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Molecular phylogeny of two slave-making ants: *Rossomyrmex* and *Polyergus* (Hymenoptera: Formicidae)

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Using mitochondrial cytochrome oxidase I gene (COI), we established the phylogenetic relationship between two slave-making genera of the tribe Formicini: *Polyergus* and *Rossomyrmex*. The resulting phylogenetic tree presents two well-defined groups: *Formica* + *Polyergus*, and *Proformica* + *Cataglyphis* + *Rossomyrmex*. Our result contradicts prior classifications which considered *Polyergus* and *Rossomyrmex* to be sister genera. These results imply that slave-making in the two taxa evolved independently.

Introduction

The genus *Polyergus* is composed of five species (Bolton 1995) endemic to the Holarctic region. In *Rossomyrmex*, only 2 species have been identified: *R. proformicarum* Arnoldi from the Tian-Shan (Kazakhstan) and Caspian steppes (Russia), and *R. minuchae* Tinaut from the Sierra Nevada mountains (Spain). *Polyergus* and *Rossomyrmex* are slave-making ants, which parasitize *Formica* and *Proformica*, respectively.

All current phylogenies consider *Polyergus* a sister genus to *Formica*, but the phylogenetic position of the genus *Rossomyrmex* has been repeatedly revised since its initial description by Arnoldi (1928). Arnoldi found similarities between *Rossomyrmex* and *Proformica*, and between *Polyergus*, *Formica* and *Cataglyphis*, but did not establish the phylogenetic relationships among these taxa (Arnoldi 1928). In sub-

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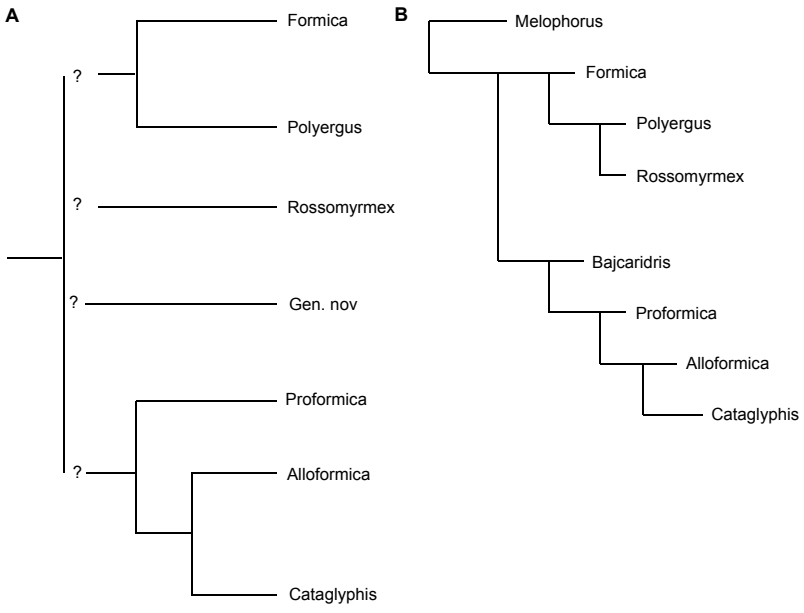

Fig. 1. Phylogenetic relationships in the tribe Formicini hypothesized by Agosti 1989 (**A**) and 1994 (**B**).

sequent papers, *Rossomyrmex* was considered derived from *Formica*-like ancestors and phyllogenetically related near *Formica* and/or *Polyergus* (Wilson 1971, Brown 1973, Buschinger 1990, Hölldobler & Wilson 1990). Agosti (1989) (Fig. 1A) placed it, with doubt, between the *Formica*/*Polyergus* group and *Proformica*, *Alloformica*, *Bajcaridris* and *Cataglyphis*. Later Agosti (1994) (Fig. 1B) changed *Rossomyrmex* to a position derived from *Polyergus*, upholding a classical opinion which considers two groups of genera with close phylogenetic ties: on one side, *Formica* L., *Polyergus* Latreille and *Rossomyrmex* Arnoldi, and, on the other, *Bajcaridris* Agosti, *Proformica* Ruzsky, *Alloformica* Dlussky and *Cataglyphis* Foerster. According to this phylogeny, the two slave-making genera, *Polyergus* and *Rossomyrmex*, are grouped together and are considered to be sister taxa. Tinaut *et al.* (1994), however, reported that, on the basis of male genitalia, *Rossomyrmex* appears to be more closely related to *Proformica* and *Cataglyphis* than to *Formica*.

