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## Diversity of non–Laboulbenialean fungi on millipedes

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### Abstract

New records are given for secondary capilliconidia of *Basidiobolus* from several species of millipedes (Diplopoda) belonging to three different orders. The anamorph ‘*Thaxteriola*’ stage of *Rhynchomyces* is recorded (with doubt) from a Brazilian millipede belonging to the order Spirostreptida. An enigmatic fungus showing characteristics of *Coreomycetopsis*, *Hormiscioideus* and *Antennopsis* is recorded from two species of Danish millipedes of the order Julida. Peculiar structures, tentatively referred to fungi are recorded from several millipede orders where they occur between micro–scutes of the external cuticle.

**Key words** – *Amphoromorpha* – *Antennopsis* – Ascomycota – *Basidiobolus* – *Coreomycetopsis* – Diplopoda – *Hormiscioideus* – Mucoromycotina – *Pyxidiophora* – *Rhynchomyces* – Zygomycota

### Introduction

Millipedes (class Diplopoda) are well known as host of Trichomycetes (Lichtward 1986, Moss & Taylor 1996), but several other kinds of fungi grow on the external cuticle of millipedes. Best known of these are Laboulbeniales, and over the last years, the diversity of Laboulbeniales on millipedes has increased significantly (Rossi & Balazuc 1977, Santamaria et al. 2014, 2016, Enghoff & Santamaria 2015). However, several types of small fungi which have nothing to do with Laboulbeniales are quite often found growing on the external cuticle of millipedes. We here present a review of the diversity of these non–laboulbenialean fungi on millipedes. Some of these can be referred to the ‘*Amphoromorpha*’ stage of the genus *Basidiobolus* Eidam, a genus currently classified in Zygomycota, Mucoromycotina, family Basidiobolaceae (Mycobank, www.mycobank.org, accessed 6 October 2017). The genus *Amphoromorpha* was described by Thaxter (1914) for minute amphora–shaped fungi found on earwigs and staphylinid beetles from the Philippine Islands. The systematic position of *Amphoromorpha* remained uncertain until Blackwell & Malloch (1989) demonstrated similarity of *Amphoromorpha* with secondary capilliconidia of *Basidiobolus*. *Amphoromorpha* is accordingly regarded as a junior synonym of *Basidiobolus*, but because the name *Amphoromorpha* so well describes the shape of these fungi in the present paper we will use ‘amphoromorph’ as a short, ‘trivial’ name for the secondary capilliconidia of *Basidiobolus*. The first record of amphoromorphs from millipedes is due to Rossi & Balazuc (1977) who reported on an Italian millipede, *Ophiulus targionii* Silvestri, 1898 (order Julida, family Julidae) which was hosting a species of Laboulbeniales as well as an amphoromorph.

Subsequently, amphoromorphs have been recorded from the Spanish *Iberoiulus cavernicola* Ceuca, 1967 (order Julida, family Blaniulidae) (Enghoff & Reboleira 2014, Enghoff & Santamaria 2015).

The genus *Thaxteriola* was described by Spegazzini (1918) for two species of tiny fungi found on earwigs (Dermaptera) and beetles (Coleoptera) in Argentina. A further three species of *Thaxteriola* were described by subsequent authors but Blackwell et al. (1986) presented evidence that *Thaxteriola* is nothing but an anamorph stage of the genus *Pyxidiophora* Bref. & Tavel, a genus which itself is now regarded a synonym of *Rhynchomyces* Sacc. & Marchal ex Marchal. (Ascomycota, www.mycobank.org, accessed 6 October 2017).

In addition to '*Amphoromorpha*' and '*Thaxteriola*' quite a number of small, simple fungi have been found on various arthropods. Thaxter (1920) coined the term '*Thaxteriolae*' for this assemblage, most of which remains very poorly known. It would be futile to try to associate the fungi described below from Danish julids with any of the named genera of '*Thaxteriolae*' and describing still another genus on the basis of our material would be irresponsible. We therefore simply present the fungi, hoping that eventually somebody will work out what they are. For the same reason, we also present images of some structures discovered during scanning electron microscopical studies of millipede cuticle which we cannot even refer to the kingdom of fungi with certainty.

