Phylogeny of the ant genus *Aphaenogaster* (Hymenoptera: Formicidae) in the Iberian Peninsula, with the description of a new species

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**Abstract**

A phylogenetic tree of the Iberian *Aphaenogaster* species - except for *A. splendida* (Roger) - and a key to the worker caste of all Iberian *Aphaenogaster* species are proposed. The position of *A. striativentris* Forel and *A. cardenai* Espadaler is discussed, stating the possibility that this second species may belong to a new, undescribed genus. *Aphaenogaster ulibeli* n. sp. is described from the Iberian Peninsula. Its closest relatives are *A. gibbosa* (Latreille) and *A. striativentris*. Its habitat seems to be restricted to caducifolia forests in the Western Central Massif.

**Materials and methods**

**Terminology and Measurements**

Specimens were examined and/or measured under a MZ 16A stereo microscope. All images were edited in Lenovo Photo Master and OpenOffice Impress, including those used from AntWeb (2016) with permission. Some specimens examined have alphanumeric codes associated with them (i.e., CASENT#, KG#, XE#) which uniquely identify the specimens for databasing purposes.

Credits of all images belong to www.antweb.org.

Available keys for the Iberian *Aphaenogaster* are almost 40 years old (Collingwood, 1978). Taxonomic changes, reidentification of former samples and revisionary works (Boer, 2013) has prompted the convenience of a new key for all the Iberian species.
Male terminology as in Boudinot (2015). Measurements and indexes as in Boer (2013). ML (Mesosoma length) and MW (Mesosoma width), as in Fig 1.

CI: Cephalic Index (CW/CL) × 100
CL: Maximum cephalic length in median line
CW: Maximum cephalic width, across eyes
EYI: Eye Index (Maximum eye diameter/CL) × 100
PSI: Propodeal Spine Index (x/y in Fig. 2) × 100
PSLWI: Propodeal Spine Length-Width Index (x/z in Figs 2 and 3) × 100
RPSI: Relative Propodeal Spine length-width Index ([x − y]/z in Figs 2 and 3) × 100
SI: Scape Index (SL/CW) × 100
SL: Maximum straight line scape length excluding articular condyle

Fig 1. Definition of ML (A) and MW (B) measurement in the queen caste.

Fig 2. Definition of x, y, z. From De Boer, 2013.

DNA barcoding

DNA extraction, PCR amplification and sequencing

Total DNA was extracted in each sample separately from three individual ants that had been preserved in 96% ethanol since the collection date.

DNA was extracted following the HotSHOT (Hot Sodium HidrOxide and Tris) method (Truett et al., 2000) using 60 µl of both alkaline lysis and neutralizing reagents. A 710 bp fragment of the 5’ region of the mitochondrial gene coding the cytochrome c oxidase subunit 1 (COI) was amplified using primers LCO1490 and HCO2198 described by Folmer et al. (1994). For the PCR reactions, 0.5 µl of the extracted DNA were used in a total reaction volume of 50 µl. Each PCR reaction also contained one unit of Taq polymerase (VWR), 1X buffer, 0.2 mM of each dNTP and 0.2 µM of each primer. PCR conditions for COI amplification were as follows: 94°C for 1 min; 40 cycles of 94°C for 30 s, 48°C for 30 s and 68°C for 30 s; a final extension step of 10 min at 68°C was included after cycling.

PCR products were purified by ammonium acetate - ethanol precipitation and reconstituted in 10 µl of LTE buffer (10mM Tris, 0.1mM EDTA). Direct Sanger sequencing of amplified fragments was done on both DNA strands using PCR primers. Sequencing was conducted using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following manufacturer’s instructions, and samples were loaded onto an ABI 3730XL automated sequencer.

Data analysis

Chromatograms were revised, PCR primers trimmed sequences corresponding to each individual ant assembled and final fasta sequences generated for each analysed ant using the Staden package v1.6.0 (Staden et al., 1998). Multiple alignment of COI sequences were done using the ClustalW program included in the MEGA 6 software (Tamura et al., 2013). The sequence of Myrmica rugiventris (accession number GQ255171) was also included in the alignment to be used as outgroup in the phylogenetic analysis.

