More than one species of *Messor* harvester ants (Hymenoptera: Formicidae) in Central Europe

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**Abstract.** It is commonly held that Central Europe harbours but a single harvester ant species, namely *Messor structor*. Recently discovered bionomic differences between two Central European populations, which may reflect interspecific variation, cast doubt on this assumption. In the present study we test alternative hypotheses — one versus two harvester ant species in Central Europe and adjacent regions — by investigating the genetic diversity of ants determined as *M. structor* or close to it ("M. cf. structor"). Sequences of the mitochondrial COI gene revealed two major lineages of different but partially overlapping geographic distributions, both occurring in Central Europe. The existence of a cryptic species within *M. cf. structor* is the most plausible interpretation, since the sequence divergence between the two major lineages equals those between *M. capitatus*, *M. concolor* and *M. bouvieri*. The phylogenetic analyses revealed a distinct substructuring for both of the detected major lineages and the possible existence of additional cryptic species.

**INTRODUCTION**

The myrmicine harvester ant genus *Messor* is mainly distributed in the Palearctic region (Bolton, 1982; Agosti & Collingwood, 1987; Cagniant & Espadaler, 1997). Bolton (1995) lists 105 recognized species worldwide. At the beginning of the last century there were a series of taxonomic revisions (Santschi, 1917, 1923, 1927; Kuznetzov-Ugamsky, 1927; Finzi, 1929). Since then modern taxonomists have conducted only partial revisions, which are confined to particular geographic regions or to certain species groups (e.g., Arnol’di, 1977; Bolton, 1982). The present taxonomic situation is thus unsatisfactory. For Central Europe (Austria, Czech Republic, Germany, Hungary, Poland, Slovak Republic and Switzerland) the occurrence of a single *Messor* species has been accepted (e.g., Stitz, 1939; Kutter, 1977; Seifert, 1996), and most of the current faunas (Gallé et al., 1998; Seifert, 2001; Czechowski et al., 2002; Steiner et al., 2003; Neumeyer & Seifert, 2005) contain only one species, *Messor structor* (Latreille, 1798). Bezděčka (1996) and Werner & Bezděčka (2001) list *Messor muticus* (Nylander, 1849), which is currently regarded as a junior synonym of *M. structor* (Atanassov & Dlušký, 1992; Table 1), and Markó & Csősز (2002) record *M. muticus* in addition to *M. structor* for Hungary. Based on current data, *M. structor*, which was originally described from Brive-la-Gaillarde, France, occurs in North Africa and the Middle East, Southern, Central and Eastern Europe, Asia Minor, the Caucasus and Central Asia (Fig. 1; Czechowski et al., 2002). The only revision of the *M. structor* species group (Bernard, 1955) is rather cursory and does not mention origin and deposition of the investigated material.

The lengthy list of junior synonyms of *M. structor* (Table 1), combined with the lack of a proper revision of these taxa, and the occasional acceptance of different synonyms as valid species (Bernard, 1967; Bezděčka, 1996; Werner & Bezděčka, 2001; Markó & Csősز, 2002) raise serious questions about the biological identity and status of *M. structor*. In addition, a recent investigation of the life history of *Messor cf. structor* (Schlick-Steiner et al., 2005b) revealed a tendency for colony organisation and behaviour among Central European harvester ants to differ. These authors found that populations in Lower Austria did not swarm and were unicolonial (sensu Wilson, 1971 and Crozier & Pamilo, 1996: ants from different nests intermingle due to the absence of clear colony boundaries). German *Messor* ants, in contrast, are reported to display swarming flight and multicoloniality, i.e., strict separation of single colonies (Heller, 1971; Seifert, 1996; Fig. 1). Such differences in life history may reflect intraspecific variation or the existence of distinct

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TABLE 1. List of taxon names currently regarded as junior synonyms or subspecies of *Messor structor* (Latreille, 1798).