## Materials & Methods

Millipedes stored in 70% ethanol in the zoological collection of the Natural History Museum of Denmark (ZMUC) were screened for fungal infestation.

One thallus from an Australian paradoxosomatid millipede was detached and mounted in Hoyer's medium with lactophenol on a slide (C-F-95160), while legs of *Xestoiulus laeticollis* (Porat, 1889) (Julidae) were temporary mounted in glycerine. Images and measurements of the fungus in the slides were made using an Olympus BX51 microscope with Differential Interference Contrast (DIC).

Specimens for Scanning Electron Microscopic (SEM) study were dried with a series of alcohol and acetone, coated with platinum/palladium and studied under with a JEOL JSM-6335F SEM. Backgrounds of the images were cleared using Adobe Photoshop software.

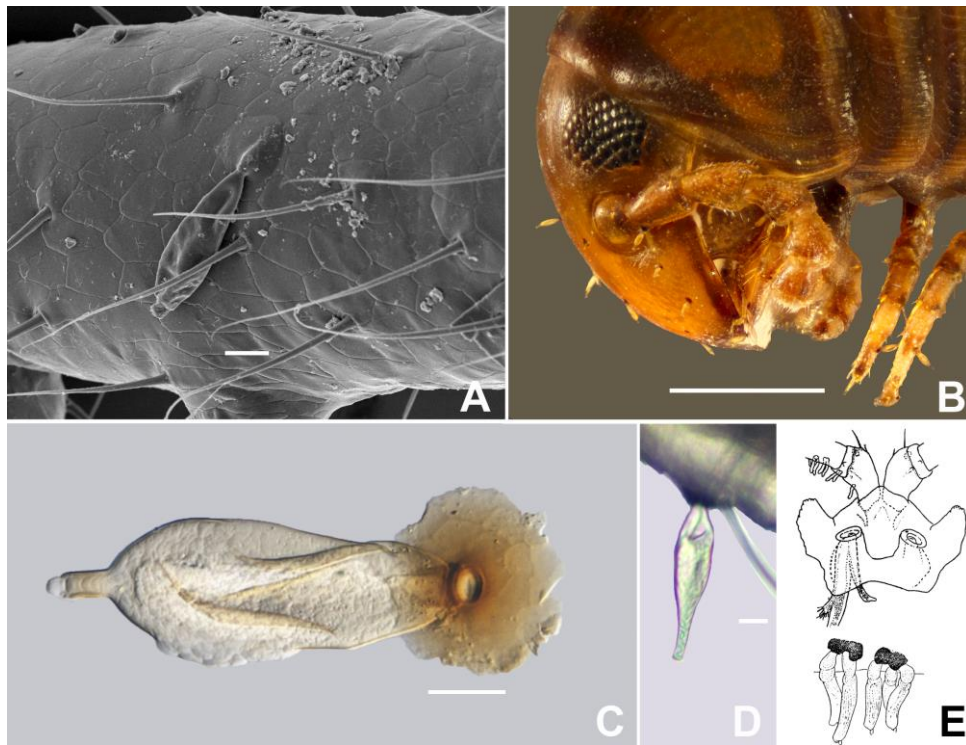
## Results

### Amphoromorphs on millipedes

We have found amphoromorphs on several millipedes, including *Boreviulisoma barrocalense* Reboleira & Enghoff 2013 (order Polydesmida, family Paradoxosomatidae) (Fig. 1A), *Acipes andalusius* Enghoff & Mauriès, 1999 (order Julida, family Blaniulidae) from Spain, an unidentified species of Spirostreptidae (order Spirostreptida) from Madagascar (Fig. 1B) and a further species of Paradoxosomatidae from Australia. The thallus of the latter (Fig. 1C) shows very close similarity with the holotype of *Amphoromorpha entomophila* Thaxter, illustrated by Blackwell & Malloch (1989): from the darkened attachment disk a bottle-shaped thallus, 114×41µm, arises, terminating in a slender, 19×7 µm tubular extension. The attachment disk shows, by transparency, imprints of the cuticular scutes that each correspond to an underlying hypodermal cell (Fusco et al. 2000). The main body of the thallus, except for its basal ¼, is tightly packed with numerous cells ('segments' in the terminology of Blackwell & Malloch, 1989). The tubular extension is open at the end, and it look as if a row of cells are on their way out of the terminal opening.

