The species of *Eucera* Scopoli, subgenus *Tetralonia* Spinola from Sardinia (Italy) with new records and *E. gennargentui* sp. nov. (Hymenoptera, Apidae)

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Abstract

In this paper, an update of the species of the genus *Eucera* Scopoli, 1770, subgenus *Tetralonia* Spinola, 1838 from Sardinia is reported, based on data collection as well as on recent survey carried out at Gennargentu Massif. Seven species are recorded, four of which are newly added: *Eucera fulvescens* (Giraud, 1863), *E. gennargentui* sp. nov. Nobile, Catania & Bella, *E. julliani* (Pérez, 1879), and *E. nana* (Morawitz, 1873). The new species, *Eucera* (*Tetralonia*) *gennargentui* Nobile, Catania & Bella is described from the high altitude of Gennargentu Massif. Details on distributions, host plants, and other biological aspects are given for each species treated. The taxonomic comparison of the taxa belonging to the subgenus *Tetralonia* from Sardinia, including *E. gennargentui* sp. nov., are discussed taking into account both morphological and COI barcode sequences. An identification key to *Eucera* (*Tetralonia*) species from Sardinia is also provided.

Keywords

Apidae, *Eucera* (*Tetralonia*), new taxon, Mediterranean bees, Sardinia
**Introduction**

Belonging to the Eucerini tribe, *Eucera* Scopoli, 1770 is a genus of bees widespread in the world (Michener 2007), with a relevant number of species in the Palaearctic region and especially in the Mediterranean Basin (Tkalcu 1979, 1984; Risch 1997, 1999, 2001, 2003; Michener 2007; Dorchin et al. 2018). In their recent taxonomic studies Dorchin et al. (2018) treated the genus *Tetraloniella* Ashmead, 1899 within *Tetralonia* Spinola, 1838, and this last as a subgenus of *Eucera*.

The subgenus *Tetralonia* includes different oligolectic species, some of them closely associated and specialised with plants of the Asteraceae family (Müller 2008). The species of this subgenus have been revised for the Afrotropical region by Eardley (1989), for the New World by LaBerge (2001), and by Tkalcu (1979, 1984) for the Palaearctic region.

*Eucera* (*Tetralonia*) includes eleven species in Italy and some endemic subspecies of the main Italian islands, such as *E. dentata* amseli (Alfken, 1938) described from Sardinia and *E. alticincta* bindai (Nobile, 1993) described from Sicily (Alfken 1938; Nobile 1993).

To date, the knowledge on Sardinian bees is still limited, and only three species of *Eucera* (*Tetralonia*) are known: *E. dentata* amseli, *E. graja* (Eversmann, 1852) and *E. malvae* (Rossi, 1790) (Comba 2019; Nobile et al. 2021). This last species was recently found 78 years after its last report (Nobile et al. 2021).

In this paper, we add a further four species of *Eucera* (*Tetralonia*) as new for Sardinia and describe a new endemic species *E. gennargentui* sp. nov. Nobile, Catania & Bella from Gennargentu Massif, based on both morphological and molecular data.

**Materials and methods**

The material examined belongs partly to the collection of Vittorio Nobile and partly comes from field collecting efforts carried out by the two other authors in Sardinia during the year 2020.

Species identification was based on the works of Tkalcu (1979, 1984) and Scheuchl (2000). Morphological terminology and taxonomic classification follow Michener (2007) and Dorchin et al. (2018). Reference was made to the online ‘Check-list of Western Palaearctic Bees’ by Kuhlmann et al. (2018) and of ‘Hymenoptera: Apoidea: Anthophila of Italy’ by Comba (2019).

Observations in the field on *Eucera gennargentui* sp. nov. were made in the first week of August 2020 in the locality of Bruncu Spina, near the town of Fonni (40°01′48.0″N, 9°17′56.6″E), in the central area of Gennargentu Massif, at 1540 m a.s.l., characterized by several endemic high mountain species plants (Bacchetta et al. 2013) (Fig. 1); observations were carried out from 09:30 to 16:30, on the flowers of the Asteraceae family.

