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Revisiting the morphological species groups of West-Palaearctic *Aphaenogaster* ants (Hymenoptera: Formicidae) under a phylogenetic perspective: toward an evolutionary classification

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Abstract

The West-Palaearctic region is a diversity hotspot for the ant genus *Aphaenogaster*. Species in this region are characterized by high morphological variation, which has led to their subdivision into different infrageneric groups. The very first classification in three subgenera, dated 1915, was gradually replaced by eight species-groups. To probe the evolutionary consistency of these species-groups, we sequenced 46 species from all eight species-groups and biogeographic sectors of the region, using one mitochondrial (COI) and six nuclear markers (EPICs), and interpreted the results by integrating qualitative morphology. Our results demonstrate the non-monophyly of all formerly recognized subgenera and species-groups, except for the *crocea* group. We use the phylogeny and morphological characters to propose a new classification of six monophyletic species-groups (*crocea*, *gibbosa*, *graeca*, *pallida*, *sardoa*, *subterranea*). The *pallida*, *subterranea* and *sardoa* (formerly *testaceopilosa*) groups attain monophyletic status by reassigning a few taxa. The *gibbosa* group is to be considered exclusively Western-Mediterranean until further assessments of similar Eastern species. The new *graeca* group is established by including former members of the *splendida* and *subterranea* groups, while the polyphyletic *ceconii*, *obsidiana*, and *splendida* groups are dismissed. Notably, the first is not part of the tropical *Deromyrma* clade as previously thought, while at least two independent clades which require further investigation are composed of species from both the *ceconii* and *splendida* groups, suggesting repeated morphological convergences based on similar ecological adaptations. Finally, *A. cardenai* is confirmed to be a significantly divergent lineage. In addition, three *Aphaenogaster* species are moved to different genera: *Messor asmaae* (Sharaf, 2018) comb. nov., *Messor isekram* (Bernard, 1977) comb. nov., and *Pheidole sarae* (Sharaf, 2018) comb. nov. Further studies should address the evolutionary relationships between the clades recovered in this study.

Keywords

apomorphy, biogeography, diversification, Mediterranean fauna, morphological convergence, plesiomorphy, Myrmicinae

1. Introduction

The ant genus *Aphaenogaster* Mayr, 1853 is part of the tribe Stenammini Ashmead, 1905, along with the genera *Goniomma* Emery, 1895, *Messor* Forel, 1890, *Novomessor* Emery, 1915, *Oxyopomyrmex* André, 1881, *Stenammina* Westwood, 1839, and *Veromessor* Forel, 1917 (Ward et al. 2015). Nowadays, *Aphaenogaster* includes 210 valid extant species and 17 subspecies, as well as 19 fossil species (Bolton 2022). Their distribution is concentrated in the subtropical regions of the Holarctic realm or in the subequatorial and equatorial areas that constitute the Indomalayan and Australasian realms, while only fewer species occur in the northern Neotropics (Central America and Caribbean), in the Nearctic realm, and in temperate regions of Australasia (Janicki et al. 2016; Guénard et al. 2017). Phylogenomic evidence, however, strongly suggests that, in its current definition, the genus is polyphyletic, and that the subequatorial and equatorial species of Asia, Australia, Madagascar, and Central America should be assigned to a separate genus, provisionally indicated as the “*Deromyrma* clade” (named after the former subgenus *Deromyrma* Forel, 1913, currently a junior synonym of *Aphaenogaster*) (Branstetter et al. 2022). The exclusively Holarctic ‘true’ genus *Aphaenogaster* is thought to have a Palearctic origin and temperate ancestral habit, and to be the sister genus of the seed-harvesting genus *Messor* Forel, 1890 (Branstetter et al. 2022). Together, the two form the “*Aphaenogaster* clade”, and while more likely considered to be a sister group to the ‘true’ *Aphaenogaster*, *Messor* may also be phylogenetically embedded within it according to some phylogenetic reconstructions (Gómez et al. 2018; Schär et al. 2020; Branstetter et al. 2022).

The type species of the genus *Aphaenogaster*, *A. sardoa* Mayr, 1853, was described from the West-Palaearctic region, specifically from the Mediterranean island of Sardinia (Mayr 1853). The Mediterranean region is a widely recognized key biodiversity hotspot for terrestrial organisms (Médail and Quézel 1999) and it hosts the largest portion of the world’s *Aphaenogaster* diversity, which becomes the vast majority of the species if the *Deromyrma* clade is not counted. In his catalogue, Borowiec (2014) presented an impressive list of 82 valid taxa, and additional species have been described since then, rising the number at over 100 taxa (Borowiec and Salata 2014; Salata and Borowiec 2016; Gómez et al. 2018; Alicata and Schifani 2019; Bračko et al. 2019; Salata et al. 2021). Interestingly, this diversity is not only a matter of species richness but also remarkable in morphological and ecological terms. The West-Palaearctic fauna ranges from long-legged ants running diurnally in open habitats of arid regions (e.g., *A. senilis* Mayr, 1853), through species with short legs and smaller eyes living in the leaf litter of forest or endogean

habitats (e.g., *A. pallida*, *A. subterranea*), and troglobiotic and hypogean species with long antennae and slow movements (e.g., *A. cardenai*, *A. ceconii*), to specialized granivorous species morphologically convergent with more typical seed-harvesting genera (*A. striativentris*) (Tinaut and Jiménez Rojas 1991; Caut et al. 2013; Borowiec and Salata 2014; Ortuño et al. 2014; Seifert 2018).

Morphological diversification in particular encouraged the introduction of different infrageneric classifications, often largely based on the West-Palaearctic fauna, the first of which was established by Emery (1915). In his work, he presented a subdivision of four subgenera: *Aphaenogaster* s. str. (comprising species characterized by reduced mesosoma in the queen caste), *Attomyrma* Emery, 1915 (a large group of species with regular-sized mesosoma for claustral-type queens and heads lacking an elongated neck, type is *A. subterranea* (Latreille, 1798) described from France), *Deromyrma* Emery, 1915 (most species having a neck-like elongation of the head, type is *A. swammerdami* Forel, 1886 described from Madagascar), and *Planimyrmica* Viehmeyer, 1914 (a few Papuan species similar to *Deromyrma* but whose males possess two spines on the mesonotum and 12 instead of 13 antennal segments, type species is *A. loriai* (Emery, 1897) described from New Guinea). Emery (1915) divided *Deromyrma* into several species-groups, including the *ceconii* group known from the Mediterranean. According to him, the West-Palaearctic fauna was split between three subgenera *Aphaenogaster* s. str., *Attomyrma*, and *Deromyrma*. This framework was gradually abandoned, and eventually *Attomyrma*, *Deromyrma*, and *Planimyrmica* were considered junior synonyms of *Aphaenogaster* (Brown 1973; Smith 1979; Bolton 1982; 1995).

Later Schulz (1994) proposed a subdivision of the very large subgenus *Attomyrma* (which he still recognized as valid) into six species-groups: *gibbosa*, *obsidiana*, *pallida*, *splendida*, *subterranea*, and *rothneyi*. All of these groups were West-Palaearctic and named after Mediterranean species, with the exclusion of the *rothneyi* group, and this new classification outlived the subgenera. However, several West-Palaearctic taxa and most of those from other regions were not mentioned by Schulz (1994), so their status in relation to his classification remained unclear. Two decades later, Boer (2013) followed the same structure as Schulz (1994), mostly focusing on Mediterranean species and entirely abandoning the subgeneric classification of Emery (1915). He replaced the former *Aphaenogaster* s. str. subgenus by splitting it into a *testaceopilosa* group, leaving *A. sardoa* as separate. He also changed the species composition of many of the groups defined by Schulz (1994) without changing their number. In the following years, a

number of other studies improved the general understanding of the Mediterranean *Aphaenogaster* diversity, kept redefining the boundaries of many groups (e.g., Salata and Borowiec 2018), and finally an eighth group was introduced (*crocea* group, see Alicata and Schifani 2019).

In the last decade, for what concerns the ‘true’ *Aphaenogaster*, there was the first influx of scattered phylogenetic data produced by the increasing use of molecular phylogenetics, mostly coming from the West-Palaearctic or Nearctic faunas (Branstetter et al. 2022; DeMarco and Cognato 2016; Lorite et al. 2017; Centorame et al. 2018; Gómez et al. 2018; Schär et al. 2020). A notable incidental result was the apparent paraphyly of the clades formed by the species of the former subgenera *Aphaenogaster* s. str. and *Attomyrma*, but no study was specifically conceived to reconstruct the phylogenetic relationships between the recognized species-groups. A large portion of these groups was not covered by any of these studies, and the consistency of the whole framework was never tested. Moreover, the relationship between the *cecconii* group and the “*Deromyrma* clade” was never investigated (Branstetter et al. 2022), despite the fact that Emery (1915) considering the first to be part of the latter.

Our aim was to finally produce a phylogenetic reconstruction covering all the species-groups recognized in the West-Palaearctic region, testing for the first time the evolutionary coherence of this framework, including clarifying the groups’ relationship with the tropical “*Deromyrma* clade”, and trying to determine whether key morphological characteristics traditionally used to characterize these groups were apomorphic or convergent.

2. Materials and methods

2.1. Study area

The West-Palaearctic boundaries are here considered to comprise the Mediterranean regions of Africa and Asia. In this definition, we followed the traditional concept of Sclater (1858), and not what was proposed more recently by Holt et al. (2013; also see Wang et al. 2022). Our reasoning behind this choice stems from the fact that the distribution of *Aphaenogaster* does not comprise the Afrotropical region (Branstetter et al. 2022), but North-Western Africa and the Eastern Mediterranean coast both host a large share of the species thought to be closely related to European taxa (e.g., Borowiec and Salata 2014; Salata and Borowiec 2018; Alicata and Schifani 2019; Salata et al. 2021). This pattern is also visible for other groups, such as vascular plants (Carta et al. 2022).

2.2. Species-groups concept

We define species-groups as mutually exclusive, monophyletic entities comprising multiple closely related species of the same genus.

2.3. Composition of the West-Palaearctic species-groups

For the composition of each species-groups, we adopted the most recently published classifications. After each taxon, we report its state-level distribution according to AntMaps (Janicki et al. 2016; Guénard et al. 2017) and recent records, if any, that have not yet been added to its database (Salata et al. 2021; Schifani et al. 2021). Taxa not found in the West-Palaearctic region, very few of which were listed by Schulz (1994), are not considered here.

2.3.1. *cecconii* group

We follow the recent definition by Borowiec and Salata (2014) and Salata and Borowiec (2016), that groups East-Mediterranean species formerly assigned to the subgenus *Deromyrma* alongside others with similar worker morphology, biogeography, and ecology, resulting in a list of 7 taxa: *A. cecconii* Emery, 1894 (Greece), *A. charresi* Salata and Borowiec, 2016 (Greece), *A. equestris* Borowiec and Salata, 2014 (Türkiye), *A. jolantae* Borowiec and Salata, 2014 (Greece), *A. lykiaensis* Borowiec and Salata, 2014 (Türkiye), *A. olympica* Borowiec and Salata, 2014 (Greece) and *A. phillipsi* Wheeler and Mann, 1916 (Egypt, Israel and Palestine, Jordan).

