

**Research article**

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***Myrmecophilus baronii* Baccetti, 1966: biogeography and genetics (Orthoptera: Myrmecophilidae)**

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**Abstract**

The presence of the ant cricket *Myrmecophilus baronii* Baccetti, 1966 is reconfirmed from the island of Pantelleria (Italy, Sicilian Channel), while new evidence of population clusters from northwest Malta is brought to light. The species is known from three separate geographical areas within the Mediterranean, spatially isolated from one another by the sea. Malta and Tunisia lie on opposite flanks of the Strait of Sicily, while Pantelleria, an island volcano of Quaternary age, lies on the graben that bisects the central Mediterranean area. The occurrence of the species in these three locations underscores the relevance of island biogeography in respect to taxa that lack the capacity to disperse over long distances unaided, as is the case of myrmecophilous crickets. The study considers passive dispersal, including sweepstakes routes, as a potential means of dispersal by *M. baronii*. It also includes an integrative systematics approach using both morphology and barcode sequencing of the 16S ribosomal RNA region and the cytochrome c oxidase subunit 1 (COI) gene. Subsequent phylogenetic analyses reveal that *Myrmecophilus baronii*, *M. orientalis* and *M. ochraceus* do not cluster with other *Myrmecophilus* species that had been DNA barcoded thus far. Given the species' conservation status, notably its Habitats Directive designation as a priority species within European territory, this work provides new insight into habitat preferences and thus provides an important basis for conservation of this species.

**Key words:** Mediterranean, Malta, Pantelleria, Tunisia, *Myrmecophilus orientalis*, *M. ochraceus*, ant cricket, dispersal, conservation.

**Introduction**

Discourses on biogeography, dispersal and evolutionary theory have captivated the attention of scientist explorers for centuries; the foundations of these were laid between the mid-18<sup>th</sup> and early 20<sup>th</sup> centuries in seminal works by individuals like Alexander von Humboldt, Alfred R. Wallace, Philip L. Sclater, Charles Darwin and Alfred Wegerer, among others (Lomolino 2010; Matthews & Triantis 2021). Robert MacArthur & Edward O. Wilson (1967) proposed and developed the classical theory of island biogeography (Whittaker & Fernández-Palacios 2007; Whittaker et al. 2008; Lomolino et al. 2010), which was elaborated further over the years (Lomolino et al. 2015; Santos et al. 2016; Graham et al. 2017; Whittaker et al. 2017; Matthews 2021; Lomolino et al. 2024; Lomolino & Whittaker 2025). While much has been achieved, unresolved questions remain (Ridley 1987;

Lomolino et al. 2015); such is the case of *Myrmecophilus baronii* Baccetti, 1966, a species of ant cricket that occurs on either side of the graben system that divides the Siculo-Tunisian strait (specifically in Malta and Tunisia) as well as on an island comprising a volcanic complex (Pantelleria), not known to have ever been connected subaerially to any other landmass (Civetta et al. 1988; Rotolo et al. 2017, 2021).

Some fifteen species of ant crickets occur within the pan-Mediterranean region, of which at least five taxa are known from the central Mediterranean area (Stalling 2015; Cigliano et al. 2025). These are *Myrmecophilus baronii* (species upon which this contribution is focused), *M. fuscus* Stalling, 2013, *M. quadrispinus* Perkins, 1899, *M. cottami* Chopard, 1922, and *M. ochraceus* Fischer, 1853 (Chopard 1943; Baccetti 1966; Harz 1969; Massa et al. 2012; Iorio et al. 2019; Cassar et al. 2023; Cigliano et al. 2023, 2025; Stalling 2024). In the case of *Myrmecophilus acervorum* (Panzer,

[1799]), it is highly likely that the records of this species from Sicily (Massa et al. 2012; Iorio et al. 2019) belong to *M. fuscus*, which appears to be widespread across localities on the northern shores of the western Mediterranean sub-basin (mainland Spain and France) and islands (extending from the sub-basin to the central Mediterranean area) including Mallorca, Corsica, Malta, and northeastern Sicily (Stalling 2015). Compared to other geographical regions, genetic studies on Mediterranean taxa, both terrestrial and marine, are, in general, quite lacking (Bartolo et al. 2020), and this also applies to species belonging to the genus *Myrmecophilus*. In fact, published material on the genetics of *Myrmecophilus* has to-date been limited to central European (Iorgu et al. 2023; Kaczmarczyk-Ziamba et al. 2024), Japanese (Komatsu et al. 2008, 2010, 2015), and Southeast Asian species (Hsu et al. 2020).