### '*Thaxteriola*' on a millipede

Luiz Iniesta has kindly provided photographs of a fungus that has the typical size (40×10µm) and shape of '*Thaxteriola*' (Fig. 1D). It was found on the antenna of *Pseudonannolene spelaea* Iniesta & Ferreira, 2013 (order Spirostreptida, family Pseudonannolenidae) from a Brazilian cave. Given the simplicity of the fungus and the limited evidence at hand, it is, however, far from certain that it is indeed a '*Thaxteriola*'.



**Fig. 1** – A Thallus of amphoromorph fungus on leg of *Boreviulisoma barrocalense* Reboleira & Enghoff, 2013. B Unidentified species of Spirostreptidae from Madagascar (Montagne d’Ambre, 12°32’ S, 49°10’ E, NOV 1993, Coddington, Scharff, Larcher, Griswold & Andriamasimanana leg.), with several thalli of amphoromorphs. C Thallus of amphoromorph from unidentified paradoxosomatid from Australia (NSW, Border Ranges NP, 22–23.iii.2010, N. Scharff & G. Hormiga leg.). D Thallus of ‘*Thaxteriola*’ from *Pseudonannolene spelaea* Iniesta & Ferreira, 2013 from a Brazilian cave. (Courtesy of L. Iniesta); B Fungi from *Stemmiulus (Nethoiulus) sjoestedti* (Brolemann, 1920) from Brolemann (1920). Scale bars: A, D = 10  $\mu$ m, B = 1 mm, C = 20  $\mu$ m.