Defining combination of morphological characters (after Borowiec and Salata 2014, all characters referred to workers): “Body surface with indistinct microsculpture, shiny across extensive areas; body coloration from yellow to black; head oval with a sharp basal carina or strongly narrowed posteriorly to a neck with a flared collar; antennal scapes long, surpassing the posterior margin of the head by at least 1/3 of their length; basal and mid antennal segments distinctively longer than wide; mesosoma narrow and elongate”

2.3.2. *crocea* group

According to its very recent definition, the Siculo-Maghrebian *crocea* group comprises 10 taxa formerly included in the *gibbosa*, *splendida* or *subterranea* groups, grouped together based on male and worker morphology and biogeography (Alicata and Schifani 2019): *A. crocea* s. str. André, 1881 (Algeria, Morocco), *A. crocea croceoides* Forel, 1890 (Algeria, Tunisia), *A. crocea lenis* Santschi, 1911 (Tunisia), *A. crocea splendidoides* Forel, 1890 (Algeria, Tunisia), *A. faureli* Cagniant, 1969 (Algeria), *A. fiorii* Emery, 1915 (Italy, Malta), *A. hesperia* Santschi, 1911 (Spain: Canary Islands), *A. sicula* Emery, 1908 (Italy), *A. strioloides* Forel, 1890 (Algeria, Italy, Tunisia), and *A. trinacriae* Alicata and Schifani, 2019 (Italy).

Defining combination of morphological characters (based on Alicata and Schifani 2019): worker pigmentation from light brownish to ferruginous or yellow (never blackish); worker appendages and mesosoma not significantly elongate, the latter lacking a deep metanotal groove in profile view; males with an anteriorly gibbous and posteriorly flat mesosoma, head approximately as long as wide.

2.3.3. *gibbosa* group

This group comprises taxa sharing similar worker and/or male morphology initially thought to be close to the *subterranea* or the *testaceopilosa* groups (Emery and Forel 1879; Dalla Torre 1893; Emery 1921). The following 9 taxa, mostly West-Mediterranean, are considered to belong to it (Salata and Borowiec 2018; Gómez et al. 2018; Alicata and Schifani 2019): *A. gibbosa* s. str. (Latreille, 1798) (France, Italy, Spain, Portugal), *A. gibbosa homonyma* Emery, 1921 (Algeria, Morocco, Tunisia), *A. italica* Bondroit, 1918 (Italy, Switzerland), *A. mauritanica* Dalla Torre, 1893 (Algeria, Morocco, Tunisia), *A. muschtaidica* Emery, 1908 (Azerbaijan, Georgia, Iran), *A. nadigi* Santschi, 1923 (Morocco), *A. striativentris* Forel, 1895 (Spain), *A. theryi* Santschi, 1923 (Morocco), and *A. ulibeli* Gómez and Espadaler, 2018 (Spain).

Defining combination of morphological characters (based on Salata and Borowiec 2018; Gómez et al. 2018; Alicata and Schifani 2019): body pigmentation of workers from dark brown to black, their head characterized by longitudinal rugae or reticulation at least on its anterior part of head dorsum, sometimes rugae and reticulation replaced or co-occurring with punctuation, surface between rugae with dense micropunctuation or smooth and shiny; in workers, funicular segments from 1.5 to 2 times longer than wide and the scapi reach at least 1/5 of its length over the occipital margin of the head; workers' propodeal spines are always present, short, triangular, inclined at an 45° angle, with a wide base. Males with an anteriorly gibbous and posteriorly flat mesosoma except for *A. ulibeli*.

2.3.4. *obsidiana* group

The *obsidiana* group was defined as a small group of 3 East-Mediterranean species based on worker morphology (Schulz 1994): *A. epirotes* Emery, 1895 (Albania, Croatia, Bosnia and Herzegovina, Bulgaria, Greece, Israel and Palestine, Italy, Montenegro, Serbia, Slovenia, Türkiye), *A. obsidiana* (Mayr, 1861) (Azerbaijan, Georgia, Iran, Russia, Türkiye), and *A. subcostata* Viehmeyer, 1922 (Greece, Türkiye).

Defining combination of morphological characters (from Schulz 1994, all characters referred to workers): “Chubby, squat species with an almost square, rounded head. Antennae just reaching the posterior edge of the head. Median funiculus segments as long as wide. Coloration light red, brown to black, sculpture deep and dense, mostly connected like a net on the head. Hairs thicker than in the previous group, protruding body hairs numerous. Spines of medium length, always prominent. Despite the deep wrinkles, the microsculpture is not developed; smooth and shiny”.

2.3.5. *pallida* group

A circum-Mediterranean group based on worker morphology, which includes 11 taxa including those presented by Kiran et al. (2008) and Boer (2013), plus their

North African subspecies or relatives defined by Cagniant (1996): *A. dulcinea* Santschi, 1919 (Italy, France, Portugal, Spain), *A. finzii* Müller, 1921 (Bosnia and Herzegovina, Croatia, Italy, Greece, Macedonia, Montenegro, Serbia), *A. foreli* Cagniant, 1996 (Algeria, Morocco), *A. holtzi* (Emery, 1898) (Iran, Türkiye), *A. lesbica* Forel, 1913 (Greece, Israel and Palestine, Montenegro), *A. leveillei* s. str. Emery, 1881 (Algeria, Morocco, Tunisia), *A. leveillei laurenti* (Santschi, 1939) (Morocco), *A. pallida* (Nylander, 1849) (Italy), *A. radchenkoi* Kiran and Aktaş, 2008 (Bulgaria, Greece, Türkiye), *A. subterraneoides* s. str. Emery, 1881 (Azerbaijan, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Greece, Israel and Palestine, Macedonia, Russia, Ukraine), and *A. subterraneoides armeniaca* Arnol'di, 1968 (Armenia).

Defining combination of morphological characters (from Schulz 1994, all characters referred to workers): “Mostly small narrow forms, with mostly smooth and glazed integument and pale yellow colouring. Epinotum usually with small tooth-like spines, or completely spineless (in *A. finzii* the spines are longer, but all other characteristics are clearly developed, which is why this species is to be classified here). Hairs long, thinner than other groups. Antennal shaft just exceeding the posterior margin. Median funiculus segments as long as wide”.

2.3.6. *splendida* group

A mostly East-Mediterranean species-groups recently redefined by Salata et al. (2021) based on worker morphology, and which counts 13 taxa: *A. aktaci* Kiran and Tezcan, 2008 (Greece, Türkiye), *A. dluskyi* Radchenko and Arakelian, 1991 (Armenia), *A. festae* Emery, 1915 (Bulgaria, Greece, Türkiye), *A. hamaensis* Salata et al., 2021 (Syria), *A. kervillei* Forel, 1910 (Israel and Palestine, Lebanon, Syria, Türkiye), *A. ovaticeps* Emery, 1898 (Albania, Bosnia and Herzegovina, Croatia, Greece, Italy, Montenegro, Serbia, Slovenia), *A. peloponnesiaca* Salata et al., 2021 (Greece), *A. schmitzi* Forel, 1910 (Israel and Palestine, Jordan, Syria, Türkiye), *A. rugosoferruginea* Forel, 1889 (Greece), *A. splendida* (Roger, 1859) (Algeria, Bosnia and Herzegovina, Cyprus, Egypt, France, Greece, Iran, Israel and Palestine, Italy, Lebanon, Libya, Macedonia, Malta, Montenegro, Portugal, Russia, Spain, Syria, Tunisia, Ukraine), *A. syriaca* Emery, 1908 (Egypt, Iran, Israel and Palestine, Lebanon, Syria), *A. transcaucasica* Karavaiev, 1926 (Azerbaijan), and *A. vohraliki* Salata et al., 2021 (Türkiye).

Defining combination of morphological characters (from Salata et al. 2021): “Workers moderate to large (mesosoma length along Weber's line: 1.5–3.0), with slim and elongated body; head always longer than wide, slim and oval to elongated (head length on head width ratio: 1.1–1.6); antennae and legs elongate; scape distinctly protruding above the head occipital margin; segments of antennal funicle always longer than wide; body usually yellow to yellowish brown, occasionally brown but never black; head sculpture distinct, with microreticulate background and more or less developed longitudinal to reticulate rugae; surface of mesosoma mostly reticulate and

with additional sculpture of longitudinal or/ and reticulate rugae, shiny areas, if present, restricted to pronotal top and sides; gaster shiny, usually smooth or with diffused microreticulation only on the first gastral tergite. Males with gibbous promesonotum, and narrow elongate propodeum, known in detail for only two species (*A. festae* and *A. splendida*)”.

2.3.7. *subterranea* group

A mostly East-Mediterranean group defined by Schulz (1994), Boer (2013), Alicata and Schifani (2019), Galkowski et al. (2019) and Bračko et al. (2019) based on worker morphology, and includes six taxa: *A. graeca* Schulz 1994 (Greece), *A. ichnusa* Santschi, 1925 (France, Italy, Spain), *A. illyrica* Bračko et al., 2019 (Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Macedonia), *A. kurdica* (Ruzsky, 1905) (Armenia, Azerbaijan, Georgia, Iran, Russia), *A. maculifrons* Kiran and Aktaş, 2008 (Türkiye), and *A. subterranea* (Latreille, 1798) (Albania, Austria, Armenia, Azerbaijan, Belgium, Bosnia and Herzegovina, Bulgaria, Czech Republic, France, Georgia, Germany, Greece, Hungary, Iran, Italy, Macedonia, Moldova, Montenegro, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Türkiye, Ukraine).

Defining combination of morphological characters (based on Schulz 1994; Bračko et al. 2019): Medium-sized forms, shallow sculpture, large parts of the body shiny. But always at least the front part of the head is dull covered with shallow wrinkles. The posterior edge of the head overruns the antennae. Median funiculus segments are about as long as wide, but not more than 1/2 times as long. Color yellowish-red to dark brown. Head rectangular rounded. Metanotal groove present, deep and narrow. Pronotum and mesonotum form regular convexity, mesonotum not raised above the surface of pronotum, propodeal spines short, not longer than half length of the first segment of antennal funiculus, mesosoma short.