In the present paper, we use molecular techniques to determine the phylogenetic position of *Rossomyrmex* and *Polyergus*.

Table 1. Species, localities and Genbank accession number.

Species	Collecting location	Genbank accesion number
Cataglyphis		
C. bicolor	Morocco	AB010942
C. floricola	Spain	AB010936
C. rosenhaueri	Spain	AB010941
C. velox	Spain	AB010933
Formica		
F. cunicularia	Spain	AB010926
F. exsecta	Poland	AB010927
F. fusca	Spain	AB010925
F. truncorum	Japan	AB010929
F. yesensis	Japan	AB010928
Lasius		
L. niger	Germany	AB007981
Plagiolepis		
P. pygmaea	Spain	AB010938
Proformica		
P. ferreri	Spain	AB010935
P. longiseta	Spain	AB010934
Polyergus		
P. rufescens	Spain	AB010931
P. samurai	Japan	AB010930
Rossomyrmex		
R. minuchae	Spain	AB010937

Materials and methods

The species

The species used and their sampling sites are given in Table 1. For outgroup species, we have considered two species: *Plagiolepis pygmaea* Latreille (tribe Plagiolepidini) and *Lasius niger* L. (tribe Lasiini), both from the Formicinae subfamily.

DNA extraction and sequencing

DNA from one individual of each species was extracted from ants preserved in 99.9% ethanol. A DNA extraction kit (DNeasy Tissue Kit, QIAGEN), was used, following the manufacturer's instructions.

We designed primers for the mitochondrial cytochrome oxidase subunit I (COI) gene by comparing the sequences of the fruit fly *Drosophila melanogaster* (de Bruijn 1983) and the honey bee *Apis mellifera* L. (Crozier & Crozier 1993). We searched for conservative regions in which more than 90% of the sequences matched between the two species. Two primer pairs were designed (given as 5´–3´):

CI13: ATAATTTTTTTTATAGTTATACC
CI14: ATTTCTTTTT TTCCTCTTTC; and
CI21: CTTTATCAACATTTATTTTGATTTTT
CI24: TCCTAA AAAATGTTGAGGAAA.

In total, we obtained 1104bp sequence within COI from these primer pairs.

The target fragment was amplified by PCR in a programmable thermal cycler (PTC-100, MJ Research Inc.). Each PCR contained a 50 μl reaction volume of the following components: 100 ng DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatine, 20 pmol of primers, and 0.4U Taq DNA polymerase (GeneTaq, NipponGene). Amplification was 30 cycles per 1 min at 94 °C, 1 min at 45 °C, and 3 min at 60 °C. Amplification products were purified with a DNA-purification kit (PCR Purification Kit, QIAGEN), and suspended in 25 μl of TE buffer. Both strands of each PCR product were sequenced using Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) on an ABI 373A automated sequencer. The accuracy of the target sequence of a species was confirmed by comparing the sequence from one of a pair of primers with the complementary sequence from the other primer.

Phylogenetic reconstruction

The phylogenetic relationship between the species was reconstructed using the software PAUP 4.0b5 (Swofford 1998). We used two different algorithms to estimate the phylogeny: neighbour joining (NJ), and maximum parsimony (MP). For NJ, we selected Kimura's two-parameter distance as an index of genetic distances between sequences. In addition, we used a gamma function with 0.5 shape variable to correct between-site-rate variations. For MP, we equally weighted all substitutions. Robustness of the resulting topologies were examined by a bootstrap test with 1000 resamplings.

Results

The target region was 1104-bp nucleotides in length, but the primer region itself and sequences near the primers could not be determined accurately. As a result, we obtained a 974-bp sequence from each species. These sequences are deposited at the DDBJ database (*see* Table 1).

Figure 2 shows NJ and MP trees with bootstrap probabilities of each node. We also estimated the maximum-likelihood tree, using the HYK 85 model. The ML estimation resulted in the same topology as the unweighted MP, and thus the ML tree is not presented. We did not provide a phylogram with branch-length information because our main concern in this study can be resolved without branch length. Branch lengths can be determined using the above-mentioned deposited sequences.