*Myrmecophilus baronii* was first collected on April 21<sup>st</sup>, 1965, by the myrmecologist Cesare Baroni Urbani in Malta, St Thomas Bay, l/o Marsascala (“Baia S. Tomaso”) and was described on the basis of three specimens, one male and two females currently deposited at the Museo Civico di Storia Naturale Giacomo Doria in Genoa (Baccetti 1966; 1973). Three female specimens were further collected from the Buskett woodlands between 1983 and 1984 [30.iv.1983: 2♀♀ with *Camponotus barbaricus* Emery, 1905; 30.iv.1984: 1♀ with *Messor structor* (Latreille, 1798) (Schembri 1984)]. Throughout this time, it was regarded as a taxon endemic to Malta. On March 30<sup>th</sup>, 1990, however, the species was found on Pantelleria, at “Lago Bagno dell’Acqua”, an endoreic saline body of water also known as “Specchio di Venere” (Baccetti et al. 1995). Following these discoveries, the species was found in Tunisia (Stalling 2014) from two localities: Bir Bou Rebka, northwest of Hammamet, and at El-Fahs, west of Zaghuan (Stalling & Cassar 2020). Subsequent to the Buskett and Lago Bagno dell’Acqua records reported respectively by Schembri (1984) and Baccetti et al. (1995), no other *M. baronii* records were noted from Malta or Pantelleria until recent years.

Dedicated searches for the species across a number of islands within the central Mediterranean area, carried out by

one of the present authors (L-FC) between 2020 and 2025, yielded new records for the main island of Malta (Cassar *in-press*) and for Pantelleria (see Results). Other islands investigated included Lampedusa, Linosa, Gozo (Ghawdex) and Comino (Kemmuna), the results of which are included in a separate contribution (Cassar, *in-press*), while random localities within Sicily’s Hyblean plateau were also subject to intensive field searches. The present paper explores the genetics of *M. baronii* from Malta, Pantelleria and Tunisia, in addition to two other species from the Mediterranean (both collected in Greece), notably *Myrmecophilus orientalis* Stalling, 2010 and *M. ochraceus*. It may be worth noting that, notwithstanding being placed within the genus *Myrmecophilus*, the three species in question do not share similar morphological traits with other taxa of the genus.

## Methodology

During field searches, ant cricket specimens were initially examined via the naked eye and subsequently determined by means of lenses, including a hand-held lens of varying magnification (10×, 20×) or a head-mounted loupe. Further examination of specimens was carried out using a pooter (aspirator with transparent tube to facilitate inspection in the field) and, whenever necessary, a hand-held field microscope (50×) connected to a tablet via Bluetooth. Searches were conducted within habitats deemed appropriate for nests of *Camponotus* species (see Results).

For the voucher specimens from Malta (Mb33M) and Pantelleria (Mb28Pnl), DNA was extracted from the sample using the QIAamp DNA tissue microkit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol and quantified using a Nanodrop 2000 spectrophotometer. A partial sequence of the mitochondrial 16S ribosomal RNA region was amplified with the primers, 16S22 and 16SR21, while the cytochrome c oxidase subunit 1 (COI) gene was amplified using the primer pairs LCO1490 and HCO2198 (Yeh & Yang 1997; Folmer et

**Table 1** – Provenance of *Myrmecophilus* spp. voucher samples, including spatial data.

Species	Specimen No.	Location	Coordinates	Specimen data
<i>M. baronii</i>	Mb33M	Ghajn Tuffieha, Malta	35°55'55"N, 14°20'40"E	18.xii.2024
<i>M. baronii</i>	Mb28Pnl	Lago Bagno dell’Acqua, Pantelleria, Italy	36°48'56"N, 11°59'03"E	x.2024
<i>M. baronii</i>	MYR0204	El Fahs, Tunisia	36°17'31"N, 9°48'33"E	12.iv.2010
<i>M. baronii</i>	MYR0210	El Fahs, Tunisia	36°17'31"N, 9°48'33"E	14.iv.2010
<i>M. baronii</i>	MYR0212	El Fahs, Tunisia	36°17'31"N, 9°48'33"E	14.iv.2010
<i>M. orientalis</i>	MYR1403	Kos, Greece	36°50'19"N, 27°12'02"E	27.v.2018
<i>M. orientalis</i>	MYR1406	Kos, Greece	36°50'19"N, 27°12'02"E	27.v.2018
<i>M. orientalis</i>	MYR1411	Kos, Greece	36°50'21"N, 27°12'00"E	27.v.2018
<i>M. orientalis</i>	MYR1412	Kos, Greece	36°50'58"N, 27°11'30"E	27.v.2018
<i>M. ochraceus</i>	MYR0196	Zakynthos, Greece	37°55'31"N, 20°42'14"E	06.x.2009

