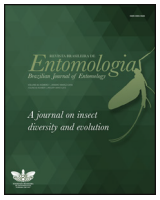
Revista Brasileira de Entomologia 66(Spe):e20220066, 2022

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution



# Setae and sensilla in the Iberian *Myrmeleon* Linnaeus, 1767 larvae (Insecta, Neuroptera: Myrmeleontidae)

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ARTICLE INFO

*Article history:*
Received 12 August 2022
Accepted 17 October 2022
Available online 28 November 2022
Associate Editor: Renato Jose Machado

*Keywords:*
Myrmeleontini
Chaetotaxy
Pit-building traps
Spain
Sensorial receptors

ABSTRACT

*Myrmeleon* (tribe Myrmeleontini) is the most successful genus within Myrmeleontidae in number of species. This is probably due to its pit-building behavior, a famous adaptation of antlion larvae but present only in a few genera of the family. In the Iberian Peninsula, where only two other genera are able to construct these traps (*Euroleon* in tribe Myrmeleontini and *Myrmecaelurus* in tribe Myrmecaelurini), five species of *Myrmeleon* are present: *M. formicarius*, *M. gerlindae*, *M. inconspicuus*, *M. almohadarum* and *M. hyalinus*. There are some useful characters to tell apart the larvae of these species using optical microscope, including the disposition of digging setae or some color spots. In this work, we study the type of setae on these species in their larval stage using SEM. The type of bristles, digging setae, and the rest of sensilla found are not different in shape, surface or structure between species. All of these confer them a great equipment in their psammophilous lifestyle.

## Introduction

The family Myrmeleontidae is the most diverse family within Neuroptera, probably due to the great adaptations of their larvae (Mansell, 1999; Badano and Pantaleoni, 2014a, 2014b; Acevedo Ramos et al., 2020). These larvae show a high diversity of lifestyles and types of habitats such as tree holes, burrows, caves, etc.; nevertheless, the psammophilous adaptations of antlions probably reached the most successful lifestyle (Mansell, 1999; Stange, 2004; Badano and Pantaleoni, 2014a, 2014b; Acevedo Ramos et al., 2020).

In the Iberian Peninsula, according to the classification of Machado et al. (2019), at least 36 species of this family are presented (Monserrat and Acevedo, 2013; Monserrat et al., 2014; Oswald, 2022), including four subfamlies, eight tribes and 19 genera. This is one of the richest European region in number of antlion species.

The most known tribe within this family is probably Myrmeleontini Latreille, 1802 (subfamily Myrmeleontinae): it has approximately 242 species within 13 genera (Oswald and Machado, 2018). Two genera belonging to this tribe has been reported in the Iberian region: *Myrmeleon* Linnaeus, 1767 and *Euroleon* Esben-Petersen, 1918 (with only *Euroleon nostras* (Fourcroy, 1785) present) (Monserrat et al., 2014). This tribe can be characterized by the behavior of constructing pitfall traps in their larval instars. However this is not an exclusive strategy of this tribe, which is also shared with *Myrmecaelurus trigrammus* (Pallas, 1781) of the tribe Myrmecaelurini Esben-Petersen, 1918 (Badano and Pantaleoni, 2014a) in the Iberian Peninsula. The genus *Myrmeleon* has a sub-cosmopolitan distribution, with approximately 180 described species (Stange, 2004; Oswald, 2022). It is represented in this region by at least five species: *Myrmeleon formicarius* Linnaeus, 1767 and *Myrmeleon gerlindae* Hölzel, 1974 of *M. formicarius*-group, *Myrmeleon inconspicuus* Rambur, 1842 and *Myrmeleon almohadarum* Badano, Acevedo, Monserrat & Pantaleoni, 2016 of *M. inconspicuus*-group, and *Myrmeleon hyalinus* Olivier, 1811. The species groups were established by Pantaleoni et al. (2010) according to adult characters, but also some larval features are useful to separate them. *M. formicarius* - group is characterized by an anterior row of digging setae composed by four bristles on the IX abdominal sternite (although *M. hyalinus* share this feature), while the larvae of *M. inconspicuus*-group are characterized by an anterior row of digging setae composed by at least six bristles on the IX abdominal sternite (Badano and Pantaleoni, 2014a; Badano et al., 2016). Apart from these species *Myrmeleon bore* (Tjerder, 1941) has also been reported in the bibliography, probably as a labeled mistake by the collector (Letardi, 1988; Monserrat and Acevedo, 2013). All larvae of these species have

