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Cryptic Diversity in the Widespread Asian Ant *Crematogaster rothneyi* (Hymenoptera: Formicidae) Inferred from Morphological and Genetic Evidence

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The myrmicine species *Crematogaster rothneyi* is one of the most widely distributed ants in Asia, but it has rarely been collected in the field. Its distribution range covers South and Southeast Asia, extending approximately 5,000 km from India in the west to Sulawesi in the east. Despite this wide distribution range, *C. rothneyi* has been treated as a single taxonomic species, and no combined morphological or molecular analysis has been conducted to assess whether any intraspecific variation exists. The sequence divergences of *C. rothneyi* populations, mainly obtained from Southeast Asia, were investigated by analyzing 387 bp and 175 bp sequences of the 12S ribosomal RNA and cytochrome *c* oxidase subunit (*COI*) genes, respectively. Phylogenetic analysis indicated that the *C. rothneyi* populations were separated into three groups: group I from Thailand and Cambodia, group II from Bangladesh and Myanmar, and group III from Krakatau and Sulawesi. Groups II and III were recovered as a single clade, sister to group I. The interspecific divergences were 7.3% to 8.5% for 12S and 14.5% to 23.3% for *COI* between most *C. rothneyi* specimens and Cambodian specimens, while divergence for 12S was 3.5% between Thai and Cambodian specimens. The Thai specimens are not morphologically differentiated, and are considered conspecific to *C. rothneyi*. We describe the Cambodian series as a new species, *Crematogaster yaharai* sp. nov., based on unique antennal morphology and mesosomal sculpture patterns with molecular evidence. *Crematogaster rothneyi civa* Forel, 1902 is synonymized under *C. rothneyi*. *Crematogaster rothneyi* is widespread and has sister species, suggesting recent diversification within the Indochinese Peninsula.

Key words: Asia, *Crematogaster rothneyi*, Geographic variation, Phylogeny, Widespread species.

BACKGROUND

Widespread species are important in species inventories because they are both easily recognizable and commonly encountered (Pfeiffer et al. 2011; Guenard and Dunn 2012; Bharti et al. 2016; Jaitrong et al. 2016). Despite their importance, relatively little attention has been paid to many of these species, and even taxonomists often accept many current taxonomies without performing comprehensive revisions (but see Seifert et al. 2017; Wagner et al. 2017). Some widespread species show geographic variations in body size and shape, but those differences have simply been treated as intraspecific variations, which can occur within a distinct species (*e.g.*, Azuma et al. 2006; Hosoishi et al. 2011). However, in some species those variations imply the existence of cryptic species, which can escape detection using traditional taxonomic

approaches based on morphology. Recent integrative taxonomic approaches employ additional methods, such as molecular phylogeny, morphometry, ethology, and phenology for species delimitation (*e.g.*, Balakirev et al. 2017).

In ant taxonomy, integrative taxonomic methods have been carried out in widespread species (*e.g.*, *Tetramorium caespitum* complex by Wagner et al. 2017; *Cardiocondyla nuda* group by Seifert et al. 2017). These studies revealed the existence of cryptic species as well as the resurrection of formerly synonymized species with distinct evidence from multiple data sources (Csősz et al. 2014). Many invasive or tramp ant species also have global distributions (*e.g.*, *Solenopsis invicta* by Morrison 2004; *Paratrechina longicornis* by Wetterer 2008; *Linepithema humile* by Wetterer et al. 2009). These species have been treated as one species, but careful consideration is required when developing appropriate biological control programs for some taxonomically difficult groups, such as *Solenopsis* species (Chialvo et al. 2018).

The genus *Crematogaster* has a global distribution, and more than 500 species have been described to date (Bolton 2019). Although no distinct tramp or invasive species have been reported in the genus, several widespread species exist around the world. For example, *Crematogaster scutellaris* is known from Western Europe (Seifert 2007 2018), and *C. crinosa*, *C. curvispinosa*, *C. distans*, and *C. rochai* from Central and South America (Longino 2003). In Asia, *C. sewardi* (Hosoishi and Ogata 2009) and *C. treubi* (Hosoishi and Ogata 2012) are known from the Indochinese Peninsula to the Sunda Islands. Among the Asian *Crematogaster* fauna, *C. rothneyi* is also considered to be widespread.

Crematogaster rothneyi was described by Mayr (1879) based on worker specimens from Calcutta, India. Two more subspecies of *C. rothneyi* have been recognized: *C. rothneyi civa* Forel, 1902 based on worker specimens from Poona, India and *C. rothneyi haputalensis* Forel, 1913 based on a queen specimen from Haputale, Sri Lanka. This species has been classified into the subgenus *Crematogaster* (Blaimer 2012), which contains more than 350 species, but *C. rothneyi* is easily distinguished from other species by its strongly sculpted body, deep metanotal groove, distinctly bilobed postpetiole and stout body setae. This species is arboreal and inhabits lowland forests.

Crematogaster rothneyi is known from South Asia to Southeast Asia (AntWeb), making it one of the most widely distributed ant species in Asia. However, the species is rarely encountered in the field. Similarly, *Oecophylla smaragdina*, *Cataulacus granulatus*, *Anochetus graeffei*, and *Carebara diversa* are also widespread in Asia but, except for a phylogeographic study of *O. smaragdina* (Azuma et al. 2006), the extent of intraspecific variation in these species has not been investigated extensively. While these species have each been treated as a distinct taxon, no further taxonomic studies have been conducted on them. Hosoishi et al. (2011) reviewed the morphology of *C. modiglianii*, which is widely distributed in Indochina, Peninsular Malaysia, Borneo, and Sumatra. Their study, which was based on workers of nest series, found only a weak geographical cline in the shape of the propodeal spine. However, morphological approaches have limitations in terms of categorizing intraspecific variation.

Mitochondrial DNA (mtDNA) is one of the most useful genetic markers for phylogeographic studies of animals because it exhibits extensive intraspecific variation (Avise 2000). Based on mtDNA gene analysis, Azuma et al. (2006) proposed that the Asian weaver ant (*Oecophylla smaragdina*) consists of two major groups with seven geographical subgroups, and inferred its dispersal history over a geological time scale. Indeed, genetic information on widely distributed species can reveal the phylogeographic history of those species and also provide useful taxonomic information for species delimitation (Steiner et al. 2010; Aguliar-Velasco et al. 2016). The 12S ribosomal RNA (12S) region has been widely used to infer the intra- and interspecific relationships of vertebrates (macaques by Evans et al. 1999, Tosi and Coke 2007, Ziegler et al. 2007, Evans et al. 2003b; rabbits by Surridge et al. 1999; squirrels by Mercer and Roth 2003; frogs by Evans et al. 2003a; tigers by Luo et al. 2004; water skinks by Honda et al. 2005; medaka fishes by Takehana et al. 2005) and insects (*Pheidole* ants by Moreau 2008; paper wasps by Santos et al. 2015; flightless moths by Liu et al. 2015; crickets by Dong et al. 2018; diving beetles by Balke et al. 2018). In this study, we use mtDNA 12S and *COI* sequences as supplementary data because sufficient data is unavailable due to a lack of fresh material.

In the present paper, we also review the *C. rothneyi* group based on the worker caste, and provide additional molecular data in the form of 12S ribosomal RNA and *COI* sequences as a useful reference for species identification.

