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First records of *Cardiocondyla* ants from Myanmar

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ABSTRACT. Numerous species of the diverse ant genus *Cardiocondyla* have been reported from countries in Southeast Asia, but as yet nothing has been known about their presence in Myanmar. A short, preliminary survey documents the occurrence of at least five species of *Cardiocondyla* in anthropogenically disturbed areas, such as gardens and parks. The species most commonly sampled has been determined as *C. tjibodana*, other specimens as *C. parvinoda, C. obscurior*, *C. itsukii* and one or two taxa of the unresolved *C. kagutsuchi* complex. Further studies, in particular in more natural habitats, will certainly reveal the presence of additional species of this highly interesting genus.

Keywords *Cardiocondyla*, biodiversity, invasive ants, Myanmar

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INTRODUCTION

The myrmicine genus *Cardiocondyla* is of considerable interest as it shows a striking diversity of life histories, reproductive strategies of its males, and ecological preferences (Heinze 2017). Including undescribed species stored in the collection of Senckenberg Museum of Natural History Görlitz, the genus is estimated to consist of about 80 valid species, which are widely distributed throughout temperate and tropical areas of Africa, Asia, Australia, and Europe (Seifert 2002). A handful of tramp species have successfully colonized anthropogenically disturbed areas in America and have also reached remote islands, such as Hawai'i, Galapagos, and St. Helena (e.g., Heinze et al. 2006; Wetterer 2012, 2014). Most *Cardiocondyla* species have wingless, "ergatoid" males, which either co-occur with or have completely replaced winged disperser males. In some species, these wingless males are fierce fighters, which engage in fatal combat for access to female sexuals in their nests (Kinomura & Yamauchi 1987; Stuart et al. 1987; Heinze et al., 1998); in other species, they defend small areas in multi-chambered nests against rivals (Jacobs and Heinze 2017), and in still others they are mutually tolerant (Lenoir et al. 2007). The recent finding that presumed female sexuals of a novel socially parasitic species *C. zoserka* (Bolton 1982) are indeed the regular winged males of the host species adds a further facet to this fascinating diversity (Heinze 2020).

According to phylogenetic studies, the origin of *Cardiocondyla* is South and Southeast Asia (Heinze 2017, but see Blaimer et al. 2018). An Asian origin is supported by the high diversity of this genus in India, Indonesia, New Guinea, and the Philippines (Seifert 2002). Nevertheless, because *Cardiocondyla* ants are tiny and their colonies usually consist only of a few dozen or hundreds of individuals, they are often missing from local inventories of ants. For example, Myanmar is presently a blank spot in the distribution map of *Cardiocondyla* and neither Bingham (1903) nor Chapman & Capco (1951) list *Cardiocondyla* for Myanmar, even though several species have been reported from neighboring countries (e.g., Forel 1903; Jaitrong & Nabhitabhata 2005, Ghosh et al. 2006; Eguchi et al. 2011; Bharti et al. 2016). A short journey to Myanmar yielded some twenty samples of *Cardiocondyla,* which by morphology and sequencing of the mitochondrial genes for cytochrome c oxidase subunits I and II were tentatively identified as belonging to at least five different taxa.

MATERIAL AND METHODS

In March 2020, foragers and dispersing alate or dealate female sexuals of *Cardiocondyla* were collected with an aspirator in private gardens, parks, and along roadsides in various places in Myanmar (Table 1) and stored in 100% EtOH. Species were identified by morphological comparison with previously collected samples. In addition, we compared sequences of the mitochondrial genes of cytochrome c oxidase subunits I and II including tRNA-Leu with sequences obtained from GenBank and earlier analyses (e.g., Heinze et al. 2005; Oettler et al. 2010; Seifert et al. 2017).

DNA was extracted following a CTAB protocol (Sambrook & Russell 2001), amplified by PCR as described before (Heinze et al. 2005; Heinze 2020), and sequenced by LGC Genomics (Berlin, Germany). The sequences of 1360 to 1460 base pairs were aligned using the CLUSTAL W Algorithm (Thompson et al. 1994) in the software BioEdit v 7.09 (Hall 1999). Mega X (Kumar et al. 2018) indicated a general time reversible model with gamma-distributed rate variation among sites and invariable sites (GTR+G+I, Nei & Kumar 2000) to best reflect the substitution pattern. A maximum likelihood phylogenetic tree was constructed using the software RAxML (Stamatakis 2014) and the graphical interface raxmlGUI2 (Edler et al. 2019). Sequences of *C. obscurior* were defined as outgroup based on previous phylogenies of the genus (Heinze et al. 2005; Oettler et al. 2010).

RESULTS

Cardiocondyla ants are very small (body size 1.5 – 3mm) and workers forage solitarily and inconspicuously. They are therefore often overlooked. Our collection revealed at least five different species of this genus, which previously had not been reported from Myanmar.

