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First record of the genus *Thyreosthenius* (Araneae: Linyphiidae) from the Iberian Peninsula with notes on the host preference of the myrmecophilic *T. biovatus*

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Abstract. The presence of the spider genus *Thyreosthenius* Simon, 1884 (Linyphiidae) is for the first time confirmed in the Iberian Peninsula with an observation of the myrmecophile species *T. biovatus* (O. Pickard-Cambridge, 1875). Four adult females and two sub-adult male specimens were collected inside mound nests belonging to the red wood ant species *Formica lugubris* Zetterstedt, 1838 in the Aragon and Catalonia regions of Spain. This represents the second known record of an occurrence of *T. biovatus* in association with *F. lugubris*, and the first for Continental Europe. In addition, the existing information on the host preference of this species is reviewed and discussed in relation to the new data from the Spanish Pyrenees.

Keywords: ant association, *Formica lugubris*, myrmecophily, new record, RWA, Spain, spider

Zusammenfassung. Erstnachweis der Gattung *Thyreosthenius* (Araneae: Linyphiidae) von der Iberischen Halbinsel mit Bemerkungen zur Wirtspräferenz der myrmekophilen Art *T. biovatus*. Die Präsenz der Spinnengattung *Thyreosthenius* Simon, 1884 (Linyphiidae) auf der Iberischen Halbinsel wird durch eine Beobachtung der myrmekophilen Art *T. biovatus* (O. Pickard-Cambridge, 1875) bestätigt. Vier adulte Weibchen und zwei subadulte männliche Exemplare wurden in Hügelnestern der Waldameisenart *Formica lugubris* Zetterstedt, 1838 im spanischen Aragonien und Katalonien gesammelt. Dies stellt den zweiten bekannten Nachweis einer Vergesellschaftung von *T. biovatus* mit *F. lugubris* dar, sowie den ersten für Kontinentaleuropa. Zusätzlich werden die vorhandenen Informationen zur Wirtspräferenz dieser Art gesichtet sowie in Zusammenhang mit den neuen Daten aus den spanischen Pyrenäen diskutiert.

The Iberian Peninsula hosts an extremely diverse spider fauna, with 1493 recorded species, 282 of which are endemic (Branco et al. 2019). Continental Spain, with 1386 species, represents one of the most spider-rich areas in Europe, ranking third after Italy and France (Nentwig et al. 2023).

Myrmecophily is defined as the ability to live in a close association with ants, from foraging alongside them to spending the whole life cycle inside ant nests (Wasmann 1894, Donisthorpe 1927, Hölldobler & Wilson 1990). Cushing (1997, 2012) reviewed this phenomenon in spiders and reported this lifestyle in 13 different spider families. Linyphiidae, the second largest family in spiders (World Spider Catalog 2023), holds the highest number of myrmecophilic taxa, with ten species from nine genera (Cushing 1997, 2012) (Tab. 1). The linyphiid genus *Thyreosthenius* Simon, 1884 includes two species, the free-living Holarctic *Thyreosthenius parasiticus* (Westring, 1851) and the myrmecophilic Palearctic *Thyreosthenius biovatus* (O. Pickard-Cambridge, 1875) (World Spider Catalog 2023). In Europe, *T. biovatus* is widespread in northern and central countries, while it has never been recorded from Iberia, the Balkans (excluding Bulgaria), Belarus, and Lithuania (Nentwig et al. 2023). This species lives inside the nests of ants belonging to the genus *Formica* Linnaeus, 1758, including mound building red wood ant species (RWA) belonging to the *Formica rufa* group, such as *Formica aquilonia* Yarrow, 1955, *Formica lugubris* Zetterstedt, 1838, *Formica polyctena* Foerster, 1850, *Formica pratensis* Retzius,1783 and *Formica rufa* Linnaeus, 1761, and the non-RWA species *Formica fusca* Linnaeus, 1758 and *Formica sanguinea* Latreille, 1798 (Bösenberg 1899, Simon 1926, Bristowe 1939, Wiehle 1960, Palmgren 1976, Robinson 1998, Parmentier et al. 2014, 2015, 2020, Castellucci et al. 2022). Due to the low amount of cuticular hydrocarbons (CHCs) registered for *T. biovatus* (Parmentier et al. 2017), it is likely that its integration within the colonies happens by means of chemical insignificance (Lenoir et al. 2001, 2013, Witte et al. 2008).

Most of the host association data available for *T. biovatus* originate from Central and Northern European countries, while little is known about its ecology at the southern limits of its distribution range. Only recently, Castellucci et al. (2022) shed light on its host preferences in the Italian Alps, recording associations with three different RWA species, including the first observation of *F. aquilonia* as a host. The only available information about this species in the Pyrenees comes from Simon (1926), who reported it living in the nests of *Formica fusca* on the French side of the mountain range. In this study, we report about the first record of the genus *Thyreosthenius* for the Iberian Peninsula, based on new data from the Spanish Pyrenees.