**Table 2** – Species and GenBank accession numbers for the sequences included in the COI and 16S datasets.

Species	Gene	Accession number	Reference
<i>Myrmecophilus manni</i>	COI	GU122504	Moulton et al. 2010
<i>Myrmecophilus balcanicus</i>	COI	MW750639	Iorgu et al. 2023
<i>Myrmecophilus balcanicus</i>	COI	MW750645	Iorgu et al. 2023
<i>Myrmecophilus gallicus</i>	COI	MW750640	Iorgu et al. 2023
<i>Myrmecophilus fuscus</i>	COI	MW750643	Iorgu et al. 2023
<i>Myrmecophilus nonveilleri</i>	COI	MW750638	Iorgu et al. 2023
<i>Myrmecophilus nonveilleri</i>	COI	MW750642	Iorgu et al. 2023
<i>Myrmecophilus nonveilleri</i>	COI	MW750641	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	COI	MW750636	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	COI	MW750637	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	COI	MW750644	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	COI	PP896884	Kaczmarczyk-Ziemba et al. 2024
<i>Myrmecophilus acervorum</i>	COI	PP896883	Kaczmarczyk-Ziemba et al. 2024
<i>Myrmecophilus acervorum</i>	COI	PP896882	Kaczmarczyk-Ziemba et al. 2024
<i>Myrmecophilus acervorum</i>	COI	PP896881	Kaczmarczyk-Ziemba et al. 2024
<i>Myrmecophilus manni</i>	16S	NC_011301	Fenn et al. 2008
<i>Myrmecophilus balcanicus</i>	16S	MW748155	Iorgu et al. 2023
<i>Myrmecophilus balcanicus</i>	16S	MW748149	Iorgu et al. 2023
<i>Myrmecophilus gallicus</i>	16S	MW748156	Iorgu et al. 2023
<i>Myrmecophilus fuscus</i>	16S	Not available	Iorgu et al. 2023
<i>Myrmecophilus nonveilleri</i>	16S	MW748154	Iorgu et al. 2023
<i>Myrmecophilus nonveilleri</i>	16S	MW748158	Iorgu et al. 2023
<i>Myrmecophilus nonveilleri</i>	16S	MW748157	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	16S	MW748153	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	16S	MW748148	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	16S	MW748159	Iorgu et al. 2023
<i>Myrmecophilus acervorum</i>	16S	PP886506	Kaczmarczyk-Ziemba et al. 2024
<i>Myrmecophilus acervorum</i>	16S	PP886507	Kaczmarczyk-Ziemba et al. 2024

al. 1994 as cited in Pradit et al. 2022). PCR amplifications were then performed in a total volume of 50  $\mu$ L, containing approximately 100 ng of DNA, a deoxynucleoside triphosphate mixture (0.2 mM each), supplemented to give a final concentration of 1.8 mM MgCl<sub>2</sub>, 0.625 U of OneTaq Quick Load 2 $\times$  Master Mix with Standard Buffer (New England Biolabs, Inc.), 0.5 pmol of each primer and 21  $\mu$ L of nuclease-free water. Amplifications were carried out in a GeneAmp thermocycler PCR system 2700 (Applied Biosystems, Foster City, CA, United States) according to the PCR programmes as per Pradit et al. (2022). PCR products were subsequently verified on 1% (w/v) agarose gel and purified using a Wizard<sup>®</sup> SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and sequenced at Genewiz (from Azenta Life Sciences) Leipzig, Germany.

For the other voucher specimens, molecular work was performed at the Biome-id in Wilhelmshaven, Germany. The COI gene was amplified using the primers jgLCO1490

and jgHCO2198 (Geller et al. 2013). PCR conditions were an initial denaturation for 5 min at 95 °C, then 35 cycles at 95 °C for 30 sec, 48 °C for 40 sec and 72 °C for 50 sec, with a final 7 min at 72 °C. All sequences were then manually checked by inspecting the chromatograms and compared to published sequences using the Basic Local Alignment Search Tool (BLAST) housed at the United States National Center of Biotechnology Information (Zhang et al. 2000).

