The complete mitochondrial genome of *Crematogaster matsumurai* (Forel 1901) (Hymenoptera: Formicidae) and its phylogenetic relationship

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**ABSTRACT**

*Crematogaster matsumurai* (Forel 1901) is an important arboreal ant species commonly found on *Phyllostachys heterocycla* (Carr.) in Lishui, Zhejiang, China. This study analyzed the mitochondrial genome sequence of *C. matsumurai* and discussed its phylogenetic relationship in Hymenoptera. The circular mitochondrial genome was 16,028 bp long, including a standard set of 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and 13 protein-coding genes (PCGs), which showed the typical insect mitochondrial genome arrangement. The AT and GC contents of the mitochondrial genome sequence were 76.92% and 23.08%, respectively. The maximum-likelihood (ML) phylogenetic analysis based on whole mitochondrial genome sequences showed that *C. matsumurai* is closest to *Crematogaster teranishii*.

*Crematogaster* (Hymenoptera: Formicidae: Myrmicinae) ants are diverse, widespread, and abundant in tropical, subtropical, and warm-temperate climates throughout the world (Blaimer 2012). Arboreal species of *Crematogaster* in particular are often dominant in the ant fauna, with polydomous and strongly territorial colonies (Dejean et al. 2010; Blaimer 2019). Species of this genus have a flat petiole and its postpetiole connected to the dorsal surface of the gaster, which is unique among all other Myrmicinae ants (Blaimer and Fisher 2013). *Crematogaster matsumurai* (Forel 1901) is an ant species commonly found in East Asia. It has been reported as a predator of some pests, such as *Halyomorpha halys* (Kamiyama et al. 2021). In the natural environment, this species mainly lives in decayed parts of relatively tall trees, such as *Acer palmatum*, *Prunus jamaica*, and *Prunus yedoensis* (Harada 2005; Hosoishi et al. 2019), and is also commonly found on *Phyllostachys heterocycla* (Carr.) in Lishui, Zhejiang, China. It builds insect nests on the leaves, stems, and branches of *P. heterocycle* (Carr.) to catch insects, such as aphids. Moreover, *C. matsumurai* develops mutualistic associations with other organisms. Despite its local abundance and important ecological interactions, the phylogenetic relationship of *C. matsumurai* within genus *Crematogaster* is controversial and in great need of modern revisionary studies (Sharaf et al. 2019). Therefore, this paper studied the mitochondrial genome of *C. matsumurai* and analyzed the evolutionary relationship between species.

Adults of *C. matsumurai* were collected from *P. heterocycle* (Carr.) forest in Longquan City (N28°14′35.12″, E119°14′30.31″), Lishui City, Zhejiang Province, China, and preserved in pure ethanol. The collected samples were identified and stored at −40°C in the Institute of Integrated Plant Protection Center, Lishui Academy of Agricultural and Forestry Sciences, Lishui, China (http://nky.lishui.gov.cn/, Jin-chao Wang, jcw199907@163.com) under the voucher number 20210805LQCM. Total genomic DNA was extracted by using E.Z.N.A.© Insect DNA kit and applied to 300 bp paired-end library construction using the Truseq SBS Kit (300 cycles) for Illumina sequencing. Sequencing was carried out on the Illumina NovaSeq 6000 platform (Biozeron Co., Ltd., Shanghai, China). A total of 5449.1 Mb of raw reads were generated, and by employing the tool Trimmomatic v0.39, the reads having adapter contamination and the small pieces less than 75 bp in length after quality trimming were removed to obtain clean reads. De novo genome assembly were conducted by SPAdes v3.14.1. The mitochondrial genes were annotated using the online MITOS tool (Bernt et al. 2013), using default parameters to predict protein-coding genes (PCGs), transfer RNA (tRNA) genes, and ribosomal RNA (rRNA) genes. The genomic sequence has been deposited in GenBank with an accession number OM328370.
The complete mitochondrial genome of *C. matsumurai* was a typical circular DNA molecule of 16,028 bp in length. A total of 37 genes were annotated, including 13 PCGs, 22 tRNAs, and two rRNAs. The AT content of the whole genome was 76.92%. 12 PCGs began with ATN as the start codon, and the start codon of NAD1 was TTG. The COI, COII, COIII, ATP6, ATP8, NAD1, NAD2, NAD3, NAD4, NAD4L, NAD5, NAD6, and COB genes were terminated with TAA as the stop codon.