C. tjibodana **Karavajev, 1935** (for an image see CASENT0916972 at antweb.org) is the sibling of the more widely distributed "lesser sneaking ant" *C. minutior* Forel 1899 (Wetterer 2014), from which it differs by narrower waist segments and larger eyes (Seifert unpublished data). Originally described from Cibodas, Java, Indonesia, *C. tjibodana* has also been recorded in Southeast Asia from the Philippines and Malaysia (Seifert 2002). In contrast to many other *Cardiocondyla*, *C. tjibodana* and *C. minutior* have both winged and wingless males (Heinze 1999; Fuessl et al. 2015).

In Myanmar, ants determined by morphology as *C. tjibodana* were found to be abundant throughout man-made parks and gardens (Table 1). In the mtDNA-based phylogeny, the material from Myanmar forms a clade separate from *C. tjibodana* from Indonesia and Australia and also from *C. minutior* from Indonesia, Japan, Taiwan, and the United States (Fig. 1). As many taxa of *Cardiocondyla* are difficult to identify based on few individuals, additional morphometry and the analysis of adequate markers of nuclear DNA might be required to solve this apparent mismatch between morphometry and molecular data.

Table 1. Collecting sites of specimens of various taxa of the ant genus *Cardiocondyla* in Myanmar.

Species	Sample ID	Collecting date	Collecting site
C. tjibodana	MM1 – MM4	6.3.20	Yangon, Shwe Taung Gyar, private garden 16°49.1‘ N 96° 08.9’E workers and dealate queen
	MM6c	7.3.20	Moeyingyi Wetland Park, entrance / museum 17°35.4’N, 96°34.5’ E
	MM7	9.3.20	Bago, hotel garden 17° 19.4’N, 96°27.4’ E
	MM8	10.3.20	Phyu Rest Area, 115 mile car park, front yard 18°30.5’N, 96°23.4’E
	MM9	10.3.20	Meiktila, hotel garden 20° 52.5’N, 95°51‘ E
	MM12	11.3.20	Nwadama, Inle Lake, edge of field 20° 35.9’N, 96°52.9’E
	MM13a	12.3.20	Nwadama, Inle Lake, hotel garden 20° 35.9’N, 96°52.8’E
	MM14 – M15	12.3.20	Nwadama, Inle Lake, hotel garden 20° 35.9’N, 96°52.8’E
	MM17	14.3.20	Bagan, hotel garden 21° 10.5’N, 94°51.9’E
	MM19 – M20	17.3.20	Yangon, Shwe Taung Gyar, private garden 16°49.1‘ N 96° 08.9’E
C. parvinoda	MM6b	7.3.20	Moeyingyi Wetland Park, entrance / museum 17°35.4’N, 96°34.5’ E
near *C. kagutsuchi* lineages A, B	MM6a	7.3.20	Moeyingyi Wetland Park, entrance / museum 17°35.4’N, 96°34.5’ E
near *C. kagutsuchi* lineage C	MM13b	12.3.20	Nwadama, Inle Lake, hotel garden 20° 35.9’N, 96°52.8’E
	MM16	13.3.20	Heho Airport, park 20° 44.6’N, 96°47.6’E
C. itsukii	MM5	7.3.20	Moeyingyi Wetland Park 17°35.4’N, 96°34.7‘ E
	MM11	11.3.20	Green Hill Valley Elephant Camp, Kalaw 20° 43.8’N, 96°29.8’E, 2 dealate queens
	MM18	14.3.20	Bagan, hotel garden 21° 10.5’N, 94°51.9’E, alate queen
C. obscurior	MM10	10.3.20	Meiktila, on tree on lake shore 20° 52.5’N, 95°51‘E

