Asellariales (Trichomycetes) from the Iberian Peninsula

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A new species of Asellariales, Asellaria saezii, is described from an Iberian locality, found in the hindgut of a terrestrial isopod, Trichoniscus pusillus. The morphological characters of its distinctive basal cell, as well as thallus features, characterise this species. Also, three previously described species of Asellariales, new for the Iberian Peninsula, are reported here: A. gramenei, A. ligiae (both found in Isopoda), and Orchesellaria mauguioi (found in a Collembola). This represents the first report of Asellariales species in the Iberian Peninsula and Balearic Islands. Morphological similarities between these and other Asellariales are discussed. Drawings and photographs are provided to illustrate these taxa, together with comments on their habitats.

Key words: gut fungi, Isopoda, taxonomy, Zygomycota

Introduction

The Asellariales is probably the Order least studied of trichomycetes (as an ecological group): the number of described species is as few as 12, including the new one documented here. The Asellariales include one single Family, Asellariaceae, and three genera: Asellaria Poisson (1932), Orchesellaria Manier ex Manier & Lichtw. (1969) and Baltomyces Cafaro, which is provisionally placed within this order though it may deserve a special classification within the Trichomycetes (Cafaro, 1999). All three genera include filamentous endosymbiotic species that reproduce asexually by arthrospore-like cells, which disarticulate from their corresponding thallus. Both Asellariales and Legeriomycetaceae (Harpellales) are branched, and both were initially placed together within the Harpellales by Manier (1950) until she reclassified the Trichomycetes some years later (Manier, 1955). Manier (1955) established the Asellariales as a distinct order at the same level as Harpellales, Eccrinales, and Amoebidiales. Both Eccrinales and Amoebidiales are considered to be related to protozoans, after molecular studies carried out

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with phylogenetic purposes (Benny and O'Donnell, 2000; Cafaro, 2003a,b, 2005). Later investigations noted the resemblance between *Harpellales* and *Asellariales* (Moss and Young, 1978; Manier, 1973, 1979; Moss, 1975; Lichtwardt, 1973). Molecular-based systematics of trichomycetes has raised a new approach to this ecological group of organisms, helping in constructing the evolutionary pathway (White and Lichtwardt, 2005), and even in the identification process in difficult genus as *Smittium* (Rizzo and Pang, 2005).

The principal character used for the *Asellariales* taxonomy is the basal cell, taking into consideration the irregularity of other characters such as arthrospore size, even within the same species or individuals. Other characters for identifying a specimen are the branching pattern of the thallus and arthrospore development. These are the features treated in this paper for the description of the new species, *A. saezii*, and for the previously known *A. gramenei* Tuzet & Manier ex Manier, *A. ligiae* Tuzet & Manier ex Manier and *Orchesellaria mauguioi* Manier, which are newly reported from Spain.

Materials and methods

Terrestrial isopods were collected in humid slopes next to water courses, by hand picking with the help of entomological forceps, under organic material (leaves, branches, etc) and rocks, in an evergreen-oak grove with acidic substrate. Aquatic Isopoda of the genus *Proasellus* were collected with the help of an aquatic net and entomological forceps. Marine isopods were collected with special nets for marine use near the seashore and in seawater ponds at low beaches. Isopods were placed in jars and transported in a cooler to the laboratory. For marine Isopods, oxygen tablets were placed in the marine water jars to prevent asphyxiation. The specimens were examined with a stereomicroscope and their guts removed with fine forceps and needles. Once the gut was cleaned with water, the gut lining and attached trichomycete thalli were placed in a drop of water on a microscopic slide for preliminary observation. This technique is recommended because the slides can be preserved with lactophenol cotton-blue for storage and for later studies (Lichtwardt et al., 2001; Valle and Santamaria, 2002). Photomicrographs were taken of living material and lactophenol cotton-blue preserved slides. Morphological criteria for rapid identification of the Iberian species of Asellaria, with measurements and drawings, are shown in Table 1. Scientific data for new species registered the Mycobank (www.mycobank.org/MycoBank.htm).

