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Original

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Integrating taxonomic, genetic and ecological data to explore the species richness of wild bees (Hymenoptera, Apoidea, Anthophila) of the Culuccia Peninsula (NE Sardinia, Italy)

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Abstract

Wild bees are essential pollinators of both native and cultivated plants, but their populations are declining worldwide. Conservation efforts are hindered by insufficient data, especially in the Mediterranean basin, which hosts some of the most diverse pollinator communities in the world. Particularly in Sardinia, the second largest island in the Mediterranean, information on the bee fauna is still limited. The aim of this work was to provide the first checklist of Apoidea Anthophila from an unexplored peninsula in north-eastern Sardinia (Italy), by combining traditional (morphologically-based) taxonomy and DNA barcoding. In addition, records of flower visits are provided and shown in a visitor network to enrich the scarce data on the associations between wild bees and plants in the Mediterranean Region. Bees were sampled from April to October 2022–2023 with two Malaise traps and nets. DNA was extracted to amplify sequences of the mitochondrial gene Cytochrome oxidase I, which were then compared with those in BOLD using the identification tool and by constructing neighbor-joining phylogenetic trees. Seventy-six different species belonging to 29 genera and six families were collected and identified. A total of 212 COI sequences were obtained for 61 different species, many of which had not yet been sequenced from Italian populations. Five of the collected taxa are Sardo-Corsican endemics, whereas six species are newly recorded from Sardinia. Finally, we highlight potential taxonomic issues and new flower visit records, emphasizing the need for further research to better understand the taxonomy and ecology of this diverse group of insects toward their conservation.

Keywords

Checklist, COI, DNA barcoding, endemism, flower visitation networks, morphological identification, new records

Introduction

Bees pollinate most of the wild and cultivated plant species, contributing to the maintenance of plant communities and agricultural production (Quaranta et al. 2004; Klein et al. 2007; Stine et al. 2015; Matias et al. 2017). The conservation of wild bees is critical for both natural ecosystems and dependent crops (Julier and Roulston 2009; Stine et al. 2015; Vaughan et al. 2015), yet several reviews have highlighted that populations are declining worldwide (Bartomeus et al. 2013; Burkle et al. 2013; Garibaldi et al. 2013). According to the IUCN European Red List of Bees, 9.2% of the assessed bee species are threatened with extinction and 5.2% are considered Near Threatened, but the status of most of the species (56.7%) remains unknown due to insufficient information (Nieto et al. 2014). Similarly, Quaranta et al. (2018) highlighted the lack of sufficient data for most Italian bee species, compromising a reliable assessment of their conservation status. Moreover, the associations between wild bees and flowering plants are insufficiently documented for many bee species, particularly in Mediterranean regions. The lack of information on floral associations, as with other ecological aspects, makes it difficult to develop effective conservation strategies.

The Mediterranean basin hosts some of the most diverse pollinator communities in the World and hosts highly diverse bee fauna (Michener 1979; Michener 2000; Nieto et al. 2014; Orr et al. 2020). Currently, published data on wild bees underline a significant gap in research efforts between the central-northern part of the European continent, where most of the taxonomic specialists are concentrated, and Mediterranean countries, which are characterised by greater species richness (Quaranta et al. 2018). This gap highlights the need to strengthen local expertise and invest in basic research on the biology and ecology of wild bees, which is essential for their effective conservation. Particularly in Sardinia, the second largest island in the Mediterranean, our knowledge of the bee fauna is still limited. This island is home to a unique flora and fauna, with many distinctive species, including endemic taxa shared with Corsica or other regions, as well as several species with a very restricted range. The checklist of Italian wild bees (Hymenoptera: Apoidea: Anthophila) of Comba (2019) reports 316 bee species in Sardinia, including several endemic taxa, such as Sardo-Corsican species or subspecies (e.g., *Panurgus corsicus* Warncke, 1972 or *Bombus terrestris sassaricus* Tournier, 1890), Tyrrhenian subspecies (e.g., *Andrena rotundata diomedea* Warncke, 1975 or *Osmia ferruginea igneopurpurea* Costa, 1882) and Italian subspecies (e.g., *Melecta leucorhyncha taormina* Strand, 1919). Recently, different new records and species have been reported for this island, such as *Hoplitis occidentalis* Müller, 2012, *Tetralonia gennargentui* (Nobile, Catania & Bella, 2021) and *Andrena antonellae* Praz & Genoud, 2022 (Müller 2012; Catania et al. 2021; Nobile et al. 2021; Praz et al. 2022). Moreover, certain areas of Sardinia such as the Culiuccia Peninsula (see Mariottini et al. 2024) where the present study was car-

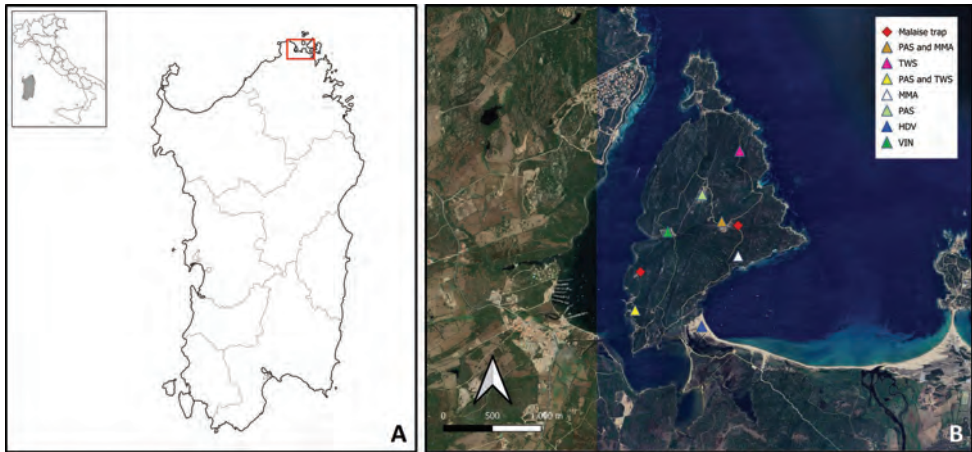


Figure 1. Position of the study area in Sardinia and in Italy (A). Satellite image of the Culuccia Peninsula and location of the sampling site (B), base map: orthophoto © 2022 Google. The red diamonds represent the position of the Malaise traps while the triangles are the main site (transects) sampled with nets. The different colours of the triangles indicate the Corine Land Cover classes in which the sampling sites were located. PAS=Pasture, MMA=Mediterranean maquis, TWS=Transitional woodland/shrub, HDV=Herbaceous dune vegetation, VIN=Vineyards.

ried out, lack information about the bee fauna. This small area of approximately 3 km² is located north-east of Sardinia (Italy), near the Maddalena archipelago, between Santa Teresa di Gallura and Palau (Fig. 1). This area shows a complex environmental mosaic that has remained relatively untouched by human activity for the past century, because of its minimal human presence compared to the neighbouring touristic coastal regions.

The main aim of this study is to provide the first checklist of bees of the Culuccia Peninsula, considering the current limited knowledge on the inventory and distribution of wild bees in the Mediterranean region. In addition, the use of a modern integrative taxonomic approach (morphological and molecular) for the accurate identification of the samples allowed to obtain the first COI sequences of the Sardinian populations for most of the species studied. Finally, we provide records of bee visits to flowers to show the interaction patterns between bees and plants on the peninsula and to enrich the limited knowledge of plant-bee associations in northern Sardinia.

Materials and methods

Study area and bee sampling

The study was carried out on the Culuccia Peninsula (Santa Teresa di Gallura, Sassari), located in north-eastern Sardinia on the edge of the Maddalena Archipelago, at 0–93 m above sea level (Fig. 1). The area, which is approximately 300 hectares,

is protected as a “Permanent Wildlife Protection and Capture Reserve”. In addition to a wide variety of natural environments, there are restricted areas focused on sustainable agri-food production, including two small vineyards, around a hundred beehives and some pastures. The environmental diversity of the area is illustrated by a detailed land use map showing 11 land cover classes, with Mediterranean Maquis (dense scrubland with woody vegetation) dominating, followed by denser vegetated areas of transitional woodland/shrub, along with other less extensive land use classes including wetlands, herbaceous dune vegetation, water bodies, bare rock, artificial, pasture, garigue (low open scrubland), beach and vineyards (Annessi et al. in press). The vegetation is mainly composed of thermophilous, psammophilous and xerophilous species, with Mediterranean scrub dominated by *Juniperus turbinata* Guss. (Cupressaceae) along with *Pistacia lentiscus* L. (Anacardiaceae), *Olea europaea* L. var. *sylvestris* (Mill.) and *Phillyrea angustifolia* L. (Oleaceae). In some areas of the peninsula, the scrub becomes tall and dense, associated with *Quercus ilex* L. (Fagaceae), whereas in other areas it gives way to garigue, characterized by discontinuous bushes with *Erica arborea* L., 1753, *E. scoparia* L., 1753 (Ericaceae), *Cistus monspeliensis* L. (Cistaceae) and *Lavandula stoechas* L. (Lamiaceae). Meadows are rich in herbaceous species, especially those belonging to the Asteraceae, Fabaceae, and Brassicaceae families. On sandy coasts there are psammophilous plants such as *Cakile maritima* Scop. (Brassicaceae), *Pancratium maritimum* L. (Amaryllidaceae) and *Eryngium maritimum* L. (Apiaceae), which give way to halophytic species (e.g. *Limonium* spp. Plumbaginaceae) in brackish wetlands.