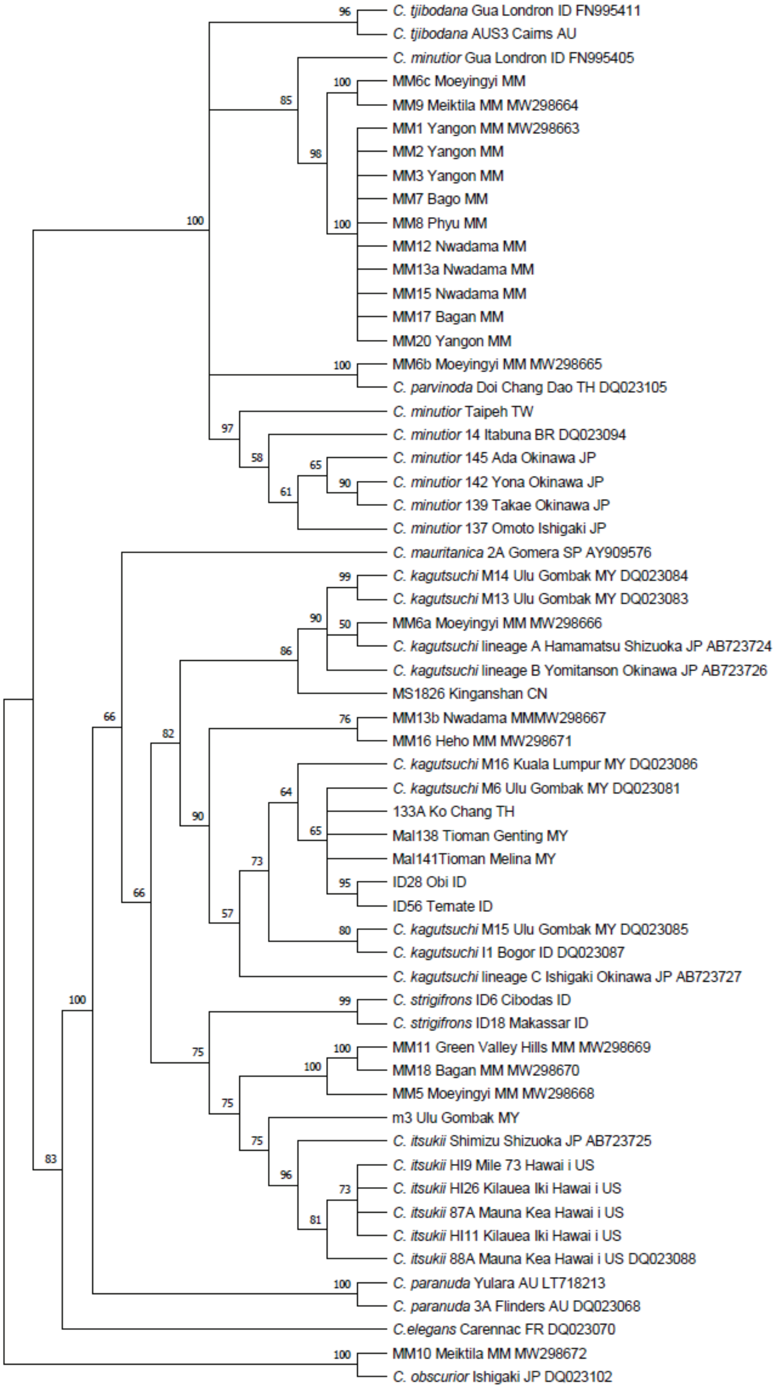

Fig. 1. Maximum likelihood tree of several taxa of the ant genus *Cardiocondyla* from Myanmar (indicated by MM) and related taxa from other areas. The phylogeny is based on 1360 – 1460 base pairs of the mitochondrial genes for cytochrome oxidase I and II, including tRNA-Leu. Sequences of *C. obscurior* were defined as outgroup. The best-scoring tree is shown and support values from 100 bootstraps are given at the nodes. Branches with a support below 50% are collapsed. For sequences from Myanmar and several other samples, GenBank accession numbers are given behind the species label. Myanmar samples without such a number have the same sequence as the closest sample from Myanmar with accession number.

C. parvinoda **Forel 1902** (for an image see CASENT0281807 at antweb.org) is a dark reddish brown ant related to *C. minutior* and *C. tjibodana* (Oettler et al. 2010). It was originally described from Pune, Maharashtra, India and has been reported also from other parts of India (Bharti et al. 2016) and Thailand (Oettler et al. 2010). Its presence on Taiwan is doubtful (Guénard & Dunn 2012). Nothing is known about male morphology in this species. A single worker of *C. parvinoda* was collected at Moeyingyi Wetland Park (Table 1). Its CO I / CO II sequence differed in only 1 of 1360 base pairs from that of a sample from Thailand (Fig. 1).

C. kagutsuchi **Terayama, 1999** (for an image see ANTWEB1041248 at antweb.org) is a complex of morphologically very similar, blackish ants in the *C. nuda* group (Seifert 2002), which are difficult to distinguish and have often been confused with *C. nuda* (Mayr 1866) and *C. mauritanica* Forel 1890. Based on sequences of the genes for mitochondrial cytochrome c oxidase subunits I and II, Okita et al. (2013, 2015) distinguished four lineages of *C. kagutsuchi* only in Japan, of which one (haplotype 2 or lineage D) has recently been described as *C. itsukii* (Seifert et al. 2017, see below). The other three lineages of *C. kagutsuchi* distinguished by Okita et al. (2013, 2015) have been collected in various parts of East and Southeast Asia but have not yet been formally described as separate species. Both male phenotypes and also intermediates have been recorded in these taxa (e.g., Yamauchi et al. 2005; Okita et al. 2013, 2015).