Multiple alignments of the COI and 16S biomarkers were performed using the MAFFT algorithm L-INS-I (Kato & Standley 2013) on the NGPhylogeny portal (Lemoine et al. 2019). A dataset based on COI (659 nt) was analysed. This included 25 nucleotide sequences from GenBank (Table 2) together with the newly produced sequences. A second dataset based on a fragment of 16S (543 nt) was analysed and included 14 nucleotide sequences from GenBank (Table 2) together with the newly produced sequences. A third dataset included concatenated COI and 16S sequences. *Myrmecophilus manni* was used as outgroup taxon.

For the separate COI, 16S datasets as well as the concatenated COI and 16S dataset, Bayesian Inference (BI) was performed using MrBayes v. 3.2.7 (Ronquist et al. 2012) on the NGPhylogeny portal (Lemoine *et al.*, 2019). Maximum Likelihood (ML) analyses were carried out using PhyML (Guindon *et al.*, 2010) on the NGPhylogeny portal (Lemoine *et al.*, 2019), with the general time reversible + gamma distribution + invariable sites model (GTR + G + I) with 1000 bootstrap replicates (Nei & Kumar, 2000). BI analyses were run with the GTR + G + I model parameters estimated independently for each partition, with four Monte Carlo Marko Chains for 2 million generations. Nodal support was assessed by calculating the posterior probability (PP) values for each node of the resulting consensus tree after a burn-in value of 25 % of the trees. Both ML and BI analyses produced trees with a similar topology. Phylogenetic trees were viewed and edited in FigTree v. 1.4.4 (Rambaut, 2012). In Mega X, separate COI, 16S datasets as well as the concatenated COI and 16S dataset, pairwise genetic distances were estimated using the Kimura 2-parameter (K2P) nucleotide substitution model with 1,000 bootstrap replicates (Kumar et al. 2018).

The omission of subgeneric names in the present work is intentional, to exclude the said taxonomic rank.

## Results

Field research on *M. baronii* in Malta over the last years has demonstrated that the species maintains clustered populations on the northwestern coast of the main island (Majjistral area), with this constituting an important biodiversity

hotspot for conservation of the species (Cassar *in-press*), while new records are also regularly being confirmed within the geographical area. However, the San Tumas type locality has not rendered any recent confirmed records, notwithstanding dedicated searches within the general area. This is probably due to urban sprawl and the overdevelopment the locality has experienced over the decades since the species was discovered. The habitat within which the species occurs on the northwest coast of Malta is typically karstic, characterised by terra rossa soils, loose rocks or discarded ashlar stones of boulder size (>256 mm) with smaller cobble-size rock fragments (80–256 mm), and colonised by garrigue and steppic biotopes forming a mosaic. *Camponotus barbaricus* nests are predictably located under boulder-size rocks, where vegetation is sparse enough to allow for the ants to be able to forage around the nest's surroundings (Fig. 1).

During field visits to Pantelleria between September and October 2024, the presence of *M. baronii* was reconfirmed for the island, from the very same locality, the endorheic waterbody known as “Lago Bagno dell’Acqua” or “Specchio di Venere”, where it was originally discovered in March of 1990 (Baccetti et al. 1995). 2♂♂ and 1♀ were eventually collected for genetic analysis (Fig. 2).

The *M. baronii* specimens from Tunisia were collected from two sites, El Fahs and Bir Bou Rebka (Stalling 2014), located north of the Tunisian Sahel. The Bir Bou Rebka site lies in the limits of the eastern coast, northwest of Hammamet, while the El Fahs site (Fig. 3) is situated further inland near Zaghouan.

Notwithstanding differences in geomorphology, all the localities (Fig. 4) from which *M. baronii* (Fig. 5) was recorded (Malta, Pantelleria and north-eastern Tunisia)



**Fig. 1** – Typical karstic terrain on the northwest coast of Malta, between Ghajn Tuffieha and Manikata (collectively referred to as the Majjistral area), where *Myrmecophilus baronii* population clusters have been recorded (Photo credit: L-F. Cassar).



**Fig. 2** – Examining a *Camponotus* sp. nest with *Myrmecophilus baronii* on the sandy grass verge of the endoreic *Lago Bagno dell'Acqua* on Pantelleria (Photo credit: J.J. Borg).