Bees were sampled from April to October 2022–2023, by capturing specimens with nets along seven fixed transects of size 200 × 1 m (Quaranta et al. 2004), each separated by at least 450 meters and located in the main different Corine Land Cover classes (Fig. 1). A total of seven sampling sessions were carried out: two in spring 2022, two in summer 2022, one in autumn 2022, one in spring 2023 and one in summer 2023. Each transect was walked once per sampling session by two people, for 50 minutes of regular walking, at different times (between 10.00 and 16.00) to capture bee species with different periods of activity throughout the day. Sampling was conducted only when the temperature was above 15 °C, the wind was light, no rain and the vegetation was dry. To ensure a comprehensive bee species richness study, additional random sampling with nets was carried out throughout the peninsula, and two Malaise traps, separated 1000 m apart, were installed and operated continuously from May to October 2022 (Fig. 1), with bee samples collected every two weeks. Each sample collected was assigned a unique identification code, date, coordinates (WGS84), Corine Land Cover class and for those collected on flowers, the plant taxa were recorded. The bee specimens were prepared and stored dry in entomological boxes and preserved in the “Museum of Zoology and Comparative Anatomy”, Department of Science, Roma Tre University (Rome, Italy). The plants were photographed in the field and identified using Cesaraccio (1990, 1992) and with the help of the botanical specialists Simona Sarmati, Alicia Teresa Rosario Acosta and Alfred Mayer.

DNA barcoding

Total genomic DNA was extracted from one leg, following the salting out protocol (Sambrook et al. 1989), and eluted in 100 μ L H₂O milliQ and stored at -20 °C. The barcode fragment of mitochondrial cytochrome c oxidase subunit I gene was amplified using the primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5' TAAACTTCAGGTGACCAAAAATCA-3') (Folmer et al. 1994). For more difficult amplifications, a “nested” protocol was performed by first using an outermost reverse primer, HCOoutout (5'-GTAAATATGRTGDGCTC-3') (Prendini et al. 2005), paired with the same forward as above, and then reapplying the primer pair by Folmer et al. (1994) to the amplification product obtained in the previous step. Finally, the primer pair LepF1 (5' ATTCAACCAATCATAAAGAT-3') and LepRI (5'-TAAACTTCTGGATGTCCAAAA-3') was used for some problematic taxa (Sheffield et al. 2009). For each PCR, an aliquot of 1–2 μ L of DNA and 23–24 μ L of reaction mixture (MIX) were used, depending on the amount of extracted DNA measured with a NanoDrop Thermo Fisher Scientific™. The MIX contained: 3 μ L of Buffer 10 \times , 1 μ L MgCl₂ 50 mM, 0.5 μ L dNTPs 10 mM, 0.5 μ L primer forward 10 mM, 0.5 μ L primer reverse 10 mM, 0.5 μ L BSA, 0.2 Taq DNA polymerase 5U/ μ L and 16.8–17.8 H₂O milliQ. The PCR cycling conditions were based on Marconi et al. (2022) whereas the protocol of Sheffield et al. (2009) was used for some samples with amplification problems, especially those from the genera *Andrena*, *Hylaeus*, and *Colletes*. Three μ L of PCR products were used to determine amplification success by agarose (1%) gel run stained with 1 μ L of SimplySafe (EurX). The amplified products were purified and sequenced by Macrogen (Madrid, Spain and Milan, Italy). The sequences were deposited in BOLD and GenBank under Acc. n° [PQ472941–PQ473152](#).

Species identification

Morphological identification was performed using dichotomous keys and other taxonomic literature (Suppl. material 3: table S1). In addition, the Meloni collection of Sardinian wild bees identified by V. Nobile and deposited in the Natural History Museum G. Doria (Genoa, Italy) was consulted. Species distribution was referenced from the online checklist of Western Palearctic Bees by Kuhlmann et al. (2018), Atlas Hymenoptera by Rasmont and Haubruge (2015), Hymenoptera: Apoidea: Anthophila of Italy by Comba (2019) and Ascher and Pickering (2020). COI sequences were edited with Geneious Prime software ver. 2023.1.2 (<https://www.geneious.com>). The BOLD identification tool (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) was used to identify our specimens. Their attribution to species was based on the Kimura 2-parameter (K2P) corrected genetic distances (Kimura 1980) with a threshold of 3% as a cut-off value (Hebert et al. 2003). The sequences were then aligned with Muscle (Edgar 2004), implemented on MEGA 7 (Kumar et al. 2015; Tamura et al. 2021). The alignment was used to construct a consensus (distance-based) neighbor-joining (NJ) phylogenetic tree (500 bootstrap replicates, K2P model) using MEGA 7 to assess cross-contamination

and correspondence between clusters and morphological species. In addition, sequences of the species with doubtful identifications were aligned (as above), with those available in BOLD for specimens of the same genus or subgenus (Suppl. material 4: table S2). The resulting alignments were used to construct consensus NJ phylogenetic trees (500 bootstrap replicates, K2P model) to evaluate the clustering of sequences in monophyletic clades within bee genera/subgenera. Identification was considered doubtful when at least one of the following conditions was met: (1) discrepancy between morphological and molecular identification, (2) morphological identification was dubious, (3) BOLD ID % similarity was < 97. Sequence selection was made for each species based on the BIN (Ratnasingham and Hebert 2013), sequence quality and country of origin of the specimen, including only sequences from the W-Palaearctic. In total, approximately three to five sequences belonging to the same BIN code and country were chosen for each species. The sequences that were too short were excluded in any case. The final assignment of the species name to each specimen was achieved by relying on its identification obtained through morphology (MORPHO ID) and BOLD (BOLD ID, % similarity), and, if necessary, considering the relative position of analysed specimens within the comprehensive NJ phylogenetic trees. In addition, bee taxonomic specialists supported the identification of uncertain taxa specimens: Achik Dorchin for *Tetralonia*, Thomas James Wood for *Andrena*, Michael Kuhlmann for *Colletes*, Holger Dathe for *Hylaeus*, Alain Pauly for *Lasioglossum*, and Romain Le Divelec for *Hoplitis*. The classification used in this paper follows that proposed in the new checklist of wild bees of Europe (Ghisbain et al. 2023), whereas at the subspecies level, the systematic framework reported in Hymenoptera: Apoidea: Anthophila of Italy by Comba (2019) was followed.

Plant-bee interaction matrix

The data collected on the interactions between bees and flowering plants were represented by a binary interaction matrix using the “bipartite” package in R 4.3.2 (Dormann et al. 2008; R Core Team 2023). Interactions are indicated by 0 and 1 to indicate the absence or presence of bee species in plant families. A broader taxonomic level (plant family rather than species or genus) was used to simplify the visualisation and mitigate potential bias due to small sample sizes for certain bee species.

Results

A total of 469 individuals were collected, 383 with nets and 86 with Malaise traps, belonging to 76 different species from 29 genera and six families (Table 1, Suppl. material 5: table S3). Of the total species, 43.4% were sampled exclusively with nets, 42.1% with both nets and Malaise traps, and 14.5% with Malaise traps only. Among the identified taxa, five are Sardo-Corsican endemics: *Bombus terrestris sassaricus*, *Bombus xanthopus* (Kriechbaumer, 1870), *Hoplitis legoffi* Le Divelec, 2024, *Panurgus corsicus* Warncke, 1972 and *Megachile sicula corsica* Benoist, 1935. Six species are reported for the first

Table 1. List of wild bee species and number of samples collected on the Culuccia Peninsula.