Table 1. Comparison of the Iberian species of *Asellaria* with their representative characters.

Species	Diameter of the main axis (μm)	Arthrospore size (μm)	Basal cell size (µm)	Basal cell figure
A. gramenei	7-8.5	32-60 × 3.5-6	36-48 × 14-26	
A. ligiae	20-22	30-42 × 14-18	30-40 × 40-50	3
A. saezii	5-5.5	(17-)30-48 × (3.5-)5-7	60-75 × 17-20	

Taxonomy

Asellaria saezii L.G. Valle, sp. nov.

(Figs. 1-8, 9)

Etymology: Saezii = In reference to Llorenç Sáez, esteemed Majorcan Botanist.

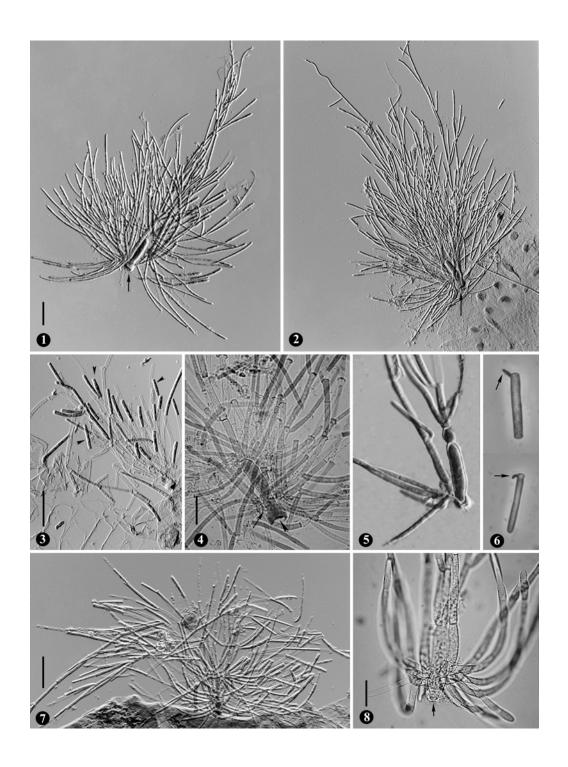
Thalli ramificantes 700-900 \times 5-5.5 μ m, cum verticillatis prominentiis in apice curvatis. Arthrosporae (17-)30(-48) \times (3.5-)5(-7) μ m. Cellula hapteroidea basalis ramosa, 60-75 μ m longitudine, 17-20 μ m diametro, latior in basi et in centro invaginata. Ad cuticulam proctodaei Isopodorum (*Trichoniscus pusillus*, Trichoniscidae) affixi.

Branched thalli of 700-900 μ m ($\bar{x}=832~\mu$ m) long and main hyphal filaments of 5-5.5 μ m ($\bar{x}=5.3~\mu$ m) wide, with verticillate projections and secondary branching (Figs. 1-3). Basal cell 60-75 × 17-20 μ m ($\bar{x}=67.5$ × 18.5 μ m), elongated-pyriform, branched, with a broad invaginated base (Fig. 4). The holdfast involves part of the hindgut lining in the central furrow (Figs. 1, 4). Profuse verticillate branches arise laterally from the basal cell (Figs. 1-2, 4-5, 8). Upper thallial branches present broad cells at the proximal zone, close to the main axis, making swollen and bulb-like structures (Figs. 1-2, 7). All these branches become disarticulated at maturity to produce arthrospores (Fig. 3). Arthrospores (17-)30-48 × (3.5-)5-7 μ m ($\bar{x}=36.4$ × 5.2 μ m), slightly bent and with a truncate apex (Fig. 6).

Habitat: Found in the hindgut lining of *Trichoniscus pusillus* Brand (Isopoda, Oniscidae).

Known distribution: Barcelona, Spain.

