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Original Article

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Integrating morphology and molecular data to explore taxonomy, evolutionary history, and conservation of Italian endemic Forficulidae (Dermaptera)

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ABSTRACT

Italy hosts 27 species of Dermaptera, five belonging to *Forficula*, which were never thoroughly investigated. We integrated morphological (morphometric measurements) and molecular data (mitochondrial COI, 16S and nuclear 28S, ITS2) to revise the Italian species, focusing on the endemic taxa, and on the diversity within *F. auricularia* to reveal the presence of cryptic species. Our data were integrated with those available for other West Mediterranean *Forficula*. Our results confirmed the taxonomic status of the endemic *F. apennina* and *F. silana*, that belong to different lineages but share some morphological traits. The endemic Pseudochelidura orsinii and *P.galvagnii* resulted nested within *Forficula,* and a new taxonomic arrangement is proposed (*Forficula orsinii*: resurrected combination and *Forficula galvagnii*: new combination). Molecular data revealed the presence in Pantelleria and Sardinia of *F. mediterranea*, cryptic with *F.auricularia* and reported only from Spain and Morocco. Morphometric data showed a distinction between West Mediterranean and Italian specimens of *F. mediterranea*, indicating a divergent selection within this species. This study provides new insights for the Italian Forficulidae, highlighting the importance of combining different approaches in the study of species diversity for their conservation. Indeed, *F.apennina* and *F. galvagnii* are high altitude species which seem to be affected by global warming so much to raise fears for their conservation.

KEYWORDS: conservation; divergent selection; *Forficula*; global warming; morphometry; molecular phylogeny; new taxonomic arrangement; *Pseudochelidura*

INTRODUCTION

With about 61 000 species, Italy is the European country with the highest animal species' richness and with a high level of endemism (Bologna *et al.* 2022). Criteria such as diversity, species' rarity, and endemism, are used for establishing conservation priorities (Reid 1998, Myers *et al.* 2000, Williams *et al.* 2002, Schmeller *et al.* 2008, Burlakova *et al.* 2011). Cryptic species represent a significant portion of undiscovered biodiversity, while, considering their limited spatial distribution and the environmental constrains often required for their survival, endemic species are among the first potential candidates for extinction, jeopardized by disturbances such as habitat degradation and destruction (Anderson 1994, Bickford *et al.* 2007, Vrijenhoek 2009, Jörger and Schrödl 2013, Nygren 2014, Struck *et al.* 2018, Li and Wiens 2023, Riccieri *et al.* 2024). Moreover, species with restricted distributions are further threatened when they live in exclusive habitats, such as high-altitude species, which are now subject to the effects of global warming (Brandmayr and Pizzolotto 2016). Indeed, high-altitude species tend to move to high elevation as temperatures rise (Scalercio *et al.* 2014). However, in mountain ranges, where the elevation reaches the limits of the alpine region, populations of high-altitude species may become extinct. Therefore, understanding which, and how many, species are present in a geographical area has an important implication in conservation (Burlakova *et al.* 2011, Hewitt 2011).

Earwigs (Dermaptera) belong to one of the smallest, though ancient, insect orders, including *c.* 2000 species distributed worldwide (Haas 1995, Jarvis *et al.* 2005, Zhang 2011). They are easily recognizable by the presence of large, pincer-shaped cerci,

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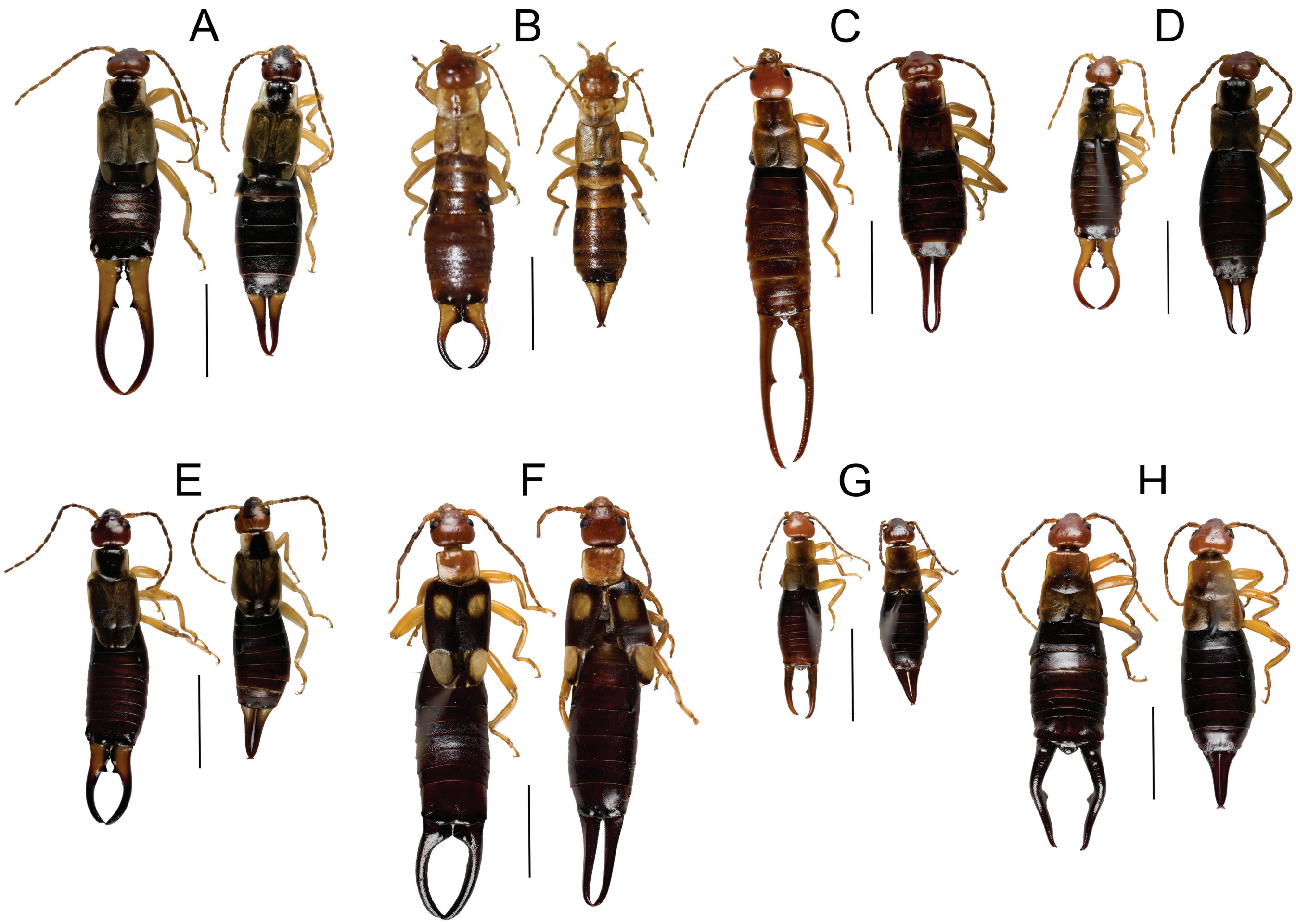

Figure 1. Male (left) and female (right) specimens of *Forficula* collected in Italy: A, *Forficula auricularia*; B, *Forficula decipiens*; C, *Forficula apennina*; D, *Forficula silana*; E, *Forficula mediterranea*; F, *Forficula smyrnensis*; G, *Pseudochelidura orsinii* (after this paper *Forficula orsinii*, see Results and Discussion); H, *Pseudochelidura galvagnii* (after this paper *Forficula galvagnii*, see Results and Discussion). Scale bar 5 mm.

shortened sclerotized forewings, and an elongate flattened body (Haas *et al.* 2000, Rankin and Palmer 2009, Fontana *et al.* 2023). Extensive research has been conducted on earwig morphology, focusing heavily on wing and genitalia features (Popham 1961, Haas and Wootton 1996, Haas *et al.* 2000, Haas and Kukalova-Peck 2001), but phylogenetic relationships among taxa remain poorly understood. Indeed, the systematic arrangement of Dermaptera is still controversial, with molecular phylogenetic results often incongruent with those from traditional morphological investigations (Kočárek *et al.* 2013).

After the Iberian Peninsula, which has 29 species, Italy is the European country with the highest number of Dermaptera species (27), belonging to four families: Anisolabididae, Labiduridae, Spongiphoridae, and Forficulidae (Engel and Haas 2007, Fontana *et al.* 2021, 2023). Within the latter, the genus *Forficula* Linnaeus, 1758 is represented in Italy by five species, while two species described and long considered as belonging to the genus *Forficula* are now referred to the genus *Guanchia* Burr, 1911: *G. obtusangula* Krauss, 1904 and *G. pubescens* Gené, 1837. The genus *Guanchia*, which has as its type species *Guanchia cabrerae* (Bolivar, 1893) from the Canary Islands, currently numbers over 30 species distributed from the Canary Islands (where many species are present), Europe, Africa, and Asia. The validity of this genus has not been, and still is not, shared by the various Dermaptera scholars, but new studies are underway aimed at its correct definition (Petr Kočárek personal communication). As far as Italy is concerned, the two species assigned to this genus are not at all common, even though they do not show particular ecological needs. During our investigations we did not find any specimens of *Guanchia* and for this reason they were not included in our analyses. The five species of *Forficula* known from Italy are: *F. auricularia* Linnaeus, 1758 (Fig. 1A), *F. decipiens* Gené, 1832 (Fig. 1B), the two endemic *F. apennina* Costa, 1882 (Fig. 1C) and *F. silana* Costa, 1882 (Fig. 1D), and the allochthonous *F. smyrnensis* Audinet-Serville, 1839 (Fig. 1F) recently found in Veneto, Emilia-Romagna, Marche, and Corsica (Fontana *et al.* 2021).

Forficula species can be distinguished by male genitalia, especially by the ratio of virga to metaparameri measurements, by the size of the pronotum, elytra, last tergite, and cerci (Cravero 1918, Maccagno 1933). According to Steinmann (1993) and Chen (2021), *Forficula* species are also distinguished by characteristic features such as normally developed, transversely truncate tegmina, first tarsomere twice as long as second, not strongly narrowed ultimate tergite, and basally dilated male forceps. In Dermaptera and in the genus *Forficula* in particular, the shape and size variability of male cerci is a very common phenomenon. The form with very developed cerci is called macrolabic, and that with small cerci is called cyclolabic (Fontana *et al.* 2021) or brachylabic. Between these two, many intermediate forms are normally present and are sometime called mesolabic. The forms with large and small cerci are known for many species such as *F. auricularia*, *F. apennina*, and *F. silana*, while this polymorphism does not seem to occur, for example, in *F. decipiens*. For this reason, morphological characteristics can prove to be misleading in discriminating cryptic species, while taxonomic molecular studies are still limited (Wirth *et al.* 1998, Guillet *et al.* 2000, González-Miguéns *et al.* 2020).