Taxon	N° of specimens
Family ANDRENIDAE Latreille, 1802	
<i>Andrena (Chlorandrena) cfr. chypella</i> Strand, 1921	3♀
<i>Andrena (Hoplandrena) nuptialis</i> Pérez, 1902	2♂
<i>Andrena (Lepiandrena) sardo</i> a Lepeletier, 1841	1♀
<i>Andrena (Melandrena) flavipes</i> Panzer, 1799	1♂; 4♀
<i>Andrena (Melandrena) morio</i> Brullé, 1832	2♂; 5♀
<i>Andrena (Melandrena) nigroaenea</i> (Kirby, 1802)	12♂; 8♀
<i>Andrena (Micrandrena) cfr. nivata</i>	6♀
<i>Andrena (Notandrena) griseobalteata</i> Dours, 1872	1♂
<i>Andrena (Plastandrena) pilipes iliensis</i> Alfken, 1938	1♀
<i>Andrena (Taeniandrena) afzeliella</i> (Kirby, 1802)	2♂; 5♀
<i>Andrena (Taeniandrena) antonellae</i> Praz & Genoud, 2022	1♂
<i>Panurgus (Panurgus) corsicus</i> Warncke, 1972	13♂; 10♀
Family APIDAE Latreille, 1802	
<i>Amegilla (Amegilla) quadrifasciata</i> (de Villers, 1789)	7♂; 16♀
<i>Amegilla (Zebramegilla) savignyi</i> (Lepeletier, 1841)	1♂; 2♀
<i>Anthophora (Anthophora) canescens</i> Brullé, 1832	1♀
<i>Anthophora (Lophanthophora) dispar</i> Lepeletier, 1841	8♂; 5♀
<i>Anthophora (Pyganthophora) sp. 1</i>	1♀
<i>Bombus (Bombus) terrestris sassaricus</i> Tournier, 1890	15♂; 19♀
<i>Bombus (Bombus) xanthopus</i> (Kriechbaumer, 1870)	3♂; 1♀
<i>Ceratina (Ceratina) cucurbitina</i> (Rossi, 1792)	2♂; 1♀
<i>Ceratina (Dalyatina) parvula</i> Smith, 1854	3♀
<i>Ceratina (Euceratina) cyanea</i> (Kirby, 1802)	4♂; 1♀
<i>Ceratina (Euceratina) saundersi</i> Daly, 1983	5♂; 1♀
<i>Epeolus variegatus</i> (Linnaeus, 1758)	1♀
<i>Eucera (Eucera) clypeata</i> Erichson, 1835	1♀
<i>Eucera (Eucera) nigrescens</i> Pérez, 1879	10♂; 17♀
<i>Melecta (Melecta) leucorhyncha taormina</i> Strand, 1919	1♀
<i>Nomada femoralis</i> Morawitz, 1869	1♀
<i>Tetralonia dentata</i> (Germar, 1839)	1♂; 1♀
<i>Tetralonia fulvescens</i> Giraud, 1863	3♂; 3♀
<i>Thyreus histrionicus</i> (Illiger, 1806)	2♂; 4♀
<i>Xylocopa (Xylocopa) violacea</i> Linnaeus, 1758	1♀
Family COLLETIDAE Lepeletier, 1841	
<i>Colletes brevigena</i> Noskiewicz, 1936	1♂
<i>Colletes maidli</i> Noskiewicz, 1936	1♂
<i>Colletes similis</i> Schenck, 1853	1♂; 2♀
<i>Hylaeus (Dentigera) deceptorius</i> (Benoist, 1959)	3♂
<i>Hylaeus (Paraprosopis) taeniolatus</i> Förster, 1871	13♀
<i>Hylaeus (Prosopis) soror</i> (Pérez, 1903)	1♂
<i>Hylaeus (Prosopis) variegatus</i> (Fabricius, 1798)	2♂; 3♀
Family HALICTIDAE Thomson, 1869	
<i>Halictus (Halictus) fulvipes</i> (Klug, 1817)	4♀
<i>Halictus (Halictus) scabiosae</i> (Rossi, 1790)	2♂; 3♀
<i>Lasioglossum (Hemihalictus) transitorium planulum</i> (Pérez, 1903)	8♀
<i>Lasioglossum (Hemihalictus) villosulum</i> (Kirby, 1802)	2♂; 4♀
<i>Lasioglossum (Lasioglossum) bimaculatum</i> (Dours, 1872)	5♀
<i>Lasioglossum (Lasioglossum) prasinum</i> (Smith, 1848)	6♀

Taxon	N° of specimens
<i>Lasioglossum (Leuchalictus) albocinctum</i> (Lucas, 1849)	3♂; 13♀
<i>Lasioglossum (Leuchalictus) leucozonium</i> (Schrank, 1781)	1♂; 3♀
<i>Lasioglossum (Sphecodogastra) malachurum</i> (Kirby, 1802)	1♀
<i>Nomiapis diversipes</i> (Latreille, 1806)	1♂
<i>Seladonia (Seladonia) submediterranea</i> Pauly, 2015	3♀
<i>Sphecodes crassanus</i> Warncke, 1992	1♂
<i>Sphecodes gibbus</i> (Linnaeus, 1758)	1♂; 1♀
Family MEGACHILIDAE Latreille, 1802	
<i>Anthidellium strigatum</i> (Panzer, 1805)	5♂; 2♀
<i>Coelioxys (Austrocleptria) afer</i> Lepeletier, 1841	1♀
<i>Heriades (Heriades) crenulata</i> Nylander, 1856	14♂; 6♀
<i>Heriades (Heriades) rubicola</i> Pérez, 1890	6♂; 8♀
<i>Hoplitis (Alcidamea) praestans</i> (Morawitz, 1893)	1♂; 1♀
<i>Hoplitis (Alcidamea) tridentata</i> (Dufour & Perris, 1840)	1♀
<i>Hoplitis (Anthocopa) fasciculata</i> (Alfken, 1934)	1♀
<i>Hoplitis (Hoplitis) bhamata</i> (Morawitz, 1867)	1♂; 15♀
<i>Hoplitis (Hoplitis) legoffi</i> Le Divelec, 2024	3♂; 3♀
<i>Megachile (Callomegachile) sicula corsica</i> Benoist, 1935	4♂; 8♀
<i>Megachile (Eutricharaea) apicalis</i> Spinola, 1808	8♂
<i>Megachile (Eutricharaea) argentata</i> (Fabricius, 1793)	5♂; 15♀
<i>Megachile (Eutricharaea) melanopyga</i> Costa, 1863	4♂; 10♀
<i>Megachile (Megachile) centuncularis</i> (Linnaeus, 1758)	1♂
<i>Osmia (Helicosmia) caerulescens</i> (Linnaeus, 1758)	1♀
<i>Osmia (Helicosmia) niveata</i> Friese, 1887	7♂; 1♀
<i>Osmia (Hoplosmia) ligurica</i> Morawitz, 1868	2♂
<i>Osmia (Osmia) cornuta</i> (Latreille, 1805)	1♂
<i>Osmia (Osmia) tricornis</i> Latreille, 1811	2♂; 2♀
<i>Osmia (Pyrosmia) ferruginea igneopurpurea</i> Costa, 1882	4♂; 4♀
<i>Osmia (Pyrosmia) submicans</i> Morawitz, 1870	8♀
<i>Rhodanthidium (Rhodanthidium) septemdentatum</i> (Latreille, 1809)	3♀
<i>Stelis (Stelidomorpha) nasuta</i> (Latreille, 1809)	1♂
Family MELITTIDAE Schenck, 1860	
<i>Dasyopoda (Dasyopoda) hirtipes</i> (Fabricius, 1793)	1♂; 1♀

time from Sardinia: *Bombus xanthopus* (Fig. 2), *Andrena griseobalteata* Dours, 1872 (Fig. 3), *Colletes brevigena* Noskiewicz, 1936 (Fig. 4), *Sphecodes crassanus* Warncke, 1992 (Fig. 5), *Hoplitis praestans* (Morawitz, 1893) (Fig. 6) and *Hoplitis tridentata* (Dufour & Perris, 1840) (Fig. 7). Bee material citations are available in Suppl. material 1, including details such as collection sites, dates, Corine Land Cover classes and associated plant taxa.

