Melitta schmiedeknechti (Hymenoptera Apoidea, Melittidae), a new species for the fauna of Italy

Simone FLAMINIO^{1,2}, Giovanni CILIA¹, Azzurra VELLA³, Marino QUARANTA¹, Gabriella LO VERDE³

¹CREA Research Centre for Agriculture and Environment, Bologna, Italy

²Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Belgium ³Department of Agricultural, Food and Forest Sciences, University of Palermo, Italy

Abstract

Melitta schmiedeknechti Friese 1899 is reported for the first time in Italy. The species was collected in two different localities, mainland Sicily and Lampedusa, expanding its known range. Localities and flora visited are reported and, in addition, barcoding of two specimens was carried out.

Key words: wild bee, Pelagie archipelago, Sicily, barcoding.

Introduction

The genus *Melitta* Kirby 1802, the most widespread and speciose genus of the family Melittidae (Michez and Eardley, 2007), includes 19 species in Europe of which, according to the bibliographic checklist of Italian wild bees (Comba, 2019), six species are reported for Italy: *Melitta dimidiata* Morawitz 1876; *Melitta haemorrhoidalis* (F. 1775); *Melitta tomentosa* Friese 1900; *Melitta leporina* (Panzer 1799); *Melitta nigricans* Alfken 1905; and *Melitta tricincta* Kirby 1802. During surveys carried out in some Sicilian areas, *Melitta schmiedeknechti* Friese 1899 was collected. The species is here reported for the first time in Italy, Sicily.

Materials and methods

The specimens were collected by sweep net during entomological surveys in Sicily, in Lampedusa Island (figure 1A) and Trapani province (figure 1B) and subsequently brought to the laboratory and identified using comparison material and taxonomic literature (Michez and Eardley, 2007). Besides, DNA barcode was performed. Examined material is preserved in the Collection of CREA-AA (Research Centre for Agriculture and Environment, Bologna, Italy).

Molecular analysis and DNA barcode

Two different methods were used to generate DNA barcodes.

Total DNA was extracted from the right hind leg (Cornalba *et al.*, 2020; Villalta *et al.*, 2021), and dissected from the female collected in Lampedusa and the male from Selinunte. Simultaneously, a sterile microbiological swab soaked with digestion buffer was gently rubbed over the sternia of each *Melitta* specimen, following the protocol described in Cilia *et al.* (2022). A yellow label with the code "MSI_001" and "MSI_002" was placed under the female and male voucher specimens, respectively. Legs and swabs from the two specimens were each placed in two 2 mL microtubes filled with 1 mL digestion buffer and incubated for 18 hours at 56 °C. Total DNA purification was performed using a phenol-chloroform extraction (UltrapureTM Phenol:Chloroform: Isoamyl Alcohol, ThermoFisher Scientific, Waltham, MA, USA), as reported by Cilia *et al.* (2022). The obtained DNAs were quantified using the spectrophotometer Infinite 200 PRO NanoQuantTM (TECAN Life Technologies, Männedorf, Switzerland) and stored at -20 °C until the analysis. Double-distilled Rnase-Dnase-free water was used as a negative control for all of these processes.

Amplification of mithocondrialDNA (*mt*DNA) was performed using primer pairs able to amplify a 710-bp fragment within the highly conserved region coding for the Cytochrome C oxidase subunit I (COI) gene: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3) (Folmer *et al.*, 1994). The PCR and amplicon visualization were performed as described in Cilia *et al.* (2022). The obtained amplicons were purified using ExoSAP-IT Express (ThermoFisher Scientific) and were then sequenced through the standard Sanger methodology. The obtained sequences were analysed using BioEdit (Hall, 1999) to create the consensus one aligning forward and reverse sequences and BLAST (using megablast algorithm) (Altschul *et al.*, 1990).

Results

Examined specimens

 $1 \bigcirc$, ITA, Lampedusa, AG, Cala Guitgia, $35^{\circ}29'56.17"N$ $12^{\circ}35'54.18"E$, 1.III.2022, Lo Verde G. leg., on *Glebionis coronaria* (L.).

1 Å, ITA, Lampedusa, AG, Cala Guitgia, 35°29'56.17"N 12°35'54.18"E, 1.III.2022, Lo Verde G. leg., on *Glebionis coronaria* (L.).