MATERIALS AND METHODS

Sources of material

Worker ants of the *Crematogaster rothneyi* group were examined based on collections from several localities in South and Southeast Asia (Fig. 1, Table 1). Specimens were examined and/or deposited in the collections listed below. Codes for public institutions

mainly follow those in Brandão (2000). BMNH: The Natural History Museum, London, U.K. CASC: California Academy of Sciences, San Francisco, USA. KUEC: Institute of Tropical Agriculture, Kyushu University, Fukuoka, Japan. MHNG: Musee d›Histoire Naturelle, Geneva, Switzerland. NHMW: Naturhistorisches Museum, Wien, Austria. THNHM: Thailand Natural History Museum, Technopolis, Khlong Luang, Pathum Thani, Thailand.

Measurements and indices

Observations and measurements were made on a Leica M205C stereomicroscope using micrometers. Images were taken using a Canon EOS 50D with a Canon MP-E 65 mm 1-5 x Macro lens, then processed using Combine ZM. All measurements are expressed in millimeters, recorded to the second decimal place. The measurements for petiole and postpetiole follow Longino (2003).

Head Width (HW): Maximum width of head in full-face view, excluding the eyes. Head Length (HL): Perpendicular distance from vertex margin to line tangent to anteriormost projections of clypeus in full-face view. Cephalic Index (CI): HW/HL × 100. Scape Length (SL): Length of the first antennal segment, excluding the neck and basal condyle. Scape Index (SI): SL/HW × 100. Eye Length (EL): Maximum length of
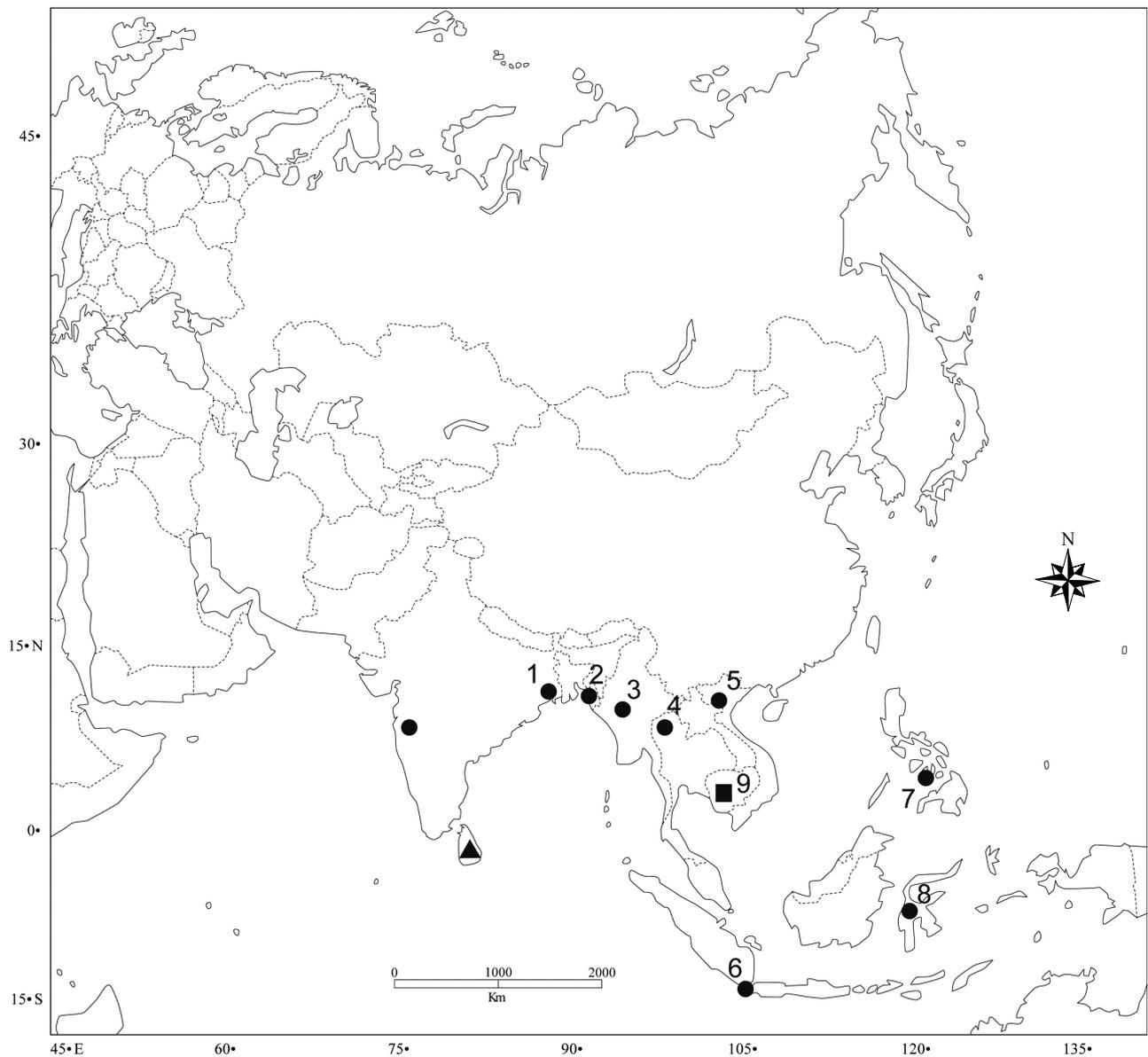
Fig. 1. Distribution map of the *Crematogaster rothneyi* group. Closed circle indicates *C. rothneyi*, closed triangle indicates *C. rothneyi haputalensis*, closed square indicates *C. yaharai*.

the compound eye. Pronotal Width (PW): Maximum width of the pronotum in dorsal view. Weber's Length of the mesosoma (WL): Diagonal length, measured in lateral view from the anterior margin of the pronotum (excluding the collar) to the posterior extremity of the propodeal lobe. Propodeal Spine Length (PSL): Measured from tip of propodeal spine to closest point on outer rim of propodeal spiracle. Petiole Length (PtL): Length of the petiole in lateral view (see Longino 2003). Petiole Width (PtW): Maximum width of petiole in dorsal view. Petiole Height (PtH): Height of the petiole in lateral view. Postpetiole Length (PpL): Length of the postpetiole in lateral view (see Longino 2003). Postpetiole Width (PpW): Maximum width of postpetiole in dorsal view, excluding the helcium. Petiole Height Index (PtHI): PtH/PtL × 100. Petiole Width Index (PtWI): PtW/PtL × 100. Postpetiole Width Index (PpWI): PpW/PpL × 100. Waist Index (WI): PpW/PtW × 100.