To reveal the phylogenetic relationship of *C. matsumurai* with other members in Hymenoptera, phylogenetic analysis was performed based on 13 mitochondrial PCGs, of which five species, *Frankliniella intonsa*, *Apolygus lucorum*, *Empoasca flavescens*, *Spodoptera frugiperda*, and *Tuta absoluta* were served as outgroups. Functional annotations were performed using sequence-similarity Blast searches with a typical cutoff E-value of $10^{-5}$ against several publicly available protein databases: NCBI non-redundant (Nr) protein database, Swiss-Prot, Clusters of Orthologous Groups (COGs), and Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) terms (Yin et al. 2012). The maximum-likelihood (ML) bootstrap analysis with 1000 replicates was performed using RaxML v8.2.12 (Stamatakis 2014). The phylogenetic tree showed that *C. matsumurai* was closely related to *C. teranishii* (Figure 1). Meanwhile, genus *Crematogaster* had a close relationship with *Ochetellus* in Hymenoptera. The genome sequence of *C. matsumurai* in this study might provide useful information for Hymenoptera researches.

**Ethical approval**

This study was approved by the Institutional Review Board (IRB) Institutional of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences. The approval code is 2021071701. The approval date is 17 July 2021.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Data availability statement**

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at [https://www.ncbi.nlm.nih.gov/](https://www.ncbi.nlm.nih.gov/) under the accession no. OM328370. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA790703, SRP352081, and SAMN24220740, respectively.

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**References**

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2): 313–319.
Blaimer BB. 2012. A subgeneric revision of *Crematogaster* and discussion of regional species-groups (Hymenoptera: Formicidae). Zootaxa. 3482(1):47–67.

Blaimer BB. 2019. Taxonomy and natural history of the *Crematogaster* (Decacrema)-group (Hymenoptera: Formicidae) in Madagascar. Zootaxa. 2714(1):1–39.

Blaimer BB, Fisher BL. 2013. Taxonomy of the *Crematogaster degeeri*-species-assemblage in the Malagasy region (Hymenoptera: Formicidae). Eur J Taxon. 51(51):1–64.

Dejean A, Fisher BL, Corbara B, Rarevohitra R, Randrianaivo R, Rajemison B, Leponce M, Moreau CS. 2010. Spatial distribution of dominant arboreal ants in a Malagasy coastal rainforest: gaps and presence of an invasive species. PLOS One. 5(2):e9319.

Harada Y. 2005. Diel and seasonal patterns of foraging activity in the arboreal ant *Crematogaster matsumurai* Forel. Entomol Sci. 8(2):167–172.

Hosoishi S, Rahman M, Murakami T, Park SH, Kuboki Y, Ogata K. 2019. Winter activity of ants in an urban area of western Japan. Sociobiology. 66(3):414–419.

Kamiyama MT, Matsuura K, Yoshimura T, Yang C. 2021. Predation of the brown marmorated stink bug, *Halyomorpha halys* by the Japanese acrobat ants, *Crematogaster matsumurai* and *Crematogaster osakensis*. Biol Control. 157:104570.

Sharaf MR, Aldawood AS, Garcia FH. 2019. Review of the Arabian *Crematogaster* Lund (Hymenoptera, Formicidae), synoptic list, distribution, and description of two new species from Oman and Saudi Arabia. Zookeys. 898:27–81.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.

Yin Y, Mao X, Yang J, Xin C, Mao F, Ying X. 2012. dbCAN: a web resource for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 40(Web Server issue):W445–W451.