| Original combination | Author | Type locality | Current status / Comments |
|----------------------|--------|---------------|--------------------------|
| *Formica structor*   | Latreille, 1798: 46 France | combination in *Messor*: Emery (1897: 238) |
| *Formica rufitarsis* | Fabricius, 1804: 406 Austria | junior synonym of *structor*: Nylander (1856: 85) |
| *Formica lapidum*    | Fabricius, 1804: 407 Austria | junior synonym of *structor*: Nylander (1856: 85) |
| *Formica aedificator*| Schilling, 1839: 56 Poland  | junior synonym of *structor*: Mayr (1855: 464) |
| *Myrmica mutica*     | Nylander, 1849: 39 Russia  | junior synonym of *structor*: Mayr (1855: 464) |
| *Stenamma (Messor) structor* var. *tyrrhena* | Emery, 1898: 141 Italy | combination in *Messor*: Emery (1908: 456); junior synonym of *structor*: Emery (1921: 210) |
| *Messor structor* var. *clivorum* | Ruzsky, 1905: 735 Turkestan | subspecies of *clivorum* Arnol’di (1977: 1644); junior synonym of *structor*: Dlussky et al. (1990: 224) |
| *Messor tataricus*   | Ruzsky, 1905: 738 Turkestan | subspecies of *clivorum* Arnol’di (1977: 1644); junior synonym of *structor*: Dlussky et al. (1990: 224) |
| *Messor platyceras*  | Crawley, 1920: 163 Persia  | subspecies of *structor*: Bolton (1995: 256) |
| *Messor platyceras* var. *rubella* | Crawley, 1920: 164 Persia | junior synonym of *clivorum*: Emery (1921: 210) |
| *Messor barbarus* subsp. *varrialei* | Emery, 1921: 215 Turkey | junior synonym of *structor*: Atanassov & Dlussky (1992: 114) |
| *Messor structor* subsp. *turanicus* | Kuznetsov-Ugamsky, 1927: 91 Uzbekistan | junior synonym of *rufitarsis* ssp. *darianus*: Arnol’di (1977: 1644) in key; of *structor*: Dlussky et al. (1990: 224) |
| *Messor structor* var. *subpolitus* | Kuznetsov-Ugamsky, 1927: 92 Turkestan | junior synonym of *clivorum*: Tarbinsky (1976: 56) |
| *Messor structor* var. *aegae* | Menozzi, 1928: 126 Turkey | first available use of *Messor barbarus* st. *structor* var. *aegae* Emery, 1921: 213 |
| *Messor structor* subsp. *novaki* | Finzi, 1929: 92 Yugoslavia | first available use of *Messor structor* st. *rufitarsis* var. *darianus* Santschi, 1926: 291; junior synonym of *structor*: Kuznetsov-Ugamsky (1927: 92); synonymy of *turanicus*: Pisarski (1967: 383) |
| *Messor rufitarsis* subsp. *darianus* | Pisarski, 1967: 383 Turkestan | first available use of *Messor rufitarsis* subsp. *darianus* Santschi, 1926: 291; junior synonym of *structor*: Kuznetsov-Ugamsky (1927: 92); synonymy of *turanicus*: Pisarski (1967: 383) |
| *Messor rufitarsis* subsp. *tadzhikorum* | Arnol’di, 1969: 79 Tadzhikistan | misspelled as *rufitarsis* *tadzhikorum*: Arnol’di (1977: 1644); subspecies of *structor*: Bolton (1995: 257) |
| *Messor rufitarsis* subsp. *jakowlevi* | Arnol’di, 1977: 1643 Ukraine | first available use of *Messor barbarus* subsp. *capitatus* var. *jakowlevi* Ruzsky, 1905: 750; junior synonym of *structor*: Dlussky et al. (1990: 224) |
| *Messor clivorum* subsp. *sevani* | Arnol’di, 1977: 1644 Caucasus | first available use of *Messor structor* subsp. *striaticeps* var. *sevani* Karawajew, 1926: 103; junior synonym of *structor*: Arakelian (1994: 39) |

Species (Bourke & Franks, 1995). Granted that species delimitation is subject to ongoing discussion (see Sites & Marshall, 2003 for review) the species category is nonetheless of unquestioned operational value for systematics and phylogenetics (Avise & Walker, 2000; Mallet, 2001). Hence in this paper we test a pair of alternative hypotheses whether the differing life histories of Central European *Messor cf. structor* harvester ants reflect (i) a single, bionomically versatile species; or (ii) two fully separated species.

