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RESEARCH ARTICLE - ANTS

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.

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Introduction

In 2007 the AIM (Asociación Ibérica de Mirmecología: http://www.mirmiberica.org/) developed an extensive pitfall sampling in 18 sites in the Iberian Peninsula. The coordination and identification of the samples was made by X. Espadaler and K. Gómez.

Two undescribed *Aphaenogaster* males were collected in a pitfall in the Sierra de Béjar (Salamanca) site by Alberto Sánchez. The males on this genus are strikingly different from one species to another, and allow identification to species level in a relatively easy way.

Reidentification of the *Aphaenogaster* workers from the site showed that samples previously identified as *A. gibbosa* were a new species indeed. The finding of new nests with workers and males together confirmed its conspecificity.

We have extracted DNA from all Iberian *Aphaenogaster* available to us and created a phylogeny to the genus in the Iberian Peninsula which shows some interesting and unexpected results.

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.

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. When specified, codenumber on image references belong

to the unique identifier in www.antweb.org database and can be accessed via https://www.antweb.org/specimenImages.do?code=CODENUMBER.

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

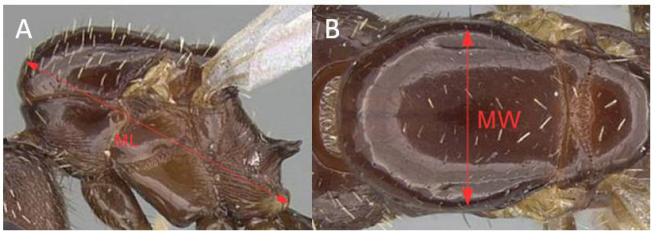

Fig 1 . Definition of ML (A) and MW (B) measurement in the queen caste.

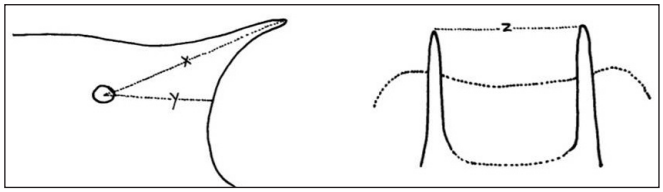

Fig 2. Definition of x, y, z. From De Boer, 2013.

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: 94ºC for 1 min; 40 cycles of 94ºC for 30 s, 48ºC for 30 s and 68ºC for 30 s; a final extension step of 10 min at 68ºC 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) 40º 22.87'N 5º 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) 40º 22.87'N 5º 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) 40º 22.87'N 5º 45.43'W 1.010m 22-24 Jun. 2007. Pitfall (Sanchez, A.). Caducifolia forest [MNHN: EY20152, 2w]
SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40º 22.87'N 5º 45.43'W 1.010m 27-29 Jul. 2007. Pitfall (Sanchez, A.). Caducifolia forest [CASC: KG02103-1, 1w]
SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40º 22.87'N 5º 45.43'W 1.010m 01 Jun. 2016. (Sanchez, A.). Caducifolia forest, Nest under stone [KGAC: KG03235-1, 3w; KG03235-3, 2w], [XEGC: KG03235-2, 3w], [CASC: KG03235-4, 1w]
SPAIN: Sierra de Gredos (Espadaler, X.) 23.vii. 1979 [XEGC: XE00200, 4w]
Paratype Queens:
SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40º 22.87'N 5º 45.43'W 1.010m 30 Jul. 2010. (Sanchez, A.). Caducifolia forest, Nest under stone [KGAC: KG02101-1, 1Q]
SPAIN: Sierra de Gredos (Espadaler, X.) 23.vii. 1979 [XEGC: XE00200, 1Q]
Paratype males: SPAIN: Salamanca, Camino Viejo de Candelario (Béjar) 40º 22.87'N 5º 45.43'W 1.010m 27-29 Jul. 2007. Pitfall (Sanchez, A.). Caducifolia forest [KGAC: KG02103-2, 1m], [XEGC: KG02103-3, 1m]

Derivationominis: 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 semierect 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

