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# A new trans-Ionian spider species for the Italian fauna: *Habrocestum graecum* Dalmas, 1920 (Araneae, Salticidae)

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**SUMMARY**

The salticid spider *Habrocestum graecum* Dalmas, 1920, until now only known from Greece, is for the first time recorded in Italy. Observations on ecology and behavior are also reported and pictures of its habitus and genitalia are provided. Furthermore, the first DNA barcode sequence for *H. graecum* is produced and made publicly available. The species has been observed in Puglia, in South-Eastern Italy, and a trans-Ionian dispersal pattern is most likely the cause of its presence both in Greece and Southern Italy, as reported for other taxa with similar distribution in different animal groups.

## INTRODUCTION

Italy holds the highest spider diversity in Europe with 53 families, 440 genera and 1700 recorded species, 224 (13%) of which are endemic to the country (Pantini and Isaia 2019). These numbers are constantly growing thank to an effort in describing new taxa (e.g., Weiss and Sarbu 2021, Bosmans and Trotta 2021, Ballarin and Pantini 2022a,b, Isaia et al. 2022), to new faunistic records (e.g., Bolognin et al. 2021, Caria et al. 2021, Dentici and Amata 2021, Dentici et al. 2022; Steinwandter et al. 2022) and to the detection of allochthonous species (e.g., Di Pompeo et al. 2011, Kulczycki et al. 2012, Nardi et al. 2019, Pantini et al. 2020). DNA barcoding relies on the analysis of a specific small genomic region (for metazoans usually a fragment of the mitochondrial cytochrome *c* oxidase subunit 1) for molecular species identification. This is particularly useful in spiders for identifying juvenile specimens, in which diagnostic morphological characters are still not visible, for matching males and females of the same species and as an aid to traditional

taxonomy when dealing with cryptic or problematic taxa (Hebert et al. 2003, Domènech et al. 2022b). In contrast to other European countries (see, e.g., Astrin et al. 2016, Domenec et al. 2022a), in Italy no projects are currently focusing on creating a DNA barcode library of Italian spider species. Despite the effort, less than 1/3 of European spider species have been barcoded so far (Nentwig et al. 2022). The salticid genus *Habrocestum* Simon, 1876 currently holds 51 described species distributed in Europe, Asia, Africa and Oceania (World Spider Catalog 2022). Ten species are known to occur in Europe, two of which are recorded in peninsular Italy: *H. latifasciatusm* (Simon, 1868) also known from Greece, Turkey, Libya and the Middle East, and *H. pullatum* Simon, 1876, distributed also in France and Spain (Nentwig et al. 2022). *Habrocestum pullatum* has been recorded in Tuscany, in Northern Italy, though with a single specimen collected in 1923 by di Caporiacco. On the other hand, *H. latifasciatum* is recorded in Puglia (di Caporiacco 1951, Kritscher 1969, Hansen 2005), in South-Eastern Italy, showing an interesting trans-Ionian distribution. Trans-Ionian and trans-Adriatic distribution patterns have been observed for vertebrate (Blain et al. 2016) and invertebrate taxa (Gridelli 1950, di Caporiacco 1951, Jesse et al. 2009, Çiplak et al. 2010, Korábek et al. 2014, Schifani and Alicata 2019, Hinojosa et al. 2021). This is most probably a result of quaternary land bridges connecting Southern Italy with the Balkans and/or of the Miocenic fragmentation of the Aegeis land (Schifani and Alicata 2019). In the present work, we report about the first observation of the once Greek endemic spider species *Habrocestum graecum* Dalmas, 1920 in Italy and provide the first genetic characterization of the species via DNA barcoding (Figure 1).