Traditional insect classification is morphology-based; in particular morphometric analyses have proved powerful (Seifert, 2002). However, groups with small interspecific and high intraspecific variation are often poorly resolved by such methods alone (for review: Wiens, 1999; for ant examples: Lucas et al., 2002; Steiner et al., 2004, 2005, 2006a; Knaden et al., 2005). A strong, gradual size polymorphism within the worker caste of Central European *Messor* ants causes pronounced allometric distortions, making morphometric differentiation more complicated than in species with monomorphic workers (Csösz et al., unpubl.). In such cases molecular genetic analyses may provide complementary information. Morphologically most similar species may differ markedly in mitochondrial DNA (mtDNA), as shown for ants, among others, by Heinze et al. (2005), Knaden et al. (2005) and Steiner et al. (2004, 2005, 2006a).

In this paper the one-or-two-species problem in *M. cf. structor* is addressed by surveying its mtDNA diversity in Central Europe. In order to evaluate geographic, intra- and interspecific variation samples from other European regions and other *Messor* species are included in the analysis.

**MATERIAL AND METHODS**

Individuals from 40 *Messor* colonies from ten European countries were studied (Table 2), including samples from German and Austrian populations, which reportedly differ bionomically (Fig. 1; Heller, 1971; Schlick-Steiner et al., 2005b). Voucher specimens were deposited in the Hungarian Natural History Museum in Budapest, the Babes-Bolyai University in Cluj-Napoca and the private collection of B.C. Schlick-
Table 2. Localities and geographic coordinates, collectors, numbered haplotypes (HT) and phylogenetic lineages of the samples of *M. cf. structor* analysed. Abbreviations: AU – Austria; BU – Bulgaria; HR – Croatia; EZ – Czech Republic; FR – France; GM – Germany; IT – Italy; RO – Romania; SI – Slovenia; SP – Spain. AMS – A.M. Stojanova; AS – A. Stradner; BM – B. Markó; BS – B. Sipos; BSFS – B.C. Schlick-Steiner & F.M. Steiner; CS – C. Stauffer; GB – G. Braéko; GH – G. Heller; HK – H. Konrad; JC – J. Casevitz-Weulersse; KG – K. Gómez; KT – K. Ticha; NW – N. Weiß-Vogtmann; PSW – P.S. Ward; SC – S. Csösz; TL – T. Ljubomirov; XE – X. Espadaler.