Phylogenetic analysis

MEGA 6 software was used for the phylogenetic analysis. The “Find Best DNA Model” option available in MEGA 6 was used to find the evolutionary model that best fit the data. A phylogenetic tree was constructed using the Maximum Likelihood method based on the General Time Reversible model [1]. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.6888)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 46.7811% sites). Node support was evaluated by the Bootstrap method (Felsenstein, 1985) using 500 pseudo replicates of the original data. Bootstrap values were included next to the branches when higher than 50%.

Collection references:

ATPC: Alberto Tinaut Personal Collection, Granada, Spain
BMNH: British Museum of Natural History, London, UK
CASC: California Academy of Sciences, California, USA
CGPC: Crisanto Gómez Personal Collection, Girona, Spain
FGPC: Federico García Personal Collection, Barcelona, Spain
JRPC: Joaquín Reyes Personal Collection, Córdoba, Spain
KGAC: Kiko Gómez Personal Collection, Barcelona, Spain
MCZC: Museum of Comparative Zoology, Harvard, USA
MNCN: Museo Nacional de Ciencias Naturales, Madrid, Spain
MNHN: Muséum National d’Histoire Naturelle, Paris, France
XEGC: Xavier Espadaler Personal Collection, Barcelona, Spain
Results

*Aphaenogaster ulibeli* Gómez & Espadaler nov. spec.

urn:lsid:zoobank.org:act:F60F14FA-748D-447F-B917-1DF22765060E

GENBANK: KY124277, MF926341, MF926344

Holotype worker, SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40° 22.87’N 5° 45.43’W 1.010 m 01 Mar. 2008. (Sanchez, A.). Caducifolia forest, Nest under stone [MNCN: KG01985-4]

Paratype workers:

Same sample than Holotype. [ATPC: KG01985-1, 2w], [JRPC: KG01985-2, 2w], [MCZC: KG01985-3, 2w].

SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40° 22.87’N 5° 45.43’W 1.010m 30 Jul. 2010. (Sanchez, A.). Caducifolia forest, Nest under stone [FGPC: KG02101-2, 3w], [CGPC: KG02101-3, 3w], [XEGC: KG02101-4, 3w], [MNCN: KG02101-5, 3w], [BMNH: KG02101-6, 3w], [KGAC: KG02101-7, 3w]

SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40° 22.87’N 5° 45.43’W 1.010m 22-24 Jun. 2007. Pitfall (Sanchez, A.). Caducifolia forest [MNHN: EY20152, 2w]

SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40° 22.87’N 5° 45.43’W 1.010m 27-29 Jul. 2007. Pitfall (Sanchez, A.). Caducifolia forest [CASC: KG02103-1, 1w], [XEGC: KG02103-2, 1m], [XEGC: KG02103-3, 1m]

Paratype Queens:

SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40° 22.87’N 5° 45.43’W 1.010m 30 Jul. 2010. (Sanchez, A.). Caducifolia forest [KGAC: KG02101-1, 1Q]

SPAIN: Sierra de Gredos (Espadaler, X.) 23.vii. 1979 [XEGC: XE00200, 4w]

Paratype males:

SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40° 22.87’N 5° 45.43’W 1.010m 27-29 Jul. 2007. Pitfall (Sanchez, A.). Caducifolia forest [KGAC: KG02103-2, 1m], [XEGC: KG02103-3, 1m]

*Derivation ominis:* Named after Ulises and Abel, first author’s sons, may the biophily be with them.

*Worker*

Holotype and Paratypes: CL 1.40-1.50, CW 1.19-1.30, SL 1.33-1.50, CI 0.85-0.90, SI 1.13-1.22, PSI 112.5-127.8, PSLWI 66.7-82.7, RPSI 10-17.25 (n=8)

HEAD: Antennae 12 segmented with 4 segmented antennal club, all segments longer than wide. Scape cylindrical, longitudinally striated, long-clearly surpassing the occipital border when laid back. Abundant greyish white semi-erect setae present in scape and funiculus, its length similar to scape maximum width. These setae decumbent in the basal zone and gradually rising to be semi-erect in the apical third.

Head clearly longer than wide with subparallel sides. Eyes located medially, well developed, much wider than scape width and with 14-20 ommatidia in its longest axis. Mandibles triangular, longitudinally striated. Dentition with 4-5 more developed teeth decreasing in size from the apical, and 4-7 smaller denticles following to the basal line. Clypeus emarginated medially, longitudinally striated. In some individuals medial portion of the clypeus between the frontal lobes are smooth and shiny.