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https://doi.org/10.1590/1806-9665-RBENT-2022-0066

been described using optical microscopy (Badano and Pantaleoni, 2014a), and also the larvae of *M. formicarius* (Lipovšek Delakorda et al., 2009), *M. inconspicuus* (Nicoli Aldini, 2007) and *M. bore* (Nicoli Aldini, 2007) have been studied by Scanning Electron Microscope (SEM), so as the other Iberian species pit-fall builders: *Myrmecaelurus trigrammus* and *Euroleon nostras* (Lipovšek Delakorda et al., 2009; Devetak et al., 2013). Apart from this, *Myrmeleon mariaemathildae* Pantaleoni, Cesaroni & Nicoli Aldini 2010 from Sardinia and Tunisia, and *M. yemenicus* Hölzel, 2002 from Arabian Peninsula have been described with both optical and electron microscopy (Pantaleoni et al., 2010; Devetak et al., 2010a). The chaetotaxy of antlion larvae has a great importance and has been remarked its phylogenetic significance (Badano et al., 2017). In this study, setae and sensilla of the five *Myrmeleon* species known for sure to be distributed in the Iberian Peninsula have been studied and compared using SEM techniques.

## Materials and methods

The study was carried out with third instar larvae. The same protocol of Devetak et al. (2010a, 2010b, 2013) was implemented. All specimens were fixed in glutaraldehyde and paraformaldehyde in PBS and washed in a buffer of 1% osmium in water. Then, they were dehydrated in increasing concentrations of ethanol and critical-point dried. They were coated with gold and studied using a JEOL JSM 6400 Scanning Electron Microscope at 15-20 kV, in *Centro Nacional de Microscopía Electrónica* in Universidad Complutense de Madrid. The same terminology employed by Lipovšek Delakorda et al. (2009), Badano and Pantaleoni (2014a) and Acevedo Ramos et al. (2020) is used. The studied specimens were collected in Spain. The collecting data is as below.

*Myrmeleon formicarius* Linnaeus, 1767

Spain: Madrid: Abantos, 4.VII.2012, 2 specimens, F. Acevedo leg.; 29.6.2013, 2 specimens, F. Acevedo leg.

*Myrmeleon gerlindae* Hölzel, 1974

Spain: Huelva: Arroyo Julianejo, 24.V.2012, 1 specimen, F. Acevedo, V. J. Monserrat legs.; Granada: La Herradura, Cerro Gordo, 2.IX.2000, 1 specimen, V. J. Monserrat leg.

*Myrmeleon hyalinus* Olivier, 1811

Spain: Almería: Rambla Torregarcía, 14.VI.2012, 2 specimens, F. Acevedo leg.; Amoladeras, 11.VII.2014, 2 specimens, F. Acevedo leg.; Rodalquilar, El Playazo, 11.VII.2014, 2 specimens, F. Acevedo leg.

*Myrmeleon inconspicuus* Rambur, 1842

Spain: Ciudad Real: El Chorro, P. N. Cabañeros, 20.VI.2014, 2 specimens, F. Acevedo leg.; Huelva: Punta Umbría, 24.V.2012, 2 specimens, F. Acevedo, V. J. Monserrat legs.

*Myrmeleon almohadarum* Badano, Acevedo, Monserrat & Pantaleoni, 2016

Spain: Cádiz: Las Cañillas, 25.V.2012, 2 specimens, F. Acevedo, V. J. Monserrat legs.; Cádiz: Bolonia, 24.V.2012, 1 specimen, F. Acevedo, V. J. Monserrat legs.

## Results

The Iberian *Myrmeleon* species of this study present the same types of bristles, plumose hairs, digging setae and sensilla, so the commented characteristics are applicable to all of these larvae (Figs. 1, 2).

The bristles differ mainly in length and width (Figs. 1A-E; 2A, 2C, 2E-F). The longest are situated laterally on thoracic and abdominal setiferous processes (Figs. 1A, 1B, 2C, 2E-F). All bristles are poorly ornamented, with stripes and not very evident spiculation, most of them clearly point-shaped (Figs. 1C-E, 2C, 2E-F). They are stouter in dorsal and ventral surfaces and in clypeo-labrum margin than in lateral parts of the body. On dorsal surfaces the larvae show higher density of bristles than in ventral surfaces. In the last sternite there are some fine bristles interspersed with stout bristles (Figs. 2E-F). The larvae also present long bristles situated on the external side of mandibles (Figs. 1A-B, 2A-B). There are some inner jaws bristles, fine but similar to the rest of bristles, that are useful for species identification depending on which teeth they reach (Figs 1B, 2A-B).