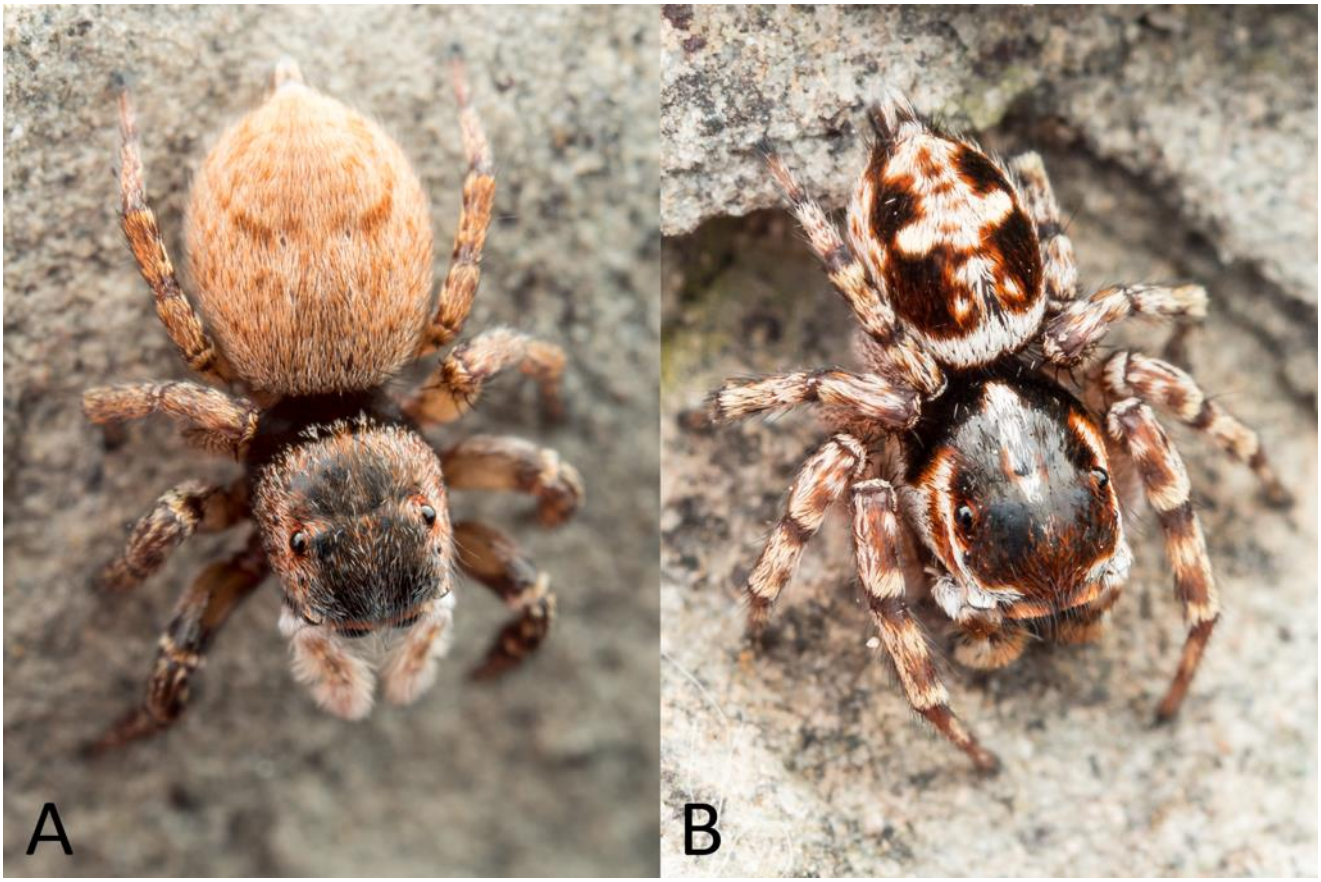


Figure 1. *Habrocestum graecum*, habitus (A-B). A: adult female; B: adult male.

## MATERIALS AND METHODS

Spiders were observed on the field, hand collected and stored in 70% and 96% ethanol. Specimens were examined with a Zeiss Stereomicroscope II and a Leica MZ16 stereoscopic microscopes and photographed with an Olympus E-M5 mkII + a Zuiko 60mm f2.8 macro lens. Identification was carried out following Metzner (1999).

Legs from one adult female specimen were used for DNA extraction using the NucleoSpin® DNA Insect kit (Macherey-Nagel) following the manufacturer protocol. The barcode region of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene was amplified via PCR with the primer couple LCO1490-HCO2198 (Folmer et al. 1994). PCR conditions where: a 3-minute denaturation step at 94°C, 35 cycles (denaturation: 50 seconds at 94°C; annealing: 45 seconds at 52°C; elongation: 50 seconds at 72°C) and a final elongation step of 5 minutes at 72°C. Quality check on the PCR products was done via gel electrophoresis on a 1% agarose gel and PCR products were cleaned with the ExoSAP-IT Product Cleanup Reagent (Thermo Fisher Scientific) prior to sequencing. Sanger sequencing was done at Macrogen Europe (Amsterdam, The Netherlands). Chromatograms were inspected using the software SeqTrace v.0.9.0 (Stucky 2012), and potential contaminants were screened using BLASTn (Zhang and Madden 1997). The obtained sequence was queried against the Bold Systems Public Record Barcode Database (Ratnasingham and Hebert 2007) and submitted to GenBank. Specimens analyzed in this study are currently deposited at MNHT, Museum of Natural History of Trieste, Trieste, Italy.


Figure 2. *Habrocestum graecum*, genitalia (A-C). A: female, epigynum; B-C: male, left pedipalp, ventral view (B), prolateral view (C).

*Habrocestum graecum* Dalmas, 1920 Figs. 1, 2.

> *Habrocestum graecum* Dalmas, 1920a: 67 (Dmf).
>
> *Habrocestum graecum* Prószyński, 1987: 30-31 (m; f=*Aelurillus* sp.).
>
> *Habrocestum graecum* Metzner, 1999: 60, f. 25a-i (m, Df).