MESOSOMA: Promesonotal line in profile view continuous. Mesosoma with clearly demarcated mesopropodeal suture. Spines smaller than the space between them, triangular, directed upwards. Metasternal process variable, a rounded lobe to sharply pointed.

METASOMA: Petiole node high, ellipsoidal. Postpetiole lower than petiole, globular slightly oriented backwards.

COLORATION: Color blackish brown to black, except mandibles and tarsi, yellowish brown. Some individuals dark brown (possibly recently hatched)

SCULPTURATION: Overall background sculpture feebly reticulated, absent on the gaster, which is smooth and shiny. Head with subparallel striae overlapping this pattern, with some transverse smaller striae present, but without creating a reticulum. These striae becoming scarcer on the posterior fourth, where only some feeble striae reach the occipital line and the background reticulation is clearly seen. Lateral sides of head and gula with some scattered striae, but most of the surface only reticulated. Pronotal dorsum mainly smooth with some faint striae present. Pleurae longitudinally striated, that continues on the propodeum as parallel transverse striae. Petiole, postpetiole and gaster smooth and shiny, without striae of any kind.

SETATION: Long, greyish white erect to semi-erect setae abundant overall including gaster tergites and lateral...
sides of the head down to the mandibular insertions, becoming scarcer only on the propodeal dorsum.

**Queen**

Paratypes: CL 1.40-1.50, CW 1.23-1.26, SL 1.20-1.26, MW 1.02-1.27, ML 2.29-2.37, CI 0.95-1.01, SI 1.03, PSI 138-175, PSLWI 64-113, RPSI 138-175 (n=2)

As in worker, but with the following differences:

**HEAD:** Four apical club clearly defined. Three ocelli present.

**MESOSOMA:** In dorsal view scutum completely covering the pronotum, with notauli present and clearly defined. Spines long and cylindrical, much more developed than in the worker caste. Wings relatively short, when laid back reaching or only slightly surpassing the gaster apex.

**METASOMA:** Petiole and postpetiole as in worker, slightly more peaked and some feeble striae maybe present in the posterior face of the petiole.

**SCULPTURATION:** Lateral sides of the head and gula striated. Dorsal surface of scutum faintly striated with irregular subconcentrical striae, lateral sides smooth, overall appearance smooth and shiny. Scutellum smooth and shiny with mesocutellum feebly striated. Anepisternum and katepisternum smooth in its anterior half and longitudinally striated in its posterior half. Pronotum transversely striated.

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Mandibles feebly striated longitudinally, margin smooth and shiny, armed with four sharp teeth, the apical long and curved, and decreasing in size to the basal tooth. Clypeus emarginated, divided in two sections, the central upper section raised. Eight long, grey hairs located basally in this upper section and covering the mandibles. Clypeal sculpture weakly reticulated, but without rugulae of any kind. Frontal ridges developed, but frontal lobes very small, so that antennal insertions clearly exposed. Eyes with microscopic hairs.

**MESOSOMA:** Mesoscutum swollen, overhanging the pronotum in dorsal view. Propodeum declivity an almost straight line (approximately 30 degrees with the horizontal) in one specimen, and with a short, vertical slightly convex face before meeting the scutellum in the other. Propodeal lobes very from reduced to non existent. Metasternal process formed by a small, blunt triangle oriented backwards. Between the second coxae and this metasternal process, another two lateral sharply pointed processes similar in size and shape.

**METASOMA:** Petiole and postpetiole low, subelliptical in profile view, the petiole slightly pedunculated.

**COLORATION:** Color brown to light brown, except mandibles, antennae and legs, light to yellowish brown.

**SCULPTURATION:** Head sculpture reticulated with a few isolated striae radiating from the ocelli. Some striae (2-4) between the lobes. Another 2-4 striae running on the frontal lobes upwards, some reaching feebly the lower ocellus. Scutoscutellar, oblique mesopleural sulcus and metapleuropropodeal suture with a transverse rugulated pattern. The rest of the body smooth and shiny.

**SETATION:** Grey to white long setae present on head, mandibles, dorsal surfaces of mesosoma, petiole, postpetiole and gaster. Absent on the genae, lateral surfaces of the mesosoma and very reduced to non existent on the propodeum.

