

Object: Fresh material from the spider genus *Mastigusa* requested for new PhD project

Dear everybody,

My name is Filippo Castellucci and I'm a PhD student at University of Bologna and at the Natural History Museum of Denmark, University of Copenhagen, working together with my supervisors, Nikolaj Scharff and Andrea Luchetti, on the spider genus *Mastigusa* Menge, 1854.



Figure 1. Male *Mastigusa arietina* from Denmark. Pictures by F.Castellucci

These spiders are fascinating for many reasons. The genital morphology of *Mastigusa* is peculiar, male spiders show remarkable pedipalps characterized by an elongated and bent conductor and a long whip-like embolus, resulting in a ram horn-like shape of the palps. Females show long and tangled fertilization ducts. Little is known about the functional morphology of this strongly modified structures and the reproductive biology of this spiders.

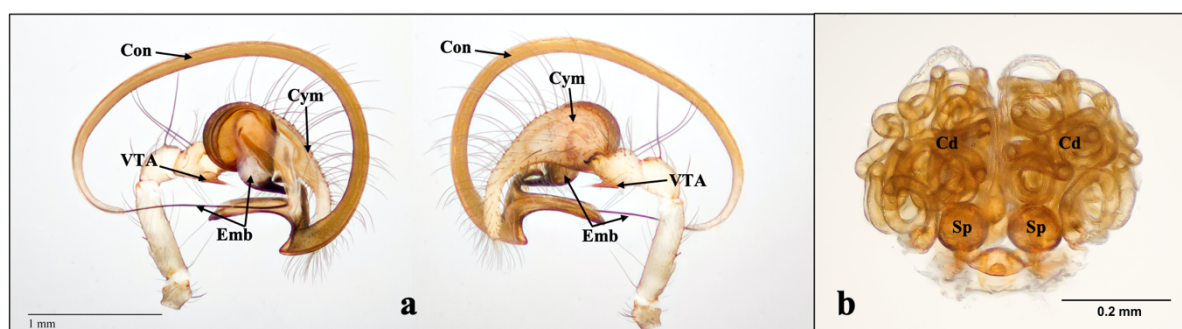


Figure 2. *a*: *Mastigusa arietina* male left palp, retrolateral (left) and prolateral (right) view. Con = conductor; Cym = cymbium; Emb = embolus; VTA = ventral tibial apophysis. Pictures by F.Castellucci. *b*: *Mastigusa arietina* exposed vulva with non-chitinous internal structures removed. Cd = copulatory ducts; Sp = spermatecha. Picture by R.A.Jensen.

The taxonomic history of *Mastigusa* has been troubled both regarding the familiar placement of the genus and the number of extant species that it should include. The WSC currently lists *Mastigusa* in Hahniidae where it was moved to, implicitly, following the move of the genus *Cicurina* from Dytinidae to Hahniidae by Wheeler et al. in 2017. However, *Mastigusa* was never included in any published phylogenetic analysis. Currently three species are recognized as valid, *M. arietina* (Thorell, 1871), *M. macrophthalma* (Kulczyński, 1897) and *M. lucifuga* (Simon, 1898), but given the unreliability of the morphological characters used to identify

them (Aagaard-Jensen, unpublished MSc-thesis) the circumscription of the species is problematic and needs to be reviewed.

The biology of *Mastigusa* is also remarkable, being mostly found in association with different ant species belonging to the genera *Formica* and *Lasius*. *Mastigusa* specimens are frequently found inside the mound nests of ants belonging to the *Formica rufa* species group, also known as red wood ants. The spiders spend all their life cycle inside the mound nests were egg-sacks, juveniles and adult specimens can be found.