Gennargentu Massif is located near the centre of the island, almost directly at 40° N. Bruncu Spina, at 1829 m a.s.l., is the second highest mountain in Sardinia,
Description of *Eucera gennargentui* from Sardinia

after Punta La Marmora (1834 m a.s.l.), and is the northernmost summit of the main Gennargentu ridge.

In order to collect biological data on the new taxon, some specimens were marked using a dye specific to bees. The date of capture, general and Italian distribution, number of specimens observed, and the plants visited are reported for all species.

Each specimen was killed by freezing at -20 °C for use in DNA analysis and was dried and identified to species level after the observation of genital structures. The specimens were preserved in the collection of the authors, and in the collection of the Department of Agriculture, Food and Environment, sect. Applied Entomology, University of Catania (Di3A).

The specimens were studied with a stereomicroscope Leica EZ4HD, the photos taken with a Leica Application Suite vs. 4.13.

Total DNA was extracted from one posterior leg using the NucleoSpin Tissue Kit (Macherey-Nagel, Germany) following the manufacturer’s instructions. Subsequently, the enzymatic amplification took place using primers LCO1490F and HC02198R (Folmer et al. 1994). PCR products were purified using ExoSAP-IT (ThermoFisher Scientific) and were sequenced at BMR genomics (Padua, Italy), and the sequences obtained were analysed using MEGA X 10.2.4 (Kumar et al. 2018).

The sequences were aligned with seven COI sequences of *Eucera* (*Tetralonia*) available in GenBank, and we also included two sequences of *Habropoda tarsata* (Spinola, 1838) (accession numbers MN919536, MN919537) as an outgroup (Table 1). Sequence divergences were calculated using the *p*-distance model (Srivathsan and Meier 2012), and a neighbour-joining (NJ) tree (Saitou and Nei 1987), as implemented in MEGA X, was used to visualize the distance matrix among taxa.

**Figure 1.** Sardinia and Gennargentu Massif, locality of Bruncu Spina, 1540 m a.s.l., the site of the samplings of *Eucera gennargentui* sp. nov.
Table 1. Analyzed sequences with accession number and origin countries.

| Species                  | Accession number | Country       |
|--------------------------|------------------|---------------|
| Eucera alticincta alticincta | MNHN1.005-20     | Luxembourg    |
| Eucera alticincta bindai  | MZ437367         | Italy, Sicily |
| Eucera dentata           | FBAPD711-11      | Germany       |
| Eucera fulvescens        | ABEE208-17       | Austria       |
| Eucera gen. nov.         | MZ437082         | Italy, Sardinia |
| Eucera graja             | MG251108         | -             |
| Eucera malvae            | FBAPC604-11      | Germany       |
| Eucera nana              | ABEE206-17       | Austria       |
| Eucera salicariae        | FBAPC749-11      | Croatia       |
| Habropoda tarsata        | MN919536         | Italy, Sicily |
| Habropoda tarsata        | MN919537         | Italy, Sicily |

Acronyms

Di3A  Department of Agriculture, Food and Environment, Catania, Italy;
RC    Roberto Catania, Catania, Italy;
SB    Salvatore Bella, Catania, Italy;
VN    Vittorio Nobile, Ragusa, Italy.

Results

Detected species

Genus Eucera Scopoli, 1770
Subgenus Tetralonia Spinola, 1838

Eucera dentata amseli (Alfken, 1938)
Fig. 2

Tetraloniella dentata amseli Alfken, 1938, Mem. Soc. Entomol. It., 16: 97–114.

Data from literature. Alfken 1938.

Material examined. • 1 ♀; Italy Sardinia, Iglesias, San Pietro (Cagliari province); 12.VII.1976.

Distribution. Sardinia and Corsica.

Range in Italy. Sardinia.

Eucera fulvescens (Giraud, 1863)

Tetralonia fulvescens Giraud, 1863, Verh. zool.-bot. Ges. Wien, 13: 42–43.