2.3.8. *testaceopilosa* group

A large Euro-Maghrebian group based on worker and queen morphology (Cagniant 1996; Boer 2013; Cagniant and Galkowski 2013) with 41 taxa: *A. afra* Santschi, 1933 (Algeria, Morocco), *A. atlantis* Santschi, 1929 (Morocco), *A. balcanica* (Emery, 1898) (Albania, Bosnia and Herzegovina, Croatia, Greece, Türkiye), *A. balcanicoides* Boer, 2013 (Greece), *A. baronii* Cagniant, 1988 (Morocco), *A. campana* Emery, 1878 (Italy), *A. curiosa* Santschi, 1933 (Morocco), *A. dejeani* Cagniant, 1982 (Morocco), *A. depilis* s. str. Santschi, 1911 (Algeria, Morocco), *A. depilis numida* Santschi, 1933 (Tunisia), *A. espadaleri* Cagniant, 1984 (Morocco), *A. fallax* Cagniant, 1992 (Algeria, Morocco), *A. gemella* s. str. (Roger, 1862) (Morocco, Algeria, Portugal, Spain), *A. gemella marocana* (Forel, 1903) (Morocco), *A. iberica* Emery, 1908 (Portugal, Spain), *A. inermia* Bolton, 1995 (Italy, Malta), *A. karpatica* Boer, 2013 (Greece), *A. koniari* Cagniant and Galkowski, 2013 (Morocco), *A. melitensis* Boer, 2013 (Malta), *A. miniata* Cagniant, 1990 (Morocco), *A. picena* Baroni Urbani,

1971 (Albania, Croatia, Italy, Slovenia), *A. praedo* s. str. Emery, 1908 (Algeria, Morocco), *A. praedo ellipsoida* Santschi, 1933 (Morocco), *A. praenoda* Santschi, 1933 (Morocco), *A. praenoda confinis* Santschi, 1933 (Morocco), *A. rifensis* Cagniant, 1994 (Morocco), *A. rupestris* Forel, 1909 (Algeria, Morocco), *A. semipolita* (Nylander, 1856) (Italy), *A. senilis* Mayr, 1853 (Algeria, France, Italy, Morocco, Portugal, Spain), *A. senilis disjuncta* Santschi, 1933 (Morocco), *A. sicardi* Cagniant, 1990 (Morocco), *A. simonellii* Emery, 1894 (Greece), *A. spinosa* Emery, 1878 (Italy, France, Switzerland), *A. sporadis* Santschi, 1933 (Cyprus, Greece, Türkiye), *A. testaceopilosa* (Lucas, 1849) (Algeria, Morocco, Spain, Tunisia), *A. testaceopilosa cabylica* Stitz, 1917 (Algeria, Morocco, Tunisia), *A. testaceopilosa canescens* (Emery, 1895) (Algeria, Tunisia), *A. tinauti* Cagniant, 1992 (Morocco), *A. torossiani* Cagniant, 1988 (Morocco), *A. weleursseae* Cagniant, 1989 (Morocco), and *A. wilsoni* Cagniant, 1988 (Morocco).

Defining combination of morphological characters (from Boer 2013): “The workers of the *A. testaceopilosa*-group have a punctate head and mesosoma, while the head is neither elongated, nor collar-shaped. This character combination is absent in the other European species of the genus *Aphaenogaster*, except for *A. sardoa*. The punctation is also present in the gyne and male, but only on certain body parts, usually the head. In most species, the anterior portion of the dorsal side of the first gastral tergite is microstriated. [...] Originally this subgenus contained all the species here placed in the *A. testaceopilosa*-group, plus *A. sardoa*. The reason to exclude this species is that *A. sardoa* differs in several characters from the other species: 1) the males of *A. sardoa* have small, short and minutely dentate mandibles, instead of dentate, broad mandibles, 2) the males and gynae of *A. sardoa* have larger eyes and ocelli, 3) they have broad spherical petioles, and 4) all castes of *A. sardoa* are yellowish, while those of the *A. testaceopilosa*-group are blackish or (particularly after preservation) reddish-brown”.

2.3.9. Species not part of any group

We consider *A. cardenai* Espadaler, 1981 (Spain), *A. sardoa* s. str. Mayr, 1853 (Algeria, Italy, Morocco, Tunisia), *A. sardoa anoemica* Santschi, 1910 (Morocco), and *A. ujhlyi* Szabó, 1910 (Tunisia) not to belong to any group. Although *A. cardenai* was until recently placed within the *splendida* group (Espadaler 1981; Boer 2013), Gómez et al. (2018) contested this position and considered it to be only distantly related to the remaining Mediterranean *Aphaenogaster* based on molecular data (though they did not test its relationship with any species of the *splendida* group). In morphological terms, *A. cardenai* is certainly unique in several aspects being characterized by very small eyes (compared to the head size, proportionally smaller than in any other species of the region), very long scapi (only comparable to those of the *ceconii* group), a significantly developed sculpture interspersed with entirely shiny areas, an elongate mesosoma and very long spines (longer than in any other species of the

region). *Aphaenogaster sardoa*, on the other hand, was long considered to form a single group with the members of the actual *testaceopilosa* group (i.e. part of the *Aphaenogaster* s. str. subgenus) until Boer (2013) separated them based on the morphological characters stated above (see under *A. testaceopilosa* group). Moreover, *A. sardoa anoemica* and *A. ujhlyi* are two valid sympatric taxa whose distinctiveness from *A. sardoa* s. str. is undemonstrated. Most likely due to their dubious status, they were not accommodated in the *sardoa* group by Boer (2013).

2.3.10. Species of uncertain position

We consider uncertain the position of *A. burri* (Donisthorpe, 1950) (Türkiye), *A. depressa* Bolton, 1995 (Türkiye), *A. pallescens* Walker, 1871 (Egypt), *A. saharensis* (Bernard, 1953) (Algeria), and *A. sangiorgii* Emery, 1901 (Greece). Morphology may suggest that the first two could be tentatively associated with the *subterranea* group, and the latter one to the *pallida* group, but their statuses are unclear since they are all known from only the holotype specimens, which in the case of *A. burri* and *A. sangiorgii* are queens (Emery 1901; Donisthorpe 1950). The identity of *A. sangiorgii* will be discussed in a dedicated paper (in prep.). Finally, *A. pallescens* is considered a species *incertae sedis* or unrecognizable taxon (Emery 1915; Bolton 1995), and the identity of *A. saharensis*, described from a single male (Bernard 1953), remains unclear.

2.3.11. Species excluded from the West-Palaearctic *Aphaenogaster* list

For reasons we detail in the results section, we do not consider *A. asmaae* Sharaf, 2018 (Oman), *A. isekram* Bernard, 1977 (Algeria), or *A. sarae* Sharaf, 2018 (Oman) as part of the West-Palaearctic *Aphaenogaster* diversity.

2.4. Molecular phylogeny

DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the protocol by Cruaud et al. (2019), on whole specimens without damaging the integument, or, in a few cases, on one of the middle legs. One mitochondrial marker (COI, 658 bp, coding for part of the cytochrome c oxidase subunit 1) and six Exon Primed Intron Crossing (“EPIC”) nuclear markers (ant.1, 373 bp; ant.263, 460 bp; ant.346, 391 bp; ant.389, 689 bp; ant.505, 521 bp; ant.1401, 935 bp) were amplified by polymerase chain reaction (PCR) using the primer pairs in Folmer et al. (1994) and Ströher et al. (2013) for COI and EPIC markers respectively. We used the EPIC markers instead of other nuclear markers more commonly used in ant phylogeny (such as those used in Ward et al. 2015) because these more commonly used markers provided little support in a previous phylogeny of the genus *Aphaenogaster* (DeMarco and Cognato

2016), or showed a lower variability and a lower amplification success than EPIC markers in preliminary tests (see also Centorame et al. 2018, where amplification success of CAD in some European *Aphaenogaster* species is only 37%). EPIC markers have been successfully used in ant phylogeny in the genera *Cataglyphis* Foerster, 1850 (Eyer et al. 2017, 2018; Kuhn et al. 2020), *Camponotus* Mayr, 1861 (Hartke et al. 2019), *Plagiolepis* Mayr, 1861 (Degueldre et al. 2021) and *Tapinoma* Foerster, 1850 (Escárraga et al. 2021), and, since they rely on universal primers, they can also be used across a broad taxonomic range. Finally, they may also allow to compare exon and intron fragments although this option was beyond the purpose of this study.

Sanger dideoxy sequencing of PCR amplicons was performed by Eurofins Genomics (Germany) in both directions using the same primers as those used for the initial amplification. Sequences were edited using CODON-CODE ALIGNER (CodonCode Corporation, Dedham, MA, USA), and contigs were built from forward and reverse sequences generated for each gene. Conflicting base calls were coded as missing. Sequences were aligned with MUSCLE (Edgar 2004) using the default settings. Alignments were inspected visually and edited manually using MESQUITE v. 3.31 (Maddison and Maddison 2017) when they could be improved. The following substitution models were selected using the Bayesian Information Criteria (BIC) implemented in JMODELTEST2 v2.1.6 (Darriba et al. 2012) run on the CIPRES Science Gateway (Miller et al. 2010): TIM2 + I + G for COI codon position 1, TIM1 + I + G for COI codon position 2, TIM2 + G for COI codon position 3, HKY + I for ant.1, ant.263 and ant.346, HKY + G for ant.389, ant.505 and ant.1401. The models TIM1 and TIM2 were replaced by the GTR model in MR-BAYES analyses. Phylogenetic reconstructions were performed using Bayesian inference with MRBAYES v. 3.2.7a (Ronquist et al. 2012) on the CIPRES Science Gateway (Miller et al. 2010). Two analyses of four chains were run for 10,000,000 generations, sampling trees every 500 generations and a 25% burn-in for each run. In addition, maximum likelihood phylogenies were constructed with PhyML 3.0 online (<http://www.atgc-montpellier.fr/phyml>), using automatic model selection by SMS (Lefort et al. 2017) and a standard bootstrap analysis. Phylogenies were produced for all markers concatenated (4027 nucleotides in total), for COI only (658 nucleotides), and for EPIC markers concatenated (3369 nucleotides).

The following numbers of new sequences were produced in this study: 90 for COI (GenBank accession numbers OM896791–OM896880), 82 for ant.1 (OM939213–OM939294), 84 for ant.263 (OM939295–OM939378), 85 for ant.346 (OM939379–OM939463), 67 for ant.389 (OM939464–OM939530), 74 for ant.505 (OM939531–OM939604), 71 for ant.1401 (OM939605–OM939675). Amplification success was 95 % for COI, 88 % for ant.1, 90 % for ant.263, 91 % for ant.346, 72 % for ant.389, 80 % for ant.505 and 76 % for ant.1401. DNA sequence alignments are provided in the Supplementary material S1, while all GenBank accession numbers are provided in the Supplementary material S2.