**Phylogenetic position**

Phylogenetic analysis. As expected, a 710 bp DNA fragment containing a portion of the mitochondrial COI gene was amplified through PCR from the ants analysed. After removing sequences corresponding to primers used in

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**Fig 4. A. ulibeli** queen paratype [KG02101-1]. www.antweb.org

Propodeum transversely striated in dorsal surface. Latero-basal part of propodeum longitudinally striated.

**Male**

Paratypes. CL 0.86-0.88, CW 0.82, SL 0.25-0.26, ML 2.41-2.58, MW 0.99-1.08, CI 0.94-0.96, SI 0.30-0.31 (n=2)

**HEAD:** Antennae 13-segmented. Five segmented apical club. Scape short, when laid back not reaching the posterior side of the eye. Decumbent to semi-erect setae abundant in the funiculus, more sparse adpressed to decumbent setae on scape.

Head subrectangular. Three ocelli present and well developed. Eyes big, located in the lower half of the head, covering almost half the head length (EI=39-42, n=4).

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**Fig 5. A. ulibeli** male paratype [KG02103-2]. www.antweb.org
the PCR reaction, the sequences obtained from each sample consisted of 658 nucleotides.

COI sequences were obtained for ants from nine *Aphaenogaster* and one *Messor* species. Sequences for the different individual ants were identical for any sample, so that a single sequence was finally assigned to each colony. Three different colonies were analyzed for *Aphaenogaster ulibeli* and *Aphaengaster subterranea*. The rest of the species are represented by one single colony. The sequences were deposited in GenBank (accession numbers on Table 1).

A phylogenetic tree for the ant genus *Aphaenogaster* in the Iberian Peninsula was obtained and is summarized in Figure 6.

**Discussion**

Our analysis support the existence of four different clades into the *Aphaenogaster* species present in the Iberian Peninsula.

Clade 2 contains the classical *Aphaenogaster s. str.* species (*A. senilis*, *A. iberica* and the mediterranean *A. spinosa*). This clade is coherent with the *A. testaceopilosa* species group defined in De Boer (2013).

Clade 3 includes *A. dulciniae* and *A. subterranea* and

![Fig 6. ML phylogenetic tree for the COI sequences obtained for species of the ant genus *Aphaenogaster* in the Iberian Peninsula. *A. splendidia* material was not available, and therefore is not included. *A. spinosa*, from continental Italy, is also included in the analysis. *M. rugiventris* is used as outgroup. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (bar corresponds to 0.02 substitutions per site). Bootstrap values are indicated on branches when higher than 50. Three of the four clades described in the text are indicated.](image)

**Table 1.** List of ant species and colonies whose COI sequence has been obtained in this study along with voucher specimen codes and relevant sampling data.

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank</th>
<th>Depository and data</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. cardenai</em></td>
<td>MF926348</td>
<td>KGAC [KG03598-1]. Spain: Jaen, Cueva pB-4 (Peal del Becerro) 01/01/2015. Hand (GEV). Cave (1w)</td>
</tr>
<tr>
<td><em>A. gibbosa</em></td>
<td>MF926349</td>
<td>Spain: Girona, Campdevanol. 15/05/2017. F. Garcia leg, det</td>
</tr>
<tr>
<td><em>A. iberica</em></td>
<td>MF926340</td>
<td>KGAC [KG03237]. Spain: Salamanca, Finca (Béjar) 1000m, 40.384, -5.7575 01/06/2016. Hand (Sanchez, A.). Caducifolia forest, Nest under stone</td>
</tr>
<tr>
<td><em>A. senilis</em></td>
<td>MF926347</td>
<td>Spain: Barcelona, Montjuich. 17/05/2017. F. Garcia leg, det</td>
</tr>
<tr>
<td><em>A. spinosa</em></td>
<td>MF926339</td>
<td>KGAC [KG03145A-1]. Italy: Umbria, Castle garden (Orvieto) 275m, 42.72262, 12.1203 04/10/2015. Hand (Gomez, K.). Urban Garden, Nest open in soil</td>
</tr>
<tr>
<td><em>A. subterranea / A</em></td>
<td>MF926338</td>
<td>KGAC [KG03147A-1]. Italy: Lazio, Camping (Lughezza) 60m, 41.93193, 12.70005 05/10/2015. Hand (Gomez, K.). Ruderal among crops, Under stone</td>
</tr>
<tr>
<td><em>A. subterranea / C</em></td>
<td>MF926350</td>
<td>Spain: Girona, Campdevanol. 15/05/2017. F. Garcia leg, det</td>
</tr>
<tr>
<td><em>A. striativentris</em></td>
<td>MF926346</td>
<td>KGAC [KG01917-1]. Spain: Cádiz, Algar, 36.65, -5.65 05/02/2007. Hand (Huertas, R.). Prado, Nest under stone</td>
</tr>
<tr>
<td><em>A. ulibeli / A</em></td>
<td>KY124277</td>
<td>KGAC [KG03235-1]. Spain: Salamanca, Camino Viejo de Candelario (Béjar) 1010m, 40.3812, -5.7572 01/06/2016. Hand (Sanchez, A.). Caducifolia forest nest under stone</td>
</tr>
<tr>
<td><em>A. ulibeli / B</em></td>
<td>MF926341</td>
<td>KGAC [KG03238-1]. Spain: Salamanca, Finca (Béjar) 1000m, 40.38306, -5.75833 01/06/2016. Hand (Sanchez, A.). Caducifolia forest, Nest under stone</td>
</tr>
<tr>
<td><em>A. ulibeli / C</em></td>
<td>MF926344</td>
<td>KGAC [KG03558-1]. Spain: Salamanca, Finca (Béjar) 1000m, 40.38306, -5.75833 01/06/2016. Hand (Sanchez, A.). Caducifolia forest, Nest under stone</td>
</tr>
<tr>
<td><em>M. capitatus</em></td>
<td>MF926342</td>
<td>KGAC [KG03248A]. Spain: Tarragona, Nby. Camping Prades Park (Serra dels Ports) 940m, 41.30938, 0.98152 22/07/2016. Hand (Gomez, K.). Disturbed Mediterranean Forest, Foraging</td>
</tr>
</tbody>
</table>
is therefore a mix of species of belonging to the pallida and subterranea groups as defined in De Boer (2013).