Forficula apennina and *F. silana* are both brachypterous mountain species but, while the first is closely linked to the highest altitudes and is recorded as a periglacial and orophilic element (Valle *et al.* 2022), the latter can be found also at lower elevations in thermophilic beech forests in southern Italy (Fontana *et al.* 2004). *Forficula apennina* is a very rare species for which small populations are known only for the Gran Sasso, Maiella (Abruzzo), Terminillo (Lazio), Pollino, and La Sila (Basilicata and Calabria) mountain ranges (Costa 1882, Ebner 1915, Maccagno 1933, Galvagni 1973, Vigna Taglianti 1994, 2005). Apart from its distribution in central-southern Italy (Abruzzo, Molise, Basilicata, Calabria, Sicily; Fontana *et al.* 2004), not much information is reported for *F. silana* in the literature, bringing attention to the need for a revision of this taxon.

Forficula auricularia, one of the most common and cosmopolitan earwig species, represents an interesting example of cryptic speciation (González-Miguéns *et al.* 2020). The presence of several species hiding within this taxon has been highlighted but never formalized by different authors (Wirth *et al.* 1998, Guillet *et al.* 2000). Only recently, four species were separated in the Western Mediterranean based on molecular and morphological data (González-Miguéns *et al.* 2020): *F. aeolica* González-Miguénes and García-París, 2020, morphologically distinguishable from the others, and the cryptic *F. mediterranea* González-Miguénes and García-París, 2020, *F. dentata* Fabricius, 1775, and *F. auricularia* Linnaeus, 1758 *ss.*. Accordingly, only the true *F. auricularia* would be present in Italy, but just a few specimens from northern regions were considered in the last study (González-Miguénes *et al.* 2020). Indeed, this complex should be deeply investigated along the whole peninsula and the close islands too, to reveal the possible presence of other cryptic species also in Italy (Fontana *et al.* 2021).

In the framework of the Italian National Biodiversity Future Center (NBFC), we focused on the study of Italian endemic insects and their conservation (e.g. Forte *et al.* 2024a, b, Poloni *et al.* 2024, Riccieri *et al.* 2024). The aim of this study is to revise the Italian species of *Forficula* by integrating morphological and molecular data. In particular, we investigated the morphological and the genetic variability of *F. auricularia* sampled throughout the peninsula and the main islands, and compared this with previously published data, to disclose the presence of other possible cryptic species. We evaluated the taxonomic status and phylogenetic relationships of the two endemic species, *F. apennina* and *F. silana*, and provided a synthesis and new information about these two understudied taxa. Finally, we point out intriguing taxonomic problems by the inclusion in the phylogenetic study of two other Apennine endemic species, *Pseudochelidura galvagnii* Vigna Taglianti, 1999 and *P. orsinii* (Gené, 1833).

MATERIALS AND METHODS

Taxon sampling

Altogether, 95 specimens of *Forficula* (*F. apennina*: nine; *F. auricularia s.l.*: 70; *F. decipiens*: two; *F. silana*: 13; *F. smyrnensis*: one) were collected by hand between 2023 and 2024 from several Italian localities (Fig. 2; Table 1). Additionally, 21 specimens of *Pseudochelidura* (*P. orsinii*: 16; *P. galvagnii*: five; Fig. 2, Table 1), and one undetermined *Chelidura* Latreille, 1825 (*Chelidura* sp.), and one of *Chelidurella* Verhoeff, 1902 (*Chelidurella vignai* Galvagni, 1995) were collected to be used as outgroups in phylogenetic analyses (Table 1; see Supporting Information, Table S1 for a taxonomic summary of the species discussed in this paper). All the specimens were preserved in ethanol 96% and stored at 4°C in the entomological collection at University Roma Tre. Species were identified by P.F. by comparison with museum samples (Galvagni's and Fontana's collections at Fondazione Museo Civico di Rovereto, Italy) and by using the following literature: Costa (1882), Maccagno (1933), Harz and Kaltenbach (1976), Albouy and Caussanel (1990), Sakai(1996), Vigna Taglianti (1999) and Fontana *et al.* (2002).

DNA extraction, sequencing, and alignment

Three legs per each specimen were processed for DNA extraction with the ©Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol. Two mitochondrial and two nuclear genes were amplified: (i) cytochrome *c* oxidase subunit I (COI; primer pairs: LCO1490/HCO2198, LCO1490/HCOoutout; Folmer *et al.* 1994, Prendini *et al.* 2005); (ii) 16S ribosomal DNA (16S rDNA; primer pair LRN13398/LRJ12887; Simon *et al.* 1994); (iii) 28S ribosomal RNA gene (28S rDNA; primer pair: 28S01/28SR01; Kim *et al.* 2000); (iv) internal transcribed spacer 2 (ITS2; primer pair: CAS5p8sFt/CAS28sB1d; Ji *et al.* 2003).

Polymerase Chain Reactions (PCRs) were carried out in a final volume of 25 μL, containing 3 μL of 10× reaction buffer, 1–3 μL of $MgCl_2$ (50 mM), 0.5–1 μL dNTPs (10 mM), 0.2 μL of TaqDNA polymerase (5 U/μL BIOTAQ DNA Polymerase, Meridian BIOSCIENCE), 0.5 μL of each primer (25 mM) and 0.5–1 μL of DNA template. Following the amplification, 3μL of PCR products underwent electrophoresis on a 1% agarose gel. Amplified products were purified and sequenced by Macrogen (Milan, Italy). In the supplementary material, primer sequences and thermal cycles are described in detail (Supporting Information, Table S2).

Sequences were checked and edited with GENEIOUS PRIME (v.2024.0.5, see https://www.geneious.com) and subsequently aligned with MAFFT (v.7, Katoh *et al.* 2019), using the E-INS-i algorithm for *COI* and ITS2 and the Q-INS-i algorithm for the two ribosomal genes 28S and 16S. We also included in the *COI* and ITS alignments sequences of *F. aeolica*, *F. auricularia*, *F. dentata*, and *F. mediterranea* published by González-Miguéns *et al.* (2020) and available in GenBank. The single specimen of *F. iberica* from González-Miguéns *et al.* (2020) was included only in the *COI* single gene tree, as it was only sequenced for that marker (see Supporting Information, Table S3).

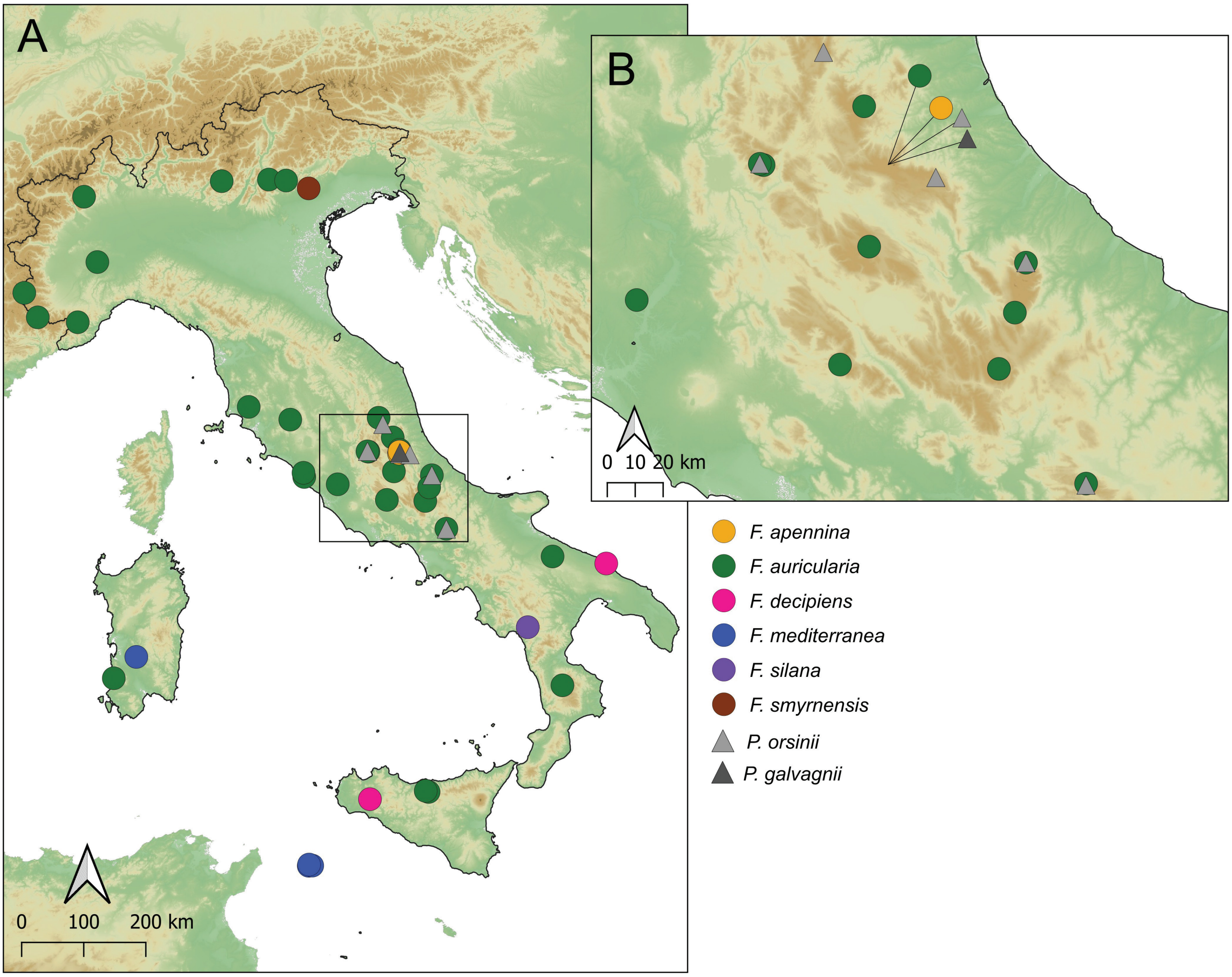

Figure 2. A, Map of collecting sites of the specimens used in this study. B, detail of the sampling sites in central Apennine: *F. auricularia*, *F. apennina*, *P. orsinii*, and *P. galvagnii* (after this paper *F. orsinii* and *F. galvagnii*, see Results and Discussion) were all collected on Monte Aquila but at different elevations (see Table 1 for more details).

Phylogenetic analyses

Maximum likelihood (ML) and Bayesian Inference (BI) trees were built for each single gene and for a multilocus dataset obtained by combining the four markers.

IQ-TREE web was used to perform the best substitution model selection through ModelFinder and the ML tree search (Trifinopoulos *et al.* 2016, Kalyaanamoorthy *et al.* 2017). The multilocus dataset was partitioned by codon position for the COI, and by genes for the remaining non-coding genes and analysed with the edge-linked model. For both single genes and the combined dataset, the number of unsuccessful iterations was set to 1000. Node support was estimated by applying 1000 replicates of ultrafast bootstrap (UFBboot2; Hoang *et al.* 2018) and Shimodaira–Hasegawa (SH)-like approximate ratio tests (SH-aLRT; Guindon *et al.* 2010).

The web-server CIPRES (Miller *et al.* 2010) was used to perform BI with MrBayes v.3.2.7 (Ronquist *et al.* 2012). ModelFinder implemented in IQ-TREE web provided the best partitioning scheme for the multilocus dataset. Two independent Markov chain Monte Carlo (MCMC) runs of 10 million generations, with four chains each and a default 25% burn-in, with the reversible jump MCMC (Huelsenbeck *et al.* 2004) were implemented for the analyses. Trees were sampled every 1000 generations and run convergence was assessed with TRACER v.1.7 (Rambaut *et al.* 2018). Trees were visualized with FigTree v.1.3.1 (Rambaut and Drummond 2009).