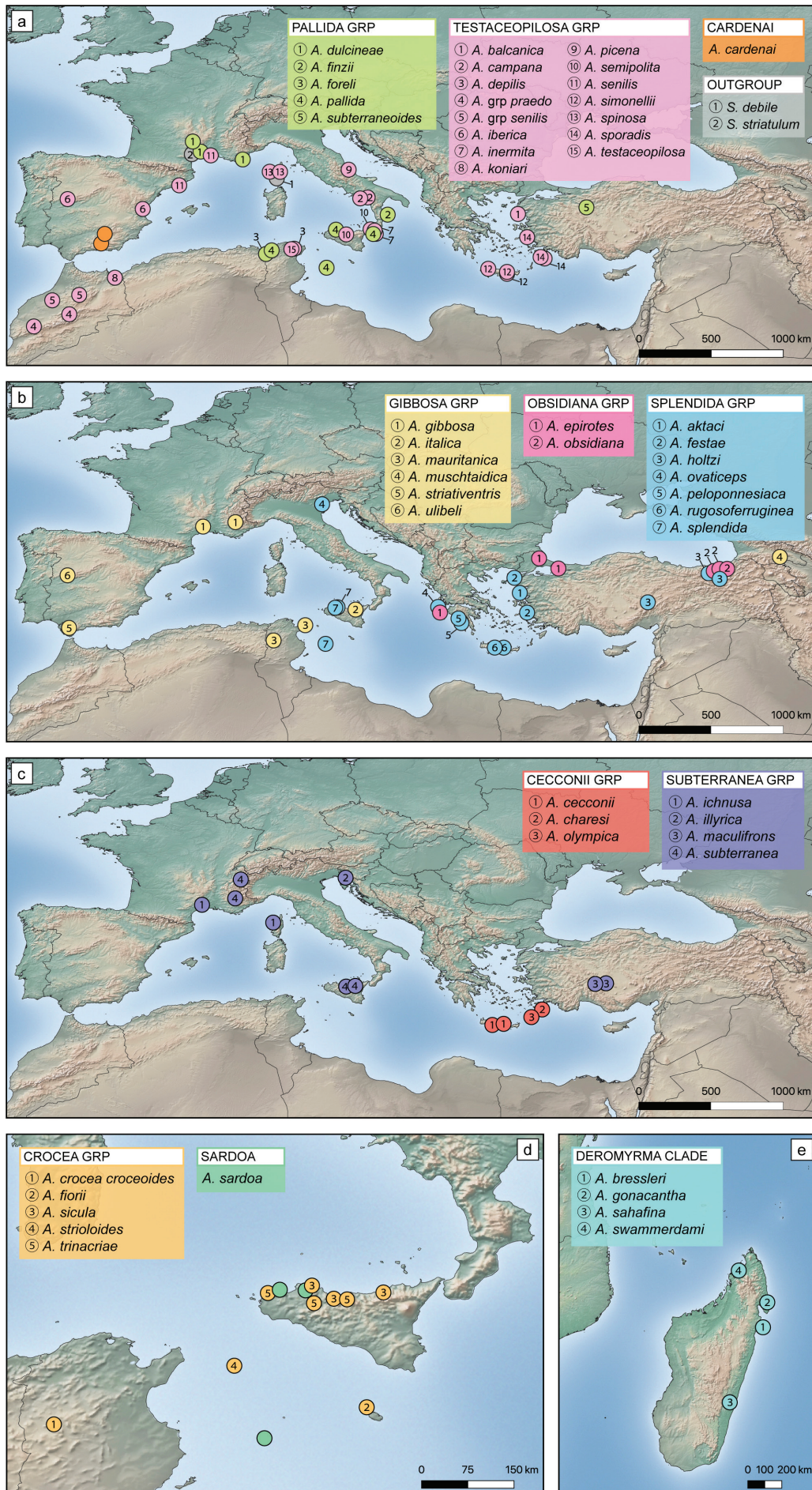


Figure 1. Identity and distribution of the samples used for the molecular analyses in this study.

2.5. Species used for the analysis

A complete list of the specimens sequenced in our investigation, including their geographic origin and collecting data is provided in Supplementary material S2, while this information is reassumed in Figure 1. The following 47 West-Palaearctic species, covering all species-groups and biogeographic sectors of the region, were used in the analyses (the % of covered species of each group is expressed in parentheses): *cecconii* group: *A. cecconii*, *A. charesi*, *A. olympica* (43%); *crocea* group: *A. crocea croceoides*, *A. fiorii*, *A. sicula*, *A. strioloides*, *A. trinacriae* (71%); *gibbosa* group: *A. gibbosa*, *A. italica*, *A. mauritanica*, *A. muschtaidica*, *A. striativentris*, *A. ulibeli* (75%); *obsidiana* group: *A. epirotes*, *A. obsidiana* (67%); *pallida* group: *A. dulcinea*, *A. finzii*, *A. foreli*, *A. holtzi*, *A. pallida*, *A. subterraneoides* (86%); *splendida* group: *A. aktaci*, *A. festae*, *A. ovaticeps*, *A. peloponnesiaca*, *A. rugosoferruginea*, *A. splendida* (50%); *subterranea* group: *A. ichnusa*, *A. illyrica*, *A. maculifrons*, *A. subterranea* (80%); *testaceopilosa* group: *A. balcanica*, *A. campana*, *A. depilis*, *A. iberica*, *A. inermis*, *A. koniari*, *A. picena*, *A. praedo* s. l., *A. semipolita*, *A. senilis*, *A. simonellii*, *A. spinosa*, *A. sporadis* (39%); species not part of any group: *A. cardenai*, *A. sardoa*.

In addition, in order to test a possible relatedness between some West-Palaearctic species and members of the tropical *Deromyrma* clade *sensu* Branstetter et al. (2022), we also sequenced the following four Malagasy taxa: *A. bressleri* Csösz and Fisher, 2021, *A. gonacantha* (Emery, 1899), *A. sahafina* Csösz and Fisher, 2021, and *A. swammerdami* Forel, 1886 (Csösz et al. 2021).

Finally, we chose as outgroups the two Stenammini species *Stenammina debile* (Foerster, 1850) and *S. striatulum* Emery, 1895 since the genus *Stenammina* is sister to both the ‘true’ *Aphaenogaster* and the *Deromyrma* clade (Branstetter et al. 2022).

Voucher specimens sequenced in this study were marked with unique identifiers which are reported in the Supplementary material S2 alongside their repositories.

2.6. Integrative revision of the species-groups classification

We modify the existing species-groups framework by interpreting the phylogenetic results in light of qualitative morphological characters of the species. Qualitative morphological characters are intended as discrete characters (presence or absence of certain traits) easily observable by trained myrmecologists without the need of detailed numerical recording (Schifani et al. 2022). Modified definitions of existing groups or new species-groups definitions are given only if a strong phylogenetic support backs a clade composed by species bonded by a distinctive set of qualitative morphological characters that should generally allow convincing hypotheses on the possible affiliation of non-sequenced species to that same clade. For the remaining clades, we offer a detailed reporting of the critical issues that must be overcome before a safe group definition can be drafted.

3. Results

3.1. Molecular phylogeny

The sequenced species from the *Deromyrma* clade (*swammerdami* group) were recovered as sister to all other investigated species. Similarly, *A. cardenai* was recovered as sister to all other Mediterranean species, and then, a well-supported clade containing *A. striativentris*, *A. gibbosa*, *A. ulibeli* and *A. mauritanica* was recovered as sister to all the remaining species. Several highly supported clades were recovered among the remaining species, but the relationships among these clades were poorly supported, hindering any inference on the phylogenetic relationships among them. Most notably, we found as well supported (posterior probability: 0.95–1), a clade containing *A. cecconii*, *A. rugosoferruginea*, *A. festae* and *A. splendida*, a clade containing *A. subterraneoides*, *A. finzii*, *A. foreli*, *A. dulcinea* and *A. pallida*, a clade containing *A. illyrica* and *A. aktaci*, a clade containing *A. strioloides*, *A. crocea croceoides*, *A. sicula*, *A. fiorii* and *A. trinacriae*, a clade containing *A. epirotes*, *A. holtzi*, *A. subterranea*, *A. maculifrons* and *A. ichnusa*, a clade containing *A. charesi*, *A. ovaticeps* and *A. peloponnesiaca*, a clade containing *A. striativentris*, *A. gibbosa*, *A. ulibeli* and *A. mauritanica*, and a clade containing all the members of the *testaceopilosa* group plus *A. sardoa*. The placement of the following species remained unresolved: *A. olympica*, *A. italica*, *A. muschtaidica* and *A. obsidiana*. This general topology was congruent in both kinds of phylogenetic reconstructions (Bayesian inference and maximum likelihood) (Fig. 2 and Supplementary material S3). The only notable difference between the two reconstruction methods was the support of the clade composed of *A. illyrica* and *A. aktaci*, which was strong with Bayesian phylogeny (posterior probability: 1) and only moderate with maximum likelihood (bootstrap value: 0.60).

An important difference between phylogenetic reconstructions based on mitochondrial and nuclear markers was that, according to EPIC markers, *A. muschtaidica* was included in the clade containing *A. epirotes*, *A. holtzi*, *A. subterranea*, *A. maculifrons* and *A. ichnusa*, whereas it was outside of this clade following the COI marker. As a whole, the phylogeny based on EPIC markers only was less resolved than that based on COI markers only, but concatenation of both types of markers provided a better result as any of the two marker types taken individually. Every species proved to be monophyletic save the case of *A. sporadis*, recovered within an unresolved clade with *A. balcanica* and *A. picena*.

We obtained the following results concerning the status of each group (Fig. 2):

3.1.1. *cecconii* group

Status: polyphyletic. The group corresponds to three independent lineages in our tree (one per each species sequenced). In two cases (*A. cecconii* and *A. charesi*), the