NJ phylogenetic trees

A total of 212 COI sequences belonging to 61 different species were successfully sequenced (Suppl. material 6: table S4). The NJ phylogenetic tree built with only our new sequences, showed correspondence between monophyletic clades and morphological



Figure 2. *Bombus xanthopus* (Kriechbaumer, 1870) male collected on the Culuccia Peninsula (Sardinia, Italy) **A** dorsal view **B** lateral view **C** head **D** genital. Scale bars: 2 mm.

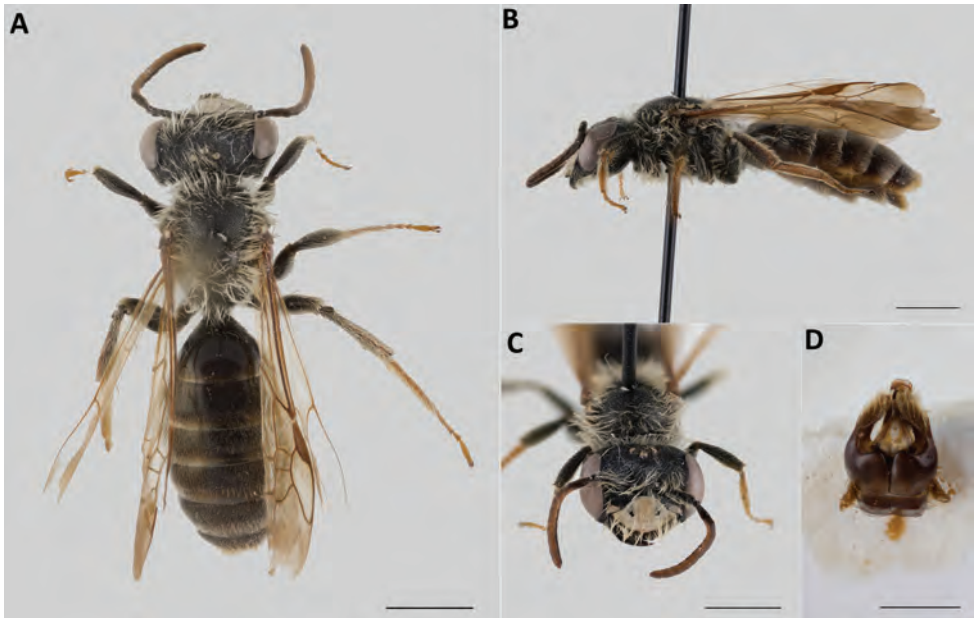


Figure 3. *Andrena griseobaltata* Dours, 1872 male collected on the Culuccia Peninsula (Sardinia, Italy) **A** dorsal view **B** lateral view **C** head **D** genital. Scale bars: 2 mm (**A–C**); 1 mm (**D**).

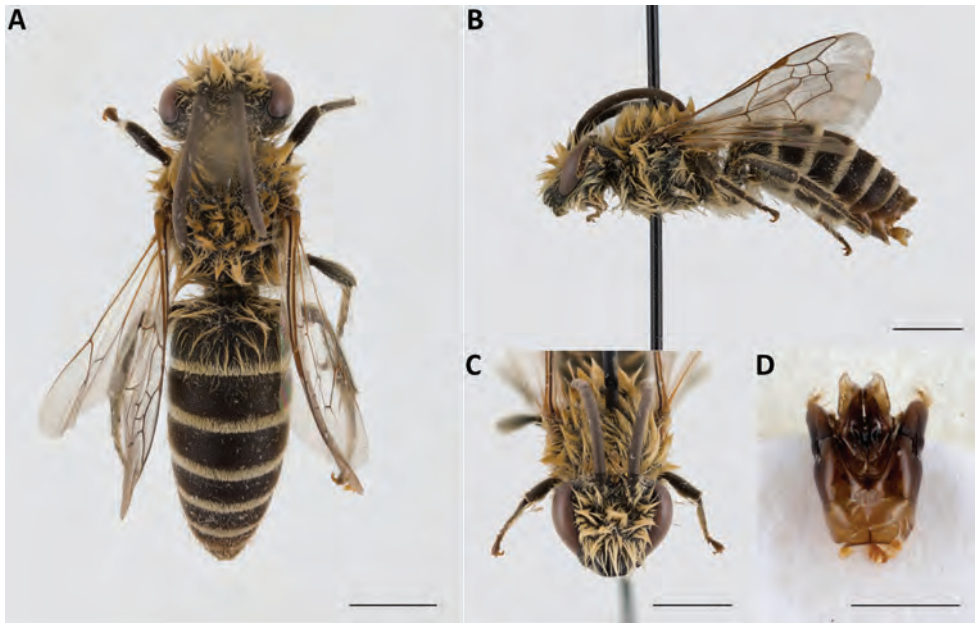


Figure 4. *Colletes brevigena* Noskiewicz, 1936 male collected on the Culuccia Peninsula (Sardinia, Italy) **A** dorsal view **B** lateral view **C** head **D** genital. Scale bars: 2 mm (**A–C**); 1 mm (**D**).

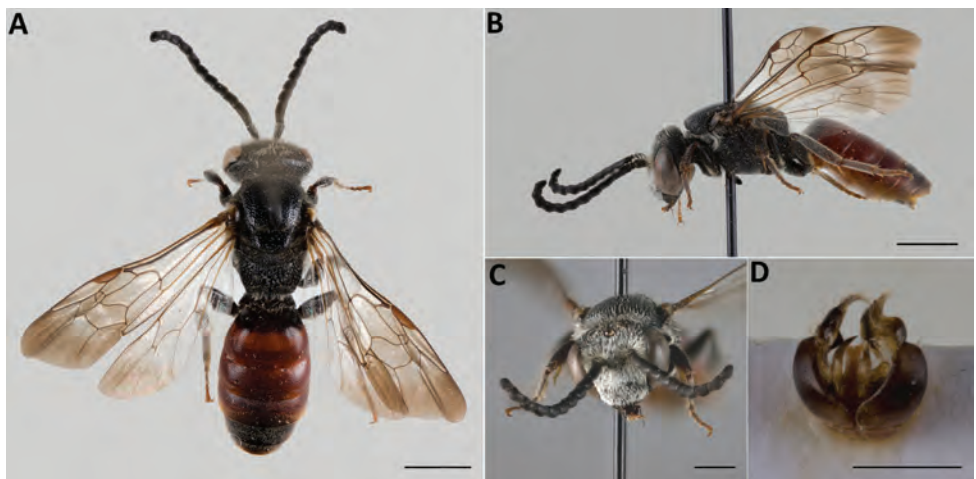


Figure 5. *Sphecodes crassanus* Warncke, 1992 male collected on the Culuccia Peninsula (Sardinia, Italy) **A** dorsal view **B** lateral view **C** head **D** genital. Scale bars: 2 mm (**A, B**); 1 mm (**C, D**).

species, except for *Bombus terrestris sassaricus* and *Bombus xanthopus*, which were grouped into a single clade (Suppl. material 2: fig. S1a). In addition, the NJ phylogenetic trees, described below, were built for the following genera and subgenera: *Anthophora*, *Andrena* (*Chlorandrena*, *Micrandrena*, *Taeniandrena*), *Bombus* (*Bombus*), *Colletes*, *Hoplitis* and *Lasioglossum* (*Hemihalictus*, *Lasioglossum*).

