, Hosoishi & Ogata (2016) described a small-eyed species, *C. ocellata* Hosoishi & Ogata, 2016, with 12–15 ommatidia. Their cladistic analysis revealed that those subterranean species belong to two different species groups, suggesting that their subterranean mode of life evolved independently as morphological convergence (Hosoishi & Ogata 2016).

Subterranean ant species have been described based on the specimens collected by Winkler extractions and subterranean pitfall traps in Asia – *Aenictus* Shuckard, 1840 by Wong & Guénard (2016); *Leptanilla* Emery, 1870 by Man et al. (2017) and Leong et al. (2018). Those sampling methods have been employed to collect rare subterranean ant taxa (Wong & Guénard 2017). During field surveys in the Indochina region, two workers of *Orthocrema* species with small eye were collected using Winkler extractions of leaf litter from lowland forests of Cambodia. The collections examined in this study are limited due to the rarity with which the subterranean species are encountered in the field. The species is morphologically similar to *C. masukoi* of the *C. biroi* group (Hosoishi & Ogata 2016), but is described here as a species new to science.

Molecular approaches including DNA barcodes have been recently used in biodiversity studies of ant species (Smith et al. 2005) and are used as an additional and powerful method in integrative taxonomy (Schlick-Steiner et al. 2010, Wang et al. 2018). They can thus provide a useful reference for the identification of Asian *Crematogaster* species (Hosoishi & Ogata 2014). Therefore, mitochondrial cytochrome oxidase I (COI) sequence data from the new species is also generated and compared with related species of the *C. biroi* group, *C. reticulata* Hosoishi, 2009. Our analysis included the 3' region of COI region. It is not the typical DNA barcoding region, but Cocuzza et al. (2015) demonstrated that not only the 5' barcoding regions but also 3' regions were useful for species delimitation in *Anuraphis* Del Guercio,1907 (Hemiptera: Sternorrhyncha: Aphididae). The sequence data provide additional information to support species delimitation.

Material and methods

Type specimens of the new species were examined and deposited in the collections listed below.

KUEC Institute of Tropical Agriculture, Kyushu University, Fukuoka, Japan;
THNHM Thailand Natural History Museum, Technopolis, Khlong Luang, Pathum Thani, Thailand.

Most observations were made on a Leica M205C stereomicroscope. Images were taken using a Canon EOS 50D with a Canon MP-E 65 mm 1–5× Macro lens and a Leica DFC 450 digital imaging system, then processed using Combine ZM (Hadley 2010).

Measurements were made under a Leica M205C stereomicroscope with Leica Application Suite V4.5 software. All measurements are expressed in millimeters, recorded to the second decimal place. The following abbreviations of measurements are used in the text:

CI cephalic index: HW/HL × 100;
EL eye length; maximum length of the compound eye;
HL head length; perpendicular distance from the vertex margin to the tangentline of the anteriormost projections of the clypeus in full-face view;
HW head width; maximum width of head in full-face view;
PpL postpetiole length; maximum length of the postpetiole in dorsal view, measured from the anterior inflection point to the posterior point;
PpW postpetiole width; maximum width of the postpetiole in dorsal view, excluding the helcium;
PpWI postpetiole width index: PpW/PpL × 100;
PSL propodeal spine length; measured from the tip of propodeal spine to the closest point on outer rim of the propodeal spiracle;
PtL petiole length; length of the petiole in lateral view, measured from the anterior inflection point to the posterodorsal margin;
PtH petiole height; height of the petiole in lateral view;
PtHI petiole height index: PtH/PtL × 100;
PtW petiole width; maximum width of the petiole in dorsal view;
PtWI petiole width index: PtW/PtL × 100;
PW pronotal width; maximum width of the pronotum in dorsal view;
SI scape index: SL/HW × 100;
SL scape length; length of the first antennal segment, excluding the neck and basal condyle;
WI waist index: PpW/PtW × 100;
WL Weber's length of the mesosoma; diagonal length, measured in lateral view from the anterior margin of the pronotum (excluding the collar) to the posterior extremity of the propodeal lobe.

