






The mitochondrial genome of a social wasp, *Vespula rufa* (Linnaeus, 1758) (Hymenoptera: Vespidae)

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ABSTRACT

For an ongoing systematic study of Korean Vespidae, we analyzed the complete mitochondrial genome of a social wasp, *Vespula rufa* (Linnaeus 1758), from the South Korea. The mitogenome is 17,521 bp in length, comprising 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and two ribosomal RNA (rRNA) genes. The nucleotide composition is 40.5% adenines, 43.0% thymines, 6.0% guanines, and 10.5% cytosines. The GC content is 16.5%. A maximum-likelihood analysis was conducted to understand phylogenetic relationships, based on 13 complete mitogenome sequences of Vespinae species. We recognized that *V. rufa* is being placed basal within the genus *Vespula*. The complete mitochondrial genome of *V. rufa* provides useful genetic information for further studies.

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Introduction

Social wasps are best-studied insect groups due to range of social behavior, diversity of nest architecture and ecological importance (Perrard et al. 2017). Also, the social wasps are important biological control agents in agroecosystems and natural ecosystems (Picanço et al. 2011). A phylogenetic study is needed to understand the origin and evolution of eusociality in Vespidae (Piekarski et al. 2018). In particular, *Vespula* is such an important group, beside *Vespa*, among social wasps because it contains infamous invasive species, such as *Vespula germanica* (Fabricius 1793) and *V. vulgaris* (Linnaeus 1758), causing ecological and economic damage. *Vespula* includes 25 species worldwide (Kumar and Carpenter 2018; Tan et al. 2018), and seven species are known from Korea (KSAE and ESK 2021).

Vespula rufa (Linnaeus 1758) is distributed in northern parts of North America, northern and central Europe, and parts of Asia. It can be distinguished from other species of the genus by usually having reddish brown areas on the 1st and 2nd tergites (Figure 1(A,B)). Lopez-Osorio et al. (2014) conducted a phylogenetic study on other species within this group, but there is no research including *V. rufa*. The complete mitochondrial genome sequences of *V. rufa* might be useful to resolve the phylogenetic relationships of the vespid species.

Materials and methods



With the consent of the Korea Forest Service, the specimen (Figure 1) was collected from Mt. Dutasan (773 m),


Pyeongchang-gun, Gangwon-do, South Korea (N37°34'18.45" E128°35'10.14") by using bee traps during 1.VII~18.VIII.2020. The specimen was identified as *Vespula rufa* female by the following morphological characteristics: clypeus with a central black stripe; yellow shield of frons extends just to antennal bases; scape black; tergite 1 with a pair of enclosed ivory basal spots; yellow coloration on 3–5 tergites without a brownish tinge (Archer 1981; Buck et al. 2008). The specimen was deposited at the Korea National Arboretum (<https://kna.forest.go.kr>, Il-Kwon Kim, ilkwons91@gmail.com) under the voucher number ves13439.

Digital images were obtained with a Fujifilm X-S1 camera (Tokyo, Japan) and a Raynox DCR-250 macro-conversion lens and stacked using Helicon Focus software[®] (Helicon Soft, Ltd., Kharkiv, Ukraine).

Total genomic DNA of the specimen was extracted from the legs of a specimen using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

The extracted DNA quality was checked with gel electrophoresis. Sequencing libraries were prepared using the TruSeq Nano DNA Sample Preparation Kit (Illumina Inc., San Diego, CA) by manufacturer's instructions. The Illumina MiSeq platform (paired-end 300 cycles) was used for sequencing at the LAS Inc. (Gimpo, South Korea). A total of 7,067,312,644 bases were generated from 11,739,722 read-pairs. To assemble the complete mitogenome, GetOrganelle v1.7.1a (Jin et al. 2020) was repeatedly conducted with five *K*-value (21, 45, 65, 85, and 105) parameters. We performed mapping with the reference data using BWA v0.7.17-r1188 (Li and Durbin 2010) to verify the