Materials and Methods

Fieldwork was conducted in 2017 and 2021 in the surroundings of Benasque, Huesca, and Toses, Girona, in the Spanish Pyrenees. A couple of liters of material were collected from the central part of RWA mound nests and sifted using an entomological sieve with an 8 × 8 mm mesh on a white fabric sheet, to facilitate the observation of myrmecophile spiders. These, and RWA workers, were then hand collected and stored in 70% or 96% ethanol. After the sifting phase, residual nest material and worker ants were carefully placed back on the mound to minimize disturbance. Spiders were examined with a stereoscopic microscope and photographed using a BK+ Imaging System from Visionary Digital equipped with a Canon EOS 7D camera. Identification was carried out following the keys by Roberts (1987). Ants were examined with a stereoscopic microscope and photographed with a Canon

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Tab. 1: List of myrmecophile Linyphiidae species, with information on their known host ant species

Species	Host ant species	References
Acartauchenius scurrilis (O. Pickard-Cambridge, 1873)	*Formica rufa* Linnaeus, 1761, *Lasius flavus* (Fabricius, 1782), *Tetramorium caespitum* (Linnaeus, 1758)	Cushing (1997)
Diastanillus pecuarius (Simon, 1884)	*Formica* cf. *fusca* (Linnaeus, 1758), *F. lemani* Bondroit, 1917	Cushing (2012)
Evansia merens O. Pickard-Cambridge, 1901	*Formica cunicularia* Latreille, 1798, *F. fusca* (Linnaeus, 1758), *F. sanguinea* Latreille, 1798, *Lasius niger* (Linnaeus, 1758)	Cushing (1997)
Grammonota pictilis (O. Pickard-Cambridge, 1875)	*Atta texana* (Buckley, 1860)	Cushing (1997)
Masoncus pogonophilus Cushing, 1995	*Pogonomyrmex badius* (Latreille, 1802)	Cushing (1997)
Masoncus sp.	*Atta texana* (Buckley, 1860)	Cushing (1997)
Pseudomaro aenigmaticus Denis, 1966	*Lasius flavus* (Fabricius, 1782)	Cushing (2012)
Thyreosthenius biovatus (O. Pickard-Cambridge, 1875)	*Formica aquilonia* Yarrow, 1955, *F. fusca* (Linnaeus, 1758), *F. lugubruis* Zetterstedt, 1838, *F. polyctena* Foerster 1850, *F. pratensis* Retzius 1783, *F. rufa* Linnaeus, 1761, *F. sanguinea* Latreille, 1798	Cushing (1997), Parmentier et al. (2020), Castellucci et al. (2022)
Scotinotylus formicarius (Dondale & Redner, 1972)	*Formica obscuripes* Forel, 1886	Cushing (1997)
Syedra myrmicarum (Kulczyński, 1882)	*Manica rubida* (Latreille, 1802), *Formica* sp.	Cushing (2012)

MP-E 65mm f/2.8 1–5 × macro lens mounted on a Canon 1300D camera. Measurements were acquired from photos using the software ImageJ (Schneider et al. 2012). Identification followed the key provided by Seifert (2021).

Results

New record for Spain

Thyreosthenius biovatus (O. Pickard-Cambridge, 1875)

(Figs 1, 2)

For a complete list of taxonomic references see the World Spider Catalog (2023).

Examined material. SPAIN: 1 ♀, Benasque, Huesca, Aragon, 42.6126°N, 0.5572°E, 1927 m a.s.l., 14. Jul. 2021, inside *F. lugubris* mound nest, F. Castellucci leg.; 3 ♀♀, 1 sub-adult ♂, Benasque, Huesca, Aragon, 42.6135°N, 0.5570°E, 1912 m a.s.l., 14. Jul. 2021, inside *F. lugubris* mound nest, F. Castellucci leg; 1 sub-adult ♂, Plans de Querol, Toses, Girona, Cataluña, 42.3123°N, 2.0310°E, 1680 m a.s.l., 13. Oct. 2017, inside *F. lugubris* mound nest, F. García leg.

Distribution. Europe, Russia (Europe to Far North East) (World Spider Catalog 2023). First record for Spain and the Iberian Peninsula.

Ecology. Spiders were collected inside mound nests belonging to the RWA species *Formica lugubris*, between 1680 and 1927 m a.s.l. in a montane pine forest (Fig. 3).