Molecular data collection

Genomic DNA was extracted from tissues rich in mitochondria (*e.g.*, legs) using a DNeasy Blood & Tissue kit (Qiagen, Maryland, USA). A 387 bp region of the mitochondrial 12S ribosomal RNA (12S) and a 175 bp region of the mitochondrial genome, 3' region of the cytochrome *c* oxidase I (*COI*) were amplified via the polymerase chain reaction (PCR) using primers (Simon et al. 1994) and programs shown in table 2. 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 2.0 μL mixture of Exonuclease I and Alkaline Phosphatase (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, Japan. Contigs were assembled using Vector NTI Advance TM ver. 11 (Invitrogen Corp.). Conserved regions were identified and aligned, and gaps were assigned to minimize changes using MEGA 5 (Tamura et al. 2011). Genetic distances were estimated using the Kimura-2-parameter (Kimura 1980) distances and the *p*-distance with MEGA 5 (Tamura et al. 2011). DNA sequence data for six individuals of two *Crematogaster*

Table 1. Specimen data and DDBJ accession numbers

Species	Locality	Code	Accession number	
			12S	*COI*
Crematogaster rothneyi	1: India, Calcutta	[1 IND]	N/A	LC369768
Crematogaster rothneyi	2: Bangladesh, Chitragong	[2 BAN]	LC369619	LC369769
Crematogaster rothneyi	3: Myanmar, Bagan	[3 MYA]	LC369620	LC369770
Crematogaster rothneyi	4: Thailand, Doi Chiang Dao	[4 THA]	LC369621	N/A
Crematogaster rothneyi	5: Vietnam, Hat Lot	[5 VIE]	N/A	LC369771
Crematogaster rothneyi	6: Indonesia, Pulau Sebesi	[6 SEB]	LC369622	LC369772
Crematogaster rothneyi	7: Philippines, Negros	[7 PHI]	N/A	LC369773
Crematogaster rothneyi	8: Indonesia, Sulawesi	[8 SUL]	LC369623	LC369774
Crematogaster yaharai	9: Cambodia, Kampong Chhnang	[9 CAM]	LC369624	LC369775
Crematogaster coriaria	Malaysia, Ulu Gombak Forest Reserve	[cor]	LC371675	N/A
Crematogaster rogenhoferi	Vietnam, Dong Nai, Dinh Quan	[rog]	LC371677	N/A
Crematogaster ferrarii	Vietnam, Cuc Phuong National Park	[fer]	LC371676	N/A
Crematogaster ferrarii	Indonesia, Sulawesi, Banti Murung		LC474370	N/A

Table 2. PCR primers and programs used to amplify gene loci

Gene	Primer	Sequence (5'–3')	Source	Amplification program
12S	12Sbi	AAGAGCGACGGGCGATGTGT	Simon et al. 1994	96°C for 3 min, 35 cycles each of 94°C for 30 s, 48°C for 1
	12Sai	AAACTAGGATTAGATACCCTATTAT	Simon et al. 1994	min, and 72°C for 1 min, finally 72°C for 10 min.
COI	CI-J-2797	CCACGACGTTATTCAGACTATC	Simon et al. 1994	94°C for 1 min, 5 cycles each of 94°C for 1 min, 48°C for
	Pat	TCCAATGCACTAATCTGCCATATTA	Simon et al. 1994	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.

species were deposited to the DNA Data Bank of Japan, DDBJ (with accession numbers shown in Table 1).

The 387 bp 12S DNA sequences were used for phylogenetic analysis; the 175 bp *COI* sequences were excluded from the analysis as they were too short to yield any meaningful data. The Indian, Vietnamese and Filipino samples were not subjected to phylogenetic analysis. Phylogeny was inferred from the 12S sequence dataset using the Maximum likelihood (ML) and Neighbor-Joining (NJ) methods (Saitou and Nei 1987) with MEGA 5 (Tamura et al. 2011) and a GTR model. Bootstrap support values were computed using 1000 replicates.

RESULTS

Molecular data

The 12S sequence comprised 387 bp and the *COI* sequences comprised 175 bp. Several sequences could not be analyzed due to the age and condition of the sample (Table 1). Since the K2P distance and *p*-distance resulted in similar values (Tables 3, 4), we used the K2P distance to illustrate all our results. Genetic distances ranged from 0.013 to 0.085 for 12S (Table 3), and 0.017 to 0.233 for *COI* (Table 4). For the 12S region, the Cambodian specimen was distantly related to most other specimens, except the one from Thailand, *i.e.*, genetic distance with Thai specimen—0.035, genetic distance range with non-Thai specimens—0.073 to 0.085. For the *COI* region, the Cambodian specimen was most distantly related to the others, with genetic distances of 0.145 to 0.233.

Since both ML and NJ analyses produced similar topologies for the *C. rothneyi* group, only the ML analysis is shown. Phylogenetic analysis of the 12S sequence data revealed three main clades (Fig. 2). Group I contained the specimens from Thailand and Cambodia, group II contained the specimens from Bangladesh and Myanmar, and group III contained the specimens from Krakatau and Sulawesi. Although the relationship was not supported by high bootstrap values, groups II and III were recovered as a clade, and as the sister group to group I (Fig. 2).

Table 3. Percent mitochondrial 12S ribosomal RNA (12S: 387 bp) sequence divergence among populations of *Crematogaster* species examined. The *p*-distance (upper right) and the K2P distance (lower left) are shown. Locality codes correspond to table 1

		[2 BAN]	[3 MYA]	[4 THA]	[6 SEB]	[8 SUL]	[9 CAM]	[cor]	[fer]	[rog]
Crematogaster rothneyi	[2 BAN]		0.018	0.066	0.050	0.050	0.080	0.196	0.208	0.192
Crematogaster rothneyi	[3 MYA]	0.019		0.058	0.047	0.042	0.069	0.193	0.208	0.192
Crematogaster rothneyi	[4 THA]	0.070	0.061		0.060	0.060	0.034	0.208	0.207	0.190
Crematogaster rothneyi	[6 SEB]	0.053	0.050	0.064		0.013	0.074	0.195	0.213	0.204
Crematogaster rothneyi	[8 SUL]	0.052	0.044	0.064	0.013		0.071	0.201	0.215	0.204
Crematogaster yaharai	[9 CAM]	0.085	0.073	0.035	0.079	0.076		0.223	0.214	0.208
Crematogaster coriaria	[cor]	0.228	0.224	0.246	0.227	0.235	0.267		0.197	0.235
Crematogaster ferrarii	[fer]	0.245	0.246	0.243	0.252	0.255	0.254	0.228		0.141
Crematogaster rogenhoferi	[rog]	0.223	0.223	0.221	0.240	0.240	0.246	0.283	0.158	

Table 4. Percent mitochondrial cytochrome *c* oxidase (*COI*: 175 bp) sequence divergence among populations of *Crematogaster* species examined. The *p*-distance (upper right) and the K2P distance (lower left) are shown. Locality codes correspond to table 1

		[1 IND]	[2 BAN]	[3 MYA]	[5 VIE]	[6 SEB]	[7 PHI]	[8 SUL]	[9 CAM]
Crematogaster rothneyi	[1 IND]		0.017	0.017	0.034	0.091	0.034	0.034	0.137
Crematogaster rothneyi	[2 BAN]	0.017		0.023	0.040	0.097	0.040	0.051	0.131
Crematogaster rothneyi	[3 MYA]	0.017	0.023		0.051	0.103	0.051	0.051	0.137
Crematogaster rothneyi	[5 VIE]	0.035	0.041	0.054		0.069	0.057	0.046	0.171
Crematogaster rothneyi	[6 SEB]	0.098	0.104	0.111	0.072		0.097	0.109	0.200
Crematogaster rothneyi	[7 PHI]	0.035	0.041	0.054	0.060	0.104		0.046	0.149
Crematogaster rothneyi	[8 SUL]	0.035	0.053	0.054	0.047	0.118	0.047		0.160
Crematogaster yaharai	[9 CAM]	0.152	0.145	0.152	0.195	0.233	0.167	0.180	