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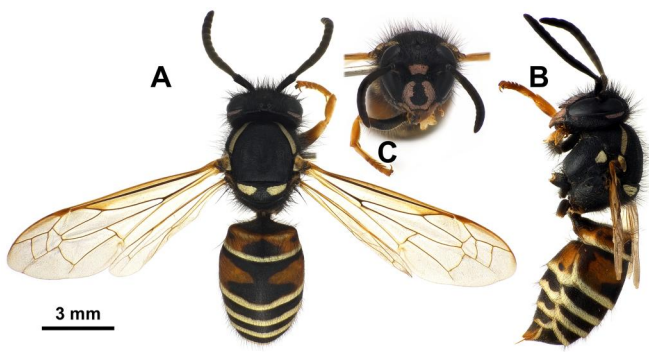


Figure 1. *Vespula rufa* female. (A) Habitus, dorsal view; (B) habitus, lateral view; (C) head, frontal view (photographed by Seung-Su Euo).

assembly quality of the assembled data. The mapping quality was confirmed using QualiMap v.2.3 and a coverage plot was visualized using the coverage data (Okonechnikov et al. 2016) (Figure S1). MITOS2 (Bernt et al. 2013) was used for gene annotations. The circular map was generated through Proksee (Grant and Stothard 2008) (Figure 2).

For phylogenetic analysis, we retrieved the complete mitochondrial genome sequences of 13 wasp species that show over 80% identity to *V. rufa* (OR074136), selected from GenBank (as of March 2024) (Table 1), using NCBI blastn (Camacho et al. 2009). *Polistes* sp. was selected as outgroup on the basis of previous morphological and molecular study (Perrard et al. 2016). The whole genome sequences were