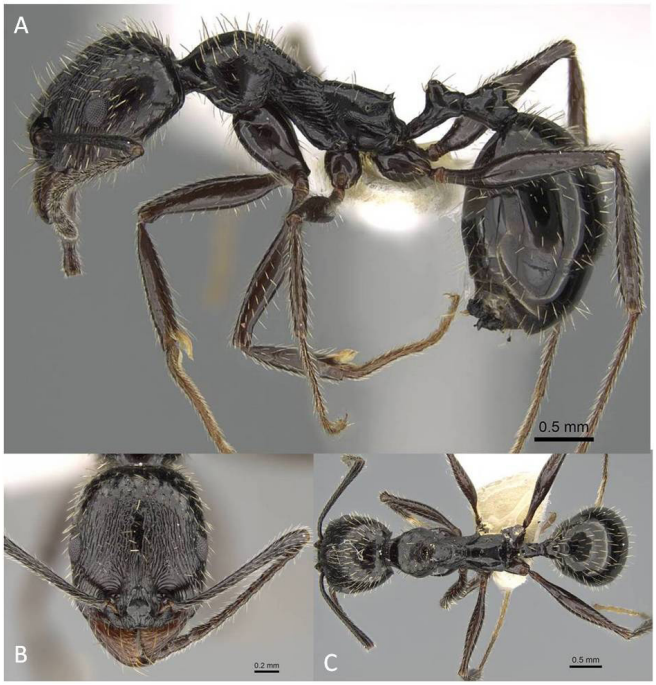

Fig 3. ***A. ulibeli*** worker paratype [KG02103-1]. www.antweb.org

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 mesoscutellum feebly striated. Anepisternum and katepisternum smooth in its anterior half and longitudinally striated in its posterior half. Pronotum transversely striated.

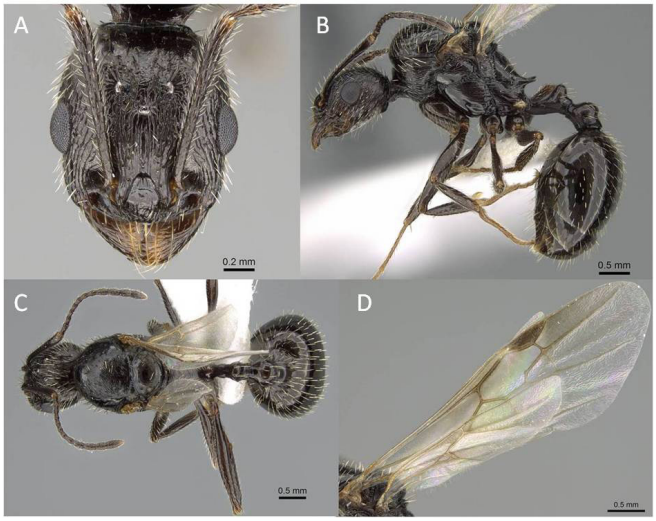

Fig 4. ***A. ulibeli*** queen paratype [KG02101-1]. www.antweb.org

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). 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

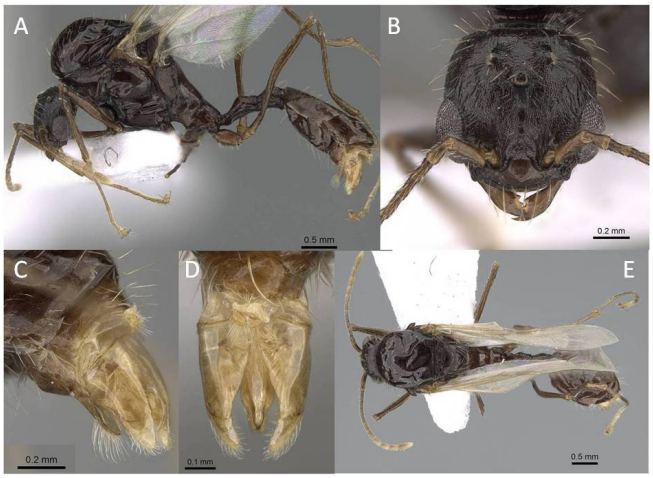

Fig 5. ***A. ulibeli*** male paratype [KG02103-2]. www.antweb.org

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 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.