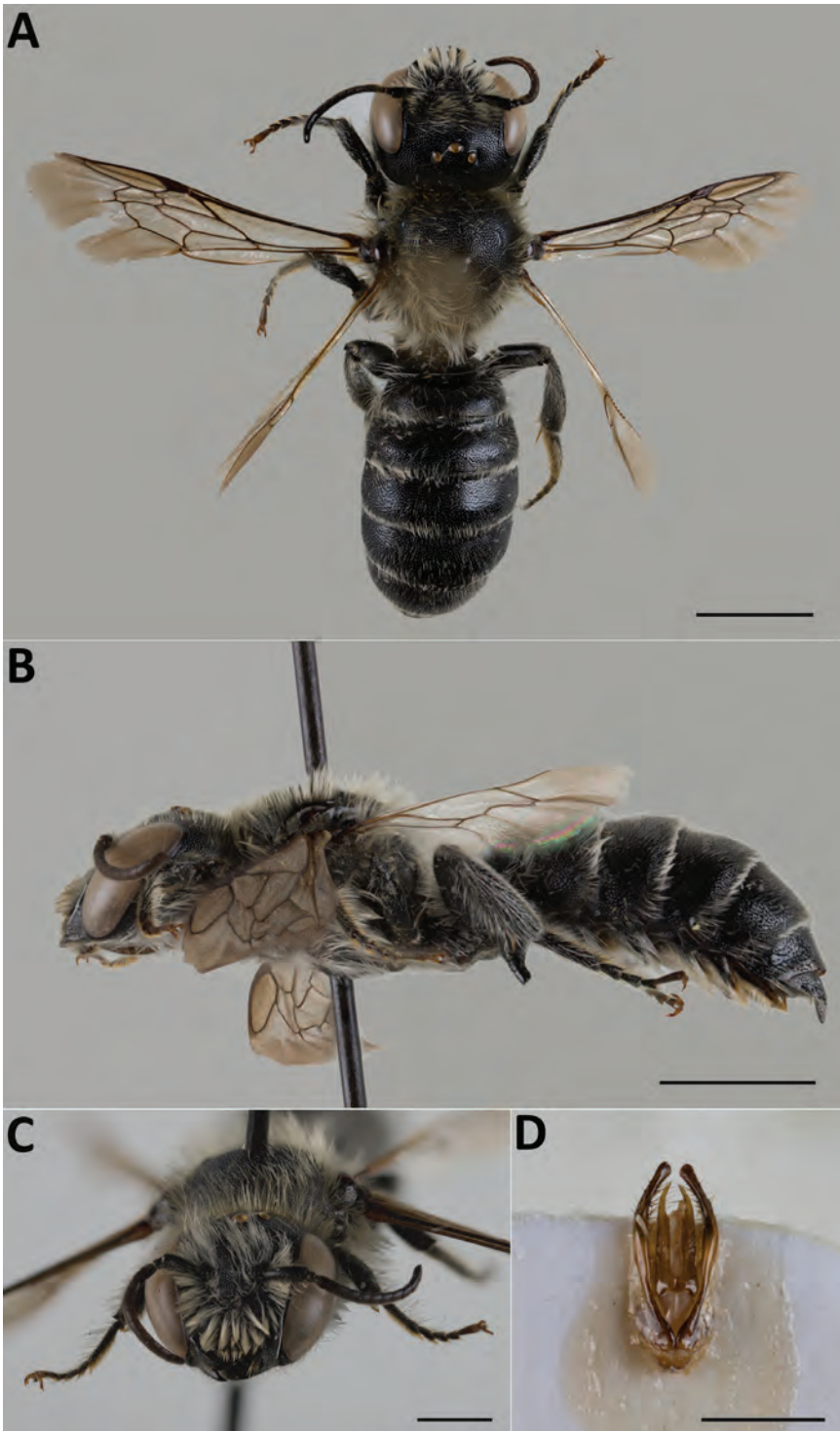


Figure 6. *Hoplitis praestans* (Morawitz, 1893) male collected on the Culuccia Peninsula (Sardinia, Italy) **A** dorsal view **B** lateral view **C** head **D** genital. Scale bars: 2 mm (**A**, **B**); 1 mm (**C**, **D**).

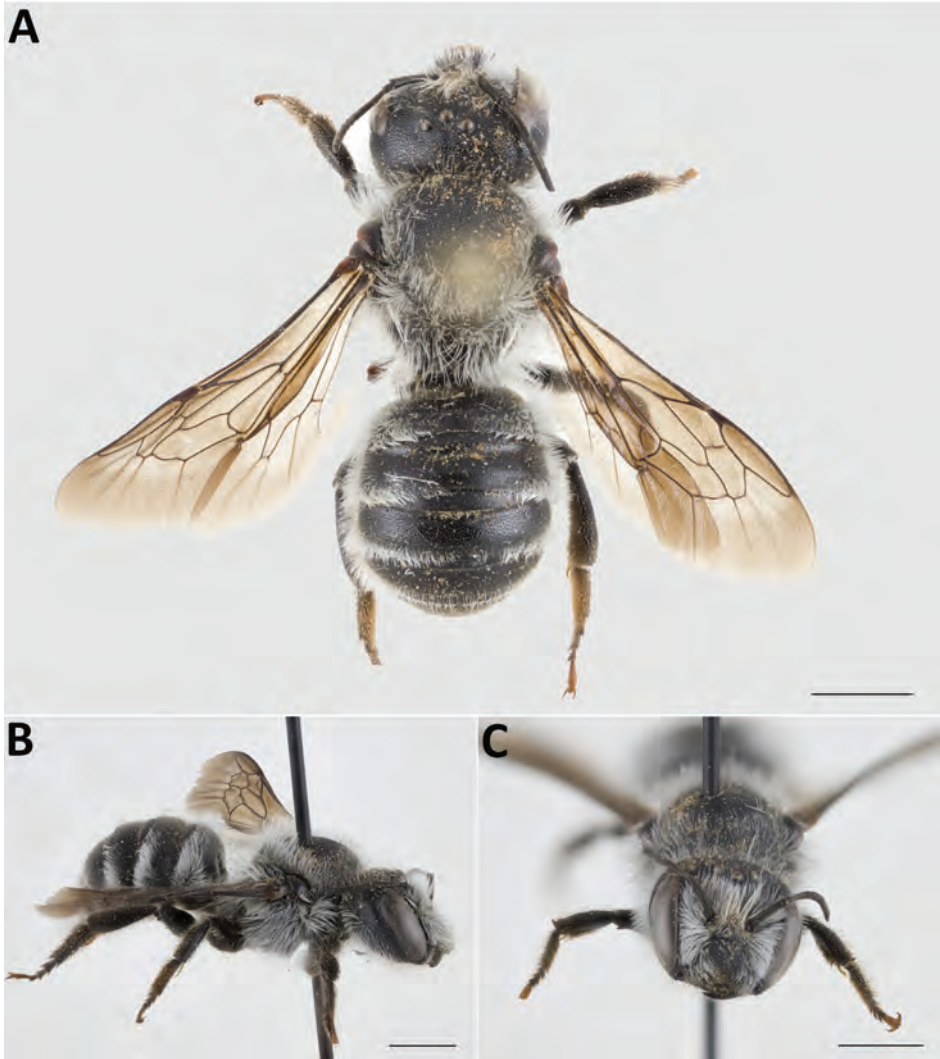


Figure 7. *Hoplitis tridentata* (Dufour & Perris, 1840) female collected on the Culuccia Peninsula (Sardinia, Italy) **A** dorsal view **B** lateral view **C** head. Scale bars: 2 mm.

Genus *Anthophora*

Anthophora sp. 1 (MB1_APCUL) clustered with *Anthophora atriceps* Pérez, 1879 from Spain (but bootstrap < 70; Suppl. material 2: fig. S1b, Fig. 8A), sharing 95.46% similarity (Suppl. material 5: table S3).

Genus *Andrena* (*Chlorandrena*, *Micrandrena*, *Taeniandrena*)

The two sequences of *A.* cfr. *clypella* diverged into two distinct clades without clustering with other species deposited in BOLD (but bootstrap < 70; Suppl. material 2:

fig. S1d, Fig. 9A). *A. cfr. niveata* (GIU9_APCUL) shared 97.81% similarity with its conspecific (Suppl. material 5: table S3), with which it formed a monophyletic clade, but with low support (bootstrap < 70; Suppl. material 2: fig. S1e, Fig. 9B). Samples attributed to *Andrena afzeliella* were included in the clade formed by conspecifics and specimens of *Andrena ovatula* (bootstrap > 70; Suppl. material 2: fig. S1f, Fig. 9C), and shared a 99.81–100% similarity with the latter (Suppl. material 5: table S3).