In the resulting trees, clades receiving SH-aLRT values ≥80% (Guindon *et al.* 2010), UFBoot (UFB) value ≥95% (Minh *et al.* 2013), and posterior probability (PP) values ≥0.95 (Erixon *et al.* 2003) were considered supported.

Haplotype networks

To better investigate relationships between the Italian specimens of *F. mediterranea* (newly discovered, see below Results and Discussion) and those from Spain and Morocco, we constructed haplotype networks for the COI and ITS2 (genes available for all the specimens) using TCS v.1.21 (Clement *et al.* 2000). Networks were then visualized in TcsBU (Múrias dos Santos *et*

Table 1. Species, Specimen code, Collection Site, Genes and GenBank accession numbers for the specimens collected for this study. For each specimen is also indicated if it was included in morphological investigations. If not, the reason why it was excluded is specified in parentheses.

Species	Code	Collection Site	COI	16S	28S	ITS2	Morphological analyses
Chelidura sp.	1158	Italy, Piemonte, CN, Bosco delle Navette	PQ561665	PQ631971	PQ631870	PQ631756	No
Chelidurella vignai	347	Italy, Veneto, VI, Altopiano di Asiago, Monte Zebio	PQ561666	PQ631972	PQ631871	PQ631757	No
Forficula apennina	308	Italy, Abruzzo, TE, Gran Sasso, Rifugio Duca degli Abruzzi, 2390 m	PQ561667	PQ631973	PQ631872	PQ631758	Yes
Forficula apennina	311	Italy, Abruzzo, TE, Gran Sasso, Corno Grande, 2740 m	PQ561668	PQ631974	PQ631873	PQ631759	Yes
Forficula apennina	312	Italy, Abruzzo, TE, Gran Sasso, Corno Grande, 2620 m	PQ561669	PQ631975	-	PQ631760	Yes
Forficula apennina	313	Italy, Abruzzo, TE, Gran Sasso, Corno Grande, 2620 m	PQ561670	PQ631976	PQ631874	PQ631761	Yes
Forficula apennina	314	Italy, Abruzzo, TE, Gran Sasso, Corno Grande, 2620 m	PQ561671	PQ631977	PQ631875	PQ631762	Yes
Forficula apennina	812	Italy, Abruzzo, AQ, Monte Aquila, 2400 m	PQ561672	PQ631978	PQ631876	PQ631763	Yes
Forficula apennina	813	Italy, Abruzzo, AQ, Monte Aquila, 2400 m	PQ561673	PQ631979	PQ631877	PQ631764	Yes
Forficula apennina	814	Italy, Abruzzo, AQ, Monte Aquila, 2400 m		PQ631980	PQ631878	PQ631765	Yes
Forficula apennina	815	Italy, Abruzzo, AQ, Monte Aquila, 2400 m	PQ561674	PQ631981	PQ631879	PQ631766	Yes
Forficula auricularia	11	Italy, Sardegna, Fluminimaggiore	PQ561675	PQ631982	PQ631880	PQ631767	Yes
Forficula auricularia	13	Italy, Toscana, GR, Massa Marittima, loc. Marsiliana	PQ561680	PQ631987	PQ631883		Yes
Forficula auricularia	14	Italy, Toscana, GR, Massa Marittima, loc. Marsiliana	PQ561684	PQ631991	PQ631887	PQ631774	Yes
Forficula auricularia	15	Italy, Toscana, GR, Massa Marittima, loc. Marsiliana	PQ561685	PQ631992	PQ631888	PQ631775	Yes
Forficula auricularia	18	Italy, Lazio, RM, Roma, Prima Porta	PQ561688	PQ631995	PQ631891	PQ631778	Yes
Forficula auricularia	19	Italy, Calabria, CS, Camigliatello Silano	PQ561691	PQ631998	PQ631894	PQ631781	Yes
Forficula auricularia	20	Italy, Lazio, RM, Allumiere	PQ561692	PQ631999	PQ631895	PQ631782	No (Juvenile)
Forficula auricularia	21	Italy, Lazio, VT, Cencelle	PQ561693	PQ632000		PQ631783	Yes
Forficula auricularia	22	Italy, Lazio, VT, Cencelle	PQ561696	PQ632003		PQ631786	No (Juvenile)
Forficula auricularia	25	Italy, Piemonte, AT, Asti	PQ561699	PQ632006	PQ631900	PQ631789	Yes
Forficula auricularia	69	Italy, Sicilia, PA, Parco delle Madonie, Piano Farina, 1350 m	PQ561713	PQ632019		PQ631802	Yes
Forficula auricularia	70	Italy, Sicilia, PA, Parco delle Madonie, Piano Farina, 1350 m	PQ561714			PQ631803	Yes
Forficula auricularia	71	Italy, Sicilia, PA, Parco delle Madonie, Piano Farina, 1350 m	PQ561715	PQ632020	PQ631912	PQ631804	Yes
Forficula auricularia	76	Italy, Sicilia, PA, Parco delle Madonie, Petralia Sottana, Piano Battaglia, 1674 m	PQ561716	PQ632021	PQ631913	PQ631805	Yes
Forficula auricularia	77	Italy, Sicilia, PA, Parco delle Madonie, Petralia Sottana, Piano Battaglia, 1674 m	PQ561717	PQ632022	PQ631914	PQ631806	Yes

Table 1. Continued

Species	Code	Collection Site	COI	16S	28S	ITS2	Morphological analyses
Forficula auricularia	78	Italy, Sicilia, PA, Parco delle Madonie, Petralia Sottana, Piano Battaglia, 1674 m	PQ561718	PQ632023	PQ631915	PQ631807	Yes
Forficula auricularia	94	Italy, Molise, CB, Monte Matese, Monte Miletto, Campitello Matese, 1504 m	PQ561731	PQ632037	PQ631928	PQ631822	No (Juvenile)
Forficula auricularia	136	Italy, Abruzzo, CH, PE, Blockhaus-Maiella, Rifugio Bruno Pomilio	PQ561681	PQ631988	PQ631884	PQ631771	Yes
Forficula auricularia	137	Italy, Abruzzo, AQ, Altopiana dell'Aremogna, 1910 m	PQ561682	PQ631989	PQ631885	PQ631772	No (Juvenile)
Forficula auricularia	138	Italy, Abruzzo, AQ, Altopiana dell'Aremogna, 1910 m	PQ561683	PQ631990	PQ631886	PQ631773	Yes
Forficula auricularia	164	Italy, Abruzzo, AQ, Maiella, Guado di Coccia, 1650 m	PQ561686	PQ631993	PQ631889	PQ631776	No (Juvenile)
Forficula auricularia	165	Italy, Abruzzo, AQ, Maiella, Guado di Coccia, 1650 m	PQ561687	PQ631994	PQ631890	PQ631777	Yes
Forficula auricularia	188	Italy, Marche, MC, Monti Sibillini, Monte Bove Nord, 1969 m	PQ561689	PQ631996	PQ631892	PQ631779	Yes
Forficula auricularia	189	Italy, Marche, MC, Monti Sibillini, Monte Bove Nord, 1969 m	PQ561690	PQ631997	PQ631893	PQ631780	Yes
Forficula auricularia	211	Italy, Abruzzo, TE, Monti della Laga, Rocca Santa Maria, Ceppo, 1864 m	PQ561694	PQ632001	PQ631896	PQ631784	Yes
Forficula auricularia	212	Italy, Abruzzo, TE, Monti della Laga, Rocca Santa Maria, Ceppo, 1864 m	PQ561695	PQ632002	PQ631897	PQ631785	Yes
Forficula auricularia	242	Italy, Abruzzo, TE, Gran Sasso, Prati di Tivo, 1620 m	PQ561697	PQ632004	PQ631898	PQ631787	Yes
Forficula auricularia	243	Italy, Abruzzo, TE, Gran Sasso, Prati di Tivo, 1620 m	PQ561698	PQ632005	PQ631899	PQ631788	Yes
Forficula auricularia	266	Italy, Abruzzo, TE, Gran Sasso, Prati di Tivo, 1570 m	PQ561700	PQ632007	PQ631901	PQ631790	Yes
Forficula auricularia	267	Italy, Abruzzo, TE, Gran Sasso, Prati di Tivo, 1570 m	PQ561701	PQ632008	PQ631902	-	Yes
Forficula auricularia	307	Italy, Abruzzo, TE, Gran Sasso, Rifugio Duca degli Abruzzi, 2390 m	PQ561702	PQ632009	PQ631903	PQ631791	Yes
Forficula auricularia	309	Italy, Abruzzo, TE, Gran Sasso, Rifugio Duca degli Abruzzi, 2390 m	PQ561703	PQ632010	PQ631904	PQ631792	Yes
Forficula auricularia	315	Italy, Lazio, RI, Monte Terminillo, Rifugio Angelo Sebastiani, 1810 m	PQ561704	PQ632011	PQ631905	PQ631793	Yes
Forficula auricularia	348	Italy, Puglia, BT, Castel del Monte	PQ561705	PQ632012	PQ631906	PQ631794	Yes
Forficula auricularia	350	Italy, Toscana, GR, Monte Amiata, Part. 20, leg. G. Caoduro	PQ561706	PQ632013		PQ631795	Yes
Forficula auricularia	353	Italy, Trentino Alto Adige, TN, Nosellari	PQ561707		PQ631907	PQ631796	Yes
Forficula auricularia	354	Italy, Trentino Alto Adige, TN, Nosellari	PQ561708	PQ632014		PQ631797	Yes
Forficula auricularia	458	Italy, Abruzzo, CH, Maielletta, Rifugio Bruno Pomilio, St. 2, 1680 m	PQ561709	PQ632015	PQ631908	PQ631798	Yes
Forficula auricularia	459	Italy, Abruzzo, CH, Maielletta, Rifugio Bruno Pomilio, St. 2, 1680 m	PQ561710	PQ632016	PQ631909	PQ631799	Yes