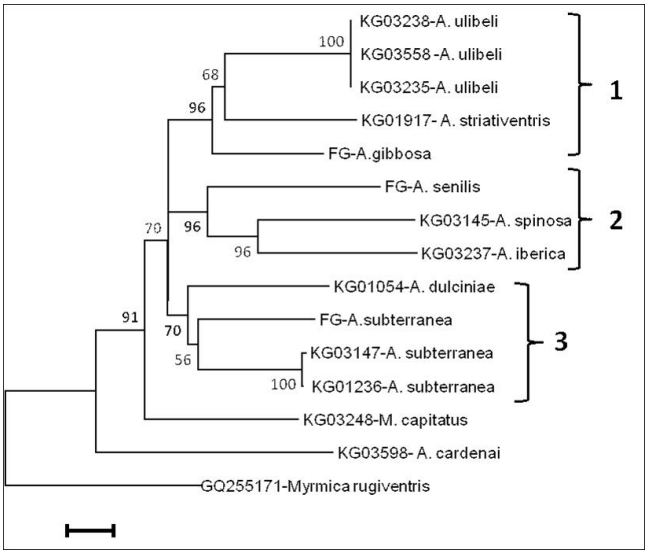

Fig 6. ML phylogenetic tree for the COI sequences obtained for species of the ant genus *Aphaenogaster* in the Iberian Peninsula. *A. splendida* material was not available, and therefore is not included. *A. spinosa*, from continental Italy, is also included in the analysis. *M. rugiventris* is used as outgroup. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site 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.

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

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.

Species	GenBank	Depository and data
A. cardenai	MF926348	KGAC [KG03598-1]. Spain: Jaen, Cueva pB-4 (Peal del Becerro) 01/01/2015. Hand (GEV). Cave (1w)
A. dulciniae	MF926345	KGAC [KG01054]. Spain: Madrid, Casa de Campo 615m, 40.42472, -3.74111 22/04/2004. Hand (Gomez, K.), Nest under stone
A. gibbosa	MF926349	Spain: Girona, Campdevanol. 15/05/2017. F. García leg, det
A. iberica	MF926340	KGAC [KG03237]. Spain: Salamanca, Finca (Béjar) 1000m, 40.384, -5.7575 01/06/2016. Hand (Sanchez, A.). Caducifolia forest, Nest under stone
A. senilis	MF926347	Spain: Barcelona, Montjuich. 17/05/2017. F. García leg, det.
A. spinosa	MF926339	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
A. subterranea / A	MF926338	KGAC [KG03147A-1]. Italy: Lazio, Camping (Lughezza) 60m, 41.93193, 12.70005 05/10/2015. Hand (Gomez, K.). Ruderal among crops, Under stone
A. subterranea / B	MF926343	KGAC [KG01236-1]. Spain: Mallorca, Randa 320m, 39.52444, 2.91917 19/07/2004. Hand (Gomez, K.). Encinar, nest under stone
A. subterranea / C	MF926350	Spain: Girona, Campdevanol. 15/05/2017. F. García leg, det.
A. striatriventris	MF926346	KGAC [KG01917-1] Spain: Cádiz, Algar , 36.65, -5.65 05/02/2007. Hand (Huertas, R.). Prado, Nest under stone
A. ulibeli / A	KY124277	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
A. ulibeli / B	MF926341	KGAC [KG03238-1] Spain: Salamanca, Finca (Béjar) 1000m, 40.38306, -5.75833 01/06/2016. Hand (Sanchez, A.). Caducifolia forest, Nest under stone
A. ulibeli / C	MF926344	KGAC [KG03558-1] Spain: Salamanca, Finca (Béjar) 1000m, 40.38306, -5.75833 01/06/2016. Hand (Sanchez, A.). Caducifolia forest, Nest under stone
M. capitatus	MF926342	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

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 madeirense* 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. rufibarbis* 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); *Myrmicaaloba* 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

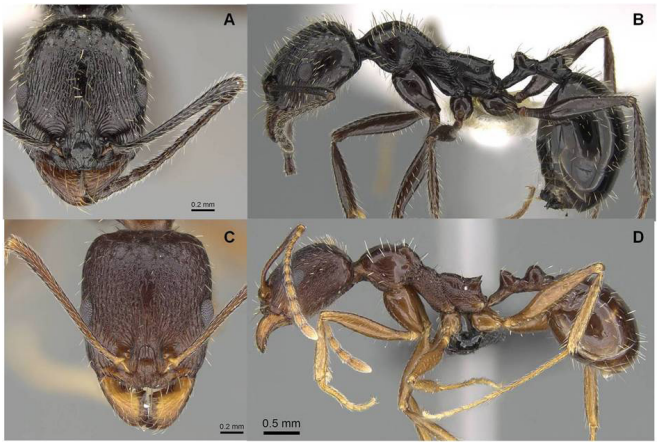

Fig 7. Comparison of ***A. ulibeli*** (A, B) and ***A. gibbosa*** workers (C, D). www.antweb.org