Plumose hairs in the genus *Myrmeleon* are very long and ramified. They are present and abundant on head, thorax and abdomen, especially on dorsal surface (Figs. 1A-E, 2C). These plumose hairs are longer, wider and more ramified dorsally than in ventral surface.

Digging setae are situated in IX sternite. Each rastra bears 4 digging setae, and also one, two or even more rows (*M. inconspicuous* and *M. almohadarum*) of digging setae are present in ventral surface on the IX abdominal segment (Figs. 1B, 2E-F). They are strong and wide, but smooth and not very long, and they are not pointed (Figs. 2E-F).

Dolichasters were not found in any species.

There are also other types of sensilla along the body of *Myrmeleon* larvae. On the head and its parts, they have many of them. Sensilla basiconica are present on the tip of the antenna (Fig. 2A) and on the last palpomere, where there is one group located on the extreme and other group subterminally (Figs. 2A-B). Sensilla coeloconica are very abundant on jaws surface (Fig. 2B), and also in the inner surface of food canal.

On the thorax, many sensilla placodea on pronotum were observed (Fig. 2C), and sensilla trichodea were found between claws of each leg (Fig. 2D).

With regard to abdomen, sensilla coeloconica are presented on the IX sternite (Figs. 2E-F), and also on X abdominal segment (Fig. 2F). Campaniform sensilla also appear on X telescopical abdominal segment, but we could only observe it on *Myrmeleon inconspicuus*, as this species was the only one in which the X abdominal segment was evaginated.

## Discussion

*Myrmeleon* is the most successful genus in Myrmeleontidae according to the number of species (Pantaleoni et al., 2010; Badano et al., 2016; Oswald and Machado, 2018; Oswald., 2022). This is probably due to their larval adaptation in psammophilous habitat and their pitfall traps (Mansell, 1999), so its chaetotaxy must show sophisticated adaptations for this lifestyle.

All species studied are recognizable using external morphological characters such as size, coloration, and distribution and abundance of bristles of the mandible external to maxilla and digging setae (Badano and Pantaleoni, 2014a; Badano et al., 2016). However, all Iberian *Myrmeleon* species of this study present the same types and shape of bristles, plumose hairs, digging setae and the rest of sensilla, as we supposed with some previous studies (Lipovšek Delakorda et al., 2009; Pantaleoni et al., 2010; Devetak et al., 2010a; Ngamo and Maoge, 2014; Acevedo Ramos et al., 2020). All types of sensilla found on previously studied antlion larvae were observed in Iberian *Myrmeleon* larvae except digitiform sensilla found in *M. formicarius* (Lipovšek Delakorda et al., 2009)