Discussion

Thyreosthenius biovatus was already known to occur in the French Pyrenees (Simon 1926). Its presence in Spain probably went unnoticed due to its myrmecophile lifestyle and the lack of focused searches carried out with the specific methods needed for inventorying ant-associates. These peculiar taxa are often neglected during classic biodiversity inventories based on the use of traditional sampling techniques such as pitfall

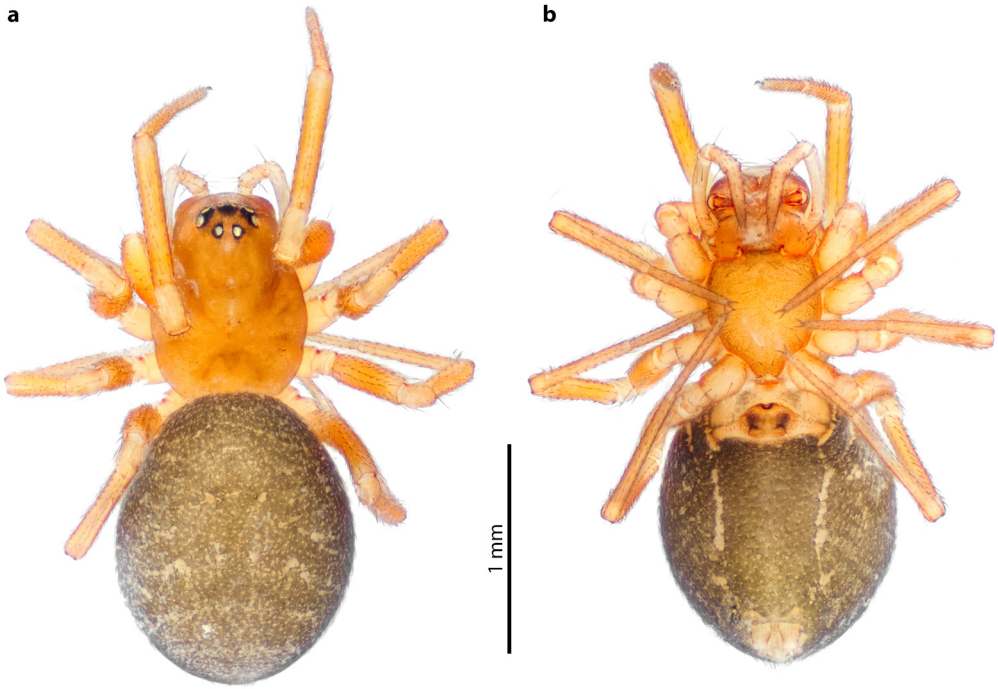

Fig. 1: *Thyreosthenius biovatus* female, habitus. **a.** dorsal view; **b.** ventral view

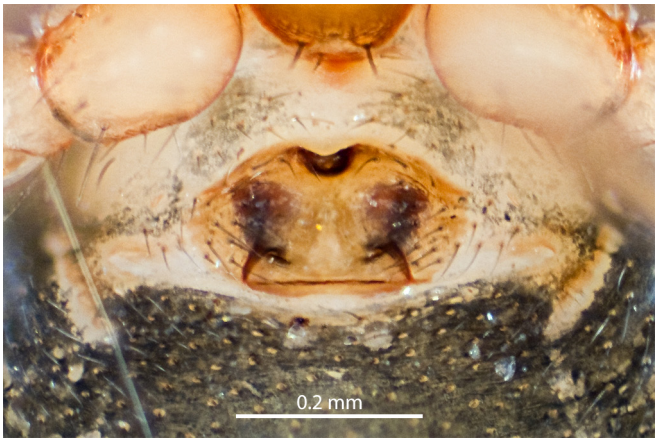

Fig. 2: *Thyreosthenius biovatus* female, epigyne

trapping, sweep netting, or the use of beating trays. Moreover, the number of studies focusing on myrmecophile spiders in Southern Europe is extremely low when compared to Central and Northern countries (Castellucci et al. 2022, Lenzini et al. 2022). Given the known presence of some of its host ant species in other regions of Spain and Portugal (Seifert 2021), it is likely that the actual distribution of *T. biovatus* in the Iberian Peninsula extends further away from the Pyrenees, and that this species is more widespread and abundant than previously thought, as observed in other countries like Belgium, Britain, Denmark and Italy after targeted searches were conducted (Donisthorpe 1927, Scharff & Gudik-Sørensen 2006, Parmentier et al. 2014, Castellucci et al. 2022). Our findings of *T. biovatus* in association with *F. lugubris* represent the only observations of co-occurrence between these two species outside the United Kingdom, where two adult males were collected in two different *F. lugubris* nests by Robinson (1998). Little is known about the host preference of this spider in the Pyrenees, with only Simon (1926) reporting its association with the non-RWA species *F. fusca* on the French side of the range. The first record of *T. biovatus* from the Iberian Peninsula suggests that the study of myrmecophile taxa in Southern Europe has long been neglected and that studies of the cryptic, but fascinating, microhabitat that ant nests represent for spiders and other invertebrate taxa may reveal many new discoveries.

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Fig. 3: *Formica lugubris* mound nest in the Spanish Pyrenees in which the myrmecophilic linyphiid *Thyreosthenius biovaticus* was found

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