| Species                      | Locality          | Coordinates       | Collector | HT | Lineage |
|------------------------------|-------------------|-------------------|-----------|----|---------|
| *Aphaenogaster iberica* Emery, 1908 | SP: vic. Los Belones | 37°37’N, 00°48’W | AS, CS     |    |         |
| *Messor bouvieri* Bondroit, 1918 | SP: Bellaterra     | 41°30’N, 02°06’E | XE        |    |         |
|                              | SP: Mallorca, Luecmajor | 39°26’N, 02°45’E | KG        |    |         |
|                              | SP: Mallorca, Luecmajor | 39°26’N, 02°45’E | KG        |    |         |
| *Messor capitatus* (Latreille, 1798) | HR: Krk, Vrbnik   | 45°04’N, 14°40’E | HK        |    |         |
|                              | IT: Elba, Monte Orello | 42°42’N, 10°19’E | GH        |    |         |
|                              | SI: Hrastovlje     | 45°30’N, 13°53’E | GB        |    |         |
|                              | SP: Oteo           | 42°43’N, 02°22’W | XE        |    |         |
| *Messor chamberlini* Wheeler, 1915 | US: California, Santa Barbara | 34°00’N, 119°44’W | PSW         |    |         |
| *Messor concolor* Santschi, 1927 | BU: Maritsa valley, Klokotnitsa | 41°58’N, 25°30’E | TL        |    |         |
|                              | BU: Maritsa valley, Klokotnitsa | 41°58’N, 25°35’E | TL        |    |         |
|                              | HR: vic. Biograd   | 43°56’N, 15°24’E | SC        |    |         |
|                              | IT: Elba, Monte Orello | 42°42’N, 10°19’E | GH        |    |         |
| *Messor lobognathus* Andrews, 1916 | US: Nevada, Washoe Co. | 41°31’N, 119°27’W | PSW         |    |         |
| *Messor cf. structor* (Latreille, 1798) | AU: Matzeisendorf | 48°12’N, 15°17’E | BSFS 9 B   |    | A       |
|                              | AU: Obernab       | 48°44’N, 15°55’E | BSFS 11 B  |    |         |
|                              | AU: Prenlenkirchen | 48°05’N, 16°57’E | BSFS 10 B  |    |         |
|                              | BU: Balick        | 43°25’N, 28°10’E | BM 1 A     |    |         |
|                              | BU: Rhodope Mts., Daskalovo | 41°46’N, 25°16’E | AMS 4 A    |    |         |
|                              | BU: Strouma valley, vic. Zemen | 42°25’N, 22°44’E | TL 3 A     |    |         |
|                              | BU: Strouma valley, vic. Zemen | 42°28’N, 22°44’E | TL 3 A     |    |         |
|                              | BU: Strouma valley, vic. Zemen | 42°27’N, 22°44’E | TL 11 B    |    |         |
|                              | EZ: Mohelno       | 49°07’N, 16°10’E | KT 11 B    |    |         |
|                              | EZ: Mohelno       | 49°07’N, 16°10’E | KT 11 B    |    |         |
|                              | FR: Les Aubersasses, Vachères | 43°55’N, 05°37’E | JC 1 A     |    |         |
|                              | FR: Rhône-Alpes   | 45°10’N, 05°20’E | NW 1 A     |    |         |
|                              | GM: Finnthen      | 49°59’N, 08°10’E | GH 1 A     |    |         |
|                              | GM: Lorchenhausen | 50°02’N, 07°46’E | GH 2 A     |    |         |
|                              | GM: Mainz         | 50°00’N, 08°16’E | GH 1 A     |    |         |
|                              | HR: Krk, Malinska | 45°07’N, 14°31’E | HK 1 A     |    |         |
|                              | IT: Verona        | 45°27’N, 11°00’E | CS 1 A     |    |         |
|                              | RO: Baile Herculan | 44°52’N, 22°24’E | BM 8 B     |    |         |
|                              | RO: Dubova        | 44°37’N, 22°16’E | BM 11 B    |    |         |
|                              | RO: Macin Mts., Pricopanu | 45°15’N, 28°09’E | BM 15 B    |    |         |
|                              | RO: Vama Vechie   | 43°45’N, 28°34’E | BM 1 A     |    |         |
|                              | RO: Vama Vechie   | 43°45’N, 28°34’E | BM 1 A     |    |         |
|                              | RO: Vama Vechie   | 43°45’N, 28°34’E | BM 5 A     |    |         |
|                              | RO: Macin Mts., Pricopanu | 45°15’N, 28°09’E | BM 14 B    |    |         |
|                              | RO: Caluseri      | 46°36’N, 24°43’E | BM 12 B    |    |         |
|                              | RO: Cluj Napoca   | 46°46’N, 23°36’E | BS 13 B    |    |         |
|                              | RO: Horia         | 45°01’N, 28°27’E | BM 16 B    |    |         |
|                              | SI: vic. Rakitovec | 45°27’N, 13°58’E | GB 7 B     |    |         |
|                              | SI: Hrastovlje    | 45°30’N, 13°53’E | GB 6 B     |    |         |

Steiner & F.M. Steiner. Workers were determined according to Agosti & Collingwood (1987). The type of *M. concolor* Santschi, 1927 (Museo Civico di Storia Naturale, Genova) was inspected for comparison. Samples keying out as *M. structor* or *M. muticus* were subsumed under *M. cf. structor*, as differential diagnostic characters (*M. muticus*: metasternal process wider; scape, relative to head width, shorter) were found to vary considerably within single colonies, and not correlated with the geographic origin of the samples. In total 29 colonies of *M. cf. structor*, four of *M. capitatus* (Latreille, 1798), four of *M. concolor* and three of *M. bouvieri* Bondroit, 1918 were analysed. *M. chamberlini* Wheeler, 1915, *M. lobognathus* Andrews, 1916, both from North America (det. P.S. Ward), and *Aphaenogaster iberica* Emery, 1908 from Spain (det. X. Espadaler), were selected as the outgroup.