Material examined: SPAIN, Barcelona, El Brull, near La Castanya stream, above the biological station La Castanya (Montseny Natural Park), 31T DG52, prepared from Trichoniscus pusillus, 28 November 2000, L.G. Valle, microscope slides BCB-Tr0420



Figs. 1-8. Asellaria saezii. **1-2.** Overview of thalli with typically ramified basal cells (arrow) (BCB-Tr0420). **3.** Thallus developing arthrospores, which contain dense cytoplasmic material (strained with cotton blue) (arrow) (BCB-Tr0353). **4.** Basal cell with lateral fasciculate ramifications (arrow) (BCB-Tr0420). **5.** Characteristic basal cell with a thick cell wall, typically invaginated at the central proximal end (BCB-Tr0419). **6.** Arthrospores undergoing the initial phases of the peculiar latero-apical germination (arrows) (BCB-Tr0421). **7.** Overview of a thallus producing arthrospores (BCB-Tr0421). **8.** Basal cell zone (arrow) with lateral branches (BCB-Tr0421). Scale bars = 50 μm in Fig. 1 (use the same bar for Figs. 1-2), $3, 7; = 25 \mu$ m in Fig. 4 (use the same bar for Figs. 4-5, 6), 8.

(holotype), BCB-Tr0418-0427 (isotypes); same locality, 3 October 2000, BCB-Tr0353; same locality, M. Cafaro, L.G. Valle and S. Santamaria, 28 August 2002, BCB-Tr1513.

Although the new *Asellaria* species described here shares with *Orchesellaria* the presence of a branched basal cell, *A. saezii* shows the typical morphology of the genus *Asellaria*. The lateral and verticillate branches, as well as the furrowed holdfast, are characters that justify the inclusion of the species in the genus *Asellaria*.

Asellaria gramenei (Tuzet & Manier) Manier, Sci. Nat. Bot. Paris 9: 93 (1968). (Figs. 9. 10-16)

■ Asellaria gramenei Tuzet & Manier, Ann. Sci. Nat. Zool. 12: 15 (1950). [nom. inval.] *Habitat*: In the hindgut lining of *Prasellus coxalis* Dollf (Isopoda, Asellidae).

Known distribution: France and Spain.

Material examined: SPAIN, Girona, La Bisbal d'Empordà, Daró river, prepared from *Proasellus coxalis*, 13 December 2000, L.G. Valle, BCB-Tr0433-0435. Amer, Brugent river, prepared from *P. coxalis*, 1 February 2001, L.G. Valle, BCB-Tr0569.

The Iberian specimens of *A. gramenei* presented branched thalli up to 2 mm long, with axial hyphae of 7-8 μ m diam. Branches arose from the base of the first thallial cells, 2-4 per verticil, with the same diameter of the axial hyphae (Figs. 10-12). Only primary branches were observed, as described by Manier (1968). Basal cells 36-48 × 14-26 μ m ($\bar{x} = 42 \times 23 \mu$ m), suborbicular at the enlarged bottom (Figs. 14-16), discrete in young specimens (Fig. 13). Arthrospores showed variable size, measuring 32-60 × 3.5-6 μ m ($\bar{x} = 47.8 \times 4.7 \mu$ m) in our collections (Figs. 9, 10).

Material of *A. gramenei* has been found in the Asellid hosts in ponds of stagnant water with low renewal flow from the Daró River. Only seven Isopods from this locality were collected, and the fungus was found infecting three of them. A single individual from Brugent River was found infected with *A. gramenei*, although the hosts were quite abundant and many were dissected.

Asellaria gramenei was described in 1950 by Tuzet and Manier, and validated by Manier in 1968, who provided a Latin diagnosis. Asellaria