SYSTEMATICS

Order Hymenoptera Linnaeus, 1758
Family Formicidae Latreille, 1809
Subfamily Myrmicinae Lepeletier de Saint-Fargeau, 1835
Genus *Crematogaster* Lund, 1831

The *Crematogaster rothneyi* group

The *Crematogaster rothneyi* group can be distinguished from other Asian *Crematogaster* species by the following characters: (1) mandible with four teeth; (2) 3-segmented antennal club; (3) a deep metanotal groove; (4) developed propodeal spines; (5) petiole broader anteriorly; (6) bilobed postpetiole with distinct longitudinal median sulcus; (7) punctuated mesosoma; (8) stout body setae. This species group is similar to *C. coriaria* in having features (3), (4), (5) and (7), but can be distinguished from *C. coriaria* by having features (1), (2), (6) and (8) (Hosoishi and Ogata 2015). *Crematogaster coriaria* is known from Peninsular Malaysia, Sumatra, Java and Borneo (Hosoishi and Ogata 2015). These large-sized *Crematogaster* ants are easily found in the field. Despite having relatively few samples, the distribution range of the two species does not appear to overlap, and the two species *C. coriaria* and *C. rothneyi* are considered to have an allopatric distribution. While *C. coriaria* typically inhabits well-developed forests and nests in dead wood (Hosoishi and Ogata 2015), the natural history of the *C. rothneyi* group is poorly known. Worker specimens of the herein described *C. yaharai* sp. nov. were collected from a disturbed community forest in Kampong Chhnang Province, Cambodia.

Synonymic list of the *Crematogaster rothneyi* group

C. rothneyi Mayr, 1879 = *C. rothneyi civa* Forel, 1902 syn. nov.
C. rothneyi haputalensis Forel, 1913
C. yaharai Hosoishi and Ogata sp. nov.

Key to species based on the worker caste

1. Antennal segments V and VI each not longer than broad. Mesopleuron densely sculptured or central region of mesopleuron smooth. Dorsal surface of propodeum sculptured reticulately. Lateral surface of propodeum sculptured reticulately or areolately .... *C. rothneyi*
-   Antennal segments V and VI each 1.5 times longer than broad. Central region of mesopleuron smooth. Dorsal surface of propodeum smooth or weakly punctuated. Lateral surface of propodeum smooth .... *C. yaharai* sp. nov.
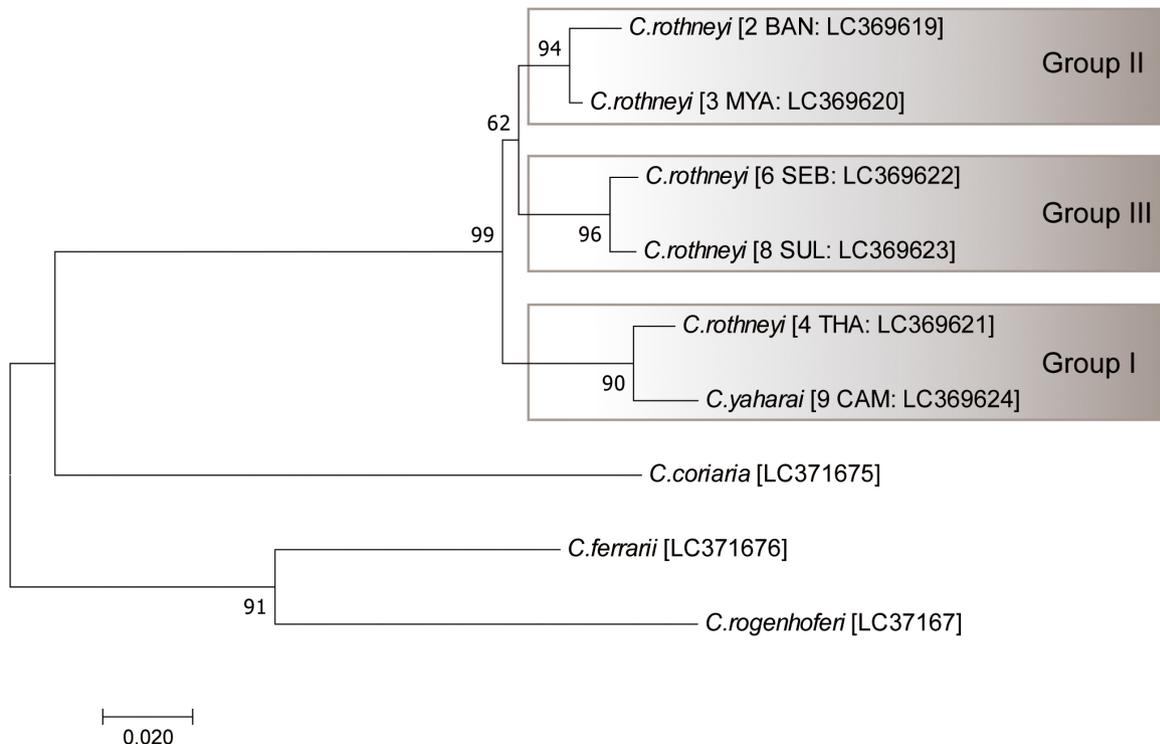
Fig. 2. Maximum likelihood tree for *Crematogaster rothneyi* and *C. yaharai* inferred from 12S rRNA sequences (12S, 387 bp). Numbers above nodes indicate the bootstrap values. Please note, only one sequence from each population was available.

Crematogaster rothneyi **Mayr, 1879**
(Figs. 3, 5A)

Crematogaster rothneyi F. Smith, 1873: viii. Nomen nudum, attributed
    to Mayr.
Crematogaster rothneyi Mayr, 1879: 685. Worker.
Crematogaster rothneyi var. *civa* Forel, 1902: 203. Worker.

Type material of Crematogaster rothneyi Mayr, 1879 (examined): Lectotype worker (by present designation) and two paralectotype workers, INDIA, Calcutta (Rothney leg.) (NHMW). Type locality: INDIA, Calcutta (Rothney) (NHMW). Karyotype by Imai et al., 1984: 6. Combination in *C.* (*Acrocoelia*) by Emery, 1922: 151; in *C.* (*Crematogaster*) by Blaimer, 2012: 55.

Type material of Crematogaster rothneyi civa Forel, 1902 (examined): Five syntype workers, INDIA, Poona (Wroughton leg.) (MHNG). Type locality: INDIA, Poona (Wroughton) (MHNG). Combination in *C.* (*Acrocoelia*) by Emery, 1922: 151. Syn. nov.