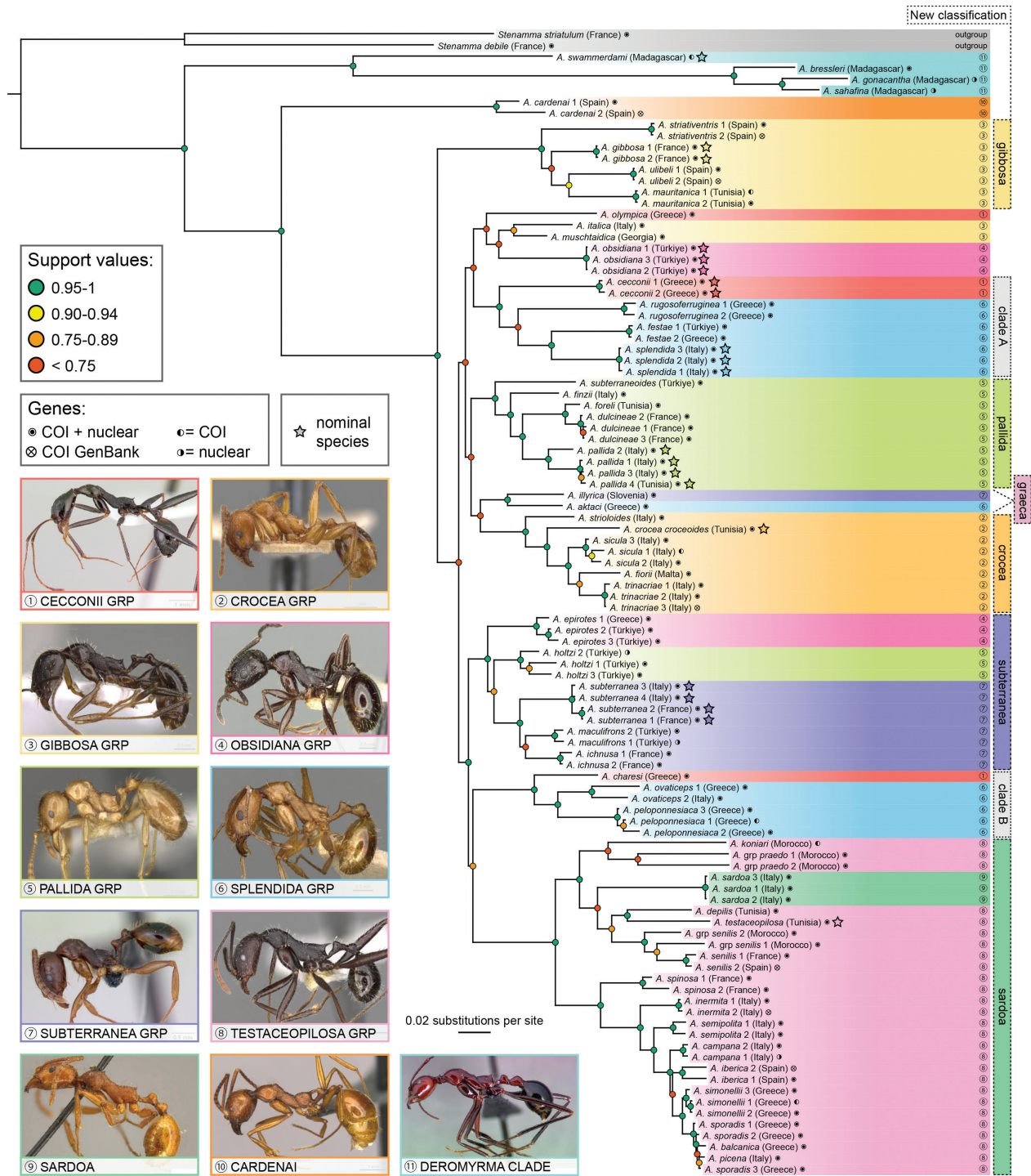


Figure 2. Phylogeny of the West-Palaearctic *Aphaenogaster* ants, including all the species-groups of the region as well as the tropical *Deromyrma* clade based on a Bayesian analysis of one mitochondrial (COI) and six nuclear (EPICs) markers. Support values represent Bayesian posterior probability values (PP). In the rightmost part of the figure, we present the new species-groups boundaries based on the interpretation of the phylogenetic results that is offered in the Discussion section. Photographs of worker specimens from *A. cecconii* (CASENT0179868, photo by Erin Prado), *A. crocea croceoides* (CASENT0907682, syntype, photo by Will Ericson), *A. gibbosa* (CASENT0914409, photo by Zach Lieberman), *A. obsidiana* (CASENT0280957, photo by Shannon Hartman), *A. dulcineae* (CASENT0280959, photo by Michele Esposito), *A. splendida* (CASENT0280965, photo by S. Hartman), *A. subterranea* (CASENT0173580, photo by April Nobile), *A. testaceopilosa* (CASENT0280966, photo by S. Hartman), *A. sardoa* (CASENT0916080, syntype, photo by Anna Pal), *A. cardenai* (CASENT0249624, photo by Z. Lieberman), *A. swammerdami* (CASENT0489647, photo by A. Nobile).

position of these species is strongly supported (posterior probability: 0.95–1) and they are placed as sister to lineages belonging to the non-monophyletic *splendida*

group, while the position of the third (*A. olympica*) as a sister to *A. italica*, *A. muschtaidica*, and *A. obsidiana* is weakly supported (posterior probability: 0.65).

3.1.2. *crocea* group

Status: monophyletic. All the sequenced species of the *crocea* group form a single monophyletic clade with strong support. The two Maghreb taxa (*A. crocea croceoides*, *A. strioloides*) are sister to the species endemic to Italy and Malta (*A. fiorii*, *A. sicula*, *A. trinacriae*).

3.1.3. *gibbosa* group

Status: polyphyletic. Most of the species are strongly supported in a single clade (posterior probability: 1), however, the position of *A. italica* and *A. muschtaidica* is separate in the tree as sister species to *A. obsidiana* with weak support (posterior probability: 0.65).

3.1.4. *obsidiana* group

Status: polyphyletic. *A. epirotas* clusters with high support (posterior probability: 1) as the sister to a group comprising *A. holtzi* (*pallida* group) plus all of the species from the *subterranea* group except *A. aktaci*. On the other hand, *A. obsidiana* is positioned separately with a low support (posterior probability: 0.52).

3.1.5. *pallida* group

Status: polyphyletic. Most species form a well-supported clade (posterior probability: 0.95–1), except *A. holtzi* which is part of the well-supported clade which is also formed by *A. epirotas* and most of the *subterranea* group (posterior probability: 1).

3.1.6. *splendida* group

Status: polyphyletic. The species of the *splendida* group form three independent clades in the tree. *Aphaenogaster aktaci* is strongly supported as the sister species of *A. illyrica* from the *subterranea* group (posterior probability: 1). The other species are divided into two well-supported clades, each with a species of the *cecconii* group as its respective sister taxon, one also formed by *A. splendida*, *A. festae*, *A. rugosoferruginea*, and one also formed by *A. ovaticeps*, *A. peloponnesiaca* (posterior probability: 0.95–1).

3.1.7. *subterranea* group

Status: non-monophyletic. All of the species form a well-supported clade except for *A. illyrica* which is clearly recognized as the sister species of *A. aktaci* from the *splendida* group (posterior probability: 1).

3.1.8. *testaceopilosa* group

Status: paraphyletic. The group is formed by a single clade consisting of two well-supported smaller clades (posterior probability: 1): one which comprises all the European taxa except *A. senilis*, and the other which is formed by all the North African taxa plus *A. senilis*. However, *A. sardoa* is also placed in the latter clade.

3.1.9. Other species

Aphaenogaster cardenai is well-supported in its position as an independent lineage from all the other West-Palaearctic *Aphaenogaster* species (posterior probability: 1). *Aphaenogaster sardoa* is placed within the *testaceopilosa* group as mentioned above.

3.1.10. *Deromyrma* clade

The Malagasy species form a well-supported clade with no close relationship with any of the W-Palaearctic *Aphaenogaster* species-groups (posterior probability: 1).

3.2. New species-groups classification

Based on the available phylogenetic and morphological evidence, we propose to recognize six West-Palaearctic species-groups of *Aphaenogaster*, while commenting the critical issues of the remaining three main clades (Fig. 2). Due to its morphological uniqueness and phylogenetic distance from the rest of the sequenced West-Palaearctic species, *A. cardenai* is kept as a species not belonging to any group. A synoptic list of the West-Palaearctic *Aphaenogaster* fauna, including the new species-groups classifications here proposed is offered in the Supplementary material S4.

3.2.1. *crocea* group (unchanged)

The definition and composition of this group previously given in the Materials and methods remains unchanged. The group is thought to extend from the Maghreb to Sicily and neighboring regions of Malta and Italy.

3.2.2. *gibbosa* group (redefined)

The existing morphological definition of the group (see Materials and methods) describes it well but also includes two species (*A. italica* and *A. muschtaidica*) whose position is unclear, but which appear to be unrelated to the group. Both have a more Eastern distribution compared to the species which are safely assigned to the group on a phylogenetic basis, as well as *A. theryi* which occurs sympatrically with *A. mauritanica*. The affiliation of *A. theryi* should be established in future studies, while the group should be considered restricted to the W-Mediterranean (Italy almost entirely excluded except for a small North-Western sector where *A. gibbosa* is thought to occur).

3.2.3. *graeca* group (newly established)

Our phylogenetic analysis strongly supports a close relatedness between *A. aktaci* (originally in the *obsidiana* group and more recently in the *splendida* group) and *A. illyrica* (originally in the *subterranea* group). *Aphaenogaster illyrica* is very similar to *A. graeca* morpho-

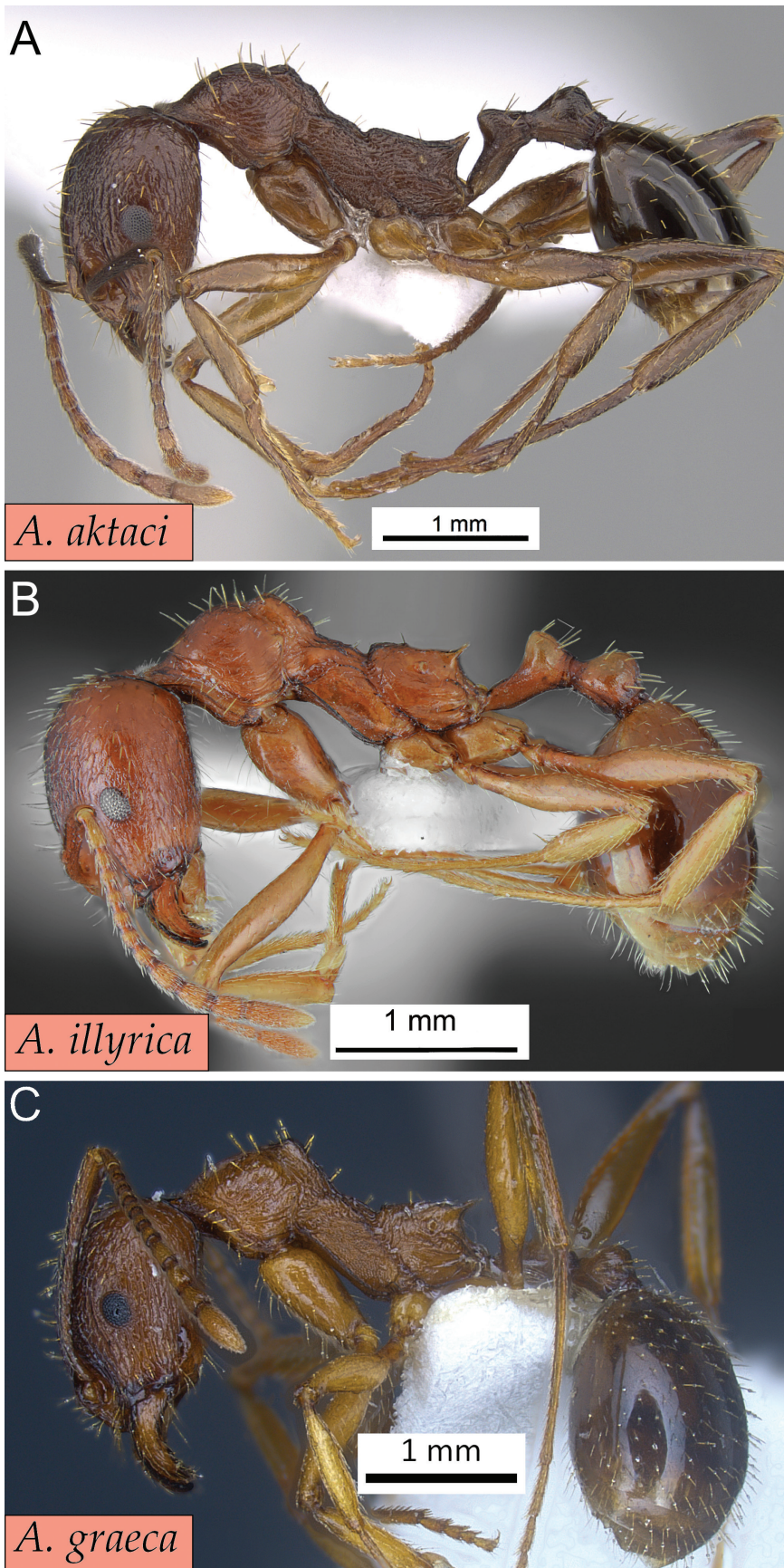


Figure 3. *Aphaenogaster* workers of species now classified into the new *graeca* species-groups: **A** *A. aktaci* (CASENT0922687, photo by M. Esposito); **B** *A. illyrica* (CASENT0872099, holotype, photo by Lech Borowiec); **C** *A. graeca* (ANTWEB1041239, paratype, photo by Roland Schultz). Photographs from www.antweb.org.

logically, so that the two were classified in the *graeca* complex within the *subterranea* group. *Aphaenogaster aktaci* shares with them a highly similar morphology, but may also somewhat resemble the darkest species from

clades B and C (*A. ovaticeps*, *A. rugosoferruginea*). We thus propose to consider the *graeca* complex as a species-groups considering its independence from the *subterranea* group and list *A. aktaci*, *A. graeca*, and *A. il-*

lyrica as its members. Further assessments regarding the phylogenetic position of species of the former *splendida* not sequenced in this study may be relevant, yet none is particularly close to the morphology of the *graeca* group species which we define according to the following combination of characters (based on workers, see Figure 2): dark brown to reddish, body elongate, appendages long, mesonotum well demarcated, medium-sized spines with a thick base, horizontal or very slightly curved upwards. Mesonotum surmounting the pronotum and separated by a strong promesonotal suture (*A. graeca*, *A. illyrica*) or propodeal dorsum lacking transverse surface sculpturing (*A. aktaci*). The group is considered to inhabit Anatolia and the Balkans.