A third, interesting clade 1 is coherent with the A. gibbosa species group (De Boer, 2013) and includes the new species with A. striativentris and A. gibbosa.

A fourth clade includes A. cardenai and is separated from the rest of Iberian species.

The phylogeny of the ant genus Aphaenogaster in the Iberian Peninsula has been recently revised (Lorite et al 2017). Six species present in the Iberian Peninsula (A. iberica Emery, A. senilis Mayr, A. gibbosa, A. subterranea (Latreille), A. dulciniae Emery and A. cardenai) and one Mediterranean species not present (A. spinosa Emery) were included in that analysis. We have analyzed the same seven species, and added A. ulibeli and A. striativentris. In our analysis we have also included Messor capitatus (Latreille). Our results are basically coincidental with those of Lorite et al (2017: Fig 3), repositioning and expanding the A. gibbosa group.

Some additional remarks to these results

Our phylogenetic analysis of COI sequences supports the definition of A. ulibeli as a new species different from the other Iberian Aphaenogaster species and divergent from A. gibbosa.

Looking deeper into this gibbosa-group, the position of A. striativentris is somewhat surprising. Its polymorphism and mandibular morphology could make us think that this species is closely related to the genus Messor. Our result, on the contrary, suggests that it is clearly imbricated into the genus Aphaenogaster, with the mandibular shape being result of convergent evolution.

Another interesting result is the position of A. cardenai, which is excluded from those three main clades and behaves like an outgroup to the genus Aphaenogaster. Its position basal to both Aphaenogaster and the Messor representative (M. capitatus) included in our study suggests that the Aphaenogaster-Messor genera structure is far from being solved. More analysis are needed to define it and they should comprise the Palaearctic Stenammini to map their real affinities.

General Ecology

This species has been found in a Mediterranean mixed forest (Castanea sativa Mill., Quercus robur L.), nesting on ground.

Alated queens and males were found into nests early in June, and males were captured in pitfall traps late in July (27th-29th), suggesting that nuptial flights occur during summer.