Table 1. Continued

Species	Code	Collection Site	COI	16S	28S	ITS2	Morphological analyses
Forficula auricularia	473	Italy, Abruzzo, CH, Maielletta, Rifugio Bruno Pomilio, St. 3, 1930–1980 m	PQ561711	PQ632017	PQ631910	PQ631800	Yes
Forficula auricularia	474	Italy, Abruzzo, CH, Maielletta, Rifugio Bruno Pomilio, St. 3, 1930–1980 m	PQ561712	PQ632018	PQ631911	PQ631801	Yes
Forficula auricularia	826	Italy, Abruzzo, AQ, Campo Imperatore, 2100 m	PQ561719	PQ632024	PQ631916	PQ631808	Yes
Forficula auricularia	827	Italy, Abruzzo, AQ, Campo Imperatore, 2100 m	PQ561720	PQ632025	PQ631917	PQ631809	Yes
Forficula auricularia	834	Italy, Abruzzo, AQ, Velino, Campo Felice, 1550 m	PQ561721	PQ632026	PQ631918	PQ631810	Yes
Forficula auricularia	835	Italy, Abruzzo, AQ, Velino, Campo Felice, 1550 m	PQ561722	PQ632027	PQ631919	PQ631811	Yes
Forficula auricularia	836	Italy, Piemonte, CN, Val Maira, Chiappera di Acceglio, 1840 m	PQ561723			PQ631812	No (Juvenile)
Forficula auricularia	837	Italy, Piemonte, CN, Val Maira, Chiappera di Acceglio, 1840 m		PQ632028		PQ631813	No (Juvenile)
Forficula auricularia	842	Italy, Lombardia, BS, Passo di Crocedomini, 1900 m	PQ561724	PQ632029	PQ631920	PQ631814	Yes
Forficula auricularia	843	Italy, Lombardia, BS, Passo di Crocedomini, 1900 m		PQ632030	PQ631921	PQ631815	Yes
Forficula auricularia	849	Italy, Veneto, VI, Gallio (sotto Malga Longara Davanti), 1540 m	PQ561725	PQ632031	PQ631922	PQ631816	Yes
Forficula auricularia	850	Italy, Veneto, VI, Gallio (sotto Malga Longara Davanti), 1540 m	PQ561726	PQ632032	PQ631923	PQ631817	Yes
Forficula auricularia	851	Italy, Piemonte, BI, Alpe Finestre, 1730 m	PQ561727	PQ632033	PQ631924	PQ631818	Yes
Forficula auricularia	852	Italy, Piemonte, BI, Alpe Finestre, 1730 m	PQ561728	PQ632034	PQ631925	PQ631819	Yes
Forficula auricularia	928	Italy, Lazio, FR, Campocatino, 1840 m	PQ561729	PQ632035	PQ631926	PQ631820	Yes
Forficula auricularia	929	Italy, Lazio, FR, Campocatino, 1840 m	PQ561730	PQ632036	PQ631927	PQ631821	Yes
Forficula auricularia	1147	Italy, Piemonte, CN, Colle della Lombarda	PQ561676	PQ631983	PQ631881	PQ631768	No (Juvenile)
Forficula auricularia	1149	Italy, Piemonte, CN, Ormea, Lago Lao, 1580 m	PQ561677	PQ631984	PQ631882	PQ631769	Yes
Forficula auricularia	1156	Italy, Molise, CB, Monte Matese, Monte Miletto, Campitello Matese, 1273 m	PQ561678	PQ631985			No (Juvenile)
Forficula auricularia	1157	Italy, Molise, CB, Monte Matese, Monte Miletto, Campitello Matese, 1273 m	PQ561679	PQ631986		PQ631770	No (Juvenile)
Forficula decipiens	12	Italy, Puglia, BA, Polignano a Mare		PQ632038		PQ631823	Yes
Forficula decipiens	349	Italy, Sicilia, TP, Poggioreale, Az. Tonnino	PQ561732	PQ632039		PQ631824	No (Juvenile)
Forficula mediterranea	4	Italy, Sicilia, TP, Pantelleria, Acropoli	PQ561736	PQ632043	PQ631932	PQ631828	Yes
Forficula mediterranea	5	Italy, Sicilia, TP, Pantelleria, Punta Spadillo	PQ561737	PQ632044	PQ631933	PQ631829	Yes
Forficula mediterranea	6	Italy, Sicilia, TP, Pantelleria, Punta Spadillo	PQ561738	PQ632045	PQ631934	PQ631830	Yes

Table 1. Continued

Species	Code	Collection Site	COI	16S	28S	ITS2	Morphological analyses
Forficula mediterranea	7	Italy, Sicilia, TP, Pantelleria, Bue Marino (Loc. Authidium)	PQ561739	PQ632046	PQ631935	PQ631831	Yes
Forficula mediterranea	8	Italy, Sicilia, TP, Pantelleria, Bue Marino (Loc. Authidium)	PQ561740	PQ632047		PQ631832	Yes
Forficula mediterranea	9	Italy, Sicilia, TP, Pantelleria, Bue Marino (Loc. Authidium)	PQ561743	PQ632050	PQ631938	PQ631835	Yes
Forficula mediterranea	10	Italy, Sicilia, TP, Pantelleria, Bue Marino (Loc. Authidium)	PQ561733	PQ632040	PQ631929	PQ631825	Yes
Forficula mediterranea	351	Italy, Sardegna, OR, Baradili	PQ561734	PQ632041	PQ631930	PQ631826	Yes
Forficula mediterranea	352	Italy, Sardegna, OR, Baradili	PQ561735	PQ632042	PQ631931	PQ631827	Yes
Forficula mediterranea	824	Italy, Sicilia, TP, Pantelleria, Punta Spadillo	PQ561741	PQ632048	PQ631936	PQ631833	No (Only three legs available)
Forficula mediterranea	825	Italy, Sicilia, TP, Pantelleria, Punta Spadillo	PQ561742	PQ632049	PQ631937	PQ631834	No (Only three legs available)
Forficula silana	816	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632051	PQ631939	PQ631836	Yes
Forficula silana	817	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632052	PQ631940	PQ631837	Yes
Forficula silana	818	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m	PQ561744	PQ632053	PQ631941	PQ631838	Yes
Forficula silana	819	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m	PQ561745	PQ632054	PQ631942	PQ631839	Yes
Forficula silana	820	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632055	PQ631943	PQ631840	Yes
Forficula silana	821a	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632056	PQ631944	PQ631841	Yes
Forficula silana	821b	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m	PQ561746	PQ632057	PQ631945	PQ631842	No (Juvenile)
Forficula silana	822a	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632058	PQ631946	PQ631843	Yes
Forficula silana	822b	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632059	PQ631947	PQ631844	Yes
Forficula silana	822c	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m	PQ561747	PQ632060	PQ631948	PQ631845	Yes
Forficula silana	823a	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632061	PQ631949	PQ631846	Yes

Table 1. Continued

Species	Code	Collection Site	COI	16S	28S	ITS2	Morphological analyses
Forficula silana	823b	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632062	PQ631950	PQ631847	Yes
Forficula silana	823c	Italy, Basilicata, PZ, Massiccio del Sirino, Lagonegro, Monte Papa N, 1750 m		PQ632063	PQ631951	PQ631848	Yes
Forficula smyrnensis	1148	Italy, Veneto, TR, Colli Asolani, Maser		PQ632064	PQ631952	PQ631849	Yes
Forficula galvagni	860	Italy, Abruzzo, AQ, Monte Aquila, 2400 m	PQ561748	PQ632065	PQ631953	PQ631850	No
Forficula galvagni	861	Italy, Abruzzo, AQ, Monte Aquila, 2400 m	PQ561749	PQ632066	PQ631954	PQ631851	No
Forficula galvagni	862	Italy, Abruzzo, AQ, Monte Aquila, 2400 m	PQ561750	PQ632067	PQ631955	PQ631852	No
Forficula galvagni	863	Italy, Abruzzo, AQ, Monte Aquila, 2400 m			PQ631956	PQ631853	No
Forficula galvagni	864	Italy, Abruzzo, AQ, Monte Aquila, 2400 m	PQ561751	PQ632068	PQ631957	PQ631854	No
Forficula orsinii	39	Italy, Marche, AP, Laghi di Pilato, Montemonaco, Loc. Foce, ParcoNazionale dei Monti Sibillini, 1800 m	PQ561754	PQ632074		PQ631858	No
Forficula orsinii	284	Italy, Abruzzo, TE, Gran Sasso (Monte Siella, Monte Tremoggia), 1916 m	PQ561752	PQ632072	PQ631959		No
Forficula orsinii	344	Italy, Lazio, RI, Monte Terminillo, 2201 m	PQ561753	PQ632073	PQ631960	PQ631857	No
Forficula orsinii	477	Italy, Abruzzo, CH, Maielletta (cima), St. 4, 2000 m	PQ561755	PQ632075	PQ631961	PQ631859	No
Forficula orsinii	478	Italy, Abruzzo, CH, Maielletta (cima), St. 4, 2000 m	PQ561756	PQ632076	PQ631962	PQ631860	No
Forficula orsinii	479	Italy, Abruzzo, CH, Maielletta (cima), St. 4, 2000 m		PQ632077	PQ631963	PQ631861	No
Forficula orsinii	480	Italy, Abruzzo, CH, Maielletta (cima), St. 4, 2000 m		PQ632078	PQ631964	PQ631862	No
Forficula orsinii	481	Italy, Abruzzo, CH, Maielletta (cima), St. 4, 2000 m		PQ632079	PQ631965	PQ631863	No
Forficula orsinii	865	Italy, Abruzzo, AQ, Campo Imperatore, 2100 m	PQ561757	PQ632080	PQ631966	PQ631864	No
Forficula orsinii	866	Italy, Abruzzo, AQ, Campo Imperatore, 2100 m	PQ561758	PQ632081	PQ631967	PQ631865	No
Forficula orsinii	867	Italy, Abruzzo, AQ, Campo Imperatore, 2100 m			PQ631968	PQ631866	No
Forficula orsinii	868	Italy, Abruzzo, AQ, Campo Imperatore, 2100 m		PQ632082	PQ631969	PQ631867	No
Forficula orsinii	869	Italy, Abruzzo, AQ, Campo Imperatore, 2100 m	PQ561759	PQ632083	PQ631970	PQ631868	No
Forficula orsinii	1152	Italy, Molise, CB, Monte Matese, Monte Miletto, Campitello Matese, 1761 m		PQ632069	PQ631958		No
Forficula orsinii	1154	Italy, Molise, CB, Monte Matese, Monte Miletto, Campitello Matese, 1441 m		PQ632070		PQ631855	No
Forficula orsinii	1155	Italy, Molise, CB, Monte Matese, Monte Miletto, Campitello Matese, 1441 m		PQ632071		PQ631856	No

al. 2016). ITS2 was initially phased using the PHASE algorithm implemented in DNAsp v.6 (Rozas *et al.* 2017).

Morphological analyses

Photographs of 78 specimens of *Forficula* [48 *F. auricularia*; nine molecularly identified as *F. mediterranea* (see Results); nine *F. apennina*; 12 *F. silana*] were taken with Visionary Digital LK Lab System (Visionary Digital, Palmyra, VA) equipped with a Canon EOS 6D mark II dSLR camera and an MP-E 65 mm *f*/2.8 1–5 × lens (Canon, Tokyo, Japan). This device allowed the automatic capture of stacks of images on different focal planes, which were then combined with the Helicon Focus 7 software. Pictures were analysed using software ImageJ (Schneider *et al.* 2012) to measure the traits considered. *Forficula decipiens* and *F. smyrnensis* were excluded from morphological investigations due to the low number of specimens available, as well as juvenile individuals of the other species.