3.2.4. *pallida* group (redefined)

The morphological definition of the group should be implemented by highlighting the lack of a strong mesoepinotal furrow which was already used by Alicata and Schifani (2019) to tell apart the *crocea* group from the *subterranea* group, and also emphasizing the importance of hairs already reported by Schulz (1994) (Figure 4): this leads to the removal of *A. holtzi* from the group and restores the monophyly of the *pallida* group in the tree, while also suggesting to remove *A. lesbica* which, like *A. holtzi*, has a strongly reduced head sculpture but in other characters is very similar to the species from the *A. subterranea* complex. The combination of short ant stout mesosoma, long and abundant hairs (especially on the head, pronotum and gaster), lack of a strong mesoepinotal furrow, shiny integument and brown, often yellowish to greenish pigmentation makes the *pallida* group one of the most easily recognizable morphologically. This group occurs in the Maghreb, Southern Europe, Anatolia, and Transcaucasia.

3.2.5. *sardoa* group (redefined)

The morphological definition of the *testaceopilosa* group *sensu* Boer (2013) is modified to restore the original boundaries defined by Emery (1915) for the former *Aphaenogaster* s. str. genus and establish a new species-groups that contains the former *testaceopilosa* group members plus *A. sardoa*. The high similarity of *A. sardoa* with the species of the former *testaceopilosa* group was already noted by Boer (2013), so that his set of diagnostic characters for the group, entirely based on workers,

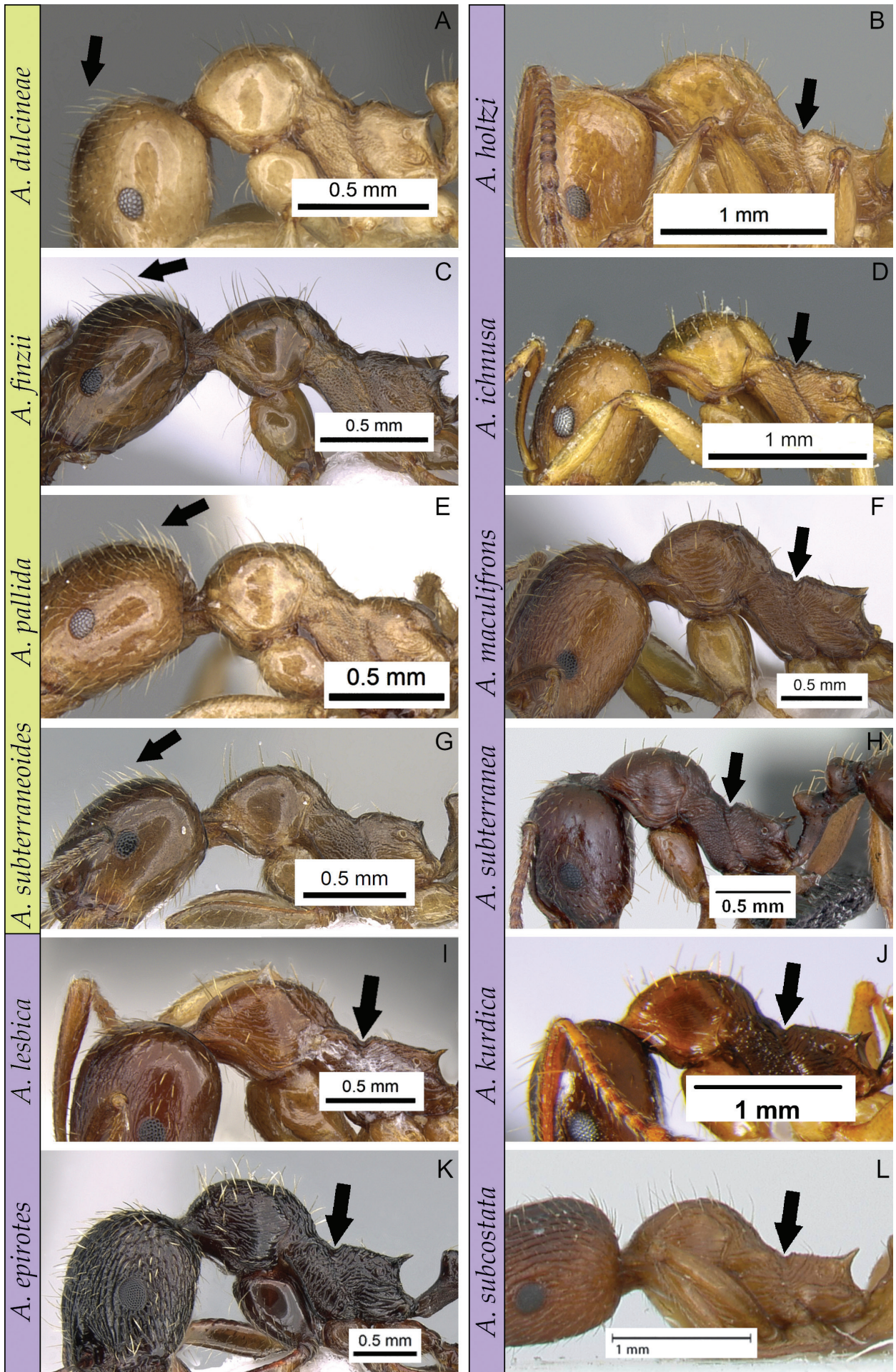
would have included *A. sardoa* and he was forced to complement it with a disclaimer to exclude *A. sardoa* based on additional characters of males and queens. We therefore refer to the worker-based definition without the complementary part to delimit the *sardoa* group. However, we also deem important to emphasize that most of the characters chosen by Boer (2013) to separate *sardoa* based on males and queens from the former *testaceopilosa* group worked only because he based his study exclusively on the European fauna and neglected the species-rich fauna of North Africa in his definition (Figure 5): it is not true that males of all the other species have larger mandibles and eyes, as the size of mandibles and eyes of *A. sardoa* is comparable to that of species such as *A. curiosa* or *A. fallax*; the notion that queens of *A. sardoa* have larger eyes compared to the other species does not seem to meet the reality in at least several cases; it is also false that all other species are black and only become reddish after preservation, as some species are genuinely red (e.g. *A. praedo*, see Cagniant 1969). It is nonetheless true that *A. sardoa* has an orange-yellowish pigmentation which is lighter than all other species of the genus, and that, as noted by Boer (2013), the petioles of workers and queens are particularly rounded. However, these characters must be autapomorphic.

The general aspect and behavior of all species belonging to the *sardoa* group makes its identification particularly easy even with the naked eye in the field by any experienced myrmecologist. The group is distributed throughout Southern Europe and the Maghreb.

3.2.6. *subterranea* group (redefined)

The morphological definition of the group should be implemented by removing the ‘shallow sculpture’ as a defining character, which allows to accommodate *A. epirotes* according to the phylogenetic results. The only species of the former *obsidiana* group we did not sequence, *A. subcostata*, shares the same general traits of the *subterranea* group definition plus a strong sculpture like that of *A. epirotes*, and is therefore reassigned to the *subterranea* group on a morphological basis (on the other hand, *A. obsidiana* lacks a deep mesoepinotal furrow) (Figure 4). Species with a short and stout mesosoma and strong mesoepinotal furrow previously included in the *pallida* group (namely *A. holtzi*, *A. lesbica*) are reassigned to the *subterranea* group. Species which lack a strong mesoepinotal furrow and/or characterized by an elongate meso-

Figure 4. Lateral profile of *Aphaenogaster* workers of species from the *pallida* and *subterranea* groups. In green, species of the *pallida* group, with arrows indicating the long and often abundant erect hairs on the dorsal side of the head. In violet, species from the *subterranea* group with arrows indicating the deep metanotal groove (including *A. holtzi* and *A. lesbica* previously attributed to the *pallida* group, *A. epirotes* and *A. subcostata* previously attributed to the *obsidiana* group). **A** *A. dulcinea* (photo by M. Esposito, CASENT0280959); **B** *A. holtzi* (CASENT0904178, syntype, photo by W. Ericson); **C** *A. finzii* (CASENT0914232, photo by M. Esposito); **D** *A. ichnusa* (CASENT0913132, syntype, photo by Z. Lieberman); **E** *A. pallida* (CASENT0280960, photo by S. Hartman); **F** *A. maculifrons* (CASENT0922688, photo by M. Esposito); **G** *A. subterraneoides* (CASENT0281536, photo by Estella Ortega); **H** *A. subterranea* (CASENT0172716, photo by A. Nobile); **I** *A. lesbica* (CASENT0907690, syntype, photo by W. Ericson); **J** *A. kurdica* (CFH000010, photo by Donat Agosti); **K** *A. epirotes* (CASENT0281535, photo by E. Ortega); **L** *A. subcostata* (FOCOL1221, syntype, photo by Christiana Klingenberg). Photographs from www.antweb.org.