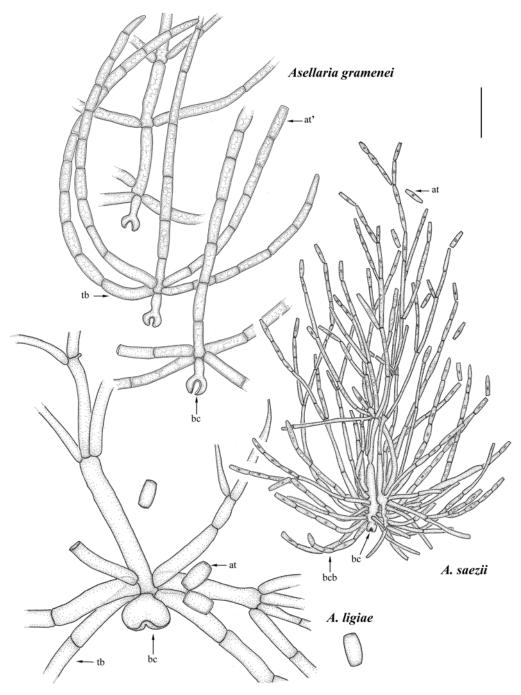


Fig. 9. Asellaria gramenei, A. saezii and A. ligiae. The species of Asellaria are here represented to easily compare shapes and sizes. Indicated here are: bc, basal cell; bcb, basal cell branches; at, arthrospores; at' arthrospores not disarticulated from the thallus; tb, thallial branches. Scale bar = $50 \mu m$ for all the figures.

gramenei and A. ligiae can be easily distinguished by the shape and size of the basal cell, being longer than wide in the former and wider than long or with similar proportions in A. ligiae. Asellaria gramenei was found by Tuzet and Manier in streams with unspecified salinity draining the French locality "Salins de Gramenet", in Asellus meridianus, a freshwater Isopod (Tuzet and Manier, 1968).

Asellaria ligiae (Tuzet & Manier) Manier, Sci. Nat. Bot. Paris 9: 93 (1968). (Figs. 9, 17-22)

= Asellaria ligiae Tuzet & Manier, Ann. Sci, Nat. Zool. Paris 12:15-23. (1950). [nom. inval.]

Habitat: In the hindgut lining of *Ligia* spp. (Isopoda, Ligiidae).

Known distribution: Europe (France, Spain, Yugoslavia), America (North Carolina, Florida, California, Hawai, Puerto Rico, Costa Rica) and Asia (Japan).

Material examined: SPAIN, Girona, Blanes, Cala Santa Cristina, prepared from Ligia italica Fabricius, 29 September 2000, L.G. Valle and A. Morton, BCB-Tr0323. Balearic Islands (Majorca), Pollença, S'Albufereta, prepared from L. italica, 25 May 2003, L.G. Valle and Ll. Sáez, Tr1797-1798. Tarragona, Móra, Punta de Na Móra, under rocks near the seashore, prepared from L. italica, 4 September 2002, M. Cafaro and L.G. Valle, BCB-Tr1527.

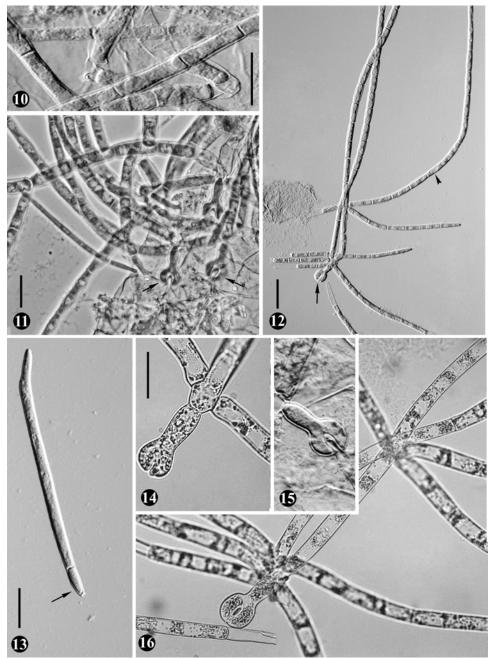
The specimens found showed thalli measuring 900-1500 μ m long (\bar{x} = 1345 μ m), densely branched at maturity, presenting 2-5 branches per verticil, with secondary, tertiary and even up to 4th order ramifications, each of them measuring less in diameter than the preceding ones (for example, 20-22 μ m the main axial cells to 8-11 μ m in the tertiary and subsequent branches) (Fig. 17). Basal cell globular, spherical, 30-38 × 40-49 μ m (\bar{x} = 35 × 45.2 μ m), invaginated at the base to form a small furrowed structure that attaches the thallus to the gut lining (Fig. 17). Hyphal branches disarticulate into arthrospores that measure 30-42 × 14-18 μ m (\bar{x} = 37.8 × 16.2 μ m) (Figs. 18-22).