The following characters were measured according to González-Miguéns *et al.* (2020) (Fig. 3; Supporting Information, Table S4): pronotum length (PL) along the anterior–posterior midline; pronotum width (PW) in a straight line across the broadest section; right tegmina as length (EL), across the middle region, starting from the visible edge further to the pronotum; and width (EW), at its widest part of the middle region; length of the posterior wing extending from the right tegmina (WL) along the inner fold. The total length (TL) of the specimen was measured starting from the epistomal suture of the head to the end of the abdomen, excluding the cerci. In addition, further traits have been explored to verify their possible relevance in species' discrimination (Fig. 3; Supporting Information, Table S4). The tegmina angle (EA) was measured considering the angle created between the inner edge of the tegmina and the closest side to the posterior wing; the total length cerci (TLC) was measured starting from the point at which the cerci fit into the end of the abdomen; the basal width cerci (BWC) was measured considering the external edge located at the end of the abdomen; maximum width of the last tergite (MWLT). Only for the male specimens were the following parameters measured:

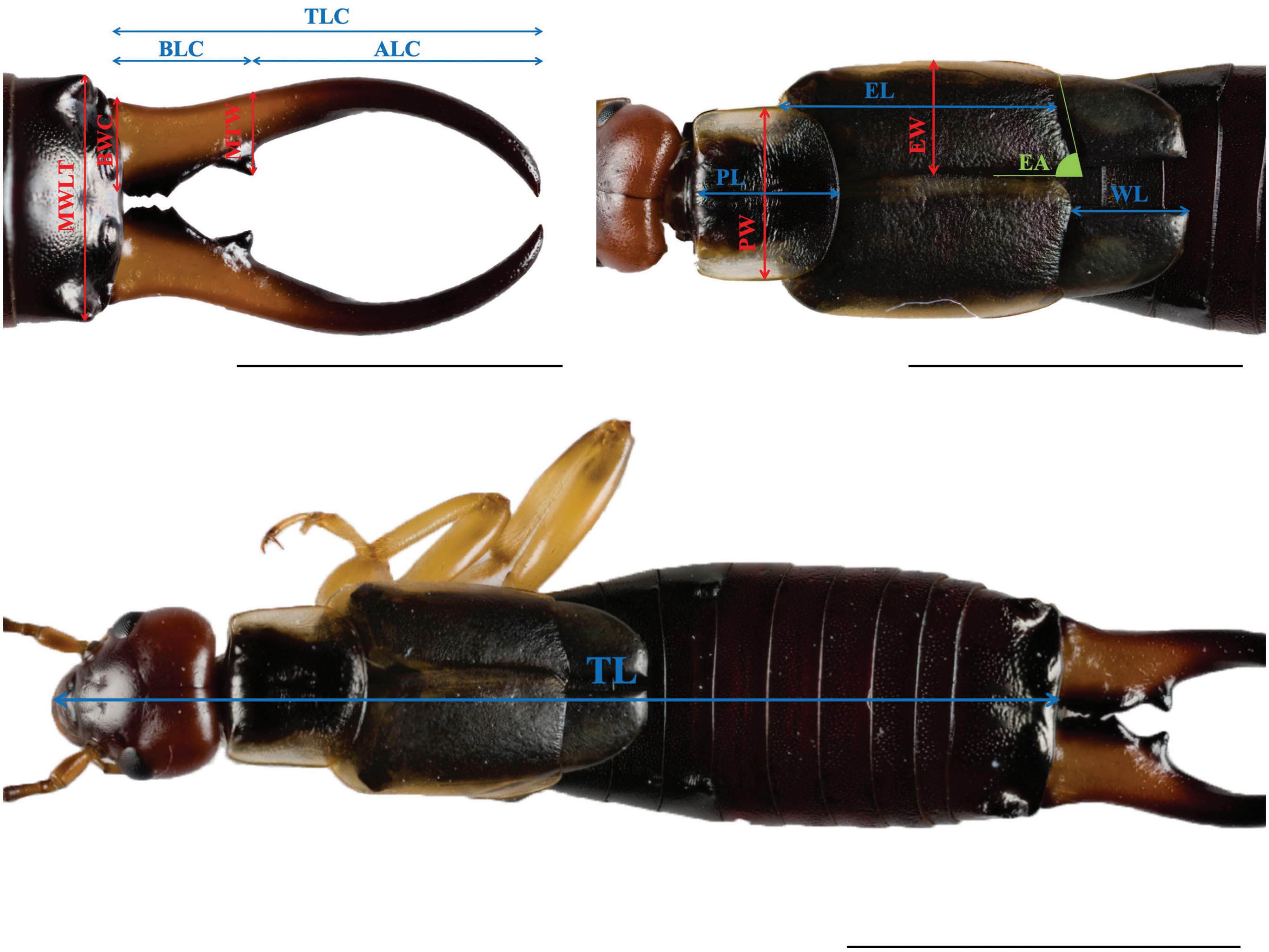

Figure 3. Disposition of the characters measured for morphometric analyses. Each figure represents a close-up of cerci (A), thorax (B) and of the entire specimen (C) of *Forficula*. Lines drawn over the specimen indicate the different measurements taken: pronotum length (PL); pronotum width (PW); tegmina length (EL), tegmina width (EW); tegmina length (WL); wing length (WL); total length (TL); tegmina angle (EA); total length cerci (TLC); basal width cerci (BWC); maximum width of the last tergite (MWLT). Only for the male specimens were measured the following parameters: basal length cerci (BLC); apical length of the cerci (ALC); median tooth width (MTW). Scale bar 5 mm.

basal length cerci (BLC), from the end of the abdomen to the median tooth of the cerci; apical length of the cerci (ALC), from the median tooth of the cerci to the apical cerci; median tooth width (MTW), from the inner peak of the median tooth to the external edge of the cerci. Our measurements were integrated with those of 114 samples of *F. dentata* (56)*, F. aeolica* (19), and *F. mediterranea* (39) from González-Miguéns *et al.* (2020) for a comparison among as many species and specimens as possible.

Morphological analyses were executed using 'R' software v.4.1.2 (RStudio Team 2020), pre-processed by an autocorrelation analysis to verify the independence of variables.

A principal component analysis (PCA) was performed with the function 'prcomp' (Pearson 1901) to reduce the dimensionality of the data and to identify the main variables contributing to the variance. A linear discriminant analysis (LDA) was performed with the packages 'MASS' (Venables and Ripley 2002) and 'caret' (Kuhn 2008) to identify linear combinations of variables that better distinguish between predefined classes. The model was validated using a K-Fold Cross-Validation approach to ensure robustness and generalizability of the findings. This approach divides the data into 10 parts (fold), using nine parts to train the model and one part to validate the model, repeating the process 10 times so that each part of the data is used once as a test set. 'ggplot2' (Wickham and Wickham 2016) was used to generate PCA and LDA scatterplots.

RESULTS

Molecular analyses results

A total of 118 specimens were successfully sequenced (95 COI, 633 bp; 113 16S, 538 bp; 101 28S, 832 bp; 113 ITS2, 374 bp). Overall, the combined dataset consisted of 159 specimens and its total length was 2377 bp (Table 1; Supporting Information, Table S3).

Both BI and ML multilocus trees showed high values of node support (i.e. UFB > 95; SH-aLRT > 80; PP > 0.95) and congruent topologies (Fig. 4; Supporting Information, Fig. S1A, B). *Forficula auricularia* was monophyletic but two specimens from Sardinia and all those sampled in Pantelleria Island (Sicily) clustered within *F. mediterranea*. The two endemics, *F. silana* and *F. apennina*, resulted monophyletic and well distinguished from the other species of *Forficula*, as well as *F. decipiens* and *F. smyrnensis*, the latter being the sister to all the other species. The two species of *Pseudochelidura*, *P. orsinii* and *P. galvagnii*, were nested within *Forficula*, were closely related to *F. apennina*, and showed few genetic differences from one another.

Networks showing relationships among *F. mediterranea* haplotypes are represented in Fig. 5. Overall, we found six haplotypes for COI, one of which was shared by most individuals, and two haplotypes for ITS2, one shared by specimens from Sardinia and Pantelleria, and the other shared by specimens from Spain and Morocco.

Morphological analyses results

PCA (Supporting Information, Fig. S2) and LDA (Fig. 6) included six predictors: PL, PW, EL, EW, WL, and TL. The characters EA, TLC, BWC, MWLT, BLC, ALC, and MTW are correlated with the other variables and were excluded from the analysis.

PCA conducted on morphometric data revealed a clear structure in the morphological variations, highlighting how some measures are more influential than others in explaining the total variance. The first three principal components (PC1, PC2, and PC3) explain a significant portion of the variance in the dataset, with the cumulative proportion of variance reaching 84.73% (Supporting Information, Fig. S2, Table S5a). This indicates that most of the morphological variation among the specimens can be effectively captured using these three components.

LDA model achieved an overall accuracy of 77.08% across the seven species. The Kappa statistic was 0.7027, indicating a great agreement between the predicted and observed species' classifications, suggesting that the LDA model performs reliably in distinguishing between the different *Forficula* species based on the selected morphological parameters. The prior probabilities of group membership reflect the distribution of each species in the training dataset (Supporting Information, Table S5b), with *F. dentata* being the most prevalent (29.17%), followed by *F. auricularia* (25.00%) and *F. mediterranea* (25.00%). The coefficients of the linear discriminants (Supporting Information, Table S5c) indicate the contribution of each morphological trait to the discriminant functions. EW had the greatest influence on the first discriminant function (LD1) followed by WL and PL, which explained 46.50% of the variance in the dataset. The second discriminant (LD2) explained a further 43.90% of the variance, where WL plays a dominant role in discriminating between species followed by PL. Together, these discriminants provide a robust classification framework for the species analysed.

The confusion matrix (Supporting Information, Table S5d) provides an overview of the LDA model's performance in predicting *Forficula* species based on morphological parameters. LDA correctly identified 100% and 89.47% of the specimens morphologically classified as *F. silana* (12 of 12) and *F. aeolica* (17 of 19), respectively, indicating that this species' morphological traits are distinctive enough to be clearly separated from the other species in this analysis. *Forficula auricularia* also exhibited high classification accuracy, with 95.83 % of specimens correctly classified; 66.66% of *F. apennina* specimens were correctly identified (six of nine). *Forficula dentata* and *F. mediterranea* exhibited more significant misclassification issues. *Forficula dentata* was misclassified into *F. mediterranea* 10 times. Conversely, *F. mediterranea* was frequently misclassified as *F. dentata* (18 instances) and *F. auricularia* (eight instances).

DISCUSSION

Before the present study, the Italian fauna of earwigs of the genus *Forficula* included five species, which were never revised with a molecular or morphometric approach. Here we integrated morphological and molecular data to investigate the taxonomic status of the two endemics *F. apennina* and *F. silana*, and to reveal the presence of cryptic species within *F. auricularia*, as recently demonstrated in the Iberian Peninsula (González-Miguéns *et al.* 2020). Collaterally, we revised the taxonomy of the two Italian endemic species *Pseudochelidura galvagnii* and *P. orsinii*, included within *Forficula* in our phylogeny.