Additional Specimens examined: INDIA: 1 worker, Calcutta, West Bengal, 23.ix.1978 (H. Imai leg.) (KUEC); BANGLADESH: 5 workers, Chitragong, FRI, 23.i.1995 (K. Ogata leg.) (KUEC); MYANMAR: 4 workers, Bagan, 23.ii.2002 (MM02-SKY-01) (Sk. Yamane leg.) (KUEC); THAILAND: 2 workers, 500-600 m alt., Doi Chiang Dao, nr Chiang Mai, 2.iv.2005 (Sk. Yamane leg.) (KUEC); 2 workers, 650-700 m alt., leaf litter, Doi Chiang Dao, nr Chiang Mai, 3.iv.2005 (Sk. Yamane leg.) (KUEC); VIETNAM: 1 worker, Hat Lot, Mai Son Dist., Son La Prov., 26.xi.1999 (K. Ogata leg.) (KUEC); INDONESIA: 4 workers, coconut log, Pulau Sebesi, Lampung Prov., Sunda Strait, 11.viii.2005 (RK05-SKY-12) (Sk. Yamane leg.) (KUEC); 4 workers, foraging on ground, Sulawesi, Mts. Tilongkabila, 100 m alt., Gorontalo Prov., 27.i.2010 (CE10-SKY-37) (Sk. Yamane leg.) (KUEC); PHILIPPINES: 1 worker, Apolong, Valencia, near Dumaguete, Negros Oriental, 30.xii.1998 (Sk. Yamane leg.) (KUEC).

Diagnosis: This species is very similar to *C. yaharai* sp. nov., but can be distinguished by the broader than long antennal segments V to VI, sculptured mesopleuron, and reticulately or areolately sculptured propodeum. The Indian specimen examined does not have distinctly developed longitudinal rugulae on the higher half of the dorsal surface of the head (Fig. 3B). The Thai specimens have broader or as broad as long antennal segments V and VI and a sculptured propodeal dorsum.

Measurements and indices of worker: HW 0.6-0.84; HL 0.63-0.85; CI 95-105; SL 0.66-0.85; SI 99-117; EL 0.14-0.22; PW 0.4-0.53; WL 0.72-1.01; PSL 0.05-0.14; PtL 0.22-0.30; PtW 0.24-0.33; PtH 0.16-0.21; PpL 0.16-0.21; PpW 0.24-0.36; PtHI 60-80; PtWI 103-132; PpWI 150-188; WI 96-116 (Fourteen workers measured).

General description of worker: Polymorphic workers with moderate size variation.

Head subquadrate in full-face view, with weakly concave posterior margin, angular posterior corners and convex sides. Occipital carinae developed. Mandible with four teeth, apical and subapical teeth large. Anterior margin of clypeus convex with slightly impressed median portion; anterolateral margins of clypeus protruded anteriorly; posterior margin of clypeus rounded between frontal lobes. Frontal carinae almost parallel. Antennae 11 segmented; relative scape length variable with worker size (SI, 99-117); SI lower in larger specimens; antennal club 3-segmented. Scape exceeding posterior corner of head by 1/4 of its length or more. Antennal segments V and VI each broader than long or as broad as long. Compound eyes distinctly projecting beyond lateral margins of head in full-face view, especially in large workers.

Pronotum and mesonotum fully fused without defined suture. In lateral view, posterior half of dorsolateral margin of mesonotum forming triangle-shaped process, anterior half forming almost flat outline. Mesothoracic spiracle reduced to form small pit dorsoventrally. Metapleural gland opening slit-shaped. Propodeal spiracle elliptical, situated at posterolateral corner, apart from metapleural gland bulla. Metanotal groove straight in dorsal view, deep and forming concave region between mesonotum and propodeum. In dorsal view, longitudinal rugulae not connecting between mesonotum and propodeum; the boundary distinct. Propodeal spines developed; length variable in several specimens.

Petiole scoop-shaped, broader anteriorly, longer than broad in dorsal view; spiracle situated at midportion between dorsal and ventral margin of petiole in lateral view, directed posterolaterally. Postpetiole with distinct longitudinal median sulcus, bilobed in dorsal view; spiracle situated anteriorly on lateral surface in lateral view.

Dorsum of head sculptured with longitudinal rugulae, but weakly developed on posterior half. Clypeus with longitudinal rugulae. Promesonotum sculptured reticulately or areolately. Mesopleuron densely sculptured in Indian specimen, or central region of mesopleuron smooth with weakly sculptured surrounding in other specimens. Dorsal surface of propodeum sculptured reticulately. Lateral surface of propodeum sculptured reticulately or areolately. Dorsal and lateral surface of petiole sculptured. Dorsal and lateral surface of postpetiole sculptured. Median sulcus of postpetiole smooth.

Standing pilosity sparse. Dorsum of head with

short and erect setae. Clypeus with some pairs of erect setae. Anterior clypeal margin with one pair of long setae mixed with short setae laterally. Mesonotal dorsum with sparse erect setae. Petiole with suberect setae. Postpetiole with suberect setae. Fourth abdominal tergite with sparse erect setae.

Body almost entirely yellow, or brown, except for antenna yellowish.

Distribution: This species is known from India, Bangladesh, Myanmar, Thailand, Vietnam, Indonesia (Pulau Sebesi, Sulawesi) and the Philippines (Fig. 1). Tiwari (1999) collected this species in Tamil Nadu, Gujarat, Maharashtra, and West Bengal States of India.

Remarks: The syntype workers of *C. rothneyi civa* match well with syntype workers of *C. rothneyi*. We treated *C. rothneyi civa* as a junior synonym of *C. rothneyi*.
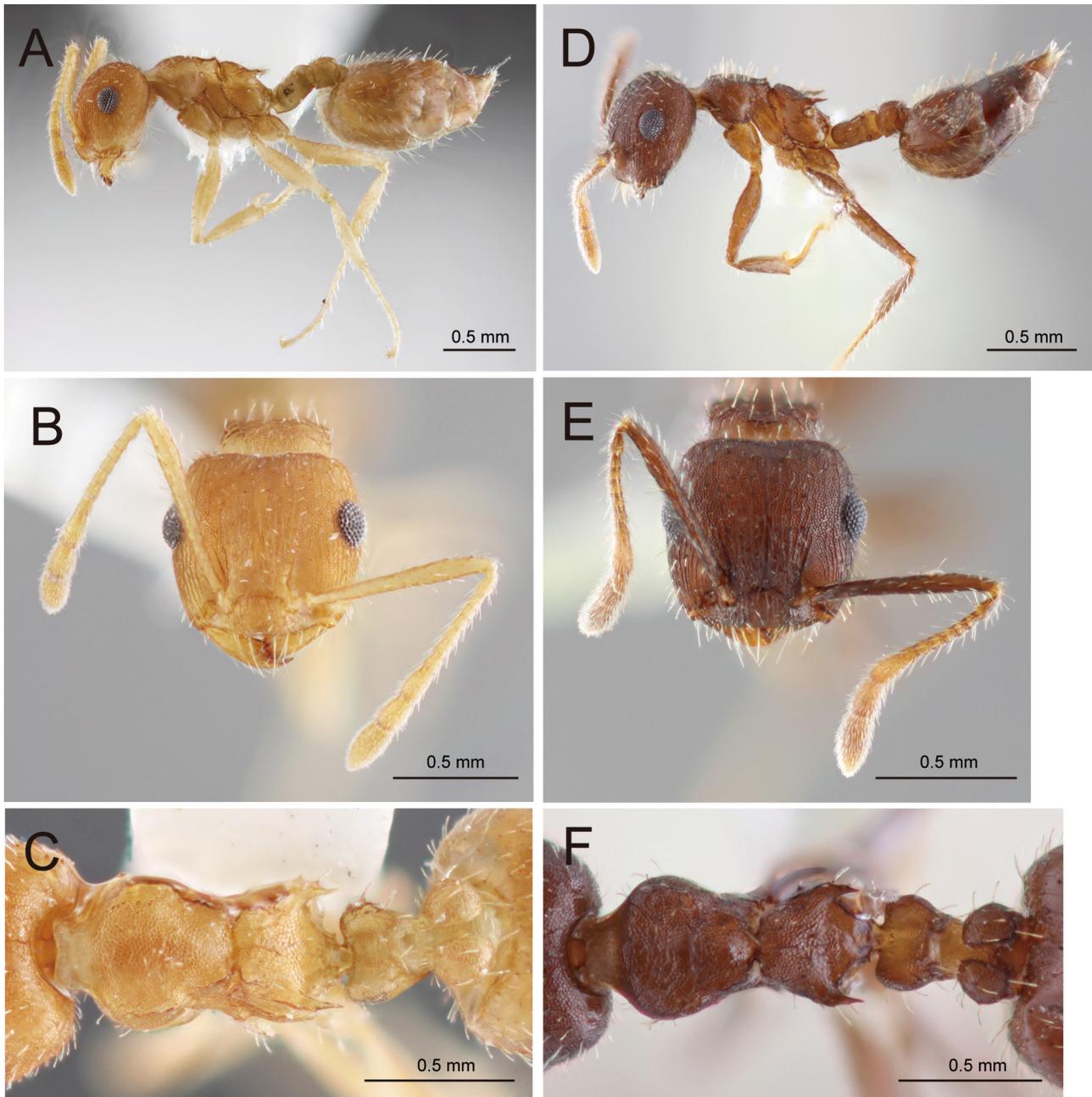
Fig. 3. *Crematogaster rothneyi*. (A, B, C) non-type worker from Calcutta, India (HW 0.8; WL 0.9). (A) body in lateral view; (B) full-face view of head; (C) dorsal view of mesosoma, petiole and postpetiole. (D, E, F) non-type worker from Sulawesi, Indonesia (HW 0.74; WL 0.88). (D) body in lateral view; (E) full-face view of head; (F) dorsal view of mesosoma, petiole and postpetiole.