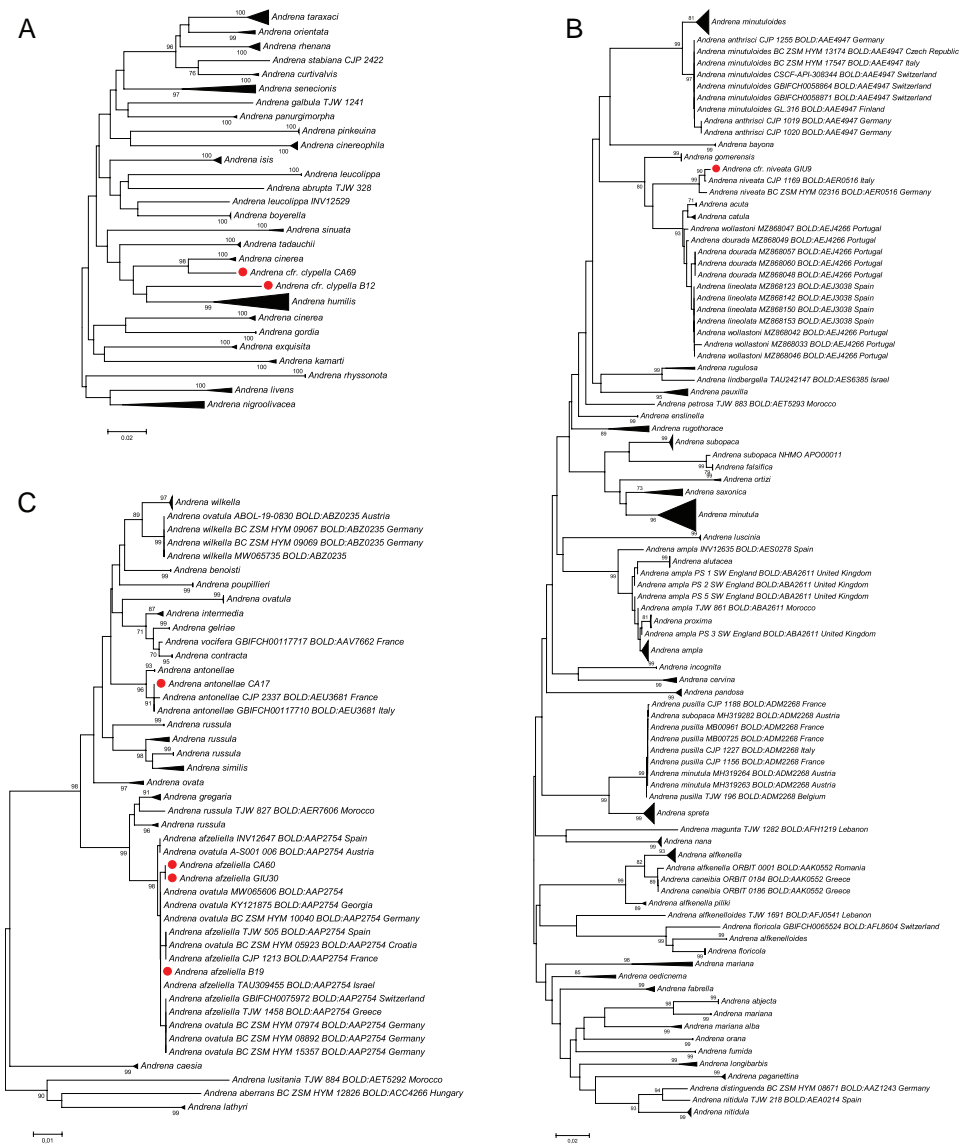


Figure 9. Neighbor Joining Trees based on the COI of three subgenera of *Andrena* **A** *Chlorandrena* **B** *Mirandrena* **C** *Taeniandrena*. Taxa marked with a red dot correspond to specimens newly sequenced in this work, while all the others were downloaded from BOLD. Only bootstrap values above 70 are shown at nodes. Collapsed clades correspond to monophyletic taxa (for a non collapsed version of the tree see Suppl. material 2).

Genus *Bombus*

Our sequences of *Bombus terrestris sassaricus* and *B. xanthopus* formed a monophyletic clade with *B. terrestris xanthopus* deposited in BOLD (bootstrap > 70; Suppl. material 2: fig. S1c, Fig. 10). However, this resulting group diverged from the only sequence of *B. terrestris sassaricus* available in the database (but bootstrap < 70; Suppl. material 2: fig. S1c, Fig. 10). Both our samples of *B. terrestris sassaricus* and *B. xanthopus* have a high similarity with *B. terrestris xanthopus* (99.83–100%; Suppl. material 5: table S3).

Genus *Colletes*

The sample attributed to *Colletes maidli* (B38_APCUL) presented 96.95% similarity with specimens of its conspecific from BOLD (Suppl. material 5: table S3). No public sequences are available to confirm such attribution, nonetheless, in the NJ phylogenetic tree our sample was isolated from all the other *Colletes* species tested (Suppl. material 2: fig. S1g, Fig. 8B).

Genus *Hoplitis*

In the NJ tree, our sequences of *Hoplitis tridentata*, *H. bihamata* and *H. praestans* diverged from conspecifics (bootstrap > 70; Suppl. material 2: fig. S1h, Fig. 8C), in agreement with the low % of similarity observed in BOLD (96.1%, 94–94.5, 93.65, respectively; Suppl. material 5: table S3). The sequence of *Hoplitis legoffi* formed a lineage close to *Hoplitis adunca* (Panzer, 1798), which was its best match on BOLD but with a low % similarity (93.78; Suppl. materials 2, 5: fig. S1h, table S3, Fig. 8C).

Genus *Lasioglossum* (*Hemihalictus*, *Lasioglossum*)

Our sequences attributed to *L. leucozonium* showed 95.62% of similarity with *L. leucozonium* specimens available in BOLD (Suppl. material 5: table S3). In the NJ phylogenetic tree, this species was paraphyletic, forming four clades, one of which was occupied by our sequences (Suppl. material 2: fig. S1i, Fig. 11A). *Lasioglossum prasinum* (GIU11_APCUL) was related to a specimen identified as a conspecific (bootstrap > 70, Suppl. material 2: fig. S1i, Fig. 11A) but their % of similarity was low (93.50, Suppl. material 5: table S3). Our specimens identified as *Lasioglossum transitorium planulum* clustered with members of this species (bootstrap > 70, Suppl. material 2: fig. S1i, Fig. 11B) with which they shared 99.66% similarity (Suppl. material 5: table S3).

Flower visitation network

The interactions between 63 wild bee species and 20 plant families are shown in the flower visitation network (Fig. 12). The plant family visited by the highest number of bee species was Asteraceae (46%), followed by Plumbaginaceae (23.8%), Cistaceae

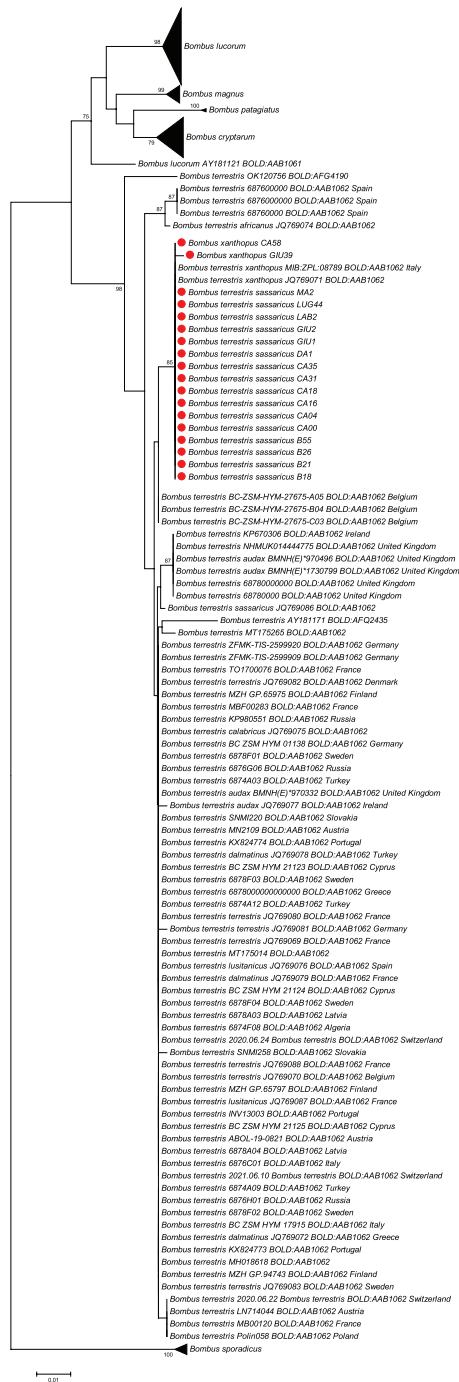


Figure 10. Neighbor Joining Tree based on the COI of *Bombus*. Taxa marked with a red dot correspond to specimens newly sequenced in this work, while all the others were downloaded from BOLD. Only bootstrap values above 70 are shown at nodes. Collapsed clades correspond to monophyletic taxa (for a non collapsed version of the tree see Suppl. material 2).