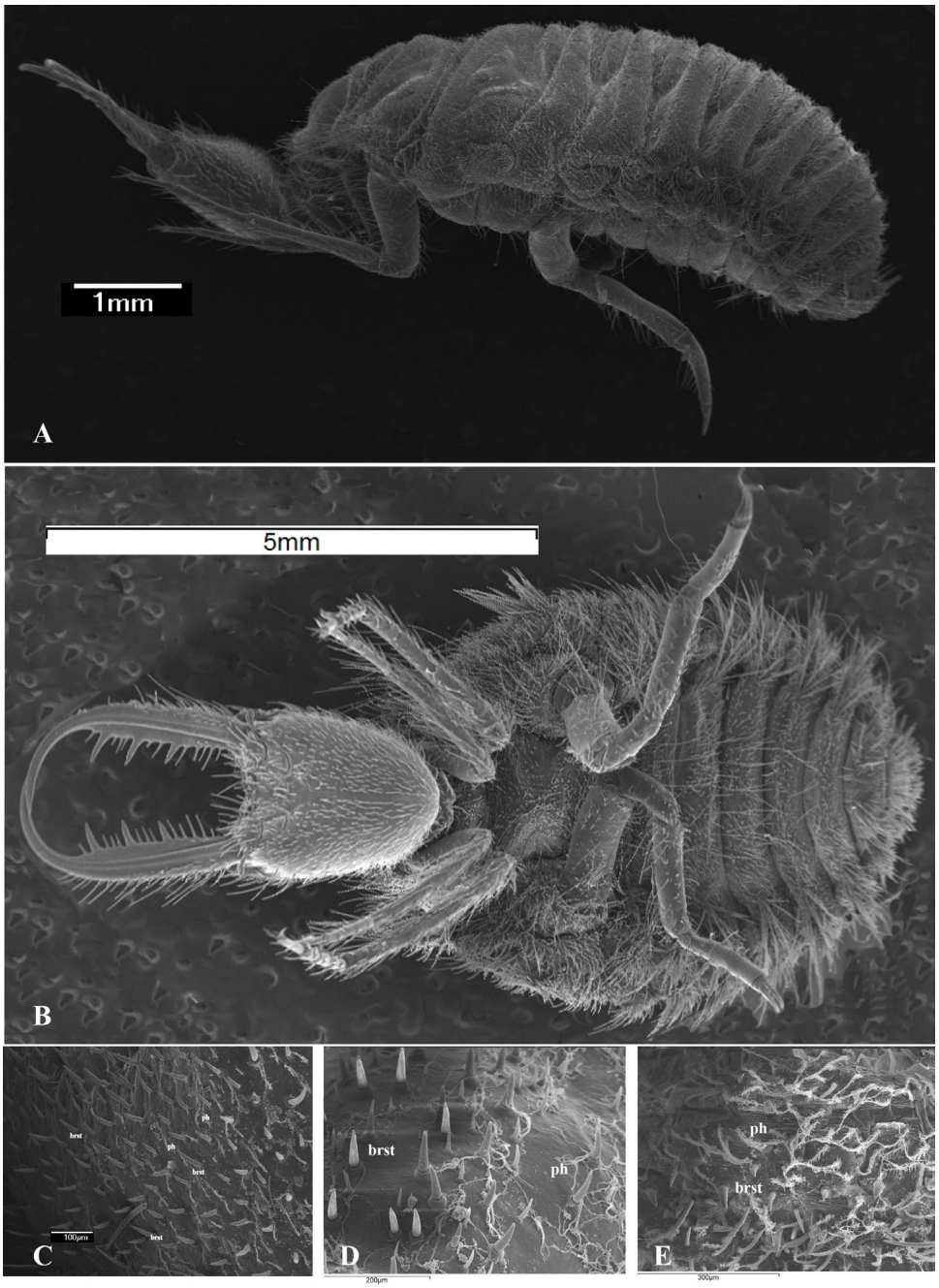


**Figure 1** A-E. SEM images of Iberian *Myrmeleon* larvae. A: *Myrmeleon hyalinus*: complete body, lateral view. B: *M. formicarius*: complete body, ventral view. C: *M. hyalinus*: head setae, dorsal view. D: *M. almohadarum*: thoracic setae, dorsal view. E: *M. gerlindae*: abdominal setae, dorsal view. **Abbreviations**: ant: antenna; brst: bristles; digs: digging setae; grs: sensilla placodea; inb: inner jaws bristles; lp: labial palp; op: odontoid process; ph: plumose hairs; sb: sensilla basiconica; sco: sensilla coeloconica; st: sensilla trichodea; stmt: stemmata; Tsp: thoracic setiferous process.

and *Myrmecaelurus trigrammus* (Devetak et al., 2013), both species with pit-building behavior. Sensilla basiconica were not observed on X abdominal segment, as were found in *Synclisis baetica* and *Macronemurus appendiculatus* (Acevedo Ramos et al., 2020).

According to their functions, bristles, plumose hairs and digging setae have a mechanosensorial role (Lipovšek Delakorda et al., 2009; Acevedo Ramos et al., 2020). Also, bristles (especially those located on head capsule and mandibles) are used to throw away sand during the construction of pitfall traps and to throw it to their preys, plumose hairs are used to retain soil particles in their body in a camouflage role, and digging setae allow to bury themselves (Lipovšek Delakorda et al., 2009; Devetak et al., 2013; Badano and Pantaleoni, 2014a; Acevedo Ramos et al., 2020). It is remarkable the absence of dolichasters, as on other genera that construct pitfall traps as *Myrmecaelurus* or *Euroleon* (Nicoli Aldini, 2007; Lipovšek Delakorda et al., 2009; Devetak et al., 2013; Badano and Pantaleoni, 2014a). Dolichasters are an specialized setae typical in Myrmeleontiformia (Badano and Pantaleoni, 2014a, 2014b) and relatively common in many Myrmeleontidae genera (antlions or owflies on traditional terms) (e.g. Tillyard, 1919; Rousset, 1973; Henry, 1976, 1978a, 1978b; Stange and Miller, 1990; Stange, 2004; Badano and Pantaleoni, 2014a, 2014b; Badano et al., 2017; etc.), and they were observed also by SEM in some larvae of Myrmeleontidae as in the antlion species *Gymnocnemia variegata* and *Megistopus flavicornis* (Cesaroni et al., 2010), *Neuroleon microstenus* (Devetak et al., 2010b),