The considerable size of this species is remarkable, both in hyphal diameter and basal cell measurements. Its large size makes it easily distinguishable from the other *Asellaria* species. The individuals of the Iberian collections share the same characteristics of the previously described specimens.

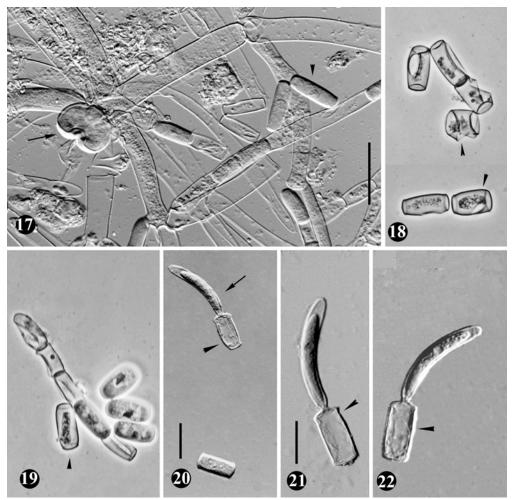
Orchesellaria mauguioi (Tuzet & Manier) Manier, Ann. Sci. Nat. Bot. Paris 10: 565 (1970). (Figs. 23-24)

Habitat: In the hindgut lining of *Collembola*.

Known distribution: Europe (France, Spain), USA (Washington, Montana).



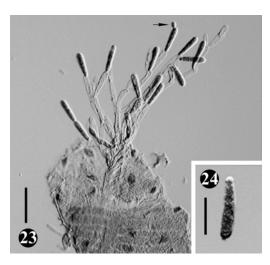
Figs. 10-16. *Asellaria gramenei.* **10.** Branches with transverse walls delimiting arthrosporecells (BCB-Tr0434). **11.** Several thalli with their corresponding basal cells (arrows) (BCB-Tr0434). **12.** Overview of a thallus with septate branches (arrowhead) and a typically lobulate basal cell (arrow). **13.** Young thalli with an incipient basal cell, not yet lobulated (BCB-Tr0434). **14-16.** Several basal cell structures (BCB-Tr0433-0434). Scale bars = 25 μm in Figs. 10-11, 13-14 (use the same bar for Figs. 14-16); = 50 μm in Fig. 12.



Figs. 17-22. Asellaria ligiae. **17.** Thalli with basal cell (arrow), and free arthrospores (arrowhead) (BCB-Tr0323). **18-19.** Arthrospores disarticulated, individually or in groups, some of them are empty and show a rupture in the cell wall (arrow) (BCB-Tr0323). **20-22.** Arthrospores (arrowhead) apically germinated (arrow) (BCB-Tr1797). Scale bars = $50 \mu m$ in Figs. 17 (use the same bar for Figs. 17-19); = $25 \mu m$ in Figs. 20, 21 (use the same bar for Figs. 21-22).

Material examined: SPAIN, Girona, Llaers, near Milany stream, prepared from Collembola, 26 March 2003, L.G. Valle, BCB-Tr1756.

We found this species in the hindgut of an unidentified Collembola. The fungus had the following characteristics: thalli basally branched, from the typical basal cell, with a thin layer of holdfast material (Fig. 23). Fertile branches disarticulate into arthrospores of $22-36 \times 3.5-8.5 \, \mu m$ ($\bar{x} = 30.1 \times 6.6 \, \mu m$) (Fig. 24). The Spanish specimens match perfectly with the original description of the species (Manier, 1964, 1970). An extremely low infection



Figs. 23-24. Orchesellaria mauguioi. 23. Overview of a thallus with arthrospores (arrow) BCB-Tr1756). 24. Detail of a free arthrospore (BCB-Tr1756). Scale bar = $50 \mu m$ in Fig. 23; = $25 \mu m$ in Fig. 24.

rate is noted, since we have dissected a large number of Collembola from different Spanish localities, and only on one occasion was the presence of any *Orchesellaria* observed. This species was previously reported from France (Manier, 1964) and the USA (Moss, 1975, 1979; Lichtwardt and Moss, 1984). This represents the first report of this species on the Iberian Peninsula.