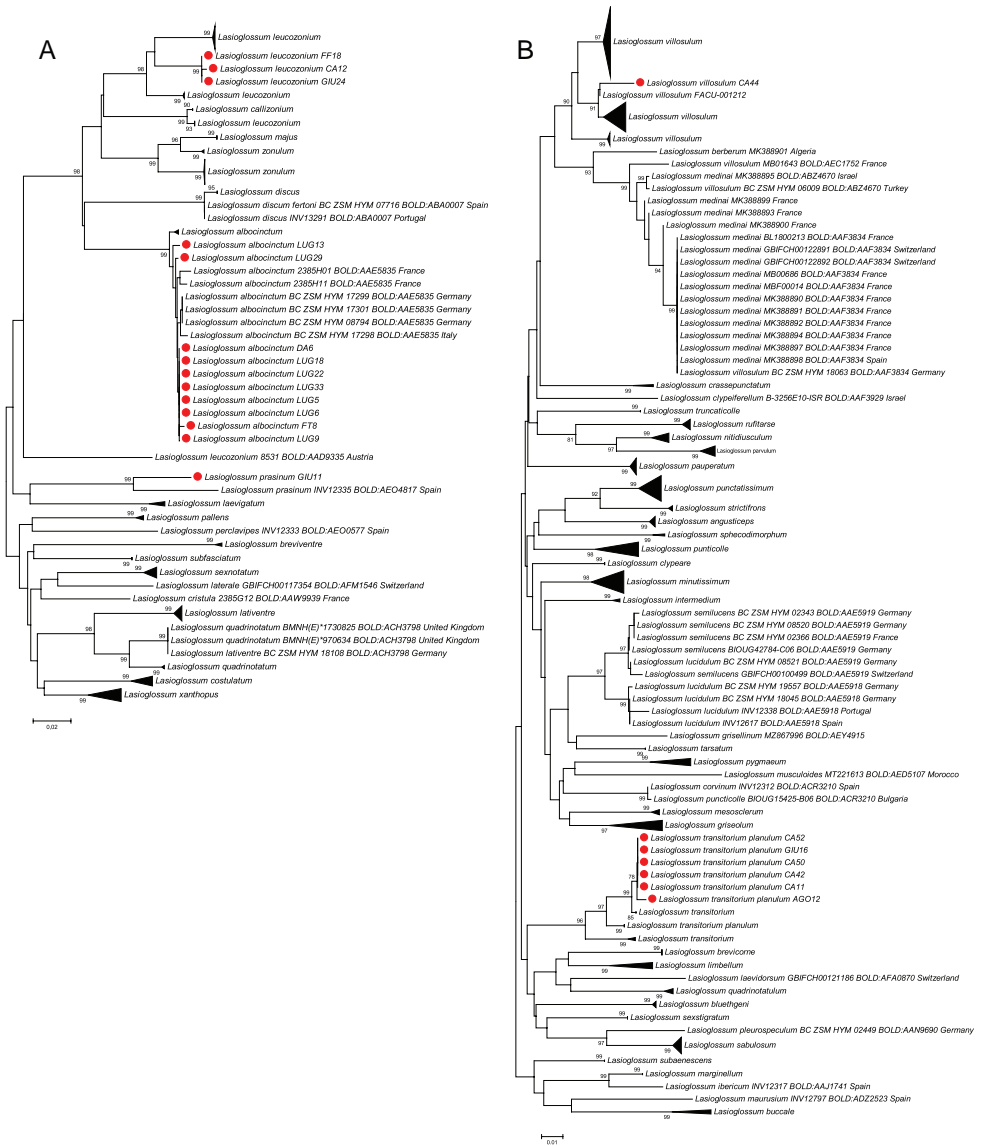


Figure 11. Neighbor Joining Trees based on the COI of two subgenera of *Laiosiglossum* **A** *Laiosiglossum* **B** *Hemihalictus*. Taxa marked with a red dot correspond to specimens newly sequenced in this work, while all the others were downloaded from BOLD. Only bootstrap values above 70 are shown at nodes. Collapsed clades correspond to monophyletic taxa (for a non collapsed version of the tree see Suppl. material 2).

(20.6%), Fabaceae (19%), Asphodelaceae (17.4%), Brassicaceae (15.9%) and Lamiaceae (15.9%) (Fig. 12). In contrast, Apiaceae, Primulaceae and Scrophulariaceae were visited by only one bee species each (Fig. 12). Suppl. material 7: table S5 lists the plant taxa visited by wild bee species.

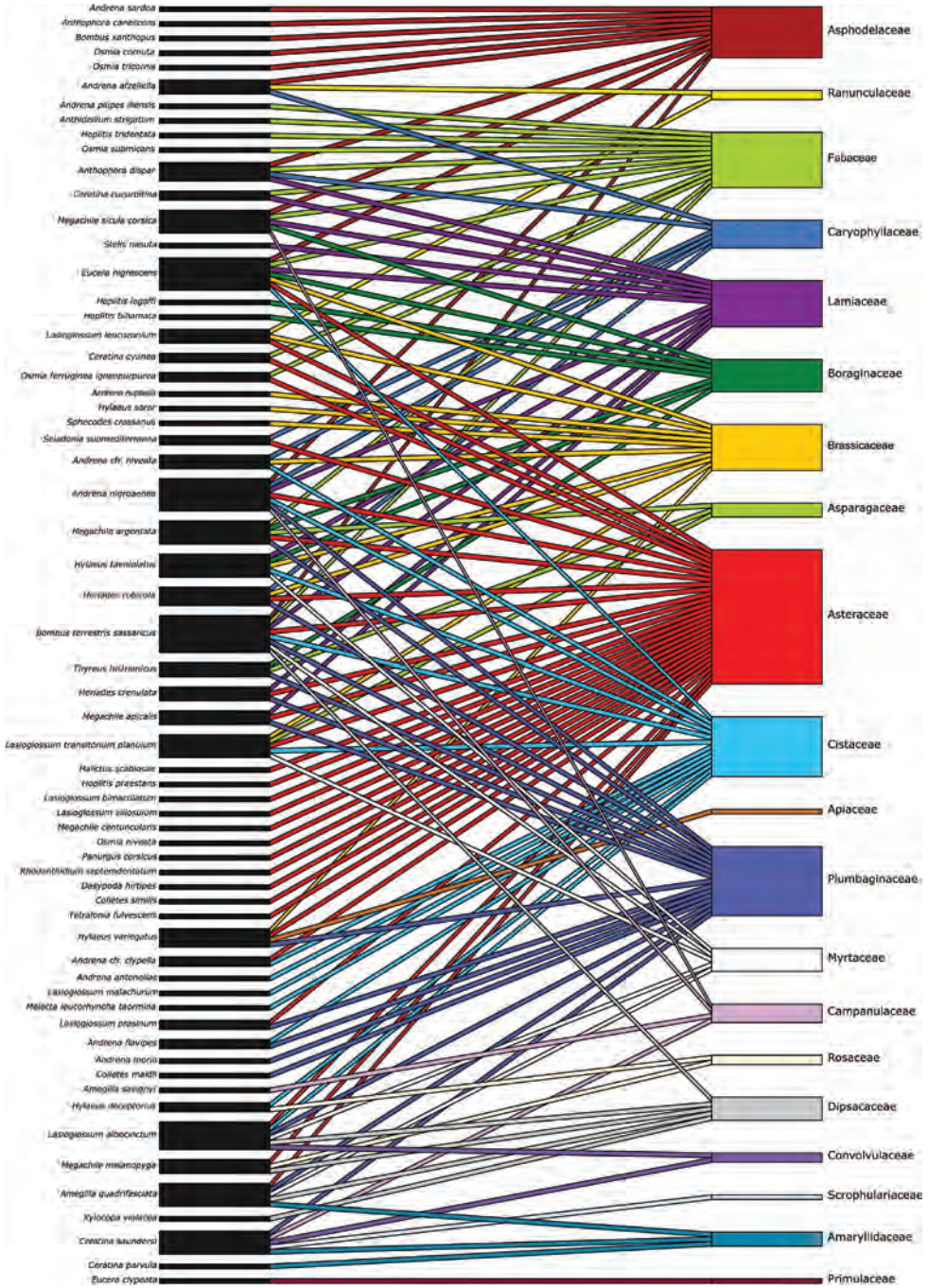


Figure 12. Flower visitation network between wild bee species and plant family on the Culuccia Peninsula (Sardinia).