Crematogaster rothneyi haputalensis Forel, 1913

Crematogaster rothneyi var. *haputalensis* Forel, 1913: 75. Queen.

Type locality: SRI LANKA, Haputale, 5000 ft. (types not found in MHNG) [not examined]. Combination in *C.* (*Acrocoelia*) by Emery, 1922: 152.

Remarks: We were not able to examine type material of *C. rothneyi haputalensis*. Forel's original description (1913) was based on a single queen collected under a stone. Forel suggested that the queen of *C. rothneyi hapulatelensis* had finer body striation and punctures than *C. rothneyi civa* did. The taxonomic status of *C. rothneyi haputalensis* will remain uncertain until the nest series become available. This taxon is known only from the type locality in Sri Lanka (Fig. 1).

Crematogaster yaharai Hosoishi and Ogata sp. nov.
(Figs. 4, 5B)
urn:lsid:zoobank.org:act:D059589E-7541-4820-860A-833B5BB6DF03

Type material: Holotype worker, CAMBODIA, Kampong Chhnang (community forest) 12.i.2010 (SH10-Cam-55) (S. Hosoishi) (THNHM). Four paratype workers same data as holotype (BMNH, CASC, KUEC, MHNG).

Etymology: The specific name is dedicated to Japanese biologist Dr. Tetsukazu Yahara, who helped our field surveys in Cambodia.

Diagnosis: This species is very similar to *C. rothneyi*, but can be distinguished by the longer than broad antennal segments V and VI, smooth mesopleuron and smooth or weakly punctuated propodeum.

Measurements and indices of holotype worker: HW 0.78; HL 0.77; CI 101; SL 0.76; SI 97; EL 0.2; PW 0.48; WL 0.89; PSL 0.11; PtL 0.26; PtW 0.33; PtH 0.17; PpL 0.2; PpW 0.33; PtHI 65; PtWI 127; PpWI 165; WI 100.

Measurements and indices of paratype workers: HW 0.63-0.76; HL 0.63-0.75; CI 99-101; SL 0.68-0.81; SI 104-115; EL 0.18-0.24; PW 0.41-0.48; WL 0.71-0.88; PSL 0.08-0.11; PtL 0.23-0.28; PtW 0.27-0.33; PtH 0.15-0.19; PpL 0.16-0.21; PpW 0.28-0.34; PtHI 64-70; PtWI 104-122; PpWI 148-175; WI 97-115 (Four paratype workers measured).

General description of worker: Polymorphic workers with moderate size variation.

Head subquadrate in full-face view, with weakly concave posterior margin, angular posterior corners and convex sides. Occipital carinae developed. Mandible with four teeth, apical and subapical teeth large. Anterior margin of clypeus convex with slightly impressed median portion; anterolateral margins of clypeus protruded anteriorly; posterior margin of clypeus rounded between frontal lobes. Frontal carinae almost parallel. Antennae 11 segmented; relative scape length variable with worker size (SI, 97-115); SI lower in larger specimens; antennal club 3-segmented. Scape exceeding posterior corner of head by 1/4 of its length or more. Antennal segments V and VI each 1.5 times longer than broad. Compound eyes distinctly projecting beyond lateral margins of head in full-face view, especially in large workers.

Pronotum and mesonotum fully fused without defined suture. In lateral view, posterior half of dorsolateral margin of mesonotum forming triangle-shaped process, anterior half forming almost flat outline. Mesothoracic spiracle reduced to form small pit dorsoventrally. Metapleural gland opening slit-shaped. Propodeal spiracle elliptical, situated at posterolateral corner, apart from metapleural gland bulla. Metanotal groove straight in dorsal view, deep and forming concave region between mesonotum and propodeum. In dorsal view, longitudinal rugulae not connecting between mesonotum and propodeum. Propodeal spines short.

Petiole scoop-shaped, broader anteriorly, longer than broad in dorsal view; spiracle situated at midportion between dorsal and ventral margin of petiole in lateral view, directed lateroposteriorly. Postpetiole with distinct longitudinal median sulcus, bilobed in dorsal view; spiracle situated anteriorly on lateral surface in lateral view.

Dorsum of head weakly sculptured with longitudinal rugulae. Clypeus with longitudinal rugulae in large workers, but weakly punctuated in small workers. Promesonotum punctuated. Central region of mesopleuron smooth, but higher anterior and lower posterior regions weakly sculptured. Dorsal surface of propodeum smooth or weakly punctuated. Lateral surface of propodeum smooth and shining. Dorsal and lateral surface of petiole sculptured. Dorsal and lateral surface of postpetiole sculptured. Median sulcus of postpetiole smooth.

Standing pilosity sparse. Dorsum of head with short and erect setae. Clypeus with some pairs of erect setae. Anterior clypeal margin with one pair of long setae mixed with short setae laterally. Mesonotal dorsum with sparse erect setae. Petiole with suberect setae. Postpetiole with suberect setae. Fourth abdominal tergite with sparse erect setae.

Body color: Yellow-brown.