Other species found nearby were: Cryptopone ochracea (Mayr, 1855); Tapinoma madeirensis Forel, 1895; T. nigerrimum (Nylander, 1856); Camponotus aethiops (Latreille, 1798); C. cruentatus (Latreille, 1802); C. fallax (Nylander, 1856); C. piceus (Leach, 1825); Cataglyphis hispanica(Emery, 1906); C. iberica(Emery, 1906); Colobopsis truncata (Spinola, 1808); Formica fusca Linnaeus, 1758; F. rubriarbis Fabricius, 1793; Lasius emarginatus (Olivier, 1792); L. grandis Forel, 1909; Proformica sp., Aphaenogaster gibbosa (Latreille, 1798); A. iberica Emery, 1908; Crematogaster auberti Emery, 1869; C. scutellaris (Olivier, 1792); Messor barbarus (Linnaeus, 1767); M. bouvieri Bondroit, 1918; M. capitatus (Latreille, 1798); Myrmicaeloba Forel, 1909; M. sabuleti Meinert, 1861; Temnothorax aveli (Bondroit, 1918); T. pardoi (Tinaut, 1987); Tetramorium forte Forel, 1904, T. sp. (caespitum group).

Differentiation between A. gibbosa and A. ulibeli

Regarding the worker and queen castes, both species are the only Iberian Aphaenogaster that share the combination

![Fig 7. Comparison of A. ulibeli (A, B) and A. gibbosa workers (C, D). www.antweb.org](image)

Table 2. Main differences between A. ulibeli and A. gibbosa workers.

<table>
<thead>
<tr>
<th>A. ulibeli</th>
<th>A. gibbosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brownish black to black</td>
<td>Brown</td>
</tr>
<tr>
<td>Head sculpture parallel striated</td>
<td>Head sculpture reticulated</td>
</tr>
<tr>
<td>Scape setae semi-erect in its apical third</td>
<td>Scape setae adpressed</td>
</tr>
<tr>
<td>Mesonotum not humped, forming a continuous line with pronotum in profile view</td>
<td>Mesonotum humped, not forming a continuous line with pronotum in profile view</td>
</tr>
<tr>
<td>Mesopleura longitudinally striated</td>
<td>Mesopleura longitudinally striated - reticulated</td>
</tr>
<tr>
<td>Petiole dome ellipsoidal, symmetrical, without an angle between anterior and posterior faces</td>
<td>Petiole dome in profile view with an anterior face straight in contrast with the posterior more curved face</td>
</tr>
</tbody>
</table>
Fig 8. Comparison of *A. ulibeli* (A, C) and *A. gibbosa* Queens (B, D). www.antweb.org

**Table 3.** Main differences between *A. ulibeli* and *A. gibbosa* queens.

<table>
<thead>
<tr>
<th><strong>A. ulibeli</strong></th>
<th><strong>A. gibbosa</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark brown to black</td>
<td>Light brown to brown</td>
</tr>
<tr>
<td>Queen and worker similar in size and smaller than <em>A. gibbosa</em> queen (ML 2.29-2.37, n=2)</td>
<td>Clearly bigger than the worker and bigger than <em>A. ulibeli</em> queen (ML 2.75-2.92, n=4)</td>
</tr>
<tr>
<td>Wing relatively smaller, reaching the gaster apex when laid back</td>
<td>Wing relatively long, overpassing the gaster by more than half the gaster length when laid back</td>
</tr>
<tr>
<td>Upper third of the head without striae, or very reduced</td>
<td>Head completely striated-reticulated</td>
</tr>
<tr>
<td>Propodeal spines cylindrical</td>
<td>Propodeal spines triangular</td>
</tr>
<tr>
<td>Petiole node ellipsoidal</td>
<td>Petiole node almost a scale</td>
</tr>
</tbody>
</table>

**Keys to Iberian *Aphaenogaster* workers**

All images in this key modified from originals at www.antweb.org, links and code numbers under the images.