Crematogaster monocula sp. nov. was sequenced from one specimen collected by Winkler extractions from Koh Kong, Cambodia, and *C. reticulata* from one nest series (SH10-Mal-84) from Ulu Gombak, Malaysia (Peninsular Malaysia). Genomic DNA was extracted from tissues rich in mitochondria (e.g., legs) using DNeasy Blood & Tissue Kit (Qiagen, Maryland, USA). A 761 bp region of the mitochondrial genome, the 3' region of cytochrome oxidase I (COI), was amplified via polymerase chain reaction (PCR) using primers (Simon et al. 1994) and program shown in Table 1. Reactions were carried out at 10 μL volumes in a PCR Thermal Cycler MP (Takara Bio Inc.). PCR products were visualized on a 1% agarose E-Gel 96-well system (Invitrogen) and then purified with 1.0 μL of ExoSAP-IT (GE Healthcare Life Sciences). All products were sequenced in both directions using BigDye Terminator v3.1 (Applied Biosystems) on an ABI 3100 Avant DNA Sequencer (Applied Biosystems) at the Faculty of Science, Kyushu University, Fukuoka. It is noted that these sequences are not the typical DNA barcoding region. DNA sequence data for one individual of each *Crematogaster monocula* sp. nov. and a related species, *C. reticulata*, were generated and deposited at DNA Data Base of Japan, DDBJ (with accession numbers: LC490807, for *C. monocula* sp. nov. and LC490808 for *C. reticulata*). Contigs were assembled using Vector NTI Advance TM ver. 11 (Invitrogen Corp.) and subsequently aligned and checked visually. Genetic distances were estimated using the Kimura-2 parameter (K2P, Kimura 1980) distances with MEGA 5 (Tamura et al. 2011).

Taxonomy

Crematogaster monocula sp. nov.

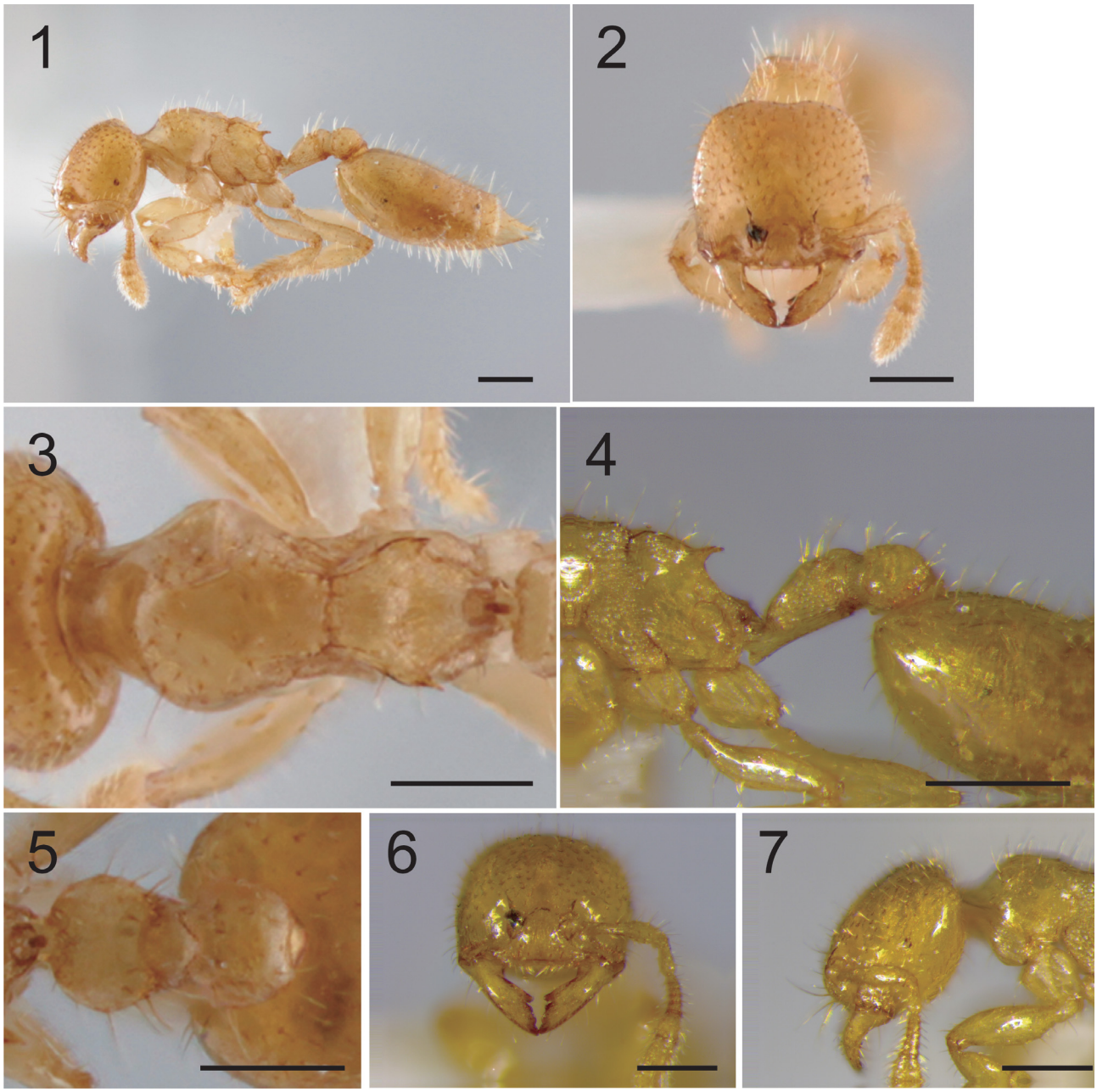
(Figs 1–7)