DNA of single individuals was extracted, using the Genelute Extraction kit (Sigma, St. Louis, USA), but applying the standard phenol-chloroform-isoamyl-alcohol protocol (Sambrook et
were used to compute a majority rule consensus tree assigning frequencies stable at 0.004), the last 2,500 trees of each run stationarity was achieved (average standard deviation of split frequency set to 100 were run twice. As after 750,000 generations how well competing substitution models fit the data. In

3.06 (Posada & Crandall, 1998), which uses hierarchical likelihood model (Tavaré, 1986; Yang, 1993) was chosen using Modeltest (100 replicates). Prior to BMCMC analysis the GTR+I+G strapping was applied for NJ (1000 replicates) and MP trees sequence replicates and the Multree option in effect. The

were generated with heuristic search using the tree bisection-reconnection (Neighbour Joining algorithm, NJ, based on Tamura-Nei distance) and character (maximum parsimony, MP; Bayesian Markov Chain Monte Carlo BMCMC) analyses were performed using PAUP* (test version 4.0b3a; Swofford, 1998) and MrBayes v3.1 (Ronquist & Huelsenbeck, 2003). For MP analysis all characters were assigned equal weights. MP trees were generated with heuristic search using the tree bisection-reconnection branch swapping with 10 random taxon addition sequence replicates and the Multree option in effect. The maximum number of trees in memory was set to 10,000. Bootstrapping was applied for NJ (1000 replicates) and MP trees (100 replicates). Prior to BMCMC analysis the GTR+I+G model (Tavaré, 1986; Yang, 1993) was chosen using Modeltest 3.06 (Posada & Crandall, 1998), which uses hierarchical likelihood ratio tests (Huelskenbeck & Rannala, 1997) to determine how well competing substitution models fit the data. In BMCMC analysis 1,000,000 generations with a sample frequency set to 100 were run twice. As after 750,000 generations stationarity was achieved (average standard deviation of split frequencies stable at 0.004), the last 2,500 trees of each run were used to compute a majority rule consensus tree assigning posterior probabilities of tree topology.

RESULTS

The 1255 bp sequences of the COI gene of all samples were deposited in GenBank under accession numbers DQ074323–DQ074365: No gaps arose in alignment. All phylogenetic analyses (Fig. 1) revealed that A. ibérica was closer to the ingroup (M. cf. structor, M. bouvieri, M. concolor and M. capitatus) than M. chamberlini and M. lobognathus. M. capitatus, M. concolor and M. bouvieri had maximum node support in all trees (bootstrap values of NJ and MP, posterior probability values of BMCMC). The order of divergence of M. capitatus, M. concolor and M. bouvieri was differently resolved by NJ/MP and BMCMC analyses. Minimum interspecific sequence divergence between these species varied from 8.5 to 11.3%. Maximum intraspecific variation within M. bouvieri (3 haplotypes), M. concolor (4) and M. capitatus (3) ranged from 0.2 to 0.6%.

Within the 29 samples determined as Messor cf. structor, mutations at 166 sites (17 mutations at the first, two at the second, and 147 at the third codon position) resulted in 16 haplotypes with a maximum sequence divergence of 9.3% (Fig. 1). All phylogenetic trees revealed the monophyly of M. cf. structor and samples determined as M. cf. structor always clustered into the same two major lineages, arbitrarily termed A and B. Number of samples in lineage A was 14 in B 15. Node support for the two lineages were maximum in all trees. Maximum sequence divergence within the lineages was 2.4% in lineage A and 5.1% in lineage B. Minimum sequence divergence between lineages was 7.1%. Moreover, in all trees the two major lineages were substructured and the sublineages were supported by very high node support values (Fig. 1). Lineage A contained two sublineages, comprising haplotypes HT1–HT2 and HT3–HT5, with a minimum divergence of 2.3% between them. Lineage B contained four sublineages, HT6–HT7, HT8–HT11, HT12–HT13 and HT14–HT16, with minimum sequence divergences between the sublineages ranging from 3.6 to 5.1%. To test for a possible saturation effect on the phylogenetic signal at the third codon position, a NJ search based only on the first and second positions of the COI data was conducted (tree not shown). The M. cf. structor lineages A and B, as well as all sublineages, were confirmed by high node support values.