<table>
<thead>
<tr>
<th>1</th>
<th>Funicular segments 2–6 short, slightly longer than broad....2</th>
<th>All of the funicular segments elongated .....3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig 10: CASENT0172716</td>
<td>Fig 11: CASENT0280964</td>
<td></td>
</tr>
<tr>
<td>2 (1)</td>
<td>Head striated-reticulated .... subterranea</td>
<td>Head punctuated, smooth ..... dulciniae</td>
</tr>
<tr>
<td>Fig 12: CASENT0172716</td>
<td>Fig 13: CASENT0280959</td>
<td></td>
</tr>
<tr>
<td>3 (1)</td>
<td>Body colour from orange-yellowish to dark orange ..... 4</td>
<td>Body colour from dark brown to black ..... 5</td>
</tr>
<tr>
<td>4 (3)</td>
<td>Propodeal spines very long, its length similar to petiole pedunculum length ..... cardenai</td>
<td>Propodeal spines very short, almost reduced to triangular teeth ..... splendida</td>
</tr>
<tr>
<td>Fig 14: CASENT0249624</td>
<td>Fig 15: CASENT0280965</td>
<td></td>
</tr>
<tr>
<td>5 (3)</td>
<td>Short and powerful mandibles, with the outer margin strongly curved towards the middle line. Major workers with square heads converging in cephalic morphology to <em>Messor</em> ..... striativentris</td>
<td>Elongated and triangular mandibles, with the outer margin not strongly curved towards the middle line. Head clearly longer than wide, oval.....6</td>
</tr>
<tr>
<td>Fig 16: CASENT0280964</td>
<td>Fig 17: KG02103-1</td>
<td></td>
</tr>
<tr>
<td>6 (5)</td>
<td>Gaster smooth and polished, shiny .....7</td>
<td>Gaster striated, at least in the base, matt.....8</td>
</tr>
<tr>
<td>Fig 18: KG02103-1</td>
<td>Fig 19: CASENT0281534</td>
<td></td>
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<tr>
<td>Figs 20-21: KG01850-2</td>
<td>Figs 22-23: KG02103-1</td>
<td></td>
</tr>
<tr>
<td>8 (6)</td>
<td>Propodeum with very reduced spines, from non-existent to blunt triangular teeth ..... gemella</td>
<td>Spines clearly developed.....9</td>
</tr>
<tr>
<td>Fig 24: CASENT0280968</td>
<td>Fig 25: CASENT0281534</td>
<td></td>
</tr>
<tr>
<td>9 (8)</td>
<td>Long propodeal spines, comparable in size to the petiole pedunculum, curved-triangular shaped and following mesosoma dorsal line, usually curved downwards. Antennal club with four segments ..... iberica</td>
<td>Propodeal spines shorter than the petiole pedunculum, cylindrical and oriented 45° with the horizontal. Antennal club with five segments ..... senilis</td>
</tr>
<tr>
<td>Fig 26: CASENT0280966</td>
<td>Fig 25: CASENT0281534</td>
<td></td>
</tr>
</tbody>
</table>

Fig 9. Comparison of *A. ulibeli* (A, B) and *A. gibbosa* Males (C, D). www.antweb.org
Table 4. Main differences between *A. ulibeli* and *A. gibbosa* males.

<table>
<thead>
<tr>
<th><em>A. ulibeli</em></th>
<th><em>A. gibbosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Promesonotum much smaller in volume than the rest of</td>
<td>Promesonotum inflated, becoming as big in volume as</td>
</tr>
<tr>
<td>the mesosoma</td>
<td>the rest of the mesosoma</td>
</tr>
<tr>
<td>Head with numerous semi-erect to erect long white</td>
<td>Head with scattered adpressed short setae, its length</td>
</tr>
<tr>
<td>setae, much longer than scape width</td>
<td>comparable to scape width</td>
</tr>
<tr>
<td>Eyes with microscopic hairs</td>
<td>Eyes without hairs</td>
</tr>
<tr>
<td>Propodeum almost a straight line</td>
<td>Propodeum ‘L’ shaped, with its anterior face vertical,</td>
</tr>
<tr>
<td>Petiole smoothly curved, without an anterior vertical</td>
<td>and its posterior half horizontal</td>
</tr>
<tr>
<td>face</td>
<td>Petiole truncated, with an anterior vertical face</td>
</tr>
<tr>
<td>Dorsal propodeal surface entirely glabrous</td>
<td>Dorsal propodeal surface with abundant erect setae</td>
</tr>
<tr>
<td>Metapleuropropodeal suture with a transverse rugulated</td>
<td>Metapleuropropodeal suture a simple line, without any</td>
</tr>
<tr>
<td>pattern</td>
<td>pattern</td>
</tr>
</tbody>
</table>

Acknowledgements

To Alberto Sánchez, for his continued help in field ant collecting, specially the samples for *A. ulibeli*. To Dr. Alberto Tinaut for kindly sending the material of *A. cardenai* workers and an early copy of the relevant Lorite et al (2017) paper. This work was partially supported by the MINECO project CGL2015-68188-P.

References


Author’s contribution

K. Gómez and X. Espadaler contributed with the specimens analysis, new species description and keys to the Iberian Fauna. D. Martinez expertise was key to the molecular and phylogenetic analysis.
Supplementary Material – Figures 10 – 27.