Examined material. ITALY: Puglia, Taranto, Martina Franca, 310 m, 40°42'27.6"N, 17°22'15.2"E, 2 ♂, 2 ♀, 23 May 2022, ibidem, 1 ♂, 1 ♀ 11 July 2022, all leg., M. Caroli (MNHT).

Remarks. The presence of this species, previously only known from Greece (World Spider Catalog 2022), is for the first time reported in Italy, were it was observed on dry stone walls surrounded by vegetation in the

countryside. In Greece it has been observed under loose pine bark, on limestone rocks directly on the coast, on river gravel and on sandy areas (Metzner 1999). In the Italian observation area, *H. graecum* specimens were not yet mature in mid-May. The first mature specimens were seen at the end of May and a mating attempt was witnessed in the wild: the male was observed crossing the legs from the first pair flat in front of the prosoma and moving left and right trying to slowly approach the female. The attempt was unsuccessful. In October, despite the unusually high temperatures, no adults or juveniles were observed in the same area. Specimens have been observed to prey mainly on Diptera, other small insects (Aphrophoridae) and other spiders, even belonging to their own species.

DNA barcode. Sequencing resulted in a 689bp fragment of the COI mitochondrial gene. No stop codons or frame shifts were observed when translating the nucleotide sequence into amino acids. Top 10 matches found in the Bold Systems Public Record Barcode Database are reported in Tab. 1. The obtained sequence was submitted to GenBank (accession number OP825024).

Table 1. Top 10 matches of the produced *H. graecum* barcode sequence with the Bold Systems Public Record Barcode Database.

| Order | Family | Genus | Species | Similarity | Status |
|---|---|---|---|---|---|
| Araneae | Salticidae | *Myrmarachne* | *M. tristis* | 89.51% | Published |
| Araneae | Salticidae | *Chapoda* | *C.* cf. *inermis* | 89.41% | Published |
| Araneae | Salticidae | *Hasarius* | *H. adansoni* | 89.39% | Published |
| Araneae | Salticidae | *Myrmarachne* | *M. robusta* | 89.20% | Published |
| Araneae | Salticidae | *Myrmarachne* | *M. robusta* | 89.20% | Published |
| Araneae | Salticidae | *Myrmarachne* | *M. robusta* | 89.20% | Published |
| Araneae | Salticidae | *Myrmarachne* | *M. melanotarsa* | 89.20% | Published |
| Araneae | Salticidae | *Myrmarachne* | *M. melanotarsa* | 89.20% | Published |
| Araneae | Salticidae | *Myrmarachne* | *M. melanotarsa* | 89.20% | Published |
| Araneae | Salticidae | *Myrmarachne* | *M. melanotarsa* | 89.20% | Published |

## RESULTS AND DISCUSSION

Even if showing a more restricted distribution range, *H. graecum* exhibits the same trans-Ionian distribution pattern that can be observed in the congeneric *H. latifasciatum*, being found in Greece and then across the Ionian Sea in the South-Eastern Italian region of Puglia. The microhabitat of the observed Italian population, the typical dry stone walls that characterize the countryside of Puglia, fits with what is known regarding the habitat requirements of the species in Greece, often found in strongly sun-lit habitats as sandy areas, river gravel and exposed rocks (Metzner 1999). Being the Mediterranean climate of Southern Italy similar to that of Greece, where *H. graecum* is widely distributed, it is likely that more populations of the species could be found in areas neighboring the locality where this first Italian population was observed. Other examples of spider species showing a similar trans-Ionian or trans-Adriatic condition are the salticid *Heliophanus equester* L. Koch, 1867, the agelenid *Maimuna vestita* (C. L. Koch, 1841) and the eresid *Eresus walckenaeri* Brullé, 1832 (Pantini and Isaia, 2019, Nentwig et al. 2022). The study of spider fauna in Southern Italy is limited if compared to that of northern regions, as an effect of the historical distribution and focus of Italian arachnologists (Pantini and Isaia 2019). This is reflected in a lower number

of species recorded in southern regions, despite the great habitat and climatic diversity that characterizes peninsular Italy. Recent faunistic works focusing on spiders in southern regions show how easily new regional and national records can originate from these areas (Ijland et al. 2012, Ijland and van Helsdingen 2019, Pantini et al. 2020, Trotta 2020, Dentici et al. 2022). Given that other species in the same family and even in the same genus show a similar distribution pattern to that observed for *H. graecum*, and that this species is not known to exhibit synanthropic habits, it is unlikely that the observed population in Italy is the result of a human-mediated introduction. The presence of this species probably went unnoticed due to the above-mentioned little attention that the arachnofauna of southern regions received historically. More detailed arachnological surveys in these areas could lead to the discovery of additional *H. graecum* populations, and even to more taxa showing a trans-Ionic or trans-Adriatic distribution, shedding light on the processes shaping the distribution of species across the Mediterranean in the last million years.

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