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

A. ulibeli	***A. gibbosa***
Brownish black to black	Brown
Head sculpture parallel striated	Head sculpture reticulated
Scape setae semi-erect in its apical third	Scape setae adpressed
Mesonotum not humped, forming a continuous line with pronotum in profile view	Mesonotum humped, not forming a continuous line with pronotum in profile view
Mesopleura longitudinally striated	Mesopleura longitudinally striated - reticulated
Petiole dome ellipsoidal, symmetrical, without an angle between anterior and posterior faces	Petiole dome in profile view with an anterior face straight in contrast with the posterior more curved face

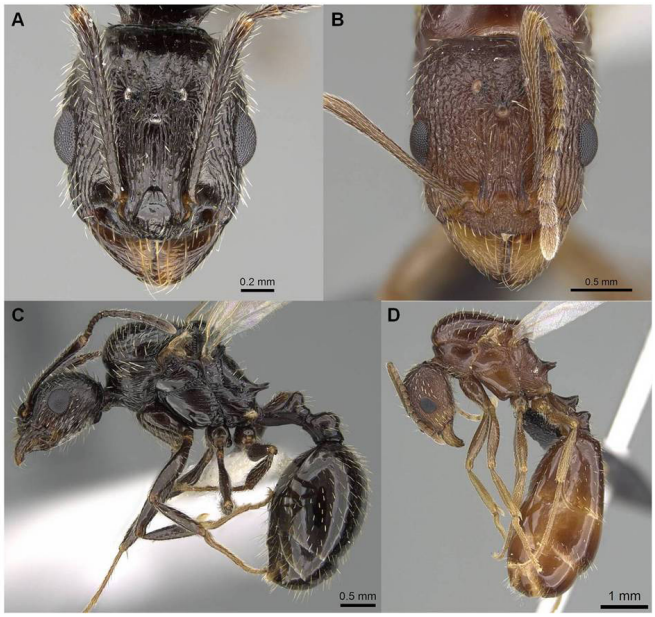

Fig 8. Comparison of ***A. ulibeli*** (A, C) and ***A. gibbosa*** Queens (B, D). www.antweb.org

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

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

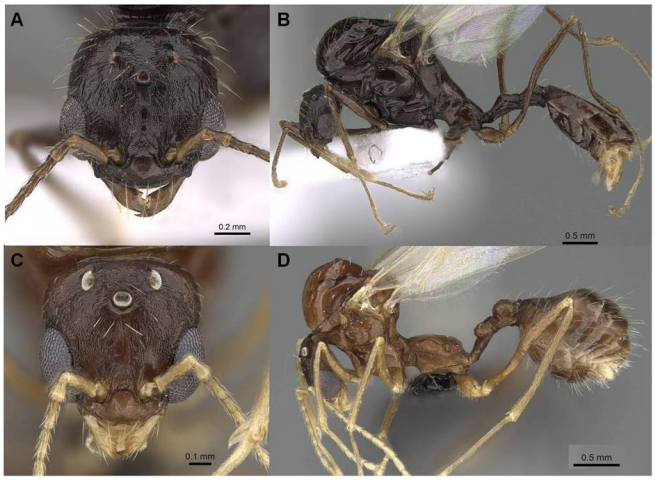

Fig 9. Comparison of ***A. ulibeli*** (A, B) and ***A. gibbosa*** Males (C, D). www.antweb.org