1 Å, ITA, Sicily, Castelvetrano, TP, Selinunte, 37°35'07.83"N 12°50'08.21"E, 13.XII.2021, Lo Verde G. leg.



Figure 1. Point of sampling (in white) of *M. schmiedeknechti* in Cala Guitgia (Lampedusa, Italy) (**A**) and in Selinunte (Sicily, Italy) (**B**). Map source: ArcGIS Online basemap.



Figure 2. *M. schmiedeknechti* female, Lampedusa (Italy). (A) dorsal habitus; (B) lateral habitus; (C) head, frontal view; (D) head, lateral view; (E) scutum; (F) propodeum. Scale bar: 2 mm.

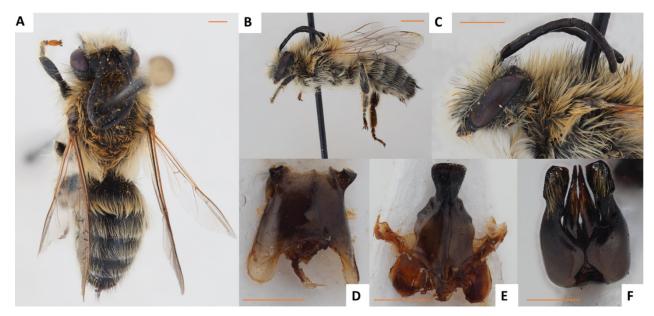


Figure 3. *M. schmiedeknechti* male, Lampedusa (Italy). (A) dorsal habitus (scale bar: 2 mm); (B) lateral habitus (scale bar: 2 mm); (C) head, lateral view (scale bar: 2 mm); (D) sternum 7 (scale bar: 0.5 mm); (E) sternum 8 (scale bar: 0.5 mm); (F) genitalia (scale bar: 0.5 mm).

Identification

M. schmiedeknechti specimens can be primarily discriminated by the labial palpus which is as long as glossa, the galea mat on the outer surface, the malar area shorter than the second antennal segment (figure 2D), the propodeal triangle as wide as metanotum (figure 2F) and the scutum, scutellum and propodeum mat and sculptured (figure 2E) (Michez and Eardley, 2007).

The female (figure 2A, 2B), in addition to the characters listed above, can be recognized, as stated by Michez and Eardley (2007), by the clypeus densely punctate and with an impunctate midline (figure 2C), the first tergum reddish and the terga 2 and 3 black and by the prepygidial fimbria black with few white hairs laterally.

The male (figure 3A, 3B), instead, can be recognized

by antennomeres from 4 to 12 slightly convex ventrally (figure 3C), the terga without apical hairbands, and the fifth sternum with a straight apical margin. Furthermore, the apicolateral processes of sternum 7 are swollen (figure 3F), and sternum 8 is almost bald with the apical area circle-shaped (figure 3E). The gonostylus is truncate apically, with the external margin shorter than the external margin of the gonocoxite (figure 3D).

According to Michez and Eardley (2007) *Melitta maura* (Perez 1896) can be excluded in female and male specimens by observing the malar area which is longer than the length of the second antennomere (malar area shorter than the length of the second antennomere). *Melitta aegyptiaca* (Radoszkowski 1891) can be excluded, in female specimens, as the apex of the clypeus is sparsely punctate and

lacks a non-punctate midline. Furthermore, in both sexes, the scutum is posteriorly shiny, whereas it is completely sculptured in *M. schmiedeknechti*.

DNA barcode

The COI region was successfully amplified and sequenced from the analysed specimens. The same sequence was obtained using both different methods. The BLAST analysis confirmed the identification, showing high similarity to the specimen of *M. schmiedeknechti* (Accession Number KC253169.1) [Query cover: 100%; E-value: 0.0; Percentage of identity: 99.30%] (Dellicour *et al.*, 2014). The sequences were deposited in GenBank (OQ625281 and OQ625282, male and female specimens, respectively).

Ecology

According to Michez and Eardley (2007) the flight period of the species is from the beginning of February to the end of April, but the male specimen collected in Sicily in December extends the flight period. The visited plants are *Oxalis pes-caprae* L. (Oxalidaceae), *Reseda* sp. (Resedaceae), Fabaceae (Michez and Eardley, 2007), and *Glebionis coronaria* (L.) Cass. ex Spach (Asteraceae) (present study).