Discussion

Asellaria saezii is most similar to A. gramenei Tuzet & Manier ex Manier, but differences in the basal cell, arthrospores and branching pattern justify this new taxon. In this new species, arthrospores germinate in a way similar to A. ligiae (Poisson, 1932; Manier, 1958, 1964, 1979), although laterally near the apex, instead of apically, as in A. ligiae. The largest branches show a sinuous and attenuated terminal apex. Asellaria gramenei is characterised by the presence of a campanulate to suborbicular basal holdfast cell (Tuzet and Manier, 1950; Manier, 1968), with an invaginated area forming a deep and narrow groove, being formed by the lateral lobules of the basal cell. The new Asellaria basal cell is different in size and shape, being longer and not so orbicularly swollen in the basal area, and with a wider indented centre that encompasses a part of the host's intestine cuticle. Both species also differ in the branching pattern, giving them a different aspect: A. gramenei has few branches (2-4 per verticil) that arise from the base of some thallial cells; normally just the first and second large cells of the main axis carry them, and never show secondary branching. The basal cell of A. gramenei never has lateral verticillate branches, which are present in A. saezii. It is noteworthy that in A. gramenei all the hyphae maintain a similar diameter, while in this new

species the difference between the axial and branching hyphae diameter is very evident.

In Spain, Asellaria ligiae was found infecting a few individuals of Ligia italica, on the marine shoreline of different peninsular localities, and in one locality from Majorca, in a seawater pond in low beaches of S'Albufereta. Although this species was initially described from the marine Isopod Ligia italica Fabricius, it has also been found in a Ligia related to the common marine Ligia exotica Roux, probably an endemism from the island of Oahu, Hawaii, which inhabited a freshwater stream (Lichtwardt, 1986). In the latter instance it could be an adaptation of both fungus and host to the freshwater environment (Lichtwardt, 1986). A similar case is reported here for Asellaria gramenei, found in Proasellus, which was first described by Tuzet and Manier from saline water (Tuzet and Manier, 1950), and now has been collected in a pair of freshwater streams. In both localities, we found the fungus in a different species from the one reported by Tuzet and Manier. The host in both freshwater localities is a native individual of Asellidae, Proasellus coxalis. Tuzet and Manier identified the host as Asellus aquaticus L., originally from Asia, introduced into Europe and a competitor of *Proasellus meridianus* Racovitza, which was identified in subsequent publications as the real host of Asellaria gramenei (Manier, 1963).

The difference in the percentage of infected individuals containing A. gramenei, in both new Spanish localities, may be due to the conditions of the aquatic habitats. In Daró river the Isopods were restricted to the bottom of small ponds connected by low water circulation, ideal conditions for transmission of the arthrospores among the small but dense Isopod population. In the locality of Riu Brugent, several Isopods were collected, buried under small rocks in the bottom of flowing water, and one might expect that arthrospores could easily be washed away by the water flow, dispersing the fungus.

The genus *Orchesellaria* includes four species (*O. lattesi* Manier ex Manier & Lichtwardt 1969; *O. mauguioi* Manier (1970), *O. podurae* Manier (1979), and *O. pelta* Lichtwardt (*in* Lichtwardt and Moss, 1984), characterised by the presence of a basal cell from which branches and thallial cells arise. They live attached to the hindgut of Collembola (Insecta) species (Lichtwardt, 1986). Of all these species, we only have found *O. mauguioi*, possibly the most common and widely distributed.

The knowledge of *Asellariales* in the Iberian Peninsula is relatively scant, if we consider that only 25% of the known taxa have been reported from this European area. More over, the global comprehension of *Asellariales* is reduced indeed. In contrast with the more common Order *Harpellales*, which

are constantly increasing the total number of known species in the vast earth geography, the *Asellariales* appear to be quite stagnant in this taxonomic aspect.

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