Two clades were well supported in all

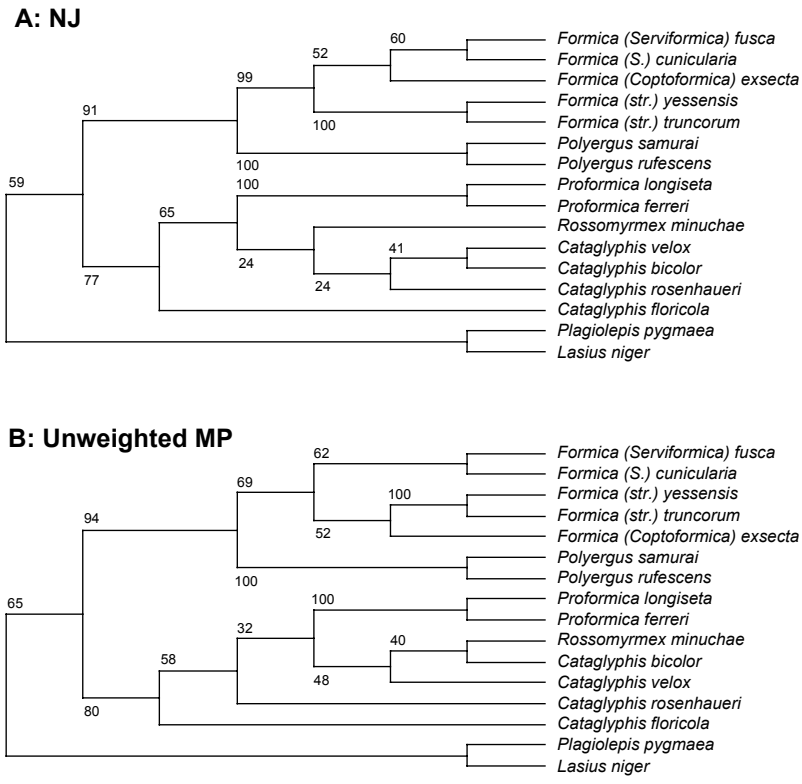

Fig. 2. The estimated phylogenetic relationships between the examined species. Topologies were derived from two estimation algorithms (**A**: NJ and **B**: unweighted MP). The numbers at branches represent bootstrap probabilities from 1000 resamplings. Descriptive statistics of the unweighted MP tree were TL = 1059, CI = 0.5467, HI= 0.4533, RI = 0.4888 and RC = 0.2673.

cases, one including *Formica* and *Polyergus* and the other consisting of *Cataglyphis*, *Proformica* and *Rossomyrmex*. The bootstrap support for both groups was 57%–94% for the *Formica*/*Polyergus* clade and 77%–85% for *Rossomyrmex*/*Proformica*/*Cataglyphis* clade (Fig. 2). In 1000 bootstrap replicates, *Rossomyrmex* and *Polyergus* spp never formed a monophyletic clade — i.e., the probability was zero for this clade.

Discussion

We found a clear relationship within the Formicini, regardless of the estimation algorithm used (Fig. 2). There is a strong likelihood that the group containing *Formica* and *Polyergus* is separate from the group containing *Cataglyphis*, *Proformica* and *Rossomyrmex*. The relationships within this clade, however, are not clear. *Rossomyrmex* may be considered derived within this clade whereas *Proformica* appears to be ancestral, although this could change with the inclusion of other genera and is not currently supported by the bootstrap analysis. Indeed the bootstrap support was low for all clades (Fig. 2), except for the one formed by *Proformica* spp. The low probabilities found between the majority of the *Cataglyphis* species appear to reflect the diversification in this genus, evident also in such morphological characters as the high variability of the male genitalia (Emery 1906, Agosti 1990, Tinaut 1990a, 1990b). It is probable that the absence, in this study, of other species-groups of the genus *Cataglyphis* contributes to the low bootstrap probability found.

Finally our results strongly suggest that *Rossomyrmex* is not the sister genus of *Polyergus* but is rather closely related to *Cataglyphis* and *Proformica*. This finding invalidates the previously hypothesized phylogenetic relationships for these genera (Hölldobler & Wilson 1990, Agosti 1994) and implies a independent origin of the slave-making in *Polyergus* and *Rossomyrmex*.

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