*Keys to Iberian **Aphaenogaster** workers*

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

1	Funicular segments 2-6 short, slightly longer than broad.....2	All of the funicular segments elongated 3
	Fig 10: CASENT0172716	Fig 11: CASENT0280964
2 (1)	Head striated-reticulated ..... ***subterranea***	Head punctuated, smooth ..... ***dulciniae***
	Fig 12: CASENT0172716	Fig 13: CASENT0280959
3 (1)	Body colour from orange-yellowish to dark orange 4	Body colour from dark brown to black 5
4 (3)	Propodeal spines very long, its length similar to petiole pedunculum length ..... ***cardenai***	Propodeal spines very short, almost reduced to triangular teeth.....***splendida***
	Fig 14: CASENT0249624	Fig 15: CASENT0280965
5 (3)	Short and powerful mandibles, with the outer margin strongly curved towards the middle line. Major workers with square heads converging in cephalic morphology to *Messor*….. ***striativentris***	Elongated and triangular mandibles, with the outer margin not strongly curved towards the middle line. Head clearly longer than wide, oval.....6
	Fig 16: CASENT0280964	Fig 17: KG02103-1
6 (5)	Gaster smooth and polished, shiny 7	Gaster striated, at least in the base, matt.....8
	Fig 18: KG02103-1	Fig 19: CASENT0281534
7 (6)	Brown. Head sculpture reticulated. Mesonotum humped, not forming a continuous line with pronotum in profile view. Mesopleurae longitudinally striated - reticulated.....***gibbosa***	Brownish black to black. Head sculpture parallel striated. Mesonotum not humped, forming a continuous line with pronotum in profile view. Mesopleura longitudinally striated ..... ***ulibeli n. sp.***
	Figs 20-21: KG01850-2	Figs 22-23: KG02103-1
8 (6)	Propodeum with very reduced spines, from non-existent to blunt triangular teeth....***gemella***	Spines clearly developed.....9
	Fig 24: CASENT0280968	Fig 25: CASENT0281534
9 (8)	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***	Propodeal spines shorter than the petiole pedunculum, cylindrical and oriented 45º with the horizontal. Antennal club with five segments.... ***senilis***
	Fig 26: CASENT0280966	Fig 25: CASENT0281534

Table 4. Main differences between ***A. ulibeli*** and ***A. gibbosa*** males.

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

Acknowledgements

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Author's contribution

K. Gómez and X. Espadaler contributed with the specimens analysis, new species description and keys to the Iberian Fauna. D. Martínez expertise was key to the molecular and phylogenetic analysis.

Supplementary Material – Figures 10 – 27.


Fig 10

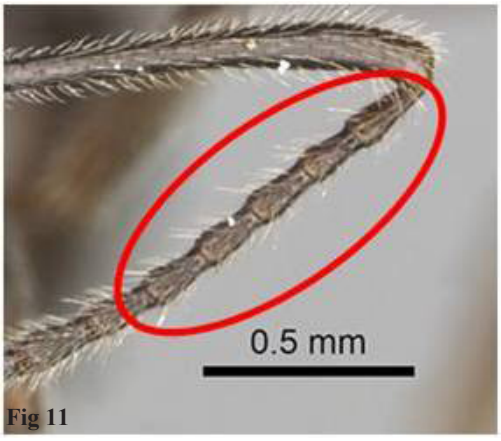

Fig 11


Fig 12


Fig 13


Fig 14


Fig 15


Fig 16


Fig 17

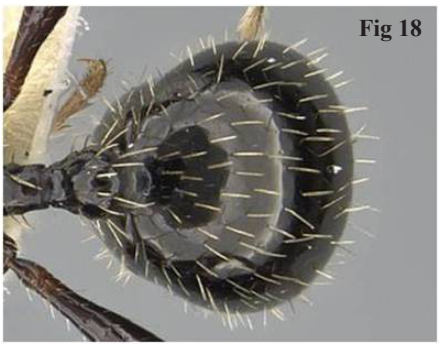
Fig 18


Fig 19

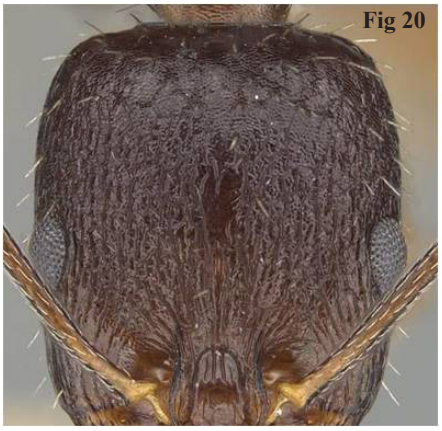
Fig 20


Fig 21


Fig 22


Fig 23


Fig 24


Fig 25


Fig 26