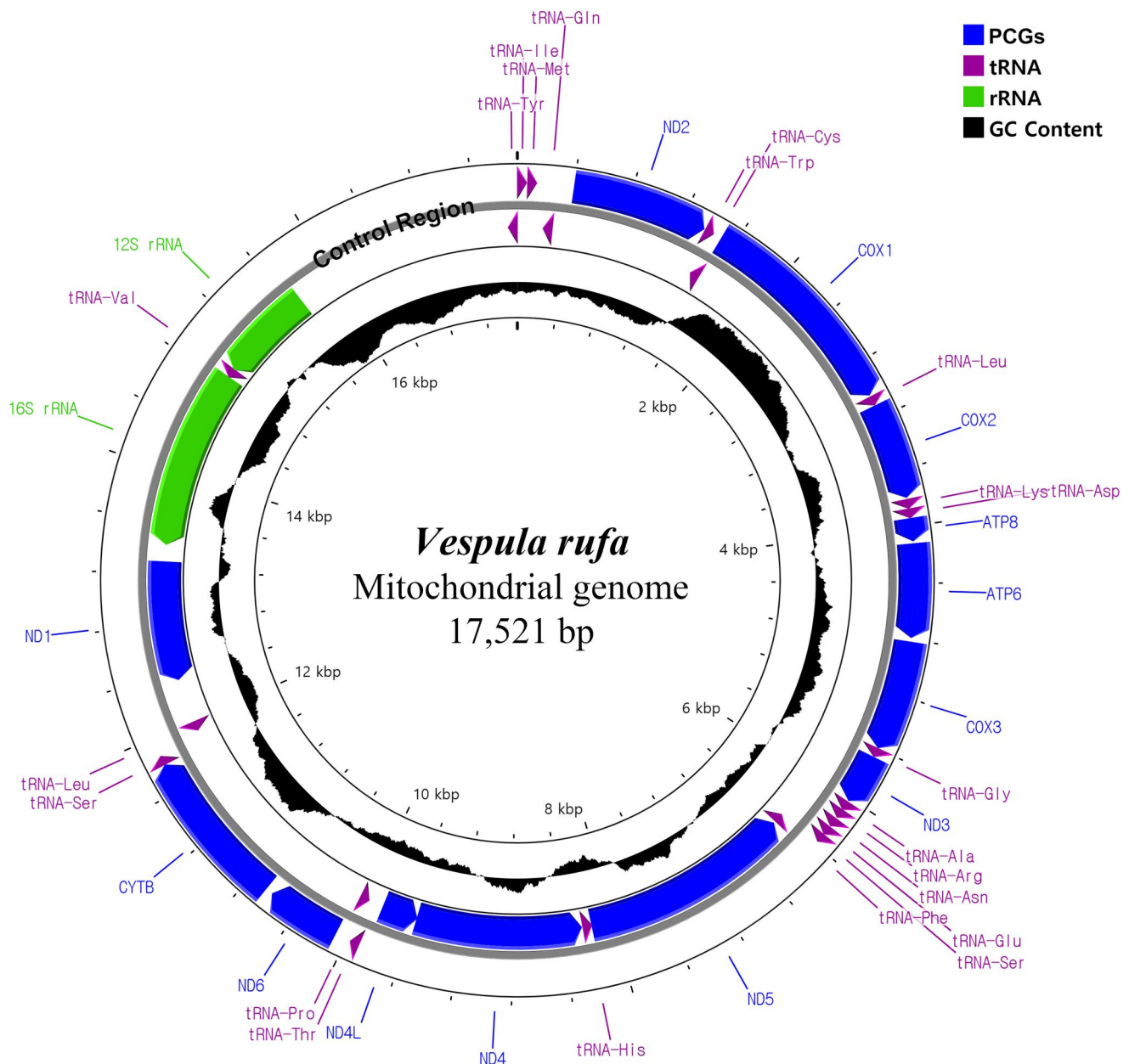
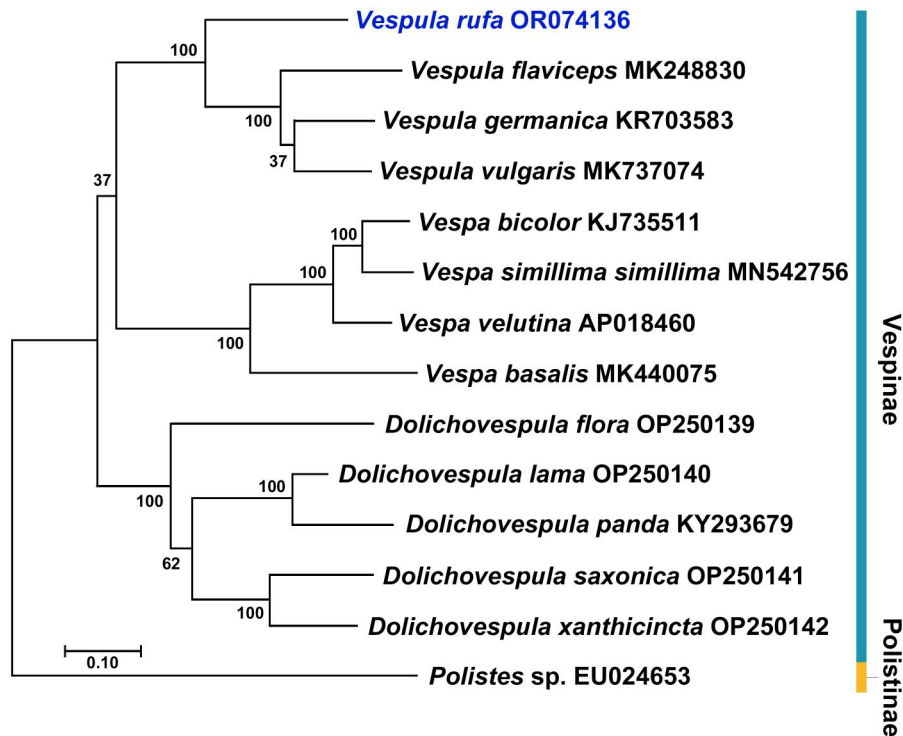


Figure 2. Circular-mapping mitochondrial genome of *Vespula rufa*. Color codes of different types of genes are shown on the legends in the map. The black bar graphs on the inner circle indicate the GC content, which shows the deviation from the average GC content.

Table 1. List of GenBank accession numbers and references for 14 sequences used to construct phylogenetic trees (Figure 3).

Species	Accession number	Percent identity to Korean <i>V. rufa</i> (%)	Reference
<i>Vespula rufa</i>	OR074136	–	This study
<i>Vespula flaviceps</i>	MK248830	84.88	Zhao, Wang, Wang, He, Xu, et al. (2019)
<i>Vespula germanica</i>	KR703583	84.38	Zhou et al. (2016)
<i>Vespula vulgaris</i>	MK737074	84.59	Dobelmann et al. (2019)
<i>Vespa bicolor</i>	KJ735511	82.92	Wei et al. (2016)
<i>Vespa simillima simillima</i>	MN542756	82.59	Choi et al. (2020)
<i>Vespa velutina</i>	AP018460	85.45	Takahashi et al. (2019)
<i>Vespa basalis</i>	MK440075	81.56	Zhao, Wang, Wang, He, Feng (2019)
<i>Dolichovespula flora</i>	OP250139	82.62	Wang et al. (2022)
<i>Dolichovespula lama</i>	OP250140	83.66	Wang et al. (2022)
<i>Dolichovespula panda</i>	KY293679	81.95	Fan et al. (2017)
<i>Dolichovespula saxonica</i>	OP250141	82.28	Wang et al. (2022)
<i>Dolichovespula xanthicincta</i>	OP250142	82.17	Wang et al. (2022)
<i>Polistes</i> sp.	EU024653	80.49	Cameron et al. (2008)

**Figure 3.** A phylogenetic tree based on the maximum-likelihood of 14 vespid complete mitochondrial genome sequences, Korean *Vespula rufa* sequence (OR074136), including 13 acquired from the NCBI (as of March 2024).

aligned using Clustal Omega 1.2.2 (Sievers et al. 2011) in Geneious Prime 2022.2.2 (Kearse et al. 2012). A maximum-likelihood analysis (bootstrap test with 500 replications) was performed in MEGA X (Kumar et al. 2018) using GTR (General Time Reversible) + G model determined using the find Best DNA/Protein Models tool (embedded in MEGA X).

Results

The complete mitochondrial genome sequence of *Vespula rufa* has 17,521 base pairs in length. The genome includes 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, and two ribosomal RNA (rRNA) genes (Figure 2). Among them, nine PCGs and 14 tRNA genes are encoded in the heavy strand, whereas four PCG (*ND5*, *ND4*, *ND4L*, and *ND1*), eight tRNA, and two rRNA genes are encoded in the light strand (Figure 2). Out of the total 13 PCGs, three PCGs (*ATP6*, *COX3*, and *ND4*) started with ATG codon. The others have the

following initiation codons: *COX1* (TTG); *COX2*, *ATP8*, *ND5*, *ND6*, and *ND1* (ATT); *ND2*, *ND3*, *ND4L*, and *CYTB* (ATA). Also, *COX3* has an incomplete stop codon, ending with T. The nucleotide composition is 40.5% adenines, 43.0% thymines, 6.0% guanines, and 10.5% cytosines. The GC content is 16.5%. The maximum-likelihood tree based on the complete mitochondrial genomes of Korean *V. rufa* and 13 other species shows that *V. rufa* is being placed basal within the genus *Vespula*. Also, each of three genera (*Vespula*, *Vespa*, and *Dolichovespula*) recovered as monophyletic lineages within Vespininae clade (Figure 3).

Discussion and conclusions

In this study, the complete mitochondrial genome of *V. rufa* was sequenced and annotated for the first time. Perrard et al. (2016) made a phylogenetic study of Vespininae with 50 Vespininae species based on morphological characters and

nine genes and recognized three subgenera within *Vespula*; *Vespula s. str.* including *V. rufa*, *Paravespula* including *V. flaviceps* (Horne and Smith 1870), *V. germanica* and *V. vulgaris*, and *Rugovespula* including *V. koreensis* (Radoszkowski 1887). Likewise, our result largely agrees well with the inferred phylogeny of Perrard et al. (2016). This study provides useful genetic information for further studies on the taxonomy, phylogeny, and evolution of the Vespidae family members.

Author contributions

SSE, JHC, MBC, IKK, and CJK conceived and planned the project. MBC and CJK performed sample collection. SSE, JHC, and IKK performed bioinformatics analyses. All the authors wrote the manuscript.

Ethical approval

No ethical approval or other relevant permission is required for this study because this species is an insect that is neither protected nor endangered species.

Disclosure statement

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

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Data availability statement

The complete mitochondrial genome sequence of *Vespula rufa* is openly available in GenBank of NCBI (<https://www.ncbi.nlm.nih.gov/>) under the accession no. OR074136 with BioSample SAMN31171325, SRA SRR21820245, and BioProject PRJNA887539.

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