Distribution

Canaries, Morocco, Tunisia, Libya (Cyrenaica), Egypt, Israel (Michez and Eardley, 2007) and Saudi Arabia (Shebl *et al.*, 2016), Italy (present study)

The disjointed distribution leads to the occurrence of two separate populations, which are considered subspecies on morphological traits (Michez and Eardley, 2007; Shebl et al., 2016): Melitta schmiedeknechti tunensis Warncke 1973 (Canary Islands, Morocco and Tunisia), which has face and scutum with yellowish or yellowishhairs and Melitta schmiedeknechti brownish schmiedeknechti Friese 1899 (Libya -Cyrenaica-, Egypt, Israel and Saudi Arabia), which instead has fuscous hairs intermixed with lighter ones on face and scutum. Given the locality of occurrence and the absence of fuscous hairs, we assign the specimens found in Sicily to the subspecies tunensis.

Discussion and conclusion

We report *M. schmiedeknechti* from Sicily and Lampedusa, two areas of particular interest concerning the Hymenoptera Apoidea fauna. Sicily is the most biodiverse region in Italy with regards to the Hymenoptera Apoidea (Comba, 2019), due to the central position of the island in the Mediterranean Basin. Despite being the subject of intensive research (Mazzeo *et al.*, 2007; 2019; Turrisi *et al.*, 2022), new species are still being reported (Bella *et al.*, 2020).

The Island of Lampedusa, regardless of being a very interesting island in terms of fauna due to its proximity to the coast of Tunisia and its geological history, has been relatively under-sampled in terms of Hymenoptera Apoidea (Pagliano and Scaramozzino, 1995; Pagliano, 2003; 2011; Romano, 2020). Our finding adds a new element regarding the ecology of the species, as it adds the Asteraceae among the botanical families visited by this species.

The discovery of *M. schmiedeknechti* in Sicily, highlights the importance of further faunistic research in Sicily and the surrounding islands. Furthermore, it raises the question of whether the species is undergoing a period of range expansion due to climate change as reported for other species in Europe (Banaszak *et al.*, 2019; Borański *et al.*, 2021; Biella *et al.*, 2022), has merely been overlooked in the past years or has arrived by accidental introduction. Further studies are therefore needed both to increase the faunal knowledge of the region and to better understand the dynamics of species movements in relation to climate change.

Acknowledgements

The authors thank Denis Michez for confirming the identification. Material from Lampedusa Island was collected during field surveys supported by the EU Project LIFE16CCA/IT/000011 "DESERT ADAPT".

This study was also supported by the project BeeNet (Italian National Fund under FEASR 2014-2020) from the Italian Ministry of Agriculture and Food Sovereignty and Forestry (MASAF).

References

- ALTSCHUL S. F., GISH W., MILLER W., MYERS E. W., LIPMAN D. J., 1990.- Basic local alignment search tool.- *Journal of Molecular Biology*, 215: 403-410.
- BANASZAK J., BANASZAK-CIBICKA W., TWERD L., 2019.- Possible expansion of the range of *Xylocopa violacea* L. (Hymenoptera, Apiformes, Apidae) in Europe.- *Turkish Journal of Zoology*, 43: 650-656.
- BELLA S., CATANIA R., NOBILE V., MAZZEO G., 2020.- New or little-known bees from Sicily (Hymenoptera: Apoidea).-*Fragmenta Entomologica*, 52: 113-117.
- BIELLA P., TOMMASI N., GUZZETTI L., PIOLTELLI E., LABRA M., GALIMBERTI A., 2022.- City climate and landscape structure shape pollinators, nectar and transported pollen along a gradient of urbanization.- *Journal of Applied Ecology*, 59: 1586-1595.
- BORAŃSKI M., CELARY W., JACHUŁA J., 2021.- First record of *Lithurgus cornutus* (Hymenoptera: Apoidea: Megachilidae) from Poland.- *Biodiversity Data Journal*, 9: e75997.
- CILIA G., FLAMINIO S., QUARANTA M., 2022.- A novel and noninvasive method for DNA extraction from dry bee specimens.- *Scientific Reports*, 12: 11679.
- COMBA M., 2019.- (Hymenoptera: Apoidea: Anthophila of Italy). Bibliographic checklist of Italian wild bees with notes on taxonomy, biology, and distribution.- [online] URL: https://digilander.libero.it/mario.comba/
- CORNALBA M., BIELLA P., GALIMBERTI A., 2020.- DNA barcoding unveils the first record of *Andrena allosa* for Italy and unexpected genetic diversity in *Andrena praecox* (Hymenoptera: Andrenidae).- *Fragmenta Entomologica*, 52: 71-75.
- DELLICOUR S., LECOCQ T., KUHLMANN M., MARDULYN P., MICHEZ D., 2014.- Molecular phylogeny, biogeography, and host plant shifts in the bee genus *Melitta* (Hymenoptera: Anthophila).- *Molecular Phylogenetics and Evolution*, 70: 412-419.

- FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R., 1994.- DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.- *Molecular Marine Biology and Biotechnology*, 3: 294-299.
- HALL T. A., 1999.- BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.- *Nucleic Acids Symposium Series*, 41: 95-98.
- MAZZEO G., SEMINARA A. R., BELLA S., FERRAUTO G., LONGO S., 2007.- Osservazioni sugli apoidei e sull'attività di *Apis mellifera* in un'area altomontana del Parco dell'Etna.- APOidea, 4: 71-75.
- MAZZEO G., LONGO S., SEMINARA A. R., BELLA S., 2019.-Faunistic and ecological studies on Apidae (Hymenoptera Apoidea) in natural and cultivated ecosystems in Sicily.- *Redia*, 102: 153-162.
- MICHEZ D., EARDLEY C., 2007.- Monographic revision of the bee genus *Melitta* Kirby 1802 (Hymenoptera: Apoidea: Melittidae).- Annales de la Société Entomologique de France, 43: 379-440.
- PAGLIANO G., 2003.- Ricerche Imenotterologiche nelle isole di Lampedusa e Pantelleria (Hymenoptera Apocrita).- *Naturalista Siciliano*, 28: 115-149.
- PAGLIANO G., 2011.- Ricerche Imenotterologiche nelle isole di Lampedusa con descrizione di una nuova specie di mutillide (Hymenoptera, Mutillidae).- *Fragmenta Entomologica*, 43: 215-224.
- PAGLIANO G., SCARAMOZZINO P. L., 1995.- Hymenoptera Gasteruptionidae, Ichneumonidae e Aculeata.- *Naturalista Siciliano*, 19 (Supplement): 723-728.
- ROMANO M., 2020.- Insects of Lampedusa, Linosa and Pantelleria: addenda to the 1995 checklist, pp. 179-207. In: *Life on islands. 1. Biodiversity in Sicily and surrounding islands. Studies dedicated to Bruno Massa* (LA MANTIA T., BADALA-MENTI E., CARAPEZZA A., LO CASCIO P., TROIA A., Eds).-Edizioni Danaus, Palermo, Italy.

- SHEBL M. A., ALQARNI A. S., ENGEL M. S., 2016.- First record of the bee genus *Melitta* from the Arabian Peninsula (Hymenoptera: Apoidea: Melittidae).- *Zoology in the Middle East*, 62: 352-357.
- TURRISI G. F., BELLA S., CATANIA R., LA GRECA P., NOBILE V., D'URSO V., 2022.- Bee diversity in fragmented areas of Volcano Etna (Sicily, Italy) at different degrees of anthropic disturbance (Hymenoptera: Apoidea, Anthophila).- Journal of Entomological and Acarological Research, 53: 10326.
- VILLALTA I., LEDET R., BAUDE M., GENOUD D., BOUGET C., CORNILLON M., MOREAU S., COURTIAL B., LOPEZ-VAAMONDE C., 2021.- A DNA barcode-based survey of wild urban bees in the Loire Valley, France.- *Scientific Report*, 11: 4770.

Authors' addresses: Giovanni CILIA (corresponding author: giovanni.cilia@crea.gov.it), Simone FLAMINIO¹, Marino QUARANTA, CREA Research Centre for Agriculture and Environment, via di Corticella 133, 40128 Bologna, Italy; Azzurra VELLA, Gabriella LO VERDE, Department of Agricultural, Food and Forest Sciences, University of Palermo, viale delle Scienze Bd. 4, 90128 Palermo, Italy.

¹ Present address: Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Place du parc 20, 7000, Mons, Belgium.

Received April 18, 2023. Accepted August 29, 2023.