Distribution: This species is only known from the type locality in Cambodia (Fig. 1).
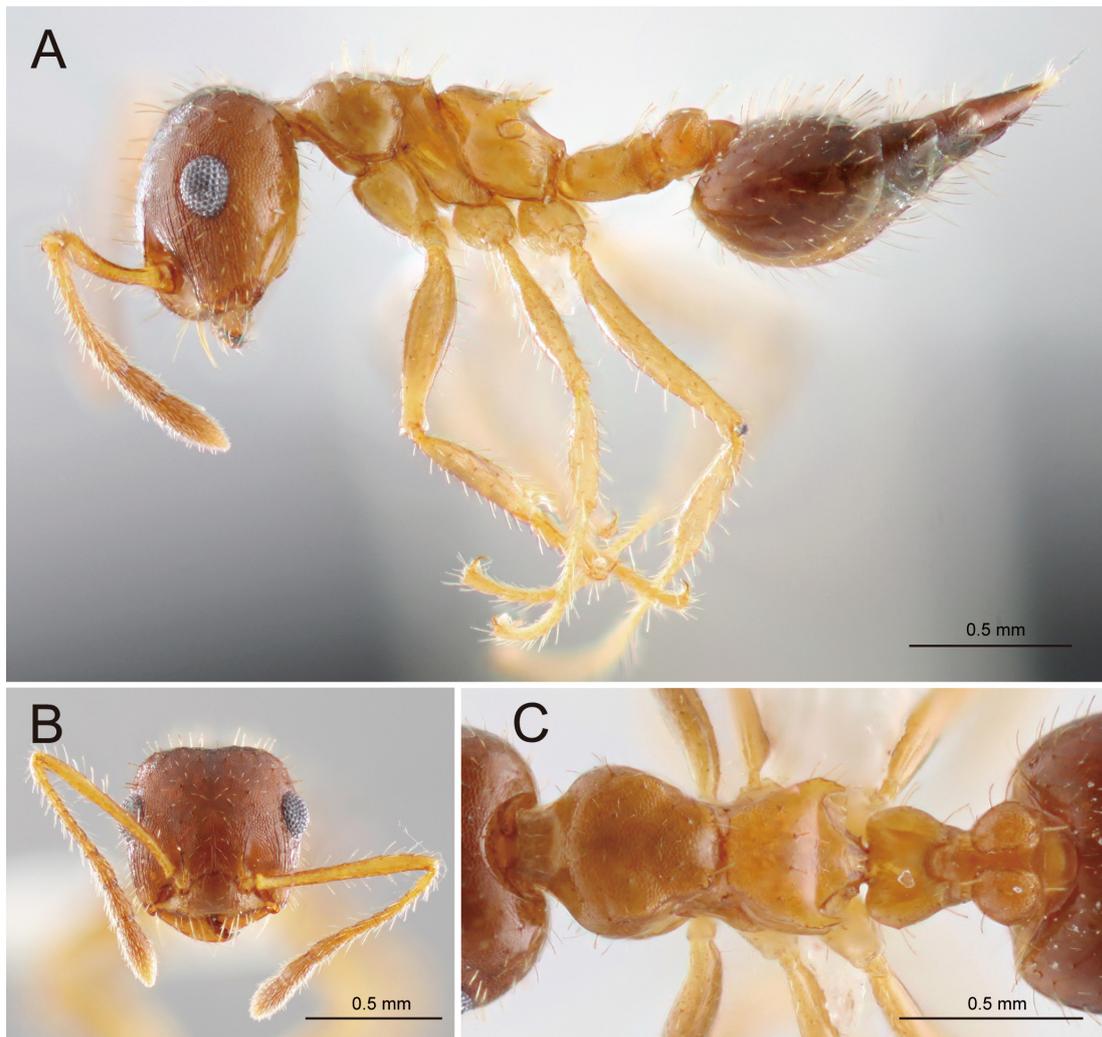
Fig. 4. *Crematogaster yaharai*, paratype worker from Cambodia (HW 0.72; WL 0.83). (A) body in lateral view; (B) full-face view of head; (C) dorsal view of mesosoma, petiole and postpetiole.
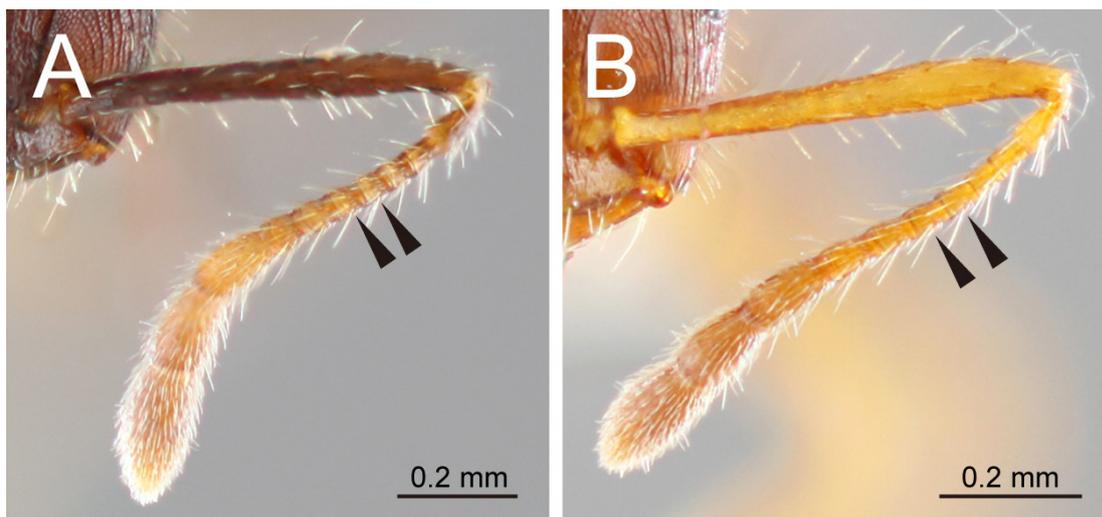
Fig. 5. Left antenna. (A) *Crematogaster rothneyi*; (B) *Crematogaster yaharai*. Arrow indicates antennal segments V and VI.

DISCUSSION

Despite the wide distribution range of *Crematogaster rothneyi*, relatively low genetic variation was observed between the Southwest and Southeast Asian populations (Tables 3, 4). For example, although Calcutta (India) is approximately 5,000 km from Sulawesi (Indonesia), the sequence divergence of *COI* sequences of specimens from these two areas was only 3.5% (K2P distance, 6/175 bp) (Table 4). Similarly, the divergence of 12S sequences of specimens from Bangladesh and Sulawesi was only 5.2% (K2P distance, 21/387 bp) (Table 3). In addition, this level of sequence divergence is considered low among allopatric populations (*e.g.*, Balke et al. 2018). Indeed, no marked morphological differences were observed in the worker caste of *C. rothneyi* in our collections. The longitudinal rugulae on the higher half of the dorsal surface of the head were not found to be distinct in the Indian specimen, but they were weakly developed in the other Southeast Asian specimens. Such slight molecular and morphological differences typically suggest fairly recent population divergence.