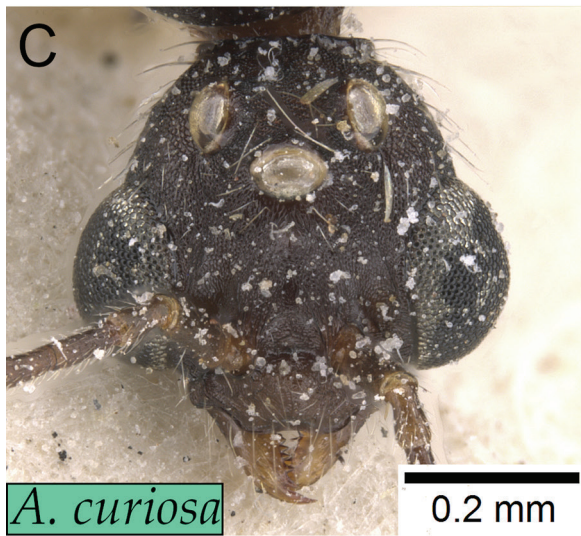
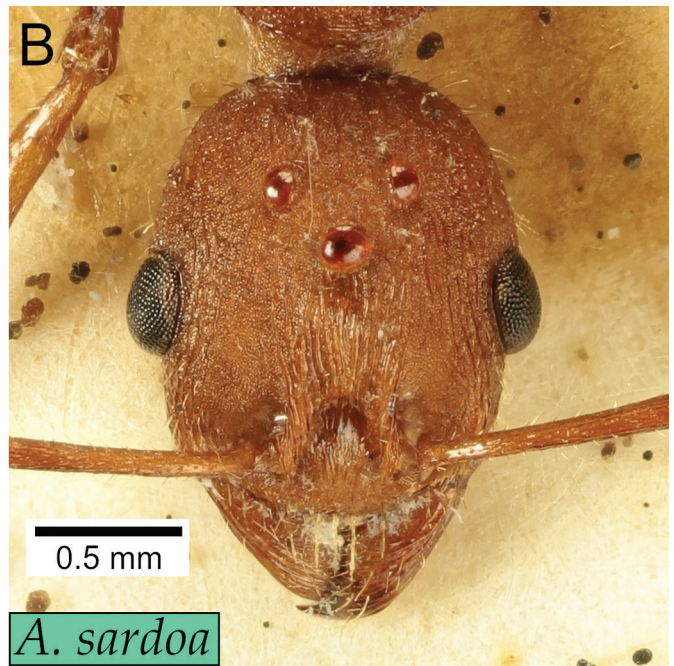
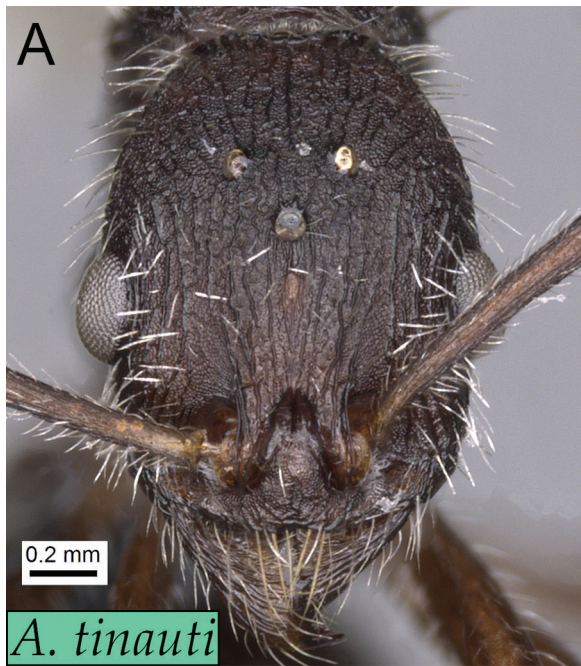


Figure 5. *Aphaenogaster* species from the *sardoa* group. Photographs B and D depict *A. sardoa* specimens from Sicily (queen and male respectively, photos by E. Schifani). Photographs A, C, E are from www.antweb.org: A *A. tinauti* queen (CASENT0913796, photo by W. Ericson); C *A. curiosa* male (CASENT0913109, syntype, photo by W. Ericson); E *A. praedo* worker (CASENT0904158, syntype, photo by Z. Lieberman). Our reassessment suggest that most characters used by Boer (2013) to keep *A. sardoa* separate from the species of the former *testaceopilosa* group are not consistent: the eyes of queens and males of *A. sardoa* are not larger compared to those of other species of the group (A, B), males with relatively small mandibles are not unique to *A. sardoa* (C, D), and workers of *A. sardoa* are not the only ones to have a naturally reddish pigmentation (E).

soma are removed (*A. graeca*, *A. illyrica*, Figs 2, 3). The group in its new definition is distributed across Southern Europe, Anatolia, and Transcaucasia, with most of its diversity concentrated in the Eastern part of this range.

3.2.7. Clade A (*A. ceconii*, *A. festae*, *A. rugosoferruginea*, *A. splendida*)

This well-supported clade (bootstrap value: 0.95–1) contains the species after which the former *ceconii* and *splendida* groups were named, as well as two other species of the *splendida* group (*A. festae* and *A. rugosoferruginea*). Within the former *ceconii* group, *A. ceconii* and two non-sequenced species, namely *A. lykiaensis* and *A. phillipsi*, are the only ones with a neck-like elongation of the head, which may represent the only criterion to tentatively hypothesize the affiliation of the other members of this former group to the clades recovered in this study (Borowiec and Salata 2014). On the other hand, within the former *splendida* group, *A. festae*, *A. rugosoferruginea* and *A. splendida* are in broad terms relatively similar one to another and to most of the species hitherto listed alongside them. It is worth noting that a certain morphological contiguity between the two former groups was already revealed by those species of the former *splendida* group with shiny integument, very long appendages and variably elongate head shape: *A. equestris*, *A. hamaensis*, *A. kervillei*, and *A. vohraliki*. This clade can be considered entirely Eastern Mediterranean based on the fact that *A. splendida* seems to be an introduced species in the Western Mediterranean basin (Salata et al. 2021).

3.2.8. Clade B (*A. charesi*, *A. ovaticeps*, *A. peloponnesiaca*)

This well-supported clade (bootstrap value: 0.95–1) contains a second group of species from the former *splendida* group (*A. ovaticeps*, *A. peloponnesiaca*) alongside another species from the former *ceconii* group (*A. charesi*). The similarities between species of the former *ceconii* and *splendida* groups mentioned for clade A should be kept in mind in this second case. In the same way, the difficulty to find morphological criteria allowing to assign the non-sequenced species of both former groups to either clade highlights the need of further molecular and morphological investigations into the composition and evolution of clade B. All species are Eastern Mediterranean.

3.2.8. Remaining species

Aphaenogaster italica, *A. obsidiana*, *A. olympica*, *A. muschtaidica* form a clade which has a very weak support (bootstrap value < 0.89 in all nodes), not allowing us to evaluate whether its members are truly closely related. Nonetheless, it is worth noting there is a substantial degree of morphological similarity with the exception of *A. olympica* (originally in the *ceconii* group). *Aphaenogaster italica* and *A. muschtaidica* (originally in the *gibbosa* group) and *A. obsidiana* (originally in the *obsidiana* group) share the following characters: black pigmentation, lack of deep metaepinotal furrow in profile view, medium to strong sculpture. For what concerns *A. obsidiana*, it is important to note that its morphology deviates in several aspects from that of *A. epirotes* and *A. subcostata* (formerly forming together the *obsidiana* group and now moved to the *subterranea* group): *i*) the background microsculpture of the dorsum of the mesonotum and the sides of the propodeum is distinctively microreticulated instead of dull and shiny (contradicting the group's definition by Schulz 1994); *ii*) the antennal scapi are longer (the ratio of their length divided by the arithmetic mean of cephalic length and width is above 0.965, while below 0.930 in *A. epirotes* and *A. subcostata*, L. Borowiec unpublished data); the legs are also longer, with the hind femora clearly longer than the first gastral tergite, while approximately as long in *A. epirotes* and *A. subcostata*. Interestingly, the presence of *A. olympica* in the same clade mirrors the presence of *A. ceconii* in clade A and of *A. charesi* in clade B, where the *ceconii*-like morphology is associated with other species which still have an elongate mesosoma and long appendages but to a lesser degree.

3.3. Reassignment of taxa to other genera

Aphaenogaster isekram and *A. asmaae* are assigned to the genus *Messor* on a morphological basis. The description of *A. isekram* as a morphologically particularly aberrant *Aphaenogaster* species did not take into account the existence of extremely similar *Messor* species such as *M. rufotestaceus* (Foerster, 1850) (Iran, but AntWeb sample CASENT0264396 from the United Arab Emirates is currently identified with the same name) and *M. lamellicornis* Arnol'di, 1968 (Kazakhstan) (Bernard 1977). The later described *M. boyeri* Cagniant, 2006 (Morocco) shares the same worker morphology (Cagniant 2006; Fig. 5), while the other castes are unknown. The morphology

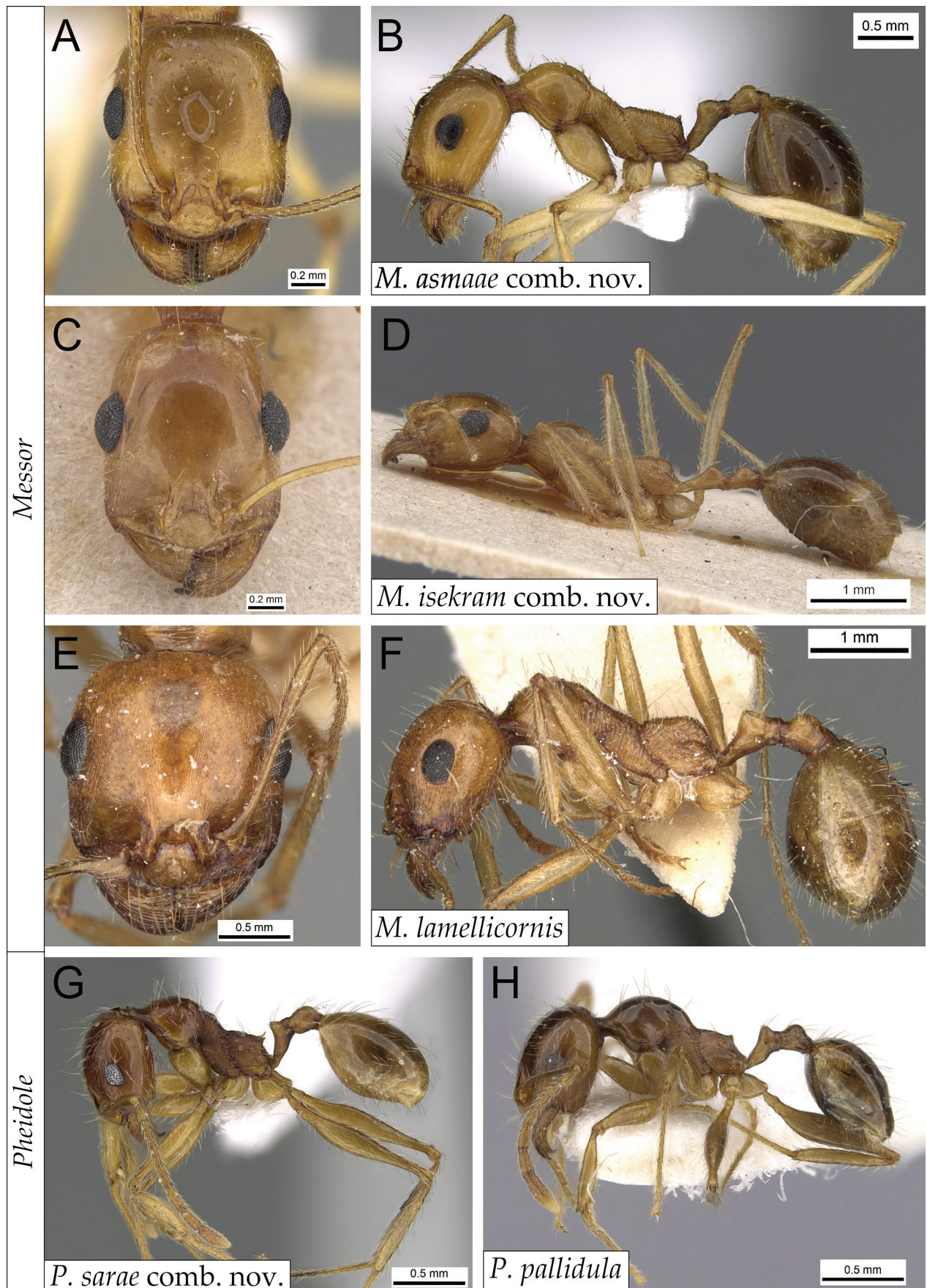


Figure 6. Names excluded from *Aphaenogaster* and examples of morphologically similar species belonging to the new assigned genus (photographs from www.antweb.org). **A, B** *Messor asmaae* comb. nov., CASENT0922290, photo by M. Esposito; **C, D** *Messor isekram* comb. nov., CASENT0913609, syntype, photo by W. Ericson; **E, F** *Messor lamellicornis*, CASENT0281598, photo by M. Esposito. **G** *Pheidole sarae* comb. nov., CASENT0922294, photo by M. Esposito; **H** *Pheidole pallidula*, CASENT0249410, photo by Shannon Hartman.