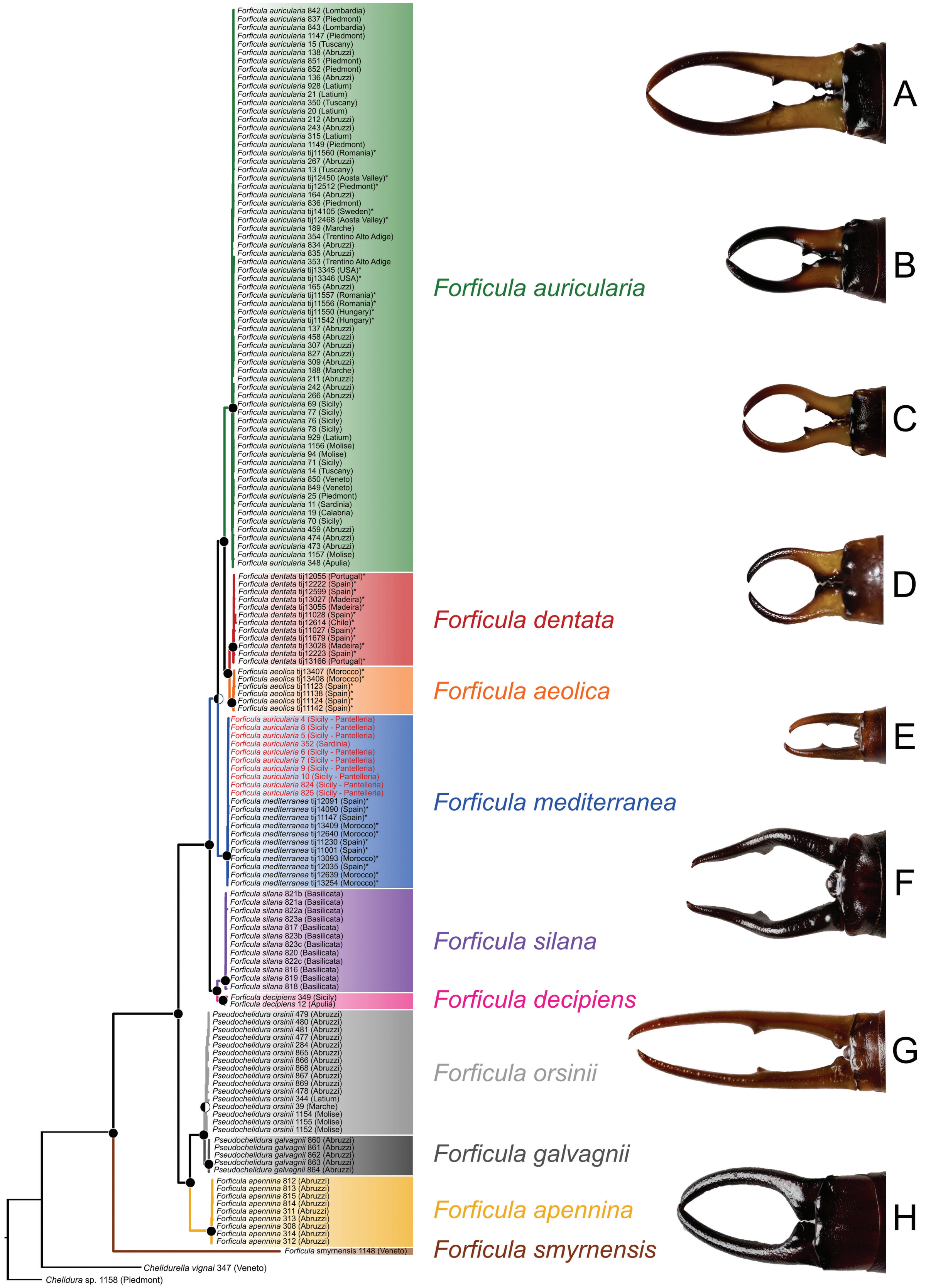

Figure 4. Multilocus (*COI*, 16S, ITS2, and 28S) phylogenetic tree. Node support from both ML and BI analyses are summarized as circles (ML: left half; BI: right half): black half circles represent highly supported values of UFB and SH-aLRT (UFB ≥ 95; SH-aLRT ≥ 80%;) and posterior probabilty (PP ≥ 0.95); white half circles represent not supported values (UFB < 95; SH-aLRT < 80%; PP < 0.90), or topological discrepancies between ML and BI trees (for more detail see supplementary Fig. S1a, b).Nodes without circles are not supported in both ML and BI, except for nodes at intraspecific level, whose support is shown in the Supporting Information (Fig. S1A, Bb). On the right side of the figure, different shapes of male's cerci of Italian species: A, *Forficula auricularia*; B, *F. silana*; C, *F. mediterranea*; D, *F. decipiens*; E, *Pseudochelidura orsinii* (after this paper *Forficula orsinii*, see Results and Discussion); F, *Pseudochelidura galvagnii* (after this paper *Forficula galvagnii*, see Results and Discussion); G, *F. apennina*; H, *F. smyrnensis*.

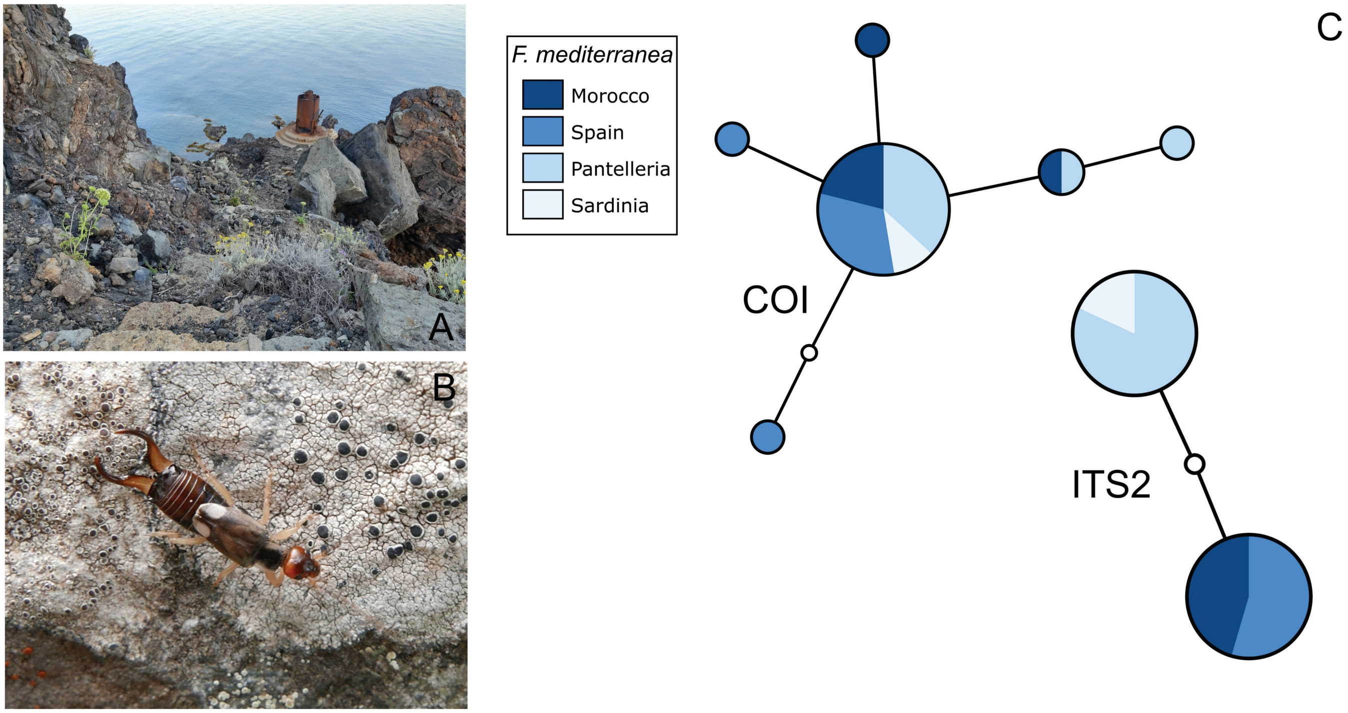

Figure 5. A, habitat of *Forficula mediterranea* in Pantelleria, Punta Spadillo (14/05/2024; Ph. Paolo Fontana). B, adult male of *F. mediterranea* found in Sardinia (Ph. Paolo Fontana). C, haplotype networks of *COI* and ITS2 of *F. mediterranea*. Not sampled intermediate haplotypes are indicated by white dots.

Morphometry, taxonomy, and phylogenetic relationships of Italian *Forficula*

The LDA (Fig. 6) performed using morphological data demonstrated overall accuracy, indicating a high level of precision in classifying the *Forficula* species. The coefficients of the linear discriminants suggest that PL, EW, and WL are particularly influential in differentiating species (Supporting Information, Table S5c). This aligns with known morphological differences among *Forficula* species, where variations in these traits are often associated with species-specific adaptations and ecological niches, such as the brachypterism of some species. Nevertheless, some specific overlaps were observed and are discussed below.

Molecular analysis showed that *F. apennina* and *F. silana* are valid species and are not related to each other (Fig. 4). *Forficula silana* is phylogenetically close to *F. decipiens*, and both belong to a broader genetic lineage, including also *F. aeolica* and the three cryptic species *F. auricularia*, *F. dentata*, and *F. mediterranea*. On its side, *F. apennina* belongs to a different lineage, but, surprisingly, it appears closely related to the two Italian endemic species of the genus *Pseudochelidura*, *P. orsinii* and *P. galvagnii*. The characters used to distinguish the genera of European Forficulidae are based more on the differences among the species present in Europe than on solid morphological differences among genera (Harz and Kaltenbach 1976). In particular, the character generally used to separate the European species of *Forficula* (and *Apterygida* Westwood, 1840) from those of the genus *Pseudochelidura* and the closely related *Eulithinus* Hincks, 1935 (Puerta-Rodríguez *et al.* 2024) is given by the conformation of the posterior margin of the tegmina, which in *Pseudochelidura* and *Eulithinus* is inclined outwards. This conformation of the tegmina can also be found in other European Forficulidae, such as *Guanchia obtusangula* (Krauss, 1904). Even with regard to the male copulatory organs, no significant differences are evident among these three genera. Already Maccagno (1933), who proposed a dichotomous key to discriminate the species based on male genitalia, had highlighted strong morphological affinities between the Italian species of the genera *Forficula* and *Pseudochelidura*. A significant difference between the Italian *Pseudochelidura* and *Forficula* could be found in the male's cerci. The first genus has a sinusoidal (S-shaped) shape when viewed from the side and, when paired and viewed from above, it forms a more or less marked '8' shape, defined by a triangular expansion located on the internal median side of the cerci.

Indeed, our findings justify the return of *Pseudochelidura orsinii* to the original combination *Forficula orsinii* Gené, 1833 (**resurrected combination**) and support the inclusion in this genus also of *Pseudochelidura galvagnii* [*Forficula galvagnii* (Vigna Taglianti, 1999) **comb. nov.**].