Interestingly, the Cambodian specimens (*C. yaharai*) were distantly related to the western and eastern populations, exhibiting a 12S sequence divergence of 7.3%–8.5% (12S, K2P distance, 387 bp) (Table 3) and a *COI* sequence divergence of 14.5%–23.3% (*COI*, K2P distance, 175 bp), although the sequence divergence for 12S was 3.5% between the Cambodian and Thai specimens (discussed below). Compared to the sequence divergence values observed for the Asian *Crematogaster* species, these levels of mtDNA sequence divergence seem to be sufficiently high for species delimitation (Hosoishi and Ogata 2014 2015 2016). For the 12S region, the sequence divergence between *C. ferraii* (Vietnamese specimen, LC371676) and its closely related but distinct species (Sulawesi specimen, LC474370) was 4.8% (370 bp). This deep genetic gap indicates that Cambodian populations have been genetically isolated from other *C. rothneyi* populations for a very long time.

The deeply divergent mtDNA lineages may be due to an introgression from related species (Toews and Brelsford 2012). The 12S sequence of the Cambodian specimen was highly divergent from the sequence of the related species *C. coriaria* (Table 3), which excludes the possibility of introgression from the related *Crematogaster* species.

The Cambodian specimen (*C. yaharai*) was both morphologically and genetically separate from *C. rothneyi*, while the Thai specimen was genetically close to the Cambodian one (*C. yaharai*) and morphologically similar to *C. rothneyi*. Such discordance between morphology and mtDNA phylogeny suggests interspecific hybridization and introgression (Goropashnaya et al. 2004; Galkowski et al. 2017). The Thai population had mitochondrial sequence typical to *C. yaharai*, presumably suggesting introgression of mtDNA from *C. yaharai* to the Thai population of *C. rothneyi*.

The deep genetic gap found between Cambodian sequences and most other Southeast Asian sequences suggests that the Cambodian populations were originally confined to smaller regions in the Indochinese Peninsula. Then they might have dispersed throughout the Indochinese Peninsula. The low genetic divergences between western and eastern populations may be because the populations spread rapidly through South- and Southeast Asia from smaller parts of the Sundaic region, when the region was connected to continental Asia by a land bridge due to low sea levels (Woodruff 2010).

Our molecular analyses revealed the existence of three clades within the *C. rothneyi* group, but we consider only the Cambodian series to be a distinct species. Since the Cambodian specimens exhibited marked genetic and morphological differences compared to *C. rothneyi*, this study describes the Cambodian specimens as a new species, *C. yaharai* sp. nov. In the obtained phylogeny, the Thai specimens were clearly separated from the clade comprising the group II and III specimens. Nevertheless, the Thai specimens did not show any distinct morphological differences in the worker caste that would warrant its separation from *C. rothneyi*; therefore, we considered that, while the Thai specimens were genetically different, they were too morphologically similar to warrant consideration as a separate taxon, even though slight morphological differences were observed. We therefore treated these specimens as conspecific to *C. rothneyi*. Recognition of *C. yaharai* within the *C. rothneyi* group rendered *C. rothneyi* paraphyletic in the material analyzed (Fig. 2). Blaimer and Fisher (2013) reported that sequence divergence between *C. degeeri* and *C. ramamy* was low (4.7%, *COI* 592 bp), and that the two species were not monophyletic. They suggested that this could be attributed to a recent or ongoing speciation event, and distinguished them clearly based on morphological characters and ecological differences. In phylogenetic studies of the ant genus *Linepithema*, Wild (2007) considered *L. neotropicum* to be paraphyletic based on the mtDNA phylogeny, but the nuclear gene phylogeny showed that the sister species were monophyletic. He suggested that mitochondrial introgression had occurred between *L. neotropicum* and *L. cerradense*. Unfortunately, due to the age and condition of the specimens used in our study, we

could not obtain nuclear gene sequences for analysis. However, a comparison between mtDNA and nuclear gene phylogenies using fresh material may reveal the relationships between these cryptic species.

Our molecular data are clearly limited, as only short lengths of the 12S and *COI* regions were sampled from each population. Phylogenetic relationships among the geographical populations should be considered in the future, when more fresh material becomes available.

Taxa that are closely related or sister to the Sundaic species have already been reported from the Indochinese Peninsula in Asian ants (*e.g.*, Ward 2001; Jaitrong and Yamane 2011; Jaitrong et al. 2012; Hosoishi and Ogata 2014 2016; Eguchi et al. 2016; Phengsi et al. 2018), but the finding that *C. yaharai* differs from those is unique to this study. According to a review by Lohman et al. (2011), terrestrial invertebrates from mainland Southeast Asian rarely show genetic differentiation (*e.g.*, weaver ant by Azuma et al. 2006; giant wood spider by Su et al. 2007), but vertebrates often do (*e.g.*, Patou et al. 2010; Chan et al. 2013). *Crematogaster rothneyi* is widely distributed in South and Southeast Asia, and its sister species, *C. yaharai* sp. nov., is found within the total distribution range of *C. rothneyi*. Its collection locality is not a geographically isolated area, such as high mountains, but lowland forest. Few similar findings have been reported in the region. Among palm civets (*Paradoxurus* spp.), *P. hermaphroditus cochinensis* is considered endemic to southern Vietnam and Cambodia, but Patou et al. (2010) found no evidence for the subspecies in their phylogeographic studies. The dolichoderine ant *Technomyrmex yamanei* is known only from northern Thailand and Vietnam, and the sister species *T. modiglianii* is widely distributed throughout the Indochinese Peninsula, Peninsular Malaysia, Borneo, Java, Sumatra and Sulawesi (Bolton 2007), but those two ant species can be clearly distinguished from each other based on morphology.

Widespread species with sister species that have a more restricted distribution are considered to reflect instances of recent speciation. Condamine et al. (2013) estimated that the sister peacock swallowtail species *Papilio arcturus* (northern India to Indochina) and *P. hopponis* (Taiwan) diverged during the Pliocene. Molecular dating analyses suggest that an ancestor or population of the butterfly *Delias hyparte* (Nepal, India, Thailand to Indonesia) colonized Sulawesi 0.90 Mya, after which time then the two related Sulawesi species, *D. rosenbergi* and *D. mitisi*, diverged (Morinaka et al. 2017). In phylogenetic studies of the Sundaic *Hylobates* gibbon species, Chan et al. (2013) suggested that the Indochinese species, *H. pileatus*, diverged initially from the other gibbons about 3 Mya, and that its distribution

then decreased while the populations of the other species expanded. Hosoishi and Ogata (2017) estimated the divergence time for the most common ancestor of the Indochinese species, *C. khmerensis*, and the Sundaic clade to be 15 Mya, in the middle Miocene. We did not estimate the divergence time for the *C. rothneyi* group due to the short length of the mtDNA sequences used in this study, but we consider it to be more recent than that proposed by Hosoishi and Ogata (2017). Indeed, the slight morphological differences suggest that speciation occurred recently, presumably in the Pleistocene. The findings of this study suggest that overlooked cryptic ant species remain to be discovered on the Indochinese Peninsula, especially among widespread Asian species. Since these species are not easily recognizable, both morphological and molecular taxonomic methods, and possibly other means of analysis, will be required to reveal the existence of cryptic species diversity.

CONCLUSIONS

Morphological and mitochondrial gene data suggest the existence of cryptic species with the widespread Asian ant *Crematogaster rothneyi*. We describe the Cambodian series as a new species, *C. yaharai* sp. nov., based on unique antennal morphology and mesosomal sculpture patterns with molecular evidence. Widespread species with sister species that have a more restricted distribution, suggesting recent diversification within the Indochinese Peninsula.

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