of the workers of these species is consistent with *Messor* and not *Aphaenogaster* in particular for what concerns the shape of the mandibles strongly curved towards the midline (as emphasized by Boer 2013), but also in the more rectangular head shape and, for what concerns *A. isekram*, the lack of propodeal spines. Worldwide, the only species of *Aphaenogaster* with similar traits of mandibles and head shape is *A. striativentris* which is known to represent an extraordinary case of evolutionary convergence (Tinaut and Jiménez-Rojas 1990; Gómez et al. 2018). On the other hand, the lack of propodeal spines is a very common feature in *Messor* (for instance, it characterizes 86% of the about 70 West-Palaearctic *Messor* species, see Borowiec 2014) but is very rare in *Aphaenogaster* (about 2%, as it is only seen in *A. inermis* and *A. pallida* in the same region). The description of *A. asmaae* ignored all the aforementioned *Messor* species, and the genus attribution by Sharaf et al. (2018) was entirely based on a comparison with *A. isekram*. Therefore, we consider these taxa as *Messor asmaae* (Sharaf, 2018) **comb. nov.**, and *Messor isekram* (Bernard, 1977) **comb. nov.** based on three elements: *i*) their morphology is convincingly congruent with traits typical of *Messor* and extremely rare in *Aphaenogaster*; *ii*) species earlier described with the same morphology were all attributed to the genus *Messor* (although their descriptors ignored it); *iii*) they live in regions outside the geographical boundaries of all other *Aphaenogaster* species (i.e., Sahara Desert and Arabian Peninsula) but well within the distribution boundaries of *Messor* spp.

In addition, *A. sarae*, according to the description and images presented by Sharaf et al. (2018) presents all key characteristics of a minor worker from the genus *Pheidole*, including the three-segmented antennal club which is distinctive of *Pheidole* against *Aphaenogaster* (the latter having 4 to 5 antennal club segments, see Boer 2013). In addition, just comparing *P. sarae* to the most well-known West-Palaearctic *Pheidole* species, *P. pallidula* (Nylander, 1849) (see Seifert 2016), it is easy to recognize the same shape and proportions of the nodes, mesosoma (with a few differences) and head, or approximately the same length and disposition of the erect setae over the body and very similar body sculpture pattern (Fig. 6). As in the previous cases, no argument was made by the descriptor to place the species in *Aphaenogaster* (Sharaf et al. 2018). Therefore, we consider it as *Pheidole sarae* (Sharaf, 2018) **comb. nov.**

Now that their generic identity has been reassessed, the status of these three taxa should be further investigated to test whether they may be synonyms of other congeneric species.

4. Discussion

While the phylogenetic relationships between many of the clades we recovered have yet to be clarified, our results demonstrate that, following all past schemes, the

great morphological diversification of the *Aphaenogaster* was largely misinterpreted in its evolutionary significance and a new perspective is needed. Almost all of the current and past infrageneric classifications largely defined non-monophyletic groups. This was also the case with the former subdivision in subgenera: *Aphaenogaster* s. str. would be monophyletic on its own, yet it is nested within *Attomyrma*, making the latter paraphyletic. The placement of *A. ceconii* within the former subgenus *Deromyrma* was also incorrect, as it should belong to *Attomyrma*, making *Deromyrma sensu* Emery (1915) polyphyletic. This implies that the morphological delimitation of the true *Deromyrma* clade *sensu* Branstetter et al. (2022) is not entirely straight-forward, and that a precise definition may require the examination of further doubtful cases (compare also with Ward and Boudinot 2021). As for the eight species-groups hitherto in use, only the *crocea* group (Alicata and Schifani 2019) was recovered as monophyletic, while the *graeca* group is added for the first time. The other pre-existing groups, albeit all non-monophyletic, were characterized by very different situations: some could be redefined with relatively minor changes, while others should be currently abandoned.

On one hand, the monophyly of the *pallida*, *subterranea* and *testaceopilosa* species-groups – the latter renamed *sardoa* group and now corresponding to the former nominotypical subgenus – was easily achieved by reassigning only a few species on clear morphological bases. The unclear clustering of the specimens identified as *A. balcanica*, *A. picena*, and *A. sporadis* emphasizes the need of a taxonomic revision of the *sardoa* group, whose boundaries are, however, very clearly defined. On the other hand, the *splendida* and *ceconii* group, highly polyphyletic, had to be dismissed. While sequencing all the species from these two groups stands as an important objective for further research, phylogenetic results revealed a very interesting relationship between the two groups: the ‘*ceconii*-like’ and the ‘*splendida*-like’ morphologies each evolved independently two or three times, and at least twice ‘*ceconii*-like’ species were sisters to ‘*splendida*-like’ species. It is possible that the peculiar ‘*ceconii*-like’ morphology represents a form of adaptation to the light-avoiding or troglomorphic lifestyle that characterizes the species of this group, since similar traits are exhibited by other troglomorphic Palearctic species, such as the Japanese *A. gamagumayaa* Naka and Maruyama, 2018 (Borowiec and Salata 2014; Salata and Borowiec 2016; Naka and Maruyama 2018). Notably, the ‘*splendida*-like’ morphology is also, albeit to a lesser degree, associated with avoidance of sunlight by the slow-moving foraging workers, accomplished by either living in shady and humid environments or foraging at dusk or nocturnally (Salata et al. 2021), and some species formerly assigned to the *splendida* group show somewhat intermediate characteristics between the two morphotypes. Finally, the possibility that the ‘*gibbosa*-like’ morphology appears in multiple separate clades remains to be further investigated. More in general, resolving the relationships between the clades will be key to un-

derstand how the remarkable morphological diversity of West-Palaearctic *Aphaenogaster* evolved, and which morphologies are ancestral.

When morphology-based infrageneric divisions were defined, in most cases no effort was made to predict which characters were apomorphic or plesiomorphic, or which ones were driven by an adaptive value and which ones were not—tasks that were very difficult or impossible to achieve in many cases without phylogenetic data, or were simply beyond the aims of the systematic works produced at that time. When the *crocea* group was split from the *subterranea* group (Alicata and Schifani 2019), subtle differences in the mesosoma of workers were interpreted as characters probably reflecting phylogenetic patterns, while their overall strong similarity was suggested to be the convergent outcome of evolutionary adaptation towards a similar lifestyle. Male morphology and biogeography were key to the formulation of this hypothesis, which ultimately proved to be correct (Alicata and Schifani 2019). Despite the great morphological diversity of *Aphaenogaster* in the West-Palaearctic, our results demonstrate that the detection of morphological characters useful to delimit monophyletic species-groups is rather difficult in many cases, and that complex patterns of evolutionary convergence or retention of ancestral traits may exist. In this environment, an integrative approach of phylogenetics and morphology appears to be necessary. The recognizable presence of a number of morphologically and phylogenetically well-delimited species-groups still represents an advantageous situation compared to other more chaotic Palaearctic ant genera (e.g. *Temnothorax*, Schifani et al. 2022). Any morphology-based hypothesis that certain West-Palaearctic groups would include species from outside the region seems very unwise for the time being given the results we obtained (e.g., placing the Nearctic *A. ashmeadi* (Emery, 1895) in the *testaceopilosa* group, the Indian *A. smythiesii* (Forel, 1902) in the *subterranea* group, and *A. pachei* (Forel, 1906) or *A. sagei* (Forel, 1902) in the *obsidiana* group, according to Schulz (1994), and Boer (2013). Notably, in the phylogeny by Branstetter et al. (2022), East-Palaearctic and Nearctic species clustered together.

Integrating phylogenomic data to address the low support of the backbone of our phylogenetic reconstruction, as well as recovering the additional West-Palaearctic species which could not be sequenced in this study would help clarify those relationships which could not be resolved here, improving our understanding of the radiation of this genus. Further investigation should also expand to the species from the other, less diverse regions (the East-Palaearctic and the Nearctic), which would be important to fully unravel the biogeographic history of the genus (Branstetter et al. 2022). However, the results of the present study offer a first comprehensive evolutionary perspective over the diversification of *Aphaenogaster* morphologies in the diversity hotspot of the genus, providing a new species-groups framework as a basis for further taxonomic, phylogenetic and evolutionary studies.

5. Authors' contributions

ES, AA and RB conceived the study and organized the selection of species to be sequenced. RB conducted the molecular analyses. ES and AA curated the morphological parts. ES prepared the first draft of the manuscript. MM and ES curated the graphic parts. All authors participated in the collection and identification of specimens and the preparation of the final draft of the manuscript.

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Supplementary material 1

Sequence alignments

Authors: Schifani E, Alicata A, Menchetti M, Borowiec L, Fisher BL, Karaman C, Kiran K, Oueslati W, Salata S, Blatrix R (2022)

Data type: .fas.(Alignments are included as FASTA files)

Explanation note: The file contains the alignments for all the genetic sequences produced in this study.

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Link: <https://doi.org/asp.80.e84428.suppl1>

Supplementary material 2

List of investigated samples

Authors: Schifani E, Alicata A, Menchetti M, Borowiec L, Fisher BL, Karaman C, Kiran K, Oueslati W, Salata S, Blatrix R (2022)

Data type: .xlsx

Explanation note: The file contains the complete list of samples used in our analyses. For each sample, we provide the following information: voucher identifier, species-level identification, species-groups (this classification does not take into account the results of the analyses), label in the phylogenetic trees, specimen code, label for extraction, latitude and longitude (decimal degrees), locality name, name of the collector(s), name of the identifier(s), Genbank accession number for COI and for the 6 EPIC genes sequenced.

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Link: <https://doi.org/asp.80.e84428.suppl2>

Supplementary material 3

Supplementary phylogenetic trees

Authors: Schifani E, Alicata A, Menchetti M, Borowiec L, Fisher BL, Karaman C, Kiran K, Oueslati W, Salata S, Blatrix R (2022)

Data type: .zip

Explanation note: **Supplementary file S3:** Maximum likelihood phylogeny based on all markers concatenated. — **Supplementary file S4:** Bayesian phylogeny based on mtCOI. — **Supplementary file S5:** Maximum likelihood phylogeny based on mtCOI. — **Supplementary file S6:** Bayesian phylogeny based on all EPIC markers concatenated. — **Supplementary file S7:** Maximum likelihood phylogeny based on all EPIC markers concatenated.

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Link: <https://doi.org/asp.80.e84428.suppl3>

Supplementary material 4

Synoptic list of species and new species-groups classification

Authors: Schifani E, Alicata A, Menchetti M, Borowiec L, Fisher BL, Karaman C, Kiran K, Oueslati W, Salata S, Blatrix R (2022)

Data type: .svg

Explanation note: The file contains a synoptic list of the West-Palaearctic *Aphaenogaster* and, for each taxon, its classification before and after this study.

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Link: <https://doi.org/asp.80.e84428.suppl4>