Material examined. • 1 ♀; Italy Sardinia, Busachi (Oristano province); 10.VI.1975; coll. C. Meloni – D. Sechi.
Description of *Eucera gennargentui* from Sardinia

**Distribution.** South Europe, North Africa, West and Central Asia (Tkalcu 1979).

**Range in Italy.** This species is discontinuously present in the Italian peninsula and Sicily.

New record for Sardinia

*Eucera gennargentui* Nobile, Catania & Bella sp. nov.

[http://zoobank.org/B12C779B-9542-4C49-ADE2-3E1F07D99A43](http://zoobank.org/B12C779B-9542-4C49-ADE2-3E1F07D99A43)

**Material examined.** 10 ♀, 1 ♂: **Holotype:** 1 ♀; Italy, Sardinia, Fonni (Nuoro province), Gennargentu Massif, Bruncu Spina, 1540 m a.s.l.; 6–8.VIII.2020; S. Bella and R. Catania leg.; Di3A. **Paratypes:** 9 ♀, 1 ♂; paratypes identical data to previous; Di3A, RC, VN, SB.

**Description.** **Female** (Fig. 3). Body length: 10 mm. Body with black integument with fine and evident punctuation, grey setae. Head. Head with wrinkled punctuation, finer at the edges and with white setae; clypeus black, with the lower half light yellow; this spot, extending upwards, and in the middle part, ends with a triangulated tip; labrum and mandibles black, antennae dorsally black, ventrally orange, with scape and
first two flagellar articles black. Mesosoma. Mesothorax with greyish setae, median part of the metathorax with dense and large punctation. Black legs with greyish setae and the last four tarsi brown; the hind legs are equipped with a uniformly yellow-rust-coloured pollen-collection system. Slightly darkened wings, with black veins and dark brown tegulae. Metasoma. Dark brown, almost black, with dense and fine punctuation. T1 with long and sparse white setae, T2–4 with basal bands of white felt, T5 with white felt on the sides and dark brown in the middle, and T6 almost completely covered with dark-brown felt. Dark-brown sternites with thick punctuation and long golden-yellow fringes.
Male (Figs 4–5). Body length: 9 mm. General appearance similar to the female, with yellow clypeus and labrum, antennae are more than half of the body length long and reddish, except for a black streak on the whole dorsal part; black scape; mandibles are black, but reddish in the distal half. Mesosoma. With long silky whitish setae. Black legs with whitish setae. The hind femur, in the lower median part, has a small pointed tubercle equipped with a tuft of brownish bristles. Metasoma. As in the female, is dark brown, but all segments are covered with white felt, and T6 on the sides has 2 prominent teeth, one on each side. The last sternites are provided with a long and deep longitudinal median sulcus. Genitalia and hidden sterna. S6 without anterolateral marginal projections; posterolateral carina curved laterally at each side. S7 with medial process rounded and attached at the posterior lobes of lateral processes; the posterior lobes of lateral processes are relatively short with straight superior margins; anterior lobes of lateral processes wide with straight superior margins and rounded lateral-inferior margins. S8 with a wide concavity between rounded apical lobes; apicoventral ventral process well defined and saddle shape, with a large depressed area at the base. Genital capsule with the apex of the gonostylus remarkably arched, and inward-facing triangular protuberance placed slightly over the central part of the gonostylus.
Difference with the closest related taxa. The new species *Eucera gennargentui*, is similar to *E. alticincta* (Lepeletier, 1841), including the nominate subspecies and *E. alticincta* *bindai*.

In the female of *E. gennargentui*, the long fringes of the sternites are yellow-gold, whereas in the closest related species *E. alticincta* *alticincta*, they are white; the scopa is yellow-rust in the new species, whitish externally and brownish internally in *E. alticincta* *alticincta*.

In the male of the new species, the long silky hairs of the mesosoma are whitish, while they are brownish-yellow in *E. alticincta* *alticincta*. Genitalia of *E. gennargentui* is very similar to that of *E. alticincta* *alticincta*, however, in the gonostylus the inward-facing triangular protuberance, that in *E. alticincta* is located in the middle part, in the new species is placed slightly over half of the gonostylus, slightly closer to the apex. Further differences can be noted in the morphology of the hidden sterna, especially for the medial and lateral processes in S7, and for apical lobes and apicomedian ventral process in S8.