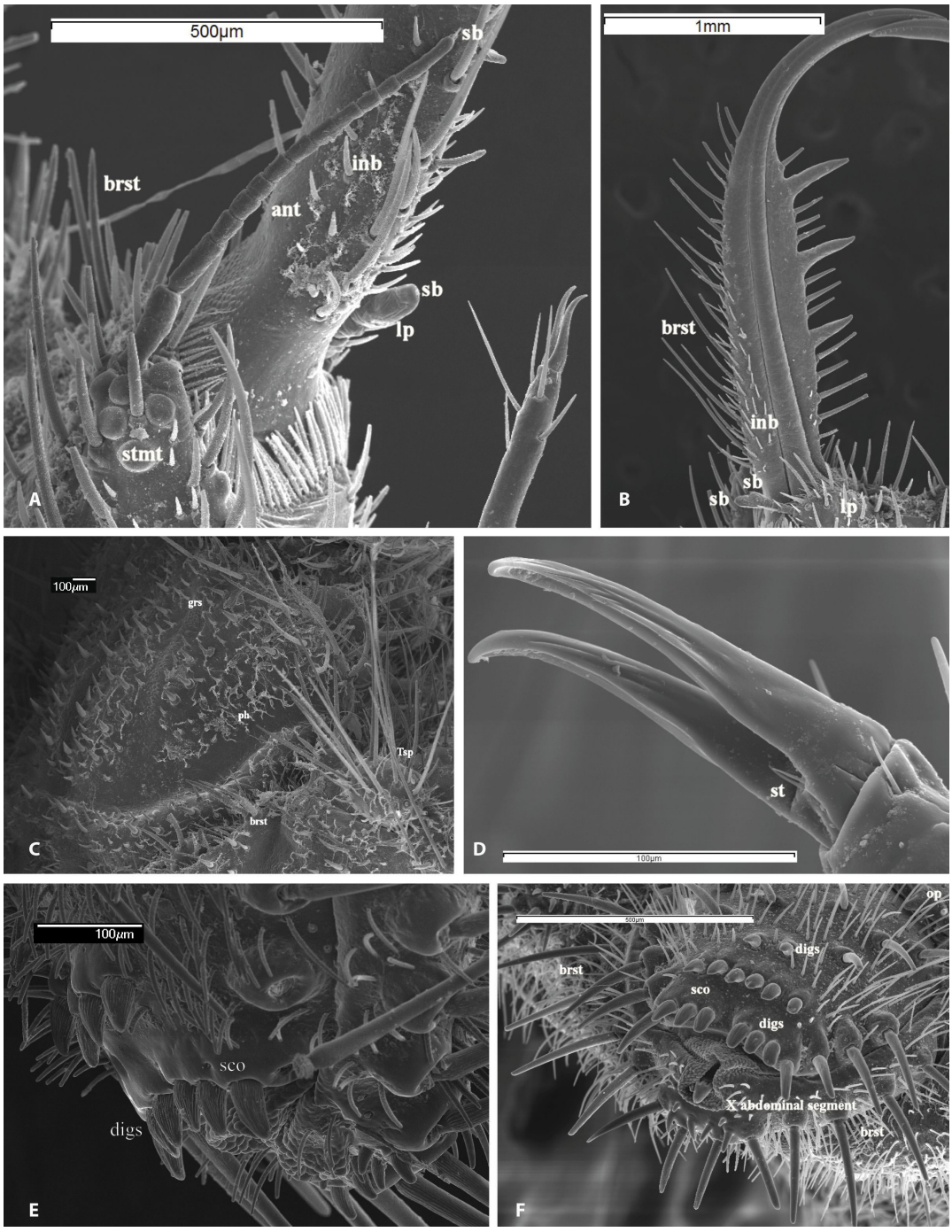


**Figure 2** A-F. SEM images of Iberian *Myrmeleon* larvae. A: *M. gerlindae*: mandibular base, eye tubercle and antenna, dorso-lateral view. B: *M. formicarius*: jaws and labial palp, ventral view. C: *M. hyalinus*: pronotum and mesothoracic setiferous process, dorso-lateral view. D: *M. hyalinus*: claws of third leg. E. *M. hyalinus*: IX sternite, lateral view. F: *M. inconspicuus*: IX sternite, postventral view. **Abbreviations**: ant: antenna; brst: bristles; digs: digging setae; grs: sensilla placodea; inb: inner jaws bristles; lp: labial palp; op: odontoid process; ph: plumose hairs; sb: sensilla basiconica; sco: sensilla coeloconica; st: sensilla trichodea; stmt: stemmata; Tsp: thoracic setiferous process.

*Solter lederi* (Satar et al., 2014a), *Gepus gibbosus* (Satar et al., 2014b), *Nemoleon notatus* and *Tricholeon relictus* (Badano et al., 2017), *Cueta lineosa* (Tusun, 2020), etc., or in owlflies species as *Deleprotophylla australis* and *Puer maculatus* (Badano et al., 2017). The only genus with pit-building behavior in which this kind of setae have been found is *Cueta* of tribe Nesoleontini, according to Tusun (2020). So it is interesting that at least the majority of genera that are able to construct these traps lack this kind of setae.

According to the rest of sensilla, we found sensilla basiconica on antennae and last palpomere (two groups) that take a chemoreceptor and olfative role, sensilla coeloconica in a great density on the surface of mandible and maxillae and in the inner of food canal with the same chemoreceptor functions, as well as sensilla placodea on pronotum (Koch, 1983; Zacharuk, 1985; Zacharuk and Shields, 1991; Römer, 2003; Devetak et al., 2013; Acevedo Ramos et al., 2020). All of them are probably related with detection of preys (Zacharuk, 1985; Devetak et al., 2013; Acevedo Ramos et al., 2020). Sensilla trichodea situated between tarsal claws have mechanosensorial functions in order to detect vibrations in the substrate (Doflein, 1916; Devetak et al., 2013; Acevedo Ramos et al., 2020). Finally, sensilla coeloconica (chemoreceptor) and campaniform

sensilla (mechanoreceptor) take a role in the excavation control and detection of an optimal substrate for construction of pitfall traps (Devetak et al., 2010b, 2013; Ramos et al., 2020). A detailed study of this topic using many psammophilous antlion species was published by Acevedo Ramos et al. (2020).