The map of where the samples of M. cf. structor (Fig. 1) were collected suggests a geographic separation of DNA lineages with partial overlaps. Lineage A has a larger distribution area; from France and Germany in the west to Bulgaria and Romania in the east. Lineage B is found from Bulgaria to Romania and Austria with its westernmost record from Slovenia. In the Dinaric and Balkan region the distribution areas overlap.

DISCUSSION

The mtDNA trees indicate that the genus Messor, as currently understood, may be polyphyletic since Aphænognaster iberica occurs closer to the Palearctic Messor than to the Nearctic outgroup species Messor lobognathus and M. chamberlini. This agrees with morphological findings (male genitalia: P.S. Ward, pers. comm.; presence / absence of strong propodeal spines in the worker caste: Csósz, unpubl.) and contributes to the dispute on the generic classification of Nearctic “Messor” species (reviewed by Brown, 1974 and Bolton, 1982).

Considerable genetic variation was found in the mitochondrial COI gene among samples determined as M. cf. structor. The two major lineages A and B are very far apart. The minimum divergence of 7.1% between the lineages is in the order of magnitude of the minimum interspecific divergences between M. bouvieri, M. concolor and M. capitatus (8.5–11.3%; Fig. 1) and that
between congeners of *Cardiocondyla*, *Cataglyphis*, *Lasius*, *Myrmica* and *Tetramorium* ants (Savolainen & Vepsäläinen, 2003; Steiner et al., 2004, 2005, 2006a, b; Knaden et al., 2005; Heinze et al., 2005). Thus, the mtDNA data are compatible with the two-species-hypothesis.

For a profound evaluation of genetic variation, however, nuclear markers should be analysed in addition to mtDNA (Beltrán et al., 2002; Lin & Danforth, 2004), and molecular data should be substantiated by other approaches such as morphology, karyology, semiochemistry and ecology (Wetterer et al., 1998; Ross, 2001; Lucas et al., 2002; Ward & Brady, 2003; Janda et al., 2004; Seifert & Goropashnaya 2004; Steiner et al., 2004; Knaden et al., 2005; Maeder et al., 2005; Schlick-Steiner et al., 2005a; Ward & Downie, 2005). This should also rule out hybridization with a hitherto not included *Messor* species and introgression of its haplotypes resulting in the observed mtDNA pattern (cf. Ross & Shoemaker, 2005).
Preliminary microsatellite data of German lineage A and Austrian lineage B populations (Arthofer et al., 2005) suggest a complete separation of allele size at one microsatellite locus (MS2D) and a private allele of the German populations at another (MS2C; Fig. 1). Current morphological analyses (Csősz et al., unpubl.) additionally corroborate that these populations belong to different species.

Overall, the evidence presented here leads us to reject the hypothesis that there is only one species of *Messor* harvester ants in Central Europe. The alternative hypothesis of two species, corresponding to lineages A and B, clearly is more plausible. *Messor cf. structor* thus encompasses a cryptic species in Central Europe. Only by in-depth analyses will it be possible to evaluate whether the distinct substructuring of A and B in the mtDNA trees is due to the existence of even more than two species. The high minimum divergence values between sublineages of A (2.3%) and B (3.6–5.1%) are compatible with this scenario.

Under these circumstances the assignment of species names to mtDNA lineages is futile. It is not even clear which lineage should bear the name *Messor structor* (Latreille, 1798), since the original description is vague and the type material lost (J. Casevitz-Weulersse, Museum of Natural History, Paris, pers. comm.). Biogeographic considerations tentatively point to lineage A because it is the only lineage presently known from the *terra typica* in France (Fig. 1). For the remaining lineages any of 19 taxon names currently classified as subspecies or junior synonyms of *Messor structor* could apply (Table 1).

From a phylogeographic point of view the distributions of lineages A and B are remarkable. The disjunction in Central Europe (Fig. 1) suggests different routes of dispersal, probably from a common Pleistocene refugium: a bypass of the Alps in the west by the ancestors of the Austrian and Czech populations, as hypothesized for the butterfly *Polyommatus cordin* (Schmitt et al., 2002) and for certain dragonflies (Sternberg, 1998), and a bypass in the east by the ancestors of the Austrian and Czech populations.

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