In both sexes of the new species, the integument and wings are considerably darker than *E. alticincta* *alticincta*, and the body length of both sexes of *E. gennargentui* is greater than that of the related species.

Further morphological and bio-ecological comparisons between *E. alticincta alticincta*, *E. alticincta* *bindai*, and *E. gennargentui* are reported in Tables 2 and 3.

**Derivatio nominis.** We named the new species after Gennargentu Massif, where the specimens were found.
Description of *Eucera gennargentui* from Sardinia

**Distribution.** Sardinia (Italy).

**Flower choices.** Specimens of the new taxon were observed on *Carlina macrocephala* Moris subsp. *macrocephala*, *Tanacetum audibertii* (Req.) DC. (Sardo-Corsican endemisms), and *Helichrysum saxatile* Moris subsp. *saxatile* (Sardinian endemism) (Asteraceae) (Fig. 6).

**Bio-ecological aspects.** A total of 22 specimens (half captured and half marked) were observed in the sampling site: 20 females and 2 males. Specimens of *E. gennargentui* were observed in activity in a restricted area of about 300 m$^2$, from 10:30 to 15:00; in particular, the females visited the flowers from 10:30 to 14:30, and the two males were observed from 13:30 to 15:00 on *Carlina macrocephala* subsp. *macrocephala*. The female specimens visited the same flowers at intervals of about ten minutes, with a preference for the flowers of *Carlina macrocephala* subsp. *macrocephala*.

To know better the flights period of *E. gennargentui*, further surveys in the same site were conducted during mid-July 2021, however no specimens were found. This confirms the relationship between the new species and its main host plant, in fact *Carlina macrocephala* subsp. *macrocephala* was at the beginning of its flowering.

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### Table 3. Geographic and bio-ecological comparison between *Eucera gennargentui* sp. nov. and the closest related taxa.

|                      | *E. alticina alticina* | *E. alticina bindai* | *E. gennargentui* sp. nov. |
|----------------------|------------------------|----------------------|-----------------------------|
| **Distribution**     | Widespread in Europe, North Africa, and western Asia (Varnava et al. 2020) | Sicily (Nobile, 1987; 1993), Nebrodi Mts (Messina province), northern slope of Mt Etna (RC, pers. observ.), and Syracuse province | Central Sardinia, Gennargentu Massif |
| **Host plants**      | oligolectic on *Inula* and *Pulicaria* (Tkalcu, 1979; Müller, 2008) | observed on *Pulicaria* (RC, pers. observ.) | oligolectic on *Carlina*, *Helichrysum*, and *Tanacetum* |
| **Environments**     | Temperate and Mediterranean | Mediterranean and Mediterranean mountains (100 – 1350 m a.s.l.) | Mediterranean high mountains (1540 m a.s.l.) |
| **Phenology (generation and months)** | probably univoltine; VII-VIII | probably univoltine; VIII-IX | probably univoltine; VIII |

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**Figure 6.** Female of *Eucera gennargentui* sp. nov. Sardinia, Gennargentu Massif, locality of Bruncu Spina (Photos by P. Niolu - R. Catania).
**Eucera graja** (Eversmann, 1852)

*Macrocera graja* Eversmann, 1852, Bull. soc. natural. Moscou. 25: 124.

**Data from literature.** Grandi 1962 (Ozieri, Sassari province); Comba 2019.

**Distribution.** South and Central Europe, and West Asia (Banaszak & Ortiz-Sánchez 1993).

**Range in Italy.** This species is discontinuously present in the Italian peninsula, Sardinia and Sicily.

**Eucera julliani** (Pérez, 1879)

*Macrocera julliani* Pérez, 1879, Act. Soc. Linn., 33: 150.

**Material examined.** • 1 ♀; Italy Sardinia, Assemini (Cagliari province); 3.IX.1990; L. Fancello leg. • 1 ♂; Sardinia, Rio Flumini Mannu (Cagliari province), 2.VII.1992; C. Meloni leg.; on *Ammi visnaga* (L.) Lam. (Apiaceae).