Similarities in some characters in groups with pit-building behavior (Myrmeleontini, Myrmecaelurini and Nesoleontini) were remarked by Badano and Pantaleoni (2014a), but they also observed that these tribes show enough differences especially on digging setae structures to suggest that this behavior evolved probably more than once in antlions. The phylogeny of the group published by Machado et al. (2019) corroborates this hypothesis. The chaetotaxy of Myrmeleontidae larvae is a great tool not only for phylogenetic and evolutionary relationships within the groups (Badano and Pantaleoni, 2014a, 2014b; Badano et al., 2017) but also for understanding the evolution of larval ecology in this family. On the other hand, the pit-building behavior is clearly a highly successful strategy not only in this family but in the order Neuroptera.

## Conclusions

This comparative study on *Myrmeleon* larvae has shown that chaetotaxy is similar between the species investigated. Bristles and plumose hairs do not show differences in shape between *Myrmeleon* species, so they seem characteristic of the genus. They are also similar to other pit-fall trap builders studied species, suggesting that this type of chaetotaxy could be adaptative for this specialized strategy. All types of bristles described and reported on antlion larvae were found in Iberian *Myrmeleon* species, except dolichasters, as with the rest of pit-fall trap builders species studied by SEM. The rest of sensilla reported on larvae of this family are present on *Myrmeleon* Iberian species, except digitiform sensilla.

## Acknowledgments

We thank Ana M. Vicente of the Centro Nacional de Microscopía Electrónica-Universidad Complutense de Madrid (CNME-UCM) for her support and help during this study.

## Conflicts of interest

The authors declare no conflicts of interest.

## Author contribution statement

FAR: conceptualization; funding acquisition; data curation; formal analysis; investigation; methodology; resources; supervision; validation; visualization; writing original draft; writing-review and editing. VJM: Conceptualization, funding acquisition; data curation; resources; supervision.

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