**Fig. 3** – *Myrmecophilus baronii* habitat at El Fahs, Tunisia. Environment is characterised by dry sandy soils, relatively low shrubbery and an extensive 'ground armour' or loose stony terrain (Photo credit: T. Stalling).



**Fig. 4** – The six localities within the central Mediterranean area, comprising Tunisia, Pantelleria (Italy) and Malta, from where *Myrmecophilus baronii* has, to-date, been recorded.



**Fig. 5** – Adult male of *Myrmecophilus baronii* from Manikata, Malta, December 2024. Preserved specimen, leg. L-F Cassar. Scale bar: 1 mm. (Photo credit: T. Stalling).

lie within the Thermo-Mediterranean zone, characterised by shrub formations adapted to mild semi-arid climates and relatively nutrient-poor soils. In the case of El Fahs in Tunisia, the vegetation is dominated by *Salvia rosmarinus* Spenn., and *Calicotome* thickets [*C. villosa* (Poir.) Link or *C. spinosa* (L.) Link] and associated floral assemblages on sandy soils, with a predominance of angular rock fragments and pre-steppic environments. The biotopes that characterise the karstland within the Majjistral area in Malta comprise a mosaic of labiate and cushion garrigues (dominated by *Thymra capitata* (L.) Cav., and *Teucrium fruticans* L., for the former and *Euphorbia melitensis* Parl., for the latter floral assemblage), with an Ermes community typified by a suite of steppic species (represented by *Asphodelus aestivus* Brot., *Carlina involuocrata* Poir., *Ferula melitensis* Brullo, C. Brullo, Cambria, Giusso, Salmeri & Bacch., and *Drimia maritima* (L.) Stearn, among other species), and interspersed with elements of the composite garrigue and the phrygana, respectively characterised by *Phagnalon rupestre* ssp. *graecum* Batt., and *Anthyllis hermanniae* ssp. *melitensis* Brullo & Giusso (Devillers-Terschuren & Devillers 2001). The predominant vegetation on the verges of the Lago Bagno dell'Acqua in Pantelleria consists of *Cynodon dactylon* (L.) Pers., indicating a degree of disturbance (predominantly through trampling by visitors to this popular, unique site) and, potentially, of eutrophication.

In total, 10 COI and two 16S sequences were obtained during the present study. Prior to this work, there was no genetic data on GenBank of the species *M. baronii*, *M. orientalis* and *M. ochraceus*; this paper thus represents the first DNA barcodes for these species. These were submitted to GenBank and assigned the accession numbers listed in Table 3.

**Table 3** – List of barcode sequences produced in this study, with corresponding NCBI accession numbers.

Specimen	Species	COI	16S
Mb33M	<i>M. baronii</i>	PV342433	PV341485
Mb28Pnl	<i>M. baronii</i>	PV342432	PV341486
MYR0204	<i>M. baronii</i>	PV330305	-
MYR0210	<i>M. baronii</i>	PV330306	-
MYR0212	<i>M. baronii</i>	PV330307	-
MYR1403	<i>M. orientalis</i>	PV330301	-
MYR1406	<i>M. orientalis</i>	PV330302	-
MYR1411	<i>M. orientalis</i>	PV330304	-
MYR1412	<i>M. orientalis</i>	PV330303	-
MYR0196	<i>M. ochraceus</i>	PV330308	-

The COI sequences for specimens Mb33M, Mb28Pnl, MYR0204, MYR210 and MYR212 of the *M. baronii* voucher specimens from Malta, Pantelleria and Tunisia did not result in a close hit with other taxa belonging to the genus *Myrmecophilus* available on GenBank. Neither did the COI sequences of the *M. orientalis* specimens (MYR1403, MYR1406, MYR1411 and MYR1412 from Greece) or of the *M. ochraceus* specimen (MYR0196 also from Greece) result in a close hit on GenBank. The closest hits were between 82-84% similar identities to non-related species; such results are considered to represent very low scoring.

The 16S sequences for voucher specimens Mb33M and Mb28Pnl from Malta and Pantelleria respectively also did not result in a close hit on GenBank. The closest hits were 88% identical to a *Myrmecophilus kinomurai* specimen from Japan, with GenBank accession number AB818352 (Komatsu et al. 2008) and 87% identical to *Myrmecophilus americanus* Saussure, 1877 from Antigua, with GenBank accession number KR903772 (Chintauan-Marquier et al. 2016), both of which are considered to be low-score hits.