**Distribution.** Turanic-South-European (Nobile 1987).

**Range in Italy.** This species is discontinuously present in the Italian peninsula, and Sicily.

New record for Sardinia

**Eucera malvae** (Rossi, 1790)

*Apis malvae* Rossi 1790, Fauna Etrusca II, p. 107.

**Data from literature.** Nobile et al. (2021).

**Material examined.** • 1 ♀, 1 ♂; Italy Sardinia, Capoterra, Rio S. Lucia (Cagliari province); 4.VI.1985; C. Meloni leg.

**Distribution.** Widespread in Europe, Eastern Mediterranean (Cyprus) and Western Asia (Turkey).

**Range in Italy.** This species is discontinuously present in the Italian peninsula, and Sicily.

**Eucera nana** (Morawitz, 1873)

*Tetralonia nana* Morawitz, 1873, Horae soc. entom. Ross. X, p. 144.

**Material examined.** • 1 ♀; Italy Sardinia, Teulada, Tuerru (South Sardinia province); 16.VII.1973; • 3 ♂♂; Sardinia, Rio Flumini Mannu (Cagliari province); 2.VII.1992; C. Meloni leg.; on *Ammi visnaga* (L.) Lam. (Apiaceae).
**Distribution.** Widespread in Europe, and Western Asia (Turkey).

**Range in Italy.** This species is known in the regions of Sardinia. New record for Sardinia.

**Identification key to *Eucera (Tetralonia)* from Sardinia**

**Female:**

1. Clypeus more or less yellow .......................................................... 2
   – Clypeus black ................................................................. 5
2. Antennae ventrally dark brown. 10,5–11,5 mm.................. *E. dentata amseli*
   – Antennae ventrally light red, except for the scape and 1–2 flagellar articles that are black .......................................................... 3
3. Scopa dark brown externally and even darker internally. 12–14 mm .............
   – Different colour of the scopa, smaller body size ...................... 4
4. Scopa whitish externally and brown internally. 8–9 mm ............ *E. julliani*
   – Scopa uniformly rusty-yellow. 9–10 mm...................... *E. gennargentui sp. nov.*
5. Orange scopa. 11–12 mm...................................................... *E. fulvescens*
   – White or whitish scopa .................................................. 6
6. Scopa with long, sparse, thin, and white setae. 8 mm .............. *E. nana*
   – Scopa with long, sparse and whitish setae. 11–13 mm ........ *E. malvae*

**Male:**

1. Antennae ventrally more or less dark brown................................. 2
   – Antennae ventrally light red except for the scape black.................. 5
2. Labrum and clypeus yellow.................................................. 3
   – Labrum and the lower part of the clypeus yellow. 11–13 mm ...... *E. malvae*
3. T6 laterally with short and stocky teeth, one on each side, facing backwards. 8 mm .......................................................... *E. nana*
   – More tergites with teeth, larger body size ................................ 4
4. In addition to clypeus and labrum, the base of the mandibles is yellow, T 5–6 with short and stocky teeth. 10 mm.................. *E. dentata amseli*
   – Mandibles black, T5–7 with slightly longer and sharper teeth. 11–12 mm ...
     ............................................................................. *E. fulvescens*
5. Clypeus, labrum, supraclypeal space and mandible yellow. 11–13 mm ......
     ............................................................................. *E. graja*
   – Mandibles and supraclypeal space black .................................. 6
6. In the genital capsule the apex of the gonostylus facing inwards are almost straight. 9–10 mm .................................................... *E. julliani*
   – In the genital capsule the apex of the gonostylus are remarkably arched. 9 mm .......................................................... *E. gennargentui sp. nov.*
Molecular evidences

High-quality DNA sequences were obtained for the two specimens analysed, one sequence of *E. gennargentui* sp. nov. from Sardinia (GenBank accession number MZ437082) and one of *E. alticincta bindai* from Sicily (1 ♀; Italy, Sicily, Mount Etna, Gurrida Lake, 25.VIII.2020 RC leg., det., and coll.) (GenBank accession number MZ437367). Both COI sequences obtained from the samples were used as queries in BOLD-IDS tools, and the system returned a match (sequence of *E. gennargentui*: 97.88%; sequence of *E. alticincta bindai*: 98.77%) with a sequence of *E. alticincta* present in GenBank.