Type material. Holotype: 1 ☿, 'CAMBODIA / Koh Kong / 141 m alt. / 11°31′N, 103°09′E // 19.V.2012 / leg. S. Hosoishi // Holotype / Crematogaster monocula **sp. nov.**' (THNHM). Paratype: 1 ☿, 'CAMBODIA / Koh Kong / 141 m alt. / 11°31′N, 103°09′E // 18.V.2012 / leg. S. Hosoishi' (KUEC).

Description of worker (Figs 1–7). Measurements and indices of workers (holotype / paratype): HW: 0.42 / 0.41; HL: 0.43 / 0.42; CI: 98 / 97; SL: 0.27 / 0.27; SI: 64 / 66; EL: 0.02 / 0.02; PW: 0.27 / 0.28; WL: 0.53 / 0.52; PSL:

Table 1. PCR primers and program used to amplify gene loci.

Gene	Primer	Sequence (5'–3')	Source	Amplification program
COI	Jerry	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)	94°C for 1 min, 5 cycles each of 94°C for 1 min, 48°C for 90 s, and 72°C for 90 s, then 30 cycles each of 94°C for 1 min, 51°C for 90 s, and finally 72°C for 90 s.
	Pat	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994)	



Figs 1–7. *Crematogaster monocula* sp. nov., holotype worker from Cambodia (HW 0.42; WL 0.53): 1 – body in lateral view; 2 – full-face view of head; 3 – dorsal view of mesosoma; 4 – lateral view of petiole and postpetiole; 5 – dorsal view of petiole and postpetiole; 6 – anterior view of mandible; 7 – lateral view of head. Scale bars = 0.2 mm.

0.04 / 0.03; PtL: 0.14 / 0.13; PtW: 0.16 / 0.16; PtH: 0.11 / 0.11; PpL: 0.12 / 0.11; PpW: 0.16 / 0.16; PtHI: 79 / 85; PtWI: 114 / 123; PpWI: 133 / 145; WI: 100 / 100.

Workers monomorphic.

Head subquadrate in full-face view. Mandible with four teeth, basal tooth arranged away from third apical one; mandibular dentition worn-out and blunt in type specimens (Fig. 6). Anterior clypeal margin almost straight in medial portion. Compound eye consisting of one ommatidium, not projecting beyond lateral margins of head in full-face view. Antenna 11-segmented. Antennal club 2-segmented. Scape not reaching posterolateral corners of head.