In general, our molecular results question the taxonomic status of the whole genus *Pseudochelidura*, which should be further investigated, including also the Iberian species attributed to it. A certain intuitive morphological separation between the Iberian species of *Pseudochelidura*, *Eulithinus*, and the Italian

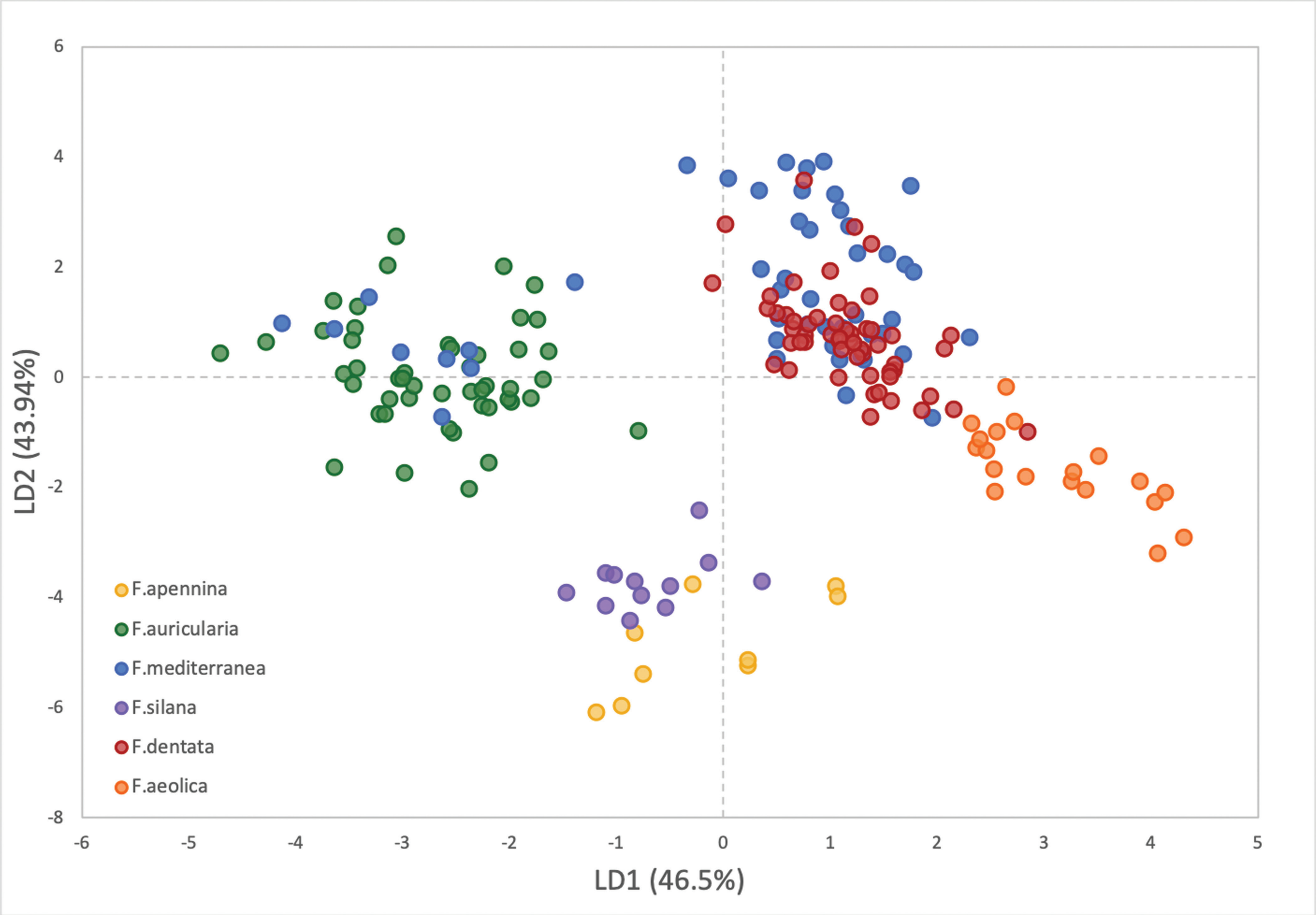

Figure 6. Scatter plot scores of linear discriminant analysis (LDA) for six species of the *Forficula* collected in Italy and Spain used in the morphometric analyses (Supporting Information, Table S2) : *F. apennina*, *F. auricularia*, *F. silana*, *F. dentata*, *F. aeolica* and *F. mediterranea*. The x-axis represents discriminant function 1 (LD1) and y-axis represents discriminant function 2 (LD2).

ones is highlighted by the different general structure of the male cerci and, above all, by the different degree of variability in the conformation of these structures (Cuesta-Segura *et al.* 2023). In the Italian species (*orsinii* and *galvagnii*), although larger, more sinuous, smaller and more simplified forms of male cerci are observed (similar, respectively, to macro- and cyclolabic forms), the manifestation of two morphological models as visibly different as in the Iberian species is not observed. This great diversity of structure in the male cerci of the macro- and cyclolabic forms has been at the basis of the taxonomic confusion within the Iberian *Pseudochelidura*, only recently unravelled thanks to molecular biology (Cuesta-Segura *et al.* 2023). A clear peculiarity of the Italian species is also implied by Puerta-Rodríguez *et al.* (2024), when they state that a more solid solution of the taxonomic status of the Iberian species belonging to the genera *Pseudochelidura* and *Eulithinus* can only be obtained with an analysis that includes the Iberian and Italian species. To date, considering our results and those of the studies cited above (Cuesta-Segura *et al.* 2023, Puerta-Rodríguez *et al.* 2024), only two species can be attributed to the genus *Pseudochelidura*, namely *P. sinuata* (Germar, 1825) from the Pyrenees (Spain, Andorra, and France) and type species of the genus, and *P. cantabrica* Cuesta-Segura *et al.*, 2023, Cantabria (Spain). As regards the identity of the genus *Eulithinus*, Puerta-Rodríguez *et al.* (2024) confirm the presence of only one species, namely *Eulithinus analis* (Rambur, 1838) from the Sierra Nevada (Spain), and highlight the great morphological similarity of this species with the Iberian *Pseudochelidura*. Based on their phylogenetic investigations, the authors prudently retain the genus *Eulithinus*, noting, however, that *E. analis* and *P. sinuata* would have isolated themselves from a common ancestor much more recently than they did with *P. cantabrica*.

Despite not being phylogenetically related (Fig. 4), *F. apennina* and *F. silana* partially overlap in morphometric results (Fig. 6), indicating some sort of morphological similarity. Indeed, a common feature between the two species (shared also by the two species previously referred to *Pseudochelidura*) is the reduction of wings that are not visible, as they are shorter than the tegmina (Fig. 1C, D). Brachypterism, typical of several mountain insects, might also explain the endemicity of these taxa, as it results in the reduction of dispersal abilities.

The widespread species *F. auricularia* is confirmed to be present in all the Italian regions here investigated (Fig. 7), but molecular data also allowed us to detect the presence of *F. mediterranea* (Figs 4, 7). Indeed, two specimens from Sardinia, and all those from Pantelleria, entered the clade with Moroccan and Spanish individuals attributed to this species. The specimens collected on Pantelleria caught the attention of one of us (P.F.) for their smaller size compared to the usual dimensions of *F. auricularia*. Nonetheless, they were preliminarily attributed to the latter species, as no other *Forficula* was known from Pantelleria. *Forficula mediterranea* was only recently described as a cryptic species within the *F. auricularia* complex, and, therefore, can only be distinguished with the support of molecular analyses (González-Miguéns *et al.* 2020). Before our study, *F. mediterranea* was known from the Mediterranean areas of Spain and Morocco, and a few other localities in the Atlantic portion of both countries (González-Miguéns *et al.* 2020). This finding represents the record of a new species for Italian fauna, and an improvement in the knowledge of the range of *F. mediterranea*, which can be considered a West-Mediterranean species (*sensu* Vigna Taglianti *et al.* 1999). Such discovery makes the distribution pattern of this species even more intriguing from a biogeographic perspective, and it would be worth investigating its presence also in Sicily, in Corsica, and in other west Mediterranean islands, as well as in other localities of North Africa (primarily in Algeria and Tunisia). Similar distributions are often attributable to that events occurred during the Messinian (Messinian Salinity Crisis, MSC), or more recent dispersal events related to marine regression during glacial cycles of the Plio-Pleistocene (Riccieri *et al.* 2017). Alternatively, as demonstrated for *F. auricularia* in several parts of the World (e.g. Zack *et al.* 2011, Quarrell *et al.* 2018, Hill *et al.* 2019, Maczey *et al.* 2019), an accidental human-mediated introduction cannot be excluded. All *Forficula* specimens collected in recent years in Pantelleria have been largely analysed with molecular techniques (thanks to which they were identified as *F. mediterranea*) and in any case show a clear morphological homogeneity, also confirmed by the specimens collected in previous years examined by the authors (Bruno Massa Collection, Palermo, Italy). Although the presence on the island of the cosmopolitan species *F. auricularia* cannot be excluded, to date *F. mediterranea* is the only species that can be attested with certainty. This fact could cast doubt on a simple case of anthropic introduction. Anyway to delineate a plausible biogeographic scenario for the origin of the distribution of *F. mediterranea* goes far beyond the scope of this study, and a more appropriate sampling would be necessary for this purpose. However, the haplotype networks based on the COI and the ITS2 (Fig. 5) seem to point to a more recent event, rather than to a relic condition that originated after the MSC and probably even after the Plio-Pleistocene marine regression. In particular, for the COI most of the individuals share a single haplotype, while only two haplotypes were recovered for the ITS2: one shared between Moroccan and Spanish individuals and one between those from Sardinia and Pantelleria. ITS2, despite being nuclear DNA, is a fast-evolving marker and might accumulate more mutations compared to the COI in the