In fact, the consensus COI tree obtained (Fig. 6) demonstrates that the specimens of *M. baronii*, *M. orientalis* and *M. ochraceus*, clustered separately to previously published sequences (Moulton et al. 2010; Iorgu et al. 2023; Kaczmarczyk-Ziemia et al. 2024) of other species within the genus *Myrmecophilus*. The same was evident in the 16S tree (Fig. 7) and concatenated (COI + 16S) tree (Fig. 8), whereby *M. baronii* clustered separately to previously published sequences of *M. acervorum*, *M. balcanicus*, *M. nonveilleri*, *M. gallicus* and *M. fuscus*.

**Table 4** – Estimates of evolutionary divergence between *Myrmecophilus* sequences. The number of base differences per site between sequences are shown. This analysis involved 16 COI nucleotide sequences.

	<i>M. baronii</i>	<i>M. orientalis</i>	<i>M. ochraceus</i>	<i>M. acervorum</i>	<i>M. gallicus</i>	<i>M. balcanicus</i>	<i>M. fuscus</i>	<i>M. nonveilleri</i>	<i>M. manni</i>
<i>M. baronii</i>	-								
<i>M. orientalis</i>	0.16	-							
<i>M. ochraceus</i>	0.18	0.17	-						
<i>M. acervorum</i>	0.20	0.18	0.21	-					
<i>M. gallicus</i>	0.20	0.21	0.21	0.12	-				
<i>M. balcanicus</i>	0.21	0.19	0.21	0.12	0.12	-			
<i>M. fuscus</i>	0.22	0.21	0.21	0.13	0.03	0.12	-		
<i>M. nonveilleri</i>	0.22	0.21	0.24	0.14	0.15	0.15	0.16	-	
<i>M. manni</i>	0.22	0.21	0.22	0.21	0.21	0.19	0.21	0.22	-

**Table 5** – Estimates of evolutionary divergence between *Myrmecophilus* sequences. The number of base differences per site between sequences are shown. This analysis involved 6 16S nucleotide sequences.

	<i>M. baronii</i>	<i>M. nonveilleri</i>	<i>M. acervorum</i>	<i>M. balcanicus</i>	<i>M. gallicus</i>	<i>M. manni</i>
<i>M. baronii</i>	-					
<i>M. nonveilleri</i>	0.21	-				
<i>M. acervorum</i>	0.23	0.08	-			
<i>M. balcanicus</i>	0.23	0.07	0.09	-		
<i>M. gallicus</i>	0.24	0.08	0.10	0.09	-	
<i>M. manni</i>	0.25	0.19	0.17	0.19	0.21	-

**Table 6** – Estimates of evolutionary divergence between *Myrmecophilus* sequences. The number of base differences per site between sequences are shown. This analysis involved 9 concatenated COI + 16S nucleotide sequences.

	<i>M. baronii</i>	<i>M. orientalis</i>	<i>M. ochraceus</i>	<i>M. acervorum</i>	<i>M. gallicus</i>	<i>M. balcanicus</i>	<i>M. fuscus</i>	<i>M. nonveilleri</i>	<i>M. manni</i>
<i>M. baronii</i>	-								
<i>M. orientalis</i>	0.17	-							
<i>M. ochraceus</i>	0.19	0.17	-						
<i>M. acervorum</i>	0.22	0.18	0.20	-					
<i>M. gallicus</i>	0.22	0.21	0.21	0.10	-				
<i>M. balcanicus</i>	0.22	0.19	0.21	0.11	0.10	-			
<i>M. fuscus</i>	0.22	0.21	0.21	0.12	0.03	0.11	-		
<i>M. nonveilleri</i>	0.22	0.21	0.24	0.11	0.12	0.11	0.16	-	
<i>M. manni</i>	0.24	0.21	0.22	0.19	0.21	0.20	0.21	0.20	-

The genetic distance among *Myrmecophilus* species, based on the COI, selected for this study varied between 0.03 and 0.22 (the lowest value of K2P distance was between *M. fuscus* and *M. gallicus*), as also reported in Kaczmarczyk-Ziemia et al. (2024). The highest was between various species (Table 4) also showing a great interspecific divergence between the species currently placed in the genus *Myrmecophilus*. The genetic distance among the species based on 16S as well as the concatenated dataset (Tables 5 and 6) also showed similar large interspecific divergence.