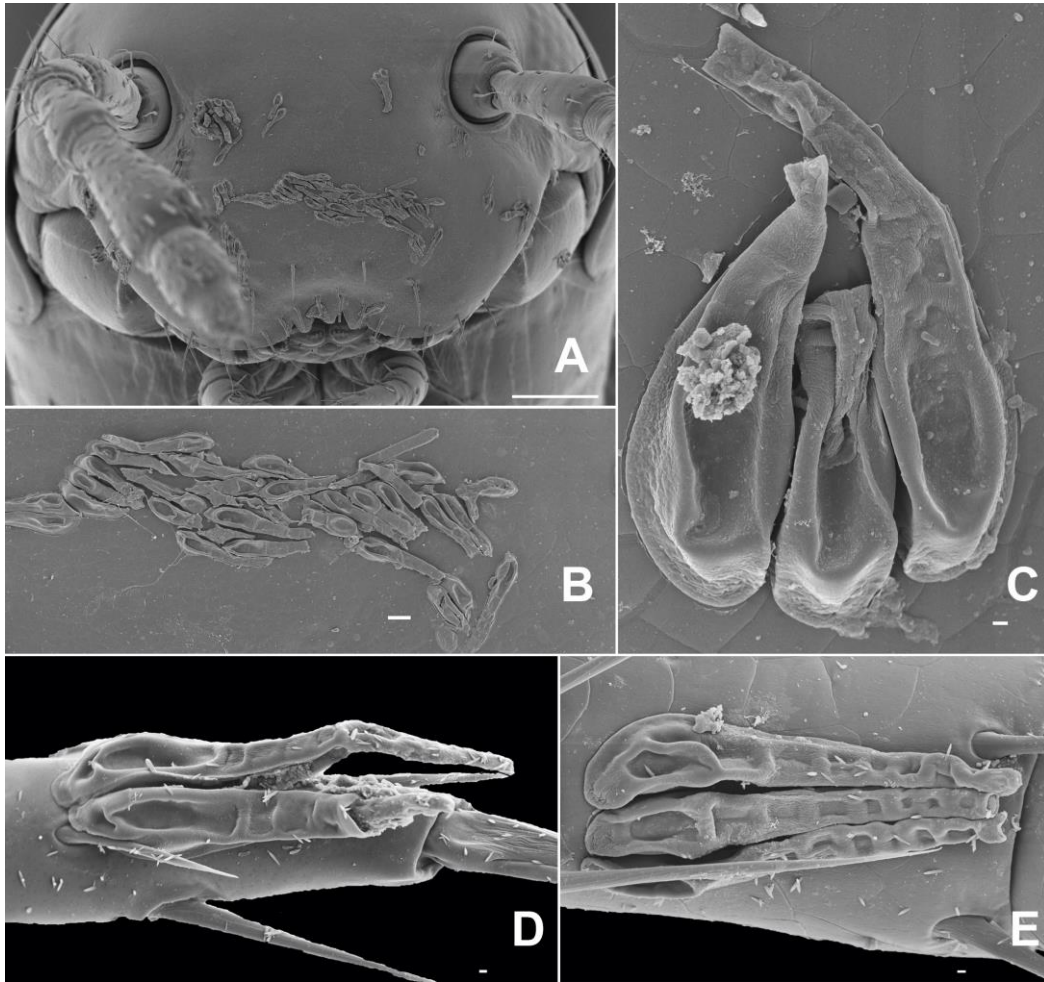
### Enigmatic fungi on millipedes

Figs 2A–C, 3 show a fungus from the head and legs of several specimens of *Xestoiulus laeticollis* (Julidae) from Denmark. Each thallus is up to ca. 140  $\mu$ m long and consists of a darkened attachment cell plus a single row of up to a dozen rectangular cells of decreasing size. The attachment cell has a circular transparent “window”, diameter ca. 2  $\mu$ m, in its upper, less darkened part (Fig. 3B, F). On some thalli, cell number six or seven from the foot bears a small spinelike appendage (Fig. 3 E) and some thalli divide into ca. three branches after cell number 5 (Fig. 3A).

Fig. 2D–E shows a somewhat similar fungus, found on a juvenile of another julid, *Ophiulus pilosus* (Newport, 1842) from Denmark. Of this fungus we only found a few thalli, occurring in pairs or triplets on the millipede’s legs. We only have SEM images; these show great similarity with SEM images of the fungus from *Xestoiulus*: a large attachment cell and a row of rectangular cells of decreasing size. We found no signs of a spinelike appendage or apical branching.

### Fungi at all?

Akkari & Enghoff (2011) first called attention to what they called “intercalary cuticular micro–scutes”: rows of tiny (from less than 1 to a few  $\mu$ m diameter) circular to ovoid structures lying in neat rows between the cuticular micro–scutes of several species of millipedes. Using scanning electron microscopy Akkari & Enghoff (2011) found these structures in several millipede families belonging to the order Polydesmida, but subsequently similar structures have been found in many species of the family Odontopygidae, order Spirostreptida) (Enghoff 2014, 2016) as well as one species of Cambalidae (order Spirostreptida) (Reboleira et al. 2015) and one species of



**Fig. 2** – Scanning electron photographs of enigmatic fungi. A–C from *Xestoiulus laeticollis* (Porat, 1889) from Denmark, (Zealand, Hesede Skov near Næstved, 55°18'N, 11°57'E, 8 MAR 2015, J. Pedersen leg.) and D–E) on *Ophiulus pilosus* (Newport 1842) from Denmark (Lolland, Næsby Strand, 54°45'N, 11°04'E, 23 FEB 2014, J. Pedersen leg.). A general view of the frontal view of the head of *X. laeticollis* with numerous thalli. B detail of a group of thalli. C thallus detail. D–E thalli on leg of *Ophiulus pilosus*. Scale bars: A = 100  $\mu$ m, B = 10  $\mu$ m, C, D, E = 1  $\mu$ m.

Dorypetalidae (order Callipodida) (Reboleira & Enghoff 2015). Fig. 4 shows a selection of these structures. They always occur along the borders between cuticular microscutes (each of which corresponds to an underlying hypodermis cell, cf. Fusco et al. 2000). Sometimes the circular-ovoid structures are connected by a band (a hypha) and sometimes appear deflated (Fig. 4E) in a single case some were borne on short stalks (Fig. 4C–D) and one of the stalked micro-spheres appeared to have broken open (Fig. 4C, arrow).

So far, these structures are known only from scanning electron micrographs. Similar structures have, to our knowledge, not been reported from other arthropods, and it is a question whether they are at all of fungal nature. Toledo et al. (2010: Fig. 1C) showed a structure which they identified as a germ tube of *Beauveria bassiana* (Bals. –Criv.) Vuill. penetrating through a pore of the wax gland of a hemipteran insect – this structure looks quite like the one shown here on Fig. 4C–D. So far this is the only “match” we have been able to find and the fungal nature of the “intercalary cuticular micro-scutes” in millipedes remains unproven.