Discussion

Faunistic studies are fundamental for understanding species distributions and developing effective conservation strategies. This is particularly important in diversity-hotspot regions with scarce faunal data, such as Sardinia, where information on bee diversity is still limited. Despite the small size of our study area, we recorded six new species for Sardinia, that were previously unreported in the checklist of Italian wild bees (Comba 2019) and in recent faunistic and ecological studies carried out on the island (Cattania et al. 2021; Nobile et al. 2021; Lezzeri et al. 2024). Our results emphasize how the knowledge of the occurrence of wild bees is still insufficient and underscore the importance of further research such as in Sardinia. Some of the new records could be explained by the geographical proximity of our study area to Corsica, where the presence of species such as *Hoplitis tridentata* and *H. praestans* was already known (Le Divelec et al. 2024) and where *Bombus xanthopus* is present and considered as endemic to this island, Capraia and Elba (Rasmont et al. 2021). However, further studies may reveal whether these species are restricted to north-eastern Sardinia or are widespread throughout the region.

The bee community sampled in the Culuccia Peninsula included both widely distributed species and taxa with restricted distribution or endemic. Some of these were assessed as being at moderate risk of extinction according to Comba (2019), such as *Colletes brevigena*, *Andrena sardoa*, *Andrena griseobaltata*, *Sphecodes crassanus*, *Panurgus corsicus*, *Hylaeus deceptorius*, *Hoplitis bihamata* and *H. fasciculata*. In addition, *LasioGLOSSUM prasinum* is classified as Near Threatened according to the European and Italian Red List of bees (Nieto et al. 2014; Quaranta et al. 2018).

We recorded several previously unreported bee floral associations, such as *Andrena antonellae* on Cistaceae, *Andrena morio* on Plumbaginaceae, *Hoplitis praestans* on Asteraceae and *Andrena afzeliella* on Asphodelaceae and Caryophyllaceae (Amiet et al. 2004; Cortis et al. 2009; Praz et al. 2022). Moreover, we provide the first observation of floral visits of the Sardo-Corsican endemic *Panurgus corsicus*, which was found only on Asteraceae species in this study, consistent with the close association with this family already known for other species of this genus (Cross 2020; Wood et al. 2022). The integrative taxonomic approach allowed the species identification of all the samples, except for two *Andrena* species and one *Anthophora* species. The latter could not be identified morphologically due to the degraded status of the specimen and its sequence did not show any similarity to any species available on BOLD that would allow a reliable molecular identification. Two *Andrena* species have not been definitively named due to uncertainties in both their morphological and molecular identification. The genetic divergence between the two sequences of *A. cfr. clypella* suggests that the samples may represent two different species, despite their strong morphological similarity. Indeed, *Chlorandrena* species may present aberrant strong divergent sequences within morphologically well-delineated groups (Wood 2023). However, it was not possible to

determine the relationship of our specimens with *A. chypella*, as no public sequences for this species were available in BOLD at the time of the NJ phylogenetic tree analysis (Suppl. material 2: fig. S1d, Fig. 9A). Conversely, the clustering of our *A. cfr. niveata* sequence with other *A. niveata* specimens lacks adequate support (Suppl. material 2: fig. S1e, Fig. 9B) and, due to uncertain morphological identification, we have refrained from assigning a definitive name.

In some cases, our samples presented low molecular similarity to their conspecifics (e.g. *Lasioglossum leucozonium*, *L. prasinum* and *Hoplitis* spp.) but firm identification was provided with the support of specialists after further morphological examination. This low similarity may be attributed to several factors, including insufficient knowledge of genetic variation among populations (i.e., intra-specific diversity), errors in DNA data repositories, and variation in the barcoding gap across all bees (Janko et al. 2024). Additionally, NJ analysis revealed distinct BINs within *Lasioglossum leucozonium* and *L. villosulum*, revealing possible cryptic species diversity as previously noted by Villalta et al. (2021) and Janko et al. (2024). A recent integrative taxonomic study employing different molecular analyses identified multiple candidate species within the *L. villosulum* complex, with morphological analysis resurrecting *L. medinae* (Vachal, 1895) and *L. berberum* (Benoist, 1941), while the remaining lineages were identified as *L. villosulum* (Pauly et al. 2019). Our sequences of *L. leucozonium* and *L. villosulum* formed distinct clusters situated between various lineages within these species complexes (Suppl. material 2: fig. S1i, l, Fig. 11A, B), suggesting that the populations in Sardinia may represent a genetically unique entity. The species *Hoplitis legoffi* was only very recently described by Le Divelec (2024), and its barcode sequence has not yet been deposited in public databases. For this reason, our sample, which was morphologically identified by the taxonomist R. Le Divelec, only matched with *H. adunca*, though with low similarity, and formed a distinct lineage in the NJ tree (Fig. 8C, Suppl. material 2: S1h).

Another point worth noting is our samples of *Bombus terrestris sassaricus* and *B. xanthopus*. These specimens were identified morphologically as two species following Rasmont et al. (2008, 2021) but both showed a high molecular affinity with sequences available in BOLD attributed to *B. terrestris xanthopus*. *Bombus terrestris sassaricus* is a subspecies endemic to Sardinia, whereas *B. xanthopus* was previously considered to be a subspecies of *B. terrestris*, restricted to Corsica, Capraia and Elba (Estoup 1996; Rasmont et al. 2021). In 2015, the latter subspecies was elevated to species status based on molecular and ecochemical characteristics (Lecocq et al. 2015) and has been considered as such in the recent literature (Rasmont et al. 2021; Ghisbain et al. 2023). However, other studies have provided new evidence that questions the specific status of this taxon. These include molecular analyses (Williams 2021) and the observation of potential hybridization (Boni et al. 2023). In this context, we have relied on morphological classification to identify our samples, pending future studies aimed at clarifying the taxonomy of this diverse and complex genus.

A similar issue was raised from the NJ phylogenetic tree of the subgenus *Taenian-drena*, in which sequences from BOLD of *A. afzeliella* and *A. ovatula*, along with our

A. afzeliella specimens, formed a well-supported monophyletic clade (Suppl. material 2: fig. S1f, Fig. 9C). However, sequences labelled as *A. ovatula* (e.g. Schmidt et al. 2015) were determined prior to the review by Praz et al. (2022), which clarified the distinction between these two taxa based on morphological and molecular differences. This explains the confounding results observed in our study, as these previous misidentifications have not been updated.

Conclusion

This work represents the first study on wild bee species richness on the Culuccia Peninsula and contributes to enrich the currently scarce knowledge on these insects in Sardinia. Furthermore, we added COI sequences to public databases (BOLD and GenBank), contributing to increase the knowledge of the genetic variability of bee species in general and, in particular, for species previously lacking sequences from Sardinia, and often from Italy as well. The integrative taxonomic approach allowed the identification of each specimen more reliably than morphological or molecular identification alone and highlighted potential taxonomic issues. For this reason, further molecular and morphological studies are needed, in particular to achieve accurate species delimitation and contribute to a lower level of understanding of phylogenetic relationships within this diverse group of insects. Finally, this study provides previously undocumented floral associations, highlighting the need for further research into the flower visitation patterns of wild bee species in other areas of the Mediterranean region.

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Supplementary material 1

Supplementary document 1

Authors: Matteo Annessi, Alessandra Ricciari, Marilena Marconi, Sabrina Rossi, Andrea Di Giulio

Data type: pdf

Explanation note: Citation of bee material collected on the Culuccia peninsula (Sardinia), including details such as collection sites, dates, Corine land cover classes and associated plant taxa.

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Link: <https://doi.org/10.3897/jhr.98.138933.suppl1>

Supplementary material 2

Neighbor-joining tree based on the COI

Authors: Matteo Annessi, Alessandra Ricciari, Marilena Marconi, Sabrina Rossi, Andrea Di Giulio

Data type: pdf

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Supplementary material 3

Main dichotomous keys and references used for the morphological identification of the bee sample, divided into genera

Authors: Matteo Annessi, Alessandra Ricciari, Marilena Marconi, Sabrina Rossi, Andrea Di Giulio

Data type: xlsx

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Supplementary material 4

List of specimens used for the construction of neighbor-joining (NJ) phylogenetic trees

Authors: Matteo Annessi, Alessandra Ricciari, Marilena Marconi, Sabrina Rossi, Andrea Di Giulio

Data type: xlsx

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Supplementary material 5

List of wild bee species sampled on the Culuccia Peninsula, identified using an integrated taxonomy approach

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Data type: xlsx

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Link: <https://doi.org/10.3897/jhr.98.138933.suppl5>

Supplementary material 6

List of sequenced specimens with reported BIN, Sample ID, Process ID, Institution Storing and Genbank accession

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Data type: xlsx

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Link: <https://doi.org/10.3897/jhr.98.138933.suppl6>

Supplementary material 7

Plant taxa visited by wild bee species on the Culuccia Peninsula

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Data type: xlsx

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