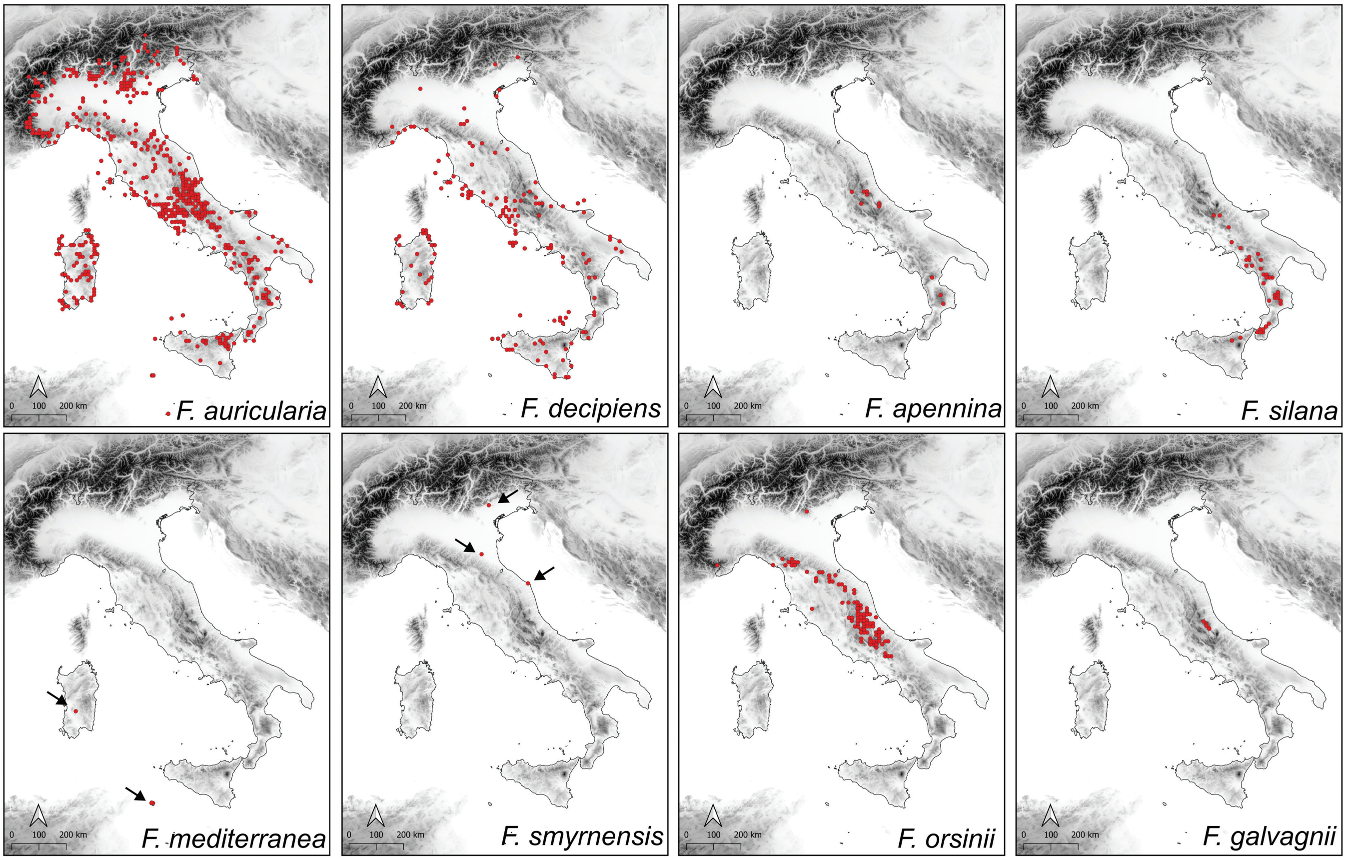

Figure 7. Updated maps of distribution for the eight species of *Forficula* recorded from Italy. Data used to build this map derived from the CKmap project—'Checklist and distribution of the Italian fauna' (Stoch 2003–04, Minelli and Stoch 2006, Ruffo and Stoch 2006) and from the Fontana Collection.

same time frame (e.g. Wang *et al.* 2023). Interestingly, the LDA based on morphometry (Fig. 6) showed that specimens of *F. mediterranea* formed two clusters: one including Moroccan and Spanish individuals, overlapping with specimens of *F. dentata* (as in: González-Miguéns *et al.* 2020), and another including individuals from Sardinia and Pantelleria, overlapping with our specimens of *F. auricularia* from Italy [no measurements were available for other specimens of *F. auricularia* used in González-Miguéns *et al.* (2020) for a comparison]. This suggests an intraspecific morphological diversity in *F. mediterranea* greater than that interspecific between Italian individuals of *F. mediterranea* and *F. auricularia* and Spanish/Moroccan individuals of *F. mediterranea* and *F. dentata*. This result seems to point to a divergent selection pattern within *F. mediterranea* that is difficult to explain in a group of cryptic species.

The findings from this study confirm that the parameters used for morphometric analyses provide a good method for distinguishing between closely related *Forficula* species but are inadequate to discriminate between cryptic species within the *F. auricularia* complex [as previously demonstrated by González-Miguéns *et al.* (2020)]. Therefore, we underline the importance of integrating different approaches (molecular and morphological) in taxonomic studies, as accurate species identification is crucial for biodiversity assessments and for its conservation.

Updated knowledge of the Italian endemic *Forficula* and considerations on its conservation

After the present study, four Italian endemic species can be attributed to the genus *Forficula*: *F. apennina*, *F. silana*, *F. galvagnii*, and *F. orsinii* (Fig. 8). As already mentioned, *F. silana* and *F. orsinii* are typically mountainous species but can also be found at lower altitudes. *Forficula apennina* and *F. galvagnii* are instead closely related to the highest altitudes, where they generally live in stony grounds like the species of the genus *Chelidura* in the Alps and Pyrenees.

Forficula apennina (Figs 1C, 8E) is a very distinct, rare species, characterized by males with very elongated and simplified cerci (with only a small internal median tooth), especially in the macrolabial forms, in which the cerci can constitute almost half of the total length of the insect. Another characteristic of this species is given by the lack of the portion of the forewings protruding from the tegmina. The species was described by Achille Costa in 1882 on specimens he collected in Calabria on the Sila Piccola and on the Sila Grande (Costa 1882), though he reported having found the species on the Gran Sasso (Abruzzo) even before his expedition to Calabria. Since its description, *F. apennina* was found in other localities, and as already mentioned in the Introduction, it is known only from the Gran Sasso, Maiella (Abruzzo), Terminillo (Lazio), Pollino, and La Sila (Calabria) (Costa 1882, Ebner 1915, Maccagno 1933, Galvagni 1973, Vigna Taglianti 1994, 2005; Fig. 7). In the Fontana collection (Fondazione Museo Civico di Rovereto) is preserved a male specimen from Monte Velino, in the Velino-Sirente massif (Abruzzo), which represents a new locality for this endemic species. Most of the reports of *F. apennina* are related to altitudes above 2300 m, even if there are rare localities, probably relict, at altitudes of 1800 m or 2000 m. The range of *F. apennina* is highly fragmented, and its presence is reported with a certain continuity only for the Gran Sasso massif, where one of the authors (P.F.) found it several times (on Monte Portella and Monte Aquila, Fig. 8E, F) from the 1990s to 2024, observing, however, a certain rarefaction of the populations and a slight rise in altitude. Indeed, in the 1990s *F. apennina* was present in a large population north of the 'Duca degli Abruzzi' refuge (Gran Sasso) at about 2300 m altitude, detected several times over the years, but since 2000 this population seems to have disappeared or has reduced so much as to be undetectable with visual search methods (the use of traps for such rare species is obviously unthinkable). In recent years the species has been found slightly higher on Monte Aquila (Gran Sasso), at about 2400–2450 m, where it had not been found before.

Forficula silana (Figs 1D, 8A) is a well-characterized species, even if at first sight it is very similar to *F. auricularia*, especially regarding the structure of the cerci of the males that also in *silana* show both the macrolabic and cyclolabic forms. It is easily distinguished from *auricularia* by the absence, as in *F. apennina*, of the portion of the forewings protruding from the tegmina. *Forficula silana* is a characteristic species of the thermophilic beech forests of southern Italy (Fontana *et al.* 2004) but it can also reach higher altitudes, e.g. on Monte Sirino (Basilicata; Fig. 8A, B) where it is present at about 1800 m above sea level or at Piani di Ruggio (Pollino), at 1600 m, in both cases at the upper edge of the beech forest, populating stony meadows. The species is endemic to the Apennines from the Meta massif (Civitella Alfedena, Abruzzo) and Molise (Monte Pagano and Monti del Matese) along the main southern mountain groups (e.g. Partenii Mountains, Monte Sirino, Pollino and Sila massifs), south to the Nebrodi Mountains (Monte Soro) in Sicily (Fig. 7).

Forficula orsinii (Figs 1G, 8C) and *F. galvagnii* (Figs 1H, 8G), are two Italian endemic species, even if the first is known for a very modest incursion into the French Alpes-Maritimes (Fig. 7), as several other insects with the same chorotype. As in *F. apennina* and *F. silana*, *F. orsinii* and *F. galvagnii* are also characterized by the absence of the portion of the forewings protruding from the tegmina. These two taxa are distinct from one another by characters of male genitalia, male and female cerci, and partially by size. *Forficula galvagnii* is larger, while in *F. orsinii* male cerci, paired and seen dorsally, determine a marked '8' conformation (Vigna Taglianti 1999). Other relevant information about the morphology of these species is given in the previous paragraph. As discussed in detail by Vigna Taglianti (1999), these two species are largely sympatric, but rarely syntopic in few areas of Central Apennines, being that *F. orsinii* is distributed in a more extended range in various ecosystems of the mountain belt, while *F. galvagnii* is stenotopyc in the cacuminal belt (Vigna Taglianti 1999, Fontana *et al.* 2021; Fig. 7). These two species probably represent the result of speciation events in refugia related to the Pleistocene glacial reduction and fragmentation of montane ecosystems in the Apennines.

Forficula orsinii is known with considerable continuity for the Apennine areas between Emilia-Romagna and Campania, and it is particularly frequent in Abruzzo. An isolated population is also known in Veneto, on the Lessini Mountains (Galvagni and Fontana 1993; Fig. 7) and, recently, the species has been confirmed, after old and sporadic observations, for the Ligurian Alps and other northern Apennine areas (Poggi 2023). It is a

Figure 8. A, adult male of *Forficula silana* found on Monte del Papa at 1700 m a.s.l. (Monte Sirino massif, Appennino Lucano, Basilicata; 21/08/2024); B, habitat of *F. silana* on Monte del Papa at 1700 m a.s.l. (Monte Sirino, Appennino Lucano, Basilicata; 21/08/2024); C, adult male of *F. orsinii* found at Campo Imperatore (Abruzzo; 06/08/2023); D, habitat of *F. orsinii* on Maielletta Blockhaus (Majella, Abruzzo; 02/08/2023); E, adult male of *F. apennina* found on Monte Aquila (Gran Sasso, Abruzzo; 10/08/2020); F, adult male of *F. galvagnii* found on Monte Aquila (Gran Sasso, Abruzzo; 07/08/2023); G, habitat of *F. apennina* and *F. galvagnii* on Monte Aquila (Gran Sasso, Abruzzo; 07/08/2023). Photo: Paolo Fontana.

mountain species, present from the lower mountain level in beech clearings to the upper mountain level in pseudo-alpine meadows, and is known from a few hundred meters above sea level up to at least 2000 m. In this sense, the presence of *F. orsinii* at Campo Imperatore at an altitude of 2150 m (Fig. 8D) is emblematic, while a few hundred meters higher, on Monte Aquila, at 2350 m, *F. galvagnii* is found (Fig. 8F).

Forficula galvagnii, described as *Pseudochelidura* by Vigna Taglianti (1999), is a species typical of the highest altitudes of central-southern Apennines, known to date only from the Sibillini Mountains (Marche), the Laga Mountains, and the Gran Sasso (Abruzzo) mountain ranges extended in two National Parks (Fig. 7). In a few localities, *F. galvagnii* lives in the same areas and environments populated by *F. apennina* and, indeed, on the Gran Sasso (Monte Aquila, Fig. 8F), specimens of *F. galvagnii* and *F. apennina* can be found under the same stone.

Forficula silana and *F. orsinii*, precisely because of their lower ecological needs and relatively larger range, do not cause any concern regarding their conservation, except for what is generally dramatically observed worldwide for biodiversity. The situation regarding *F. apennina* and *F. galvagnii* is different. Considering the few locations for which these two species are known and their very clear ecological preferences for high-altitude habitats, which also perfectly coincide, their conservation, e.g. through the protection of their habitats, should be taken into account. Clearly global warming is the biggest problem for these two endemic species, but we should not underestimate tourist exploitation (mass hiking), which is increasingly affecting the most charismatic and evocative peaks, such as the few stations for which *F. apennina* and *F. galvagnii* are known.

SUPPLEMENTARY DATA

Supplementary data are available at *Zoological Journal of the Linnean Society* online.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

DATA AVAILABILITY

Sequences alignments are available on figshare (10.6084/m9.figshare.28408202). Additional information is available upon request.

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