## Discussion and Conclusion

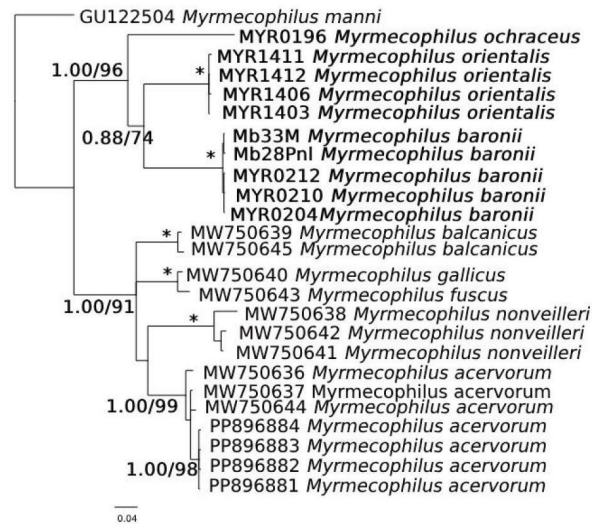
The COI consensus phylogenetic tree (Fig. 6) shows that *Myrmecophilus acervorum*, *M. fuscus*, *M. gallicus* and *M. nonveillieri*, all of which occur in the Mediterranean, clustered together. With the exception of *M. fuscus*, which occurs on the northern shores of the western sub-basin as well as within the central Mediterranean area, the provenance of all other three species extends northward, from the northern Mediterranean shores to the central European landmass, particularly for *M. acervorum*, whose range extends to northern Europe as well as to central Asia. The geographical range of *M. baronii* and *M. orientalis*, in contrast, is restricted to the central Mediterranean area and the easternmost boundaries of the Eastern Mediterranean sub-basin respectively (Stalling 2010, 2020; Cigliano et al. 2025), while that of *M. ochraceus* is pan-Mediterranean (Cigliano et al. 2025).

The genetic results of the present work demonstrate a degree of disparity between *M. baronii* and *M. orientalis* with the former cluster. Interestingly, both species also exhibit morphometric traits that are incongruous with those of the species that clustered, suggesting a somewhat ambiguous taxonomic relationship with other members of the genus *Myrmecophilus* (see also Fig. 7 and Fig. 8). At this juncture, the present authors favour the adoption of a conservative approach, consequently referring to these taxa as the ‘*baronii* species group’. This issue exists with respect to many species of the genus *Myrmecophilus*, and a future extensive review that includes species outside the Mediterranean region is needed to clarify the situation.

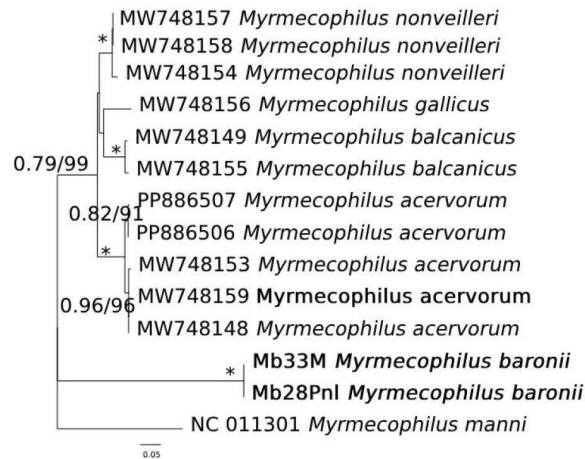
It would also be interesting to establish dispersal timelines. *M. baronii* has, so far, been recorded from (i) the North African mainland, located on the southern flank of the Siculo-Tunisian strait; (ii) Malta, an island located on the northern flank of the graben, which was definitely connected intermittently to Sicily via terrestrial corridors on a number of occasions during the Pleistocene Epoch; and (iii) Pantelleria, a volcanic island that lies between Sicily and Tunisia, never known to have been connected with another landmass. It is worth adding that although Pantelleria presently lies closer to Tunisia (70 km) than to Sicily (<100 km), during the Quaternary lowstands it would have been closer to Sicily when the Adventure Bank platform and associated seamounts