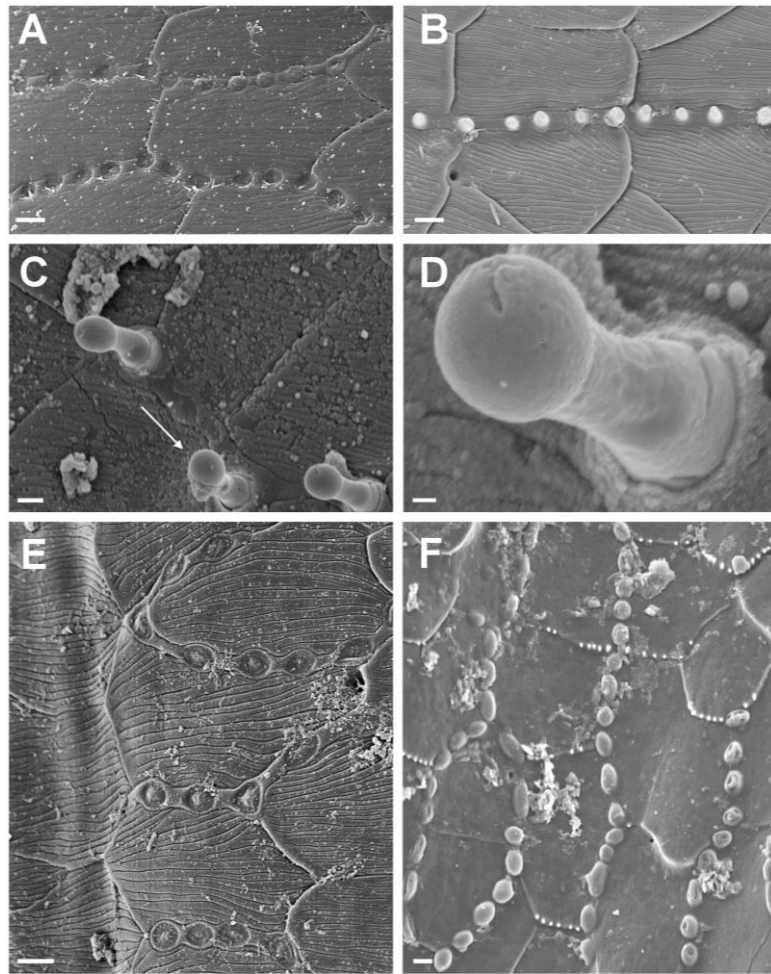


**Fig. 3** – Enigmatic fungi from *Xestoiulus laeticollis* from Denmark (Zealand, Hesede Skov near Næstved, 55°18'N, 11°57'E, 8 MAR 2015, J. Pedersen leg.). Scale bars: A, C, D = 20  $\mu$ m, B, E = 10  $\mu$ m.

### Discussion

Our observations on amphoromorphs are in agreement with previous studies on *Basidiobolus/Amphoromorpha*. Dykstra & Bradley–Kerr (1994) showed that the attachment disk is formed by an adhesive droplet. It seems that amphoromorphs do not invade the host cuticle: after removal of an amphoromorph fungus from a spider, the underlying cuticle showed no signs of penetration (Henriksen et al. 2017). In fact, an image of an amphoromorph on a millipede was presented by Reboleira & Enghoff (2013: their fig. 8) in the description of *Boreviulisoma barrocalense*, however without pointing out the fungus. The enigmatic fungus from *Xestoiulus laeticollis* “seems to be most like a combination of *Coreomycetopsis* Thaxt. (for the attachment cell) and *Hormiscioideus* M. Blackw. & Kimbr. or *Antennopsis* R. Heim. (for the filamentous part of the thallus). This is of course not really possible, so it could be new” (Meredith Blackwell in litt.). *Coreomycetopsis*, *Hormiscioideus*. and *Antennopsis* have all been described from termites (Blackwell & Kimbrough 1976, 1978). The very small fungi described by Brolemann (1920) from *Stemmiulus (Nethoiulus) sjoetedi* