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MITOGENOME ANNOUNCEMENT

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Complete mitochondrial genome of the H3 haplotype Argentine ant *Linepithema humile* (Mayr, 1868) (Formicidae; Hymenoptera)

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ABSTRACT

The argentine ant, *Linepithema humile* (Mayr, 1867), is an invasive ant species that has spread across the world. We have determined the mitochondrial genome of *L. humile* collected in South Korea, which is 15,934 bp containing 10 SNPs and 5 INDELs compared to the previous mitogenome. Most SNPs were found in *cox3*, followed by *cytb*. From SNPs our mitogenome was identified as a H3 haplotype, which was previously recorded in Japan and the U.S. while the previous mitogenome was H1 haplotype. Phylogenetic analysis was congruent to previous study within the tribe Leptomyrmecini but not between other tribes of subfamily Dolichoderinae.

Linepithema humile, belonging to Formicidae family, more often called 'Argentine ants' is an infamous tramp ant species named after its neotropical origin. They have a unique feature of 'unicoloniality' where multiple colonies cooperate forming so called supercolonies (Sunamura et al. 2009). Mating occurs within colonies and new colonies are made when small fractions of the mother colony bud out forming smaller colonies (Keller and Passera 1992). While these super-colonies remained insignificant in native ranges, genetic bottleneck of initial introduction and following 'genetic cleansing' presumably by annual queen executions (Keller et al. 1989; Inoue et al. 2015) caused extreme relatedness within introduced populations which allowed massive super-colonies distributed over multiple continents (Tsutsui et al. 2003). In Korea, Argentine ants have just arrived in southern ports of Busan and Kwangyang (Lee et al. 2020). Securing the mitogenome of the newly introduced populations will not only increase our understandings of its genetic background but aid the identification and managements of invasive species (Park et al. 2020).

Worker ants of *L. humile* were collected in Busan station, Busan, Republic of Korea (35°07′25.8″N 129°02′55.0″E). The ants were yellowish brown, about 2.5 mm from tip to tip, and were covered in light pubescence. They were easily identified as *L. humile* since no other dolichoderine ants in Korea had such large eyes located close to the anterior margin, elongated body structure with the propodeum much lower compared to the pronotum and mesonotum (Dong 2017). Total DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). Raw sequences obtained from Illumina NovaSeq6000 (350-bp paired-end library) at Macrogen Inc.,

Korea, were filtered by Trimmomatic v0.33 (Bolger et al. 2014), *de novo* assembled and confirmed by Velvet v1.2.10 (Zerbino and Birney 2008), SOAPGapCloser v1.12 (Zhao QY et al. 2011), BWA 0.7.17 (Li et al. 2009), and SAMtools v1.9 (Song and Liang 2013) under the environment of Genome Information System (GeIS; http://geis.infoboss.co.kr). Geneious R11 v11.1.5 (Biomatters Ltd, Auckland, New Zealand) was used for annotations based on the mitogenome (NC_045057; Zhao E et al. 2017). DNA sample and specimen (95% ethanol) were deposited in InfoBoss Cyber Herbarium (IN; http://herbarium.infoboss.co.kr; Contact: Suhyeon Park, shpark817@infoboss.co.kr; KFDS00420).

The new mitogenome of *L. humile* (GenBank accession is MT890564) is 15,934 bp long, 5 bp longer than the previous mitogenome due to 5 insertion and deletions (INDELs): three in control region and one each in *tRNA-Ile* and *tRNA-Cys*. Gene components and order of 37 genes, 13 protein-coding genes (PCGs), 2 rRNAs, and 22 tRNAs, were conserved compared to other dolichoderine mitogenomes. Ten single nucleotide polymorphisms (SNPs) were identified between the two mitogenomes, all in PCGs: five in *cox3*, 2 in *cytb,* and 1 in *cox1, cox2,* and *nad4*.

To investigate the genetic properties of Argentine ants supercolonies, fractions of *cox1, cox2,* and *cytb* genes were developed as mitochondrial markers suggesting 19 different haplotypes in both native and introduced ranges (Vogel et al. 2009, 2010; Inoue et al. 2013). Our mitogenome was identical to the H3 haplotype while the previous mitogenome was H1 haplotype (LH3 and LH1 in Inoue et al. 2013). H1 was the intercontinental supercolony's haplotype while H3 was a minority found in Bermuda, Chile, Ecuador, North Carolina
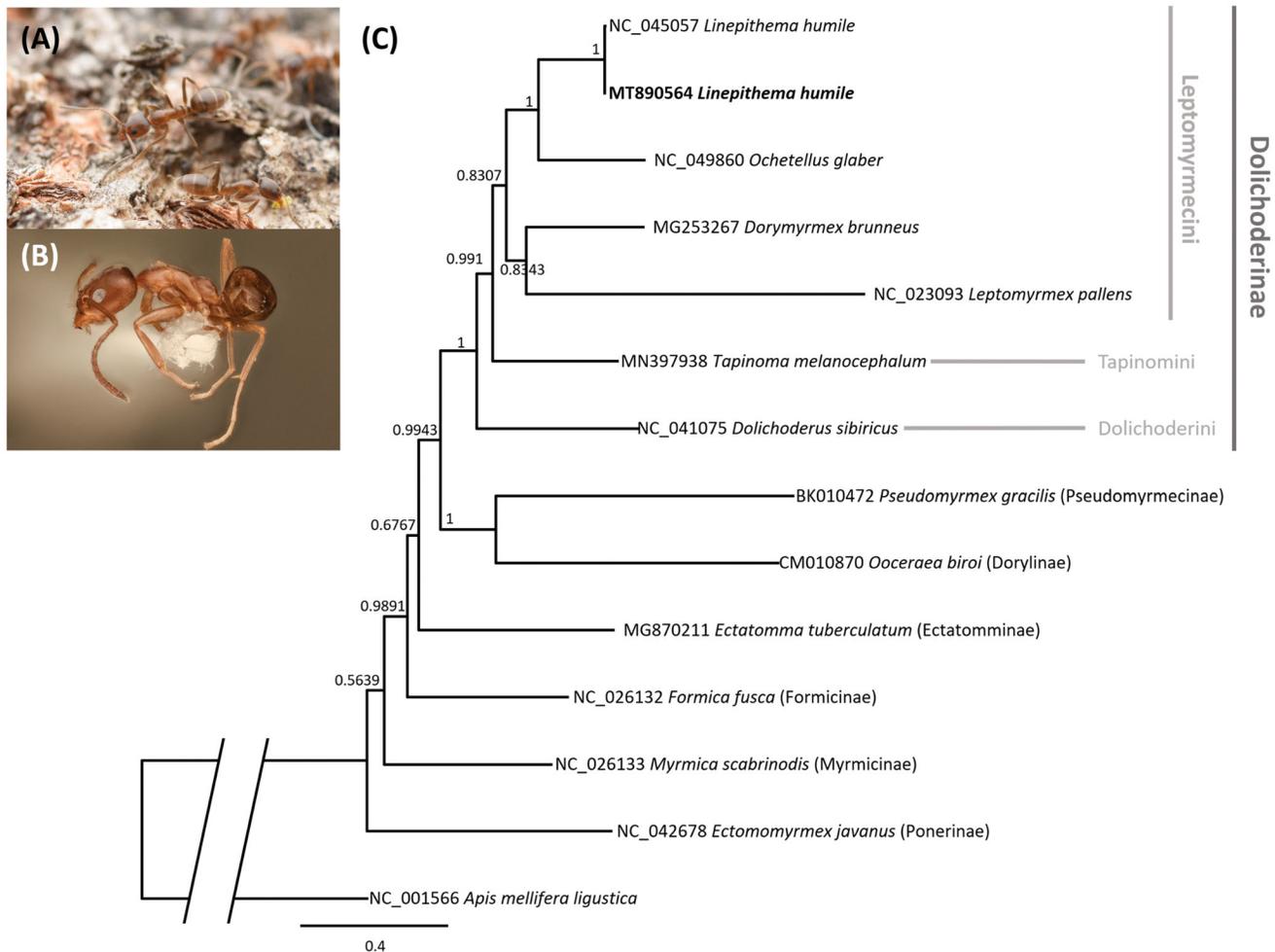

Figure 1. (A) Photo of *Linepithema humile* workers foraging in the collected site of Busan, South Korea. (B) Lateral view of *Linepithema humile* collected in Busan, South Korea. (C) Bayesian inference (1,100,000 generations) phylogenetic tree of 13 ant mitochondrial genomes. The numbers above branches indicate the posterior probabilities of the Bayesian inference tree.

and the lesser supercolonies of California and Kobe (Vogel et al. 2010; Inoue et al. 2013). The last two countries, Japan and the U.S., could be suspected as the origin of Korean population as they are the third and second largest countries in imports of Korea (Choi and Choi 2017).

Thirteen PCGs from 13 ants consisting of 7 dolichoderine ants, representative ants to each subfamily, and an outgroup species, *Apis mellifera ligustica*, were aligned independently using MAFFT v7.450 (Katoh and Standley 2013) and were concatenated for phylogenetic purposes. Bayesian inference tree was generated using MrBayes v3.2.6 (Ronquist et al. 2012). In the phylogenetic tree, two *L. humile* was grouped with *Ochetellus glaber* and two remaining leptomyrmecine species were also grouped (Figure 1), congruent to the previous phylogeny of the tribe (Ward et al. 2010). Relationship between the tribes, however, was incongruent to the previous study as tribe Leptomyrmecini was grouped with tribe Tapinomini not Dolichoderini based on nuclear genes (Ward et al. 2010). In addition, the two nodes showing low supportive values (Figure 1) are also congruent to the previous studies (Park et al. 2020c, 2020a, 2020b), requiring additional mitogenomes to improve them.

Our findings show only two of ten SNPs were used as haplotype markers, suggesting further utilization on other regions of mitogenome will be useful. The highly variable *cox3* could be utilized for further dividing haplotypes, which could separate supercolonies distinguishable in behavioral test but not in haplotypes (e.g., the minor supercolonies of California). It also shows that the upstream region amplified by LCO1490-HCO2198 universal marker (Folmer et al. 1994) can be considered additional marker region as the only SNP was found on *cox1*.

Disclosure statement

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Data availability statement

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