According to Numeric Morphology-Based Alpha-Taxonomy (NUMOBAT, Seifert 2008), lineages A and B match the type of *C. strigifrons* Viehmeyer 1922 from Malang, Java, Indonesia. This, however, is not fully reflected in mtDNA phylogenies (Seifert et al. 2017 and Fig. 1), probably because of incomplete lineage sorting or introgression. In Myanmar, a sample collected at Moeyingyi Wetland Park had a mtDNA sequence close to *C. kagutsuchi* lineages A and B from Japan, while samples collected at Heho Airport and in Nwadama were more similar to *C. kagutsuchi* lineage C (Table 1, Fig. 1).

C. itsukii **Seifert et al., 2017** (for an image see CASENT0922311 at antweb.org) is widely distributed throughout South and East Asia and also known from Hawai'i and other Pacific islands. It apparently lacks winged males and all males that have been found so far were wingless ergatoids (Seifert et al. 2017). Sequences of a dispersing queen from a forest at Green Hill Valley and workers from Moeyingyi Wetland Park and Bagan clustered with samples of *C. itsukii* and *C. strigifrons* Viehmeyer 1922 (Table 1, Fig. 1).

C. obscurior **Wheeler, 1929** (for an image see CASENT0103429 at antweb.org) is a well-studied tramp species with an extremely wide cosmotropical distribution. In contrast to most other species of the genus, which build their nests in the soil, *C. obscurior* lives on trees. Both male phenotypes do occur. The ergatoid males of *C. obscurior* are characterized by long, sabre-shaped mandibles, which they use to eliminate rivals from their nests (Kinomura & Yamauchi 1987; Stuart et al. 1987). A single worker was collected from a tree on the shore of Meiktila Lake. Its sequence was completely identical to that of a sample from Ishigaki, Okinawa, Japan (Table 1, Fig. 1).

DISCUSSION

Our short survey documents that at least five species of *Cardiocondyla* occur in anthropogenically disturbed places in Myanmar. The worldwide revision of *Cardiocondyla* by BS is still in progress and we cannot yet give final species identities to some of the specimens. As can be seen in the mtDNA-based phylogeny in Fig. 1, several clades of *Cardiocondyla* are quite jumbled. As a consequence, future attempts of delimiting species have to focus on informative markers of nuclear DNA and its expression products (e.g., Seifert 2020). Light-microscopic investigation of the architecture of phenotype, as one of the most informative expression products of nuclear DNA, is the only method allowing a non-destructive investigation of name-giving type specimens of these tiny ants. This is mandatory if we want to have testable and stable name allocations of taxonomically available names to genetically delimited clusters (Steiner et al. 2009; Seifert 2018)

A species recognized by morphometry as *C. tjibodana* proved to be particularly common in Myanmar and was found almost everywhere in parks or gardens. Typically, soil-nesting *Cardiocondyla* live close to humid places, such as river banks, irrigation ditches, or beaches (Seifert 2002; Heinze 2017). Not surprisingly, foragers were frequently observed near regularly watered lawns, flower gardens, or the edge of pools. Specimens of the *C. kagutsuchi* complex were also quite widely distributed, while only one worker each of *C. parvinoda* and *C. obscurior* could be detected.

Unfortunately, we could not yet collect in more remote, undisturbed areas, e.g., rainforests or dry forests. The large diversity of ecoregions in Myanmar, from coastal mangroves and freshwater swamp forests to Eastern Himalayan alpine meadows, makes it likely that future studies will reveal many more species of *Cardiocondyla* in this country. For example, in other parts of Southeast Asia and New Guinea, rainforests are habitat of species of the *C. wheeleri, C. sima*, and *C. "argentea"* groups (e.g., Heinze et al. 2005, 2016; Oettler et al. 2010; Seifert & Frohschammer 2013) and it is likely that related species will also be present in Myanmar.

It is surprising that our study did not reveal any samples of *C. emeryi* Forel 1881, a widely-distributed tramp species of African origin, which according to Wetterer (2012) occurs in Thailand, Indonesia, India, and the Philippines. Even though the easy spreading of *Cardiocondyla* through human activities makes it possible that *C. emeryi* have reached urban areas in Myanmar, we cannot preclude the possibility of a misidentification, in particular as proper species-level determination of *Cardiocondyla* requires meticulous measurement of nest samples at high magnification (Seifert 2008, 2009). Indeed, Clouse (2007) suggested samples of *C. emeryi* from Guam to be in fact *C. tjibodana* (but see Wetterer 2012).

To conclude, further examinations are needed to fully unravel the diversity of *Cardiocondyla* in Myanmar and also its neighboring countries. Given the large variation of the colony structures and reproductive strategies in this genus, such an endeavor will certainly yield surprising new insights into the complex evolution of the life histories of this often overlooked genus.

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