Pronotal collar with almost straight anterior margin in dorsal view, distinctly lower than pronotal dorsum in lateral view. Pronotal dorsum without distinct ridges laterally in dorsolateral view. Mesonotal dorsum without lateral ridges. Pronotum and mesonotum in lateral view forming slightly convex, continuous dorsal outline. Metanotal groove in dorsal view transverse, almost straight in median portion, forming deep concavity that is laterally margined by thin lamellate ridges. Propodeal spiracle oval, situated at posterolateral corners of propodeum, touching metapleural gland bullae. Propodeal spine shorter than maximum diameter of propodeal spiracle, in dorsal view directed posteriorly.

Petiole in dorsal view with weakly convex sides and narrowed anteriorly, longer than broad (Fig. 5). Posterior portion of petiole without distinct process in lateral view. Subpetiolar process developed, acute apically (Fig. 4).

Postpetiole in lateral view with dorsum weakly convex and as high as petiole, in dorsal view as wide as petiole, weakly bilobed posteriorly, but without longitudinal sulcus. Subpostpetiolar process developed as small process (Fig. 4).

Integument weakly sculptured. Dorsal surface of head generally smooth, but with rugulae on region surrounding antennal sockets. Mandibles smooth. Clypeus smooth and shining without distinct longitudinal rugulae. Dorsal and lateral surfaces of pronotum smooth and shining; anterolateral shoulders of pronotum without rugulae. Mesopleuron weakly sculptured. Longitudinal rugulae on higher portion of mesopleuron developed. One pair of rugulae running from metanotal groove extending posteriorly and diverging to propodeal spines in dorsal view. Dorsal surface of propodeum with feeble rugulae. Lateral surface of propodeum mostly smooth and shining, but weakly sculptured with rugulae on anterior areas. Dorsal surface of petiole generally smooth. Lateral surface of petiole sculptured. Dorsal surface of postpetiole smooth and shining. Lateral surface of postpetiole smooth.

Standing pilosity sparse. Dorsal face of head with sparse erect and stout setae. Clypeus with two pairs of long and stout setae in anterior portion, one directed upward and the other downward. Anterior clypeal margin with one pair of long setae (0.15 mm) medially, and a few pairs of short setae laterally. Scape with sparse erect to suberect setae. Mesosoma with four pairs of long erect and stout setae on pronotal shoulders, anterior and posterior mesonotal ridges, near base of propodeal spines that are much longer than other erect setae. Posterolateral tubercles of petiole posteriorly with three pairs of long and stout setae. Postpetiole with two pairs of long and stout setae on disc anterolaterally, posteriorly. Fourth abdominal tergite with sparse erect setae, no decumbent to appressed setae.

Body yellow. All flagellar segments yellow.

Differential diagnosis. *Crematogaster monocula* sp. nov. is a member of the *C. biroi* group of *Orthocrema* and shares all the group-specific characters (Hosoishi & Ogata 2016). This species is well distinguished from all other Asian species of this species group by having one ommatidium and a relatively short scape (SI 64–66). *Crematogaster monocula* sp. nov. is similar to *C. masukoi*, but can be distinguished by having only one ommatidium, almost straight anterior clypeal margin and sculptured petiole.

The COI divergence between *C. monocula* sp. nov. (Cambodia: Koh Kong specimen) and *C. reticulata* (Malaysia: Ulu Gombak specimen) was relatively high (17.8% K2P distance). In comparison, the COI divergence between the two related Asian *Crematogaster* species was 9.4% (8.1–10.8%) for *C. chhangi* Hosoishi & Ogata, 2014 (Accession numbers: AB828274, AB828377, AB828264) and *C. fraxatrix* Forel, 1911 (AB828275–AB828280, AB828381–AB828378, AB828265–AB828270) (Hosoishi & Ogata 2014), and 17.4% for *C. coriaria* Mayr, 1872 (LC068833) and *C. modiglianii* Emery, 1900 (LC068834) (Hosoishi & Ogata 2015).

Etymology. The species name refers to one ommatidium ('mono' and 'oculus' are Latin words for 'one' and 'eye', respectively). Noun in apposition.

Biological notes. The two worker specimens were collected from a lowland forest by Winkler extraction of leaf litter.

Distribution. This species is only known from the type locality in Cambodia.

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