Sequence divergences calculated using the *p*-distance model are reported in Table 4, and the neighbor-joining tree showing the relationships between the new species and other related species of *Eucera* (*Tetralonia*) are reported in Fig. 7.

### Table 4. *p*-distance and nucleotide divergences (expressed as percentages) of *Eucera gennargentui* sp. nov., *E. alticincta bindai*, and *Eucera* (*Tetralonia*) species with *Habropoda tarsata* used as the outgroup. Average distance: 12.25.

| Species  | *p*-distance | Nucleotide divergence |
|----------|--------------|-----------------------|
| *E. gennargentui* sp. nov. | 2.21 | |
| *E. alticincta bindai* | 2.08 | 1.21 |
| *E. fulvescens* | 4.09 | 3.55 | 3.41 |
| *E. graja* | 7.65 | 7.31 | 7.47 | 7.47 |
| *E. dentata* | 8.34 | 8.0 | 8.05 | 8.68 | 8.34 |
| *E. salicariae* | 7.30 | 6.95 | 6.68 | 6.82 | 7.65 | 8.20 |
| *E. nana* | 7.69 | 8.84 | 8.07 | 8.84 | 9.61 | 8.84 | 6.92 |
| *E. malvae* | 7.82 | 8.0 | 8.51 | 7.44 | 6.43 | 8.81 | 6.38 | 6.92 |
| *Habropoda tarsata* MN919536 | 22.61 | 23.29 | 24.73 | 24.92 | 23.29 | 23.96 | 23.05 | 24.61 | 23.05 |
| *Habropoda tarsata* MN919537 | 22.61 | 23.29 | 24.06 | 24.24 | 22.76 | 23.44 | 22.36 | 24.61 | 22.51 | 0 |

**Figure 7.** Neighbor-Joining tree showing relationships among *Eucera gennargentui* sp. nov., and *Eucera* (*Tetralonia*) species with *Habropoda tarsata* used as the outgroup.
Description of *Eucera gennargentui* from Sardinia

**Conclusion**

In the current study, *Eucera fulvescens*, *E. julliani*, and *E. nana* are reported for the first time for Sardinia, and *E. gennargentui* sp. nov. is described from Gennargentu Massif.

This new species shows a larger body size and a darker appearance than the closest related taxa *E. alticincta alticincta* and *E. alticincta bindai*, and other different characters reported in this work. Furthermore, following our field observations, we ascertained how the new species was linked to three Sardo-Corsican and Sardinian endemic botanical species of the flora of Gennargentu Massif.

The barcode sequence of the new species *Eucera gennargentui* diverged from that of *E. alticincta alticincta* from 2.08% and from that of *E. alticincta bindai* by 2.21%.

In Europe, wild bees are threatened by habitat changes resulting from human activities, e.g., urban expansion, agricultural practices, habitat destruction or changes, and climate changing (Matheson et al., 1996; Steffan-Dewenter et al., 2005; Nieto et al. 2014; Bella et al. 2020; Fisogni et al. 2020). There was poor information for the majority of the species (56.7%) to determine their conservation status (Nieto et al. 2014).

In Italy, the availability of information on bees is particularly scarce, especially in southern and insular regions, so it is important to increase research to better understand the distribution and the population tendencies, especially for localised taxa.

More observations are necessary to understand the conservation status of this new species and of the other *Eucera* (*Tetralonia*) species present in Sardinia. However, the area of Gennargentu where *Eucera gennargentui* was found is rather restricted and subject to disturbing factors due to strong grazing regimes and reckless reforestation that inexorably damage the grasslands where the endemic host plants live. The frequency of numerous fires that devastate natural ecosystems and rural areas in Sardinia (Capra et al. 2018; Salis et al. 2021) represents a further important threat to the diversity of flora and consequently of wild bees.

The present study greatly improves the Sardinian bee fauna, showing that this Mediterranean island will surely deserves further new acquisitions, in order to increase our knowledge of its still underestimated pollinator fauna.

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