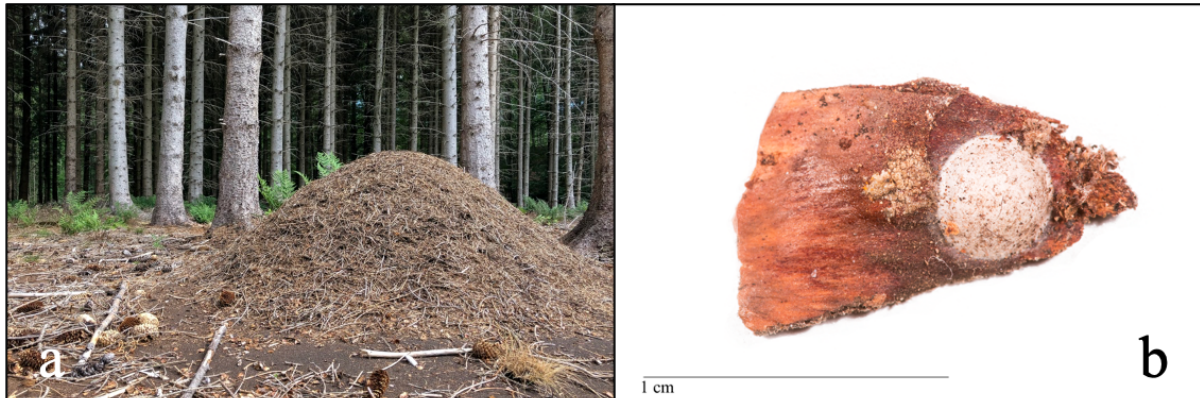


Figure 3. *a:* red wood mound nest in a Danish forest; *b:* a *Mastigusa* egg-sack laid on a small piece of wood found inside a red wood ant nest. Pictures by F.Castellucci.

Little material is usually present in collections for this genus and *Mastigusa* is for this reason considered rare in many countries, but this is probably a bias derived from the myrmecophilic habits of this spiders. Both in Denmark and Italy, when we started looking for *Mastigusa* in *Formica rufa* nests, the spider turned out to be widespread and pretty common.

Two MSc students of N.Scharff have studied the morphology of *Mastigusa* and some of you may have contributed specimens to these morphological studies. We are working on a manuscript and will soon be able to publish these results. You will all receive copies of the publication.

The goal of my PhD project is to explore different aspects related to the biology of *Mastigusa*. To determine the phylogenetic placement of *Mastigusa* and determine the status of the three recognized species using both morphology and molecular data, analyzing the genetic variability of different European populations and get a better understanding of the mechanisms underlying their relationship with ants.

For molecular work, the availability of fresh or 96% ethanol preserved specimens is fundamental and getting material from as many European areas as possible would be particularly useful for understanding how the genetic variability is distributed throughout the continent.

During this difficult COVID-19 period, when movements between different countries are restricted, any help for acquiring fresh specimens would be highly appreciated and if anybody is willing to try collecting this myrmecophile spider in their country, I here include a brief guide to the techniques that we use for collecting them from within the *Formica rufa* mound nests.

Thank you all for the attention. For any additional information or questions you can write me at filippo.castellucci2@unibo.it

Collecting *Mastigusa* in *Formica rufa* nests

What you need:

- Entomological sifter (8mm-1cm mesh)
- White plastic tray/white entomological sheet
- Soft forceps/pooter
- Vials
- Ethanol (70%, 96%)

Method:

Start digging inside the ant mound until you reach moist nest material. We know from experience that spiders tend to move to the moist part of the nests and don't seem to tolerate dry conditions. In order not to disturb the nest more than necessary, you use your hands to 'dig' into the mound. The mounds of *Formica rufa* consist of loose material which you can easily penetrate with your hand and thereby reach into the nest until you can feel nest material inside the mound. Collect some hands full of material and sift it on the tray/sheet while trying to spread it as much as possible. Then inspect the sifted material to spot spiders and other myrmecophilic arthropods. In fact different arthropod taxa live inside such a mound nests, including other spiders, pseudoscorpions, isopods, staphylinid beetles and other ants. This could be a good opportunity to collect other arthropod species that are usually overlooked with classic collecting methods such as pitfall trapping and sweep netting and to share the material with taxonomical experts in other groups. Many interesting discoveries await you! After collecting the specimens, the nest material should be returned to the mound, thereby trying to leave it as intact as possible.

If spiders are present in the nest, a hint is given by the presence of egg-sacks, which can be easily spotted among the nest material. They are white, circular and flat, with a diameter of 4-5mm and are laid on hard surfaces present inside the nest such as pieces of wood and bark, pine cones or rocks. The egg-sacks are similar to the ones produced by gnaphosids.

Red wood ants are not particularly "friendly" animals, so they won't be happy when material is removed from the nest and will try to bite and spray formic acid. Long trousers and long-sleeved shirts are recommended, and gloves can also be used to avoid bites on the hands, but the ants are more of an annoyance and does not pose any kind of danger to the collector. Ants are particularly active in the hottest part of the day and tend to be more docile early in the morning and in the late afternoon/evening, and this is something that could be considered when planning the fieldwork.