were exposed subaerially (Grasso et al. 1985). Nevertheless, there is no evidence of any physical connection, such as via a terrestrial isthmus between the Sicilian coast and the island volcano throughout the latter’s entire subaerial existence of >300,000 years (Villari 1974; Civetta et al. 1988; Rotolo et al. 2021). Nevertheless, the species maintains an established presence in all three locations. Whether or not the distribution of *M. baronii* was more widespread (for example prior to the Zanclean Flood at the onset of the Pliocene Epoch) and then experienced a regression in its range as environmental conditions changed, as do paleoendemic taxa, or whether it actually is more widespread and overlooked (e.g. an undiscovered presence on Sicily, among other locations), the species’ presence on Pantelleria is a question that remains unresolved. That ant crickets are an elusive group, there is little doubt; they are small, burrowing organisms without the capacity to fly (apterous) nor travel significant distances independently and unaided. Ant crickets are also obligate kleptoparasites, which means they are not expected to journey far from their ant hosts. Their mode of dispersal therefore remains enigmatic, although assumed to occur via passive means, either through sweepstakes routes or as a result of kleptoparasite-host exploitation. In the case of *M. baronii*, its ant host predominantly comprises the large and relatively robust *Camponotus* group. However, it is not envisaged that species like *Camponotus barbaricus*, notwithstanding its size, is capable of transporting adult or nymph specimens of *M. baronii* over long distances. Might nuptial flights in autumn (annual swarming rituals when members of a colony undertake a mating flight and disperse widely over the terrain to establish new colonies between September and October) aid the dispersal of ant crickets? If so, what morphological mechanisms exist to facilitate such a ‘hitch-hike’ mode of dispersal? Could ova of myrmecophilous crickets somehow attach themselves to ants prior to undertaking nuptial flights? Another possibility would be drifting across the sea in ant nests inside plant parts (e.g. torn off root stumps of trees with soil material), or through agriculture-associated trade, vis-à-vis the commercial transportation of fruit trees in large pots (even if the notion of *Camponotus barbaricus* establishing a nest in potted plants is atypical). As indicated above, the proposed establishment of dispersal timelines through further comprehensive genetic analysis can shed light on the various pending questions.

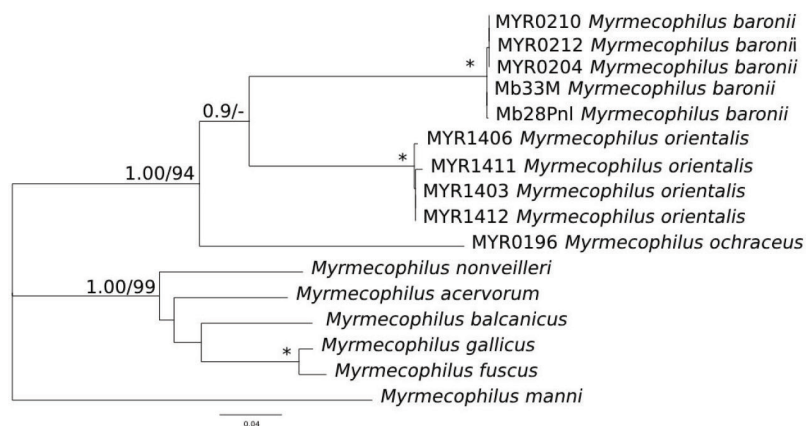
In addition to the results on genetics and biogeography presented herein, the findings are also remarkable from a nature conservation perspective. *M. baronii* is listed in Annex 2 of the Habitats Directive (Council Directive 92/43/EEC of May 21, 1992, on the conservation of natural habitats and of wild fauna and flora) as a priority species and is therefore of high conservation interest. The current records provide an important basis for proactive protection of this species; enhanced knowledge of the species’ habitat requirements and methods used to find specimens can also contribute to conservation efforts.



**Fig. 6** – Consensus phylogenetic tree of species of *Myrmecophilus* species inferred from COI sequences. The numbers on branches are Bayesian posterior probabilities (BPP) and bootstrap (BS) values (> 0.7 and 70% respectively). Asterisk (\*) indicates full support (= 1.00 and 100%). The scale bar represents number of substitutions per site.



**Fig. 7** – Consensus phylogenetic tree of species of *Myrmecophilus* species inferred from 16S sequences. The numbers on branches are Bayesian posterior probabilities (BPP) and bootstrap (BS) values (> 0.7 and 70% respectively). Asterisk (\*) indicates full support (= 1.00 and 100%). The scale bar represents number of substitutions per site.



**Fig. 8** – Consensus phylogenetic tree of species of *Myrmecophilus* species based on concatenated sequences of COI and 16S mtDNA. The numbers on branches are Bayesian posterior probabilities (BPP) and bootstrap (BS) values (> 0.7 and 70% respectively). Asterisk (\*) indicates full support (= 1.00 and 100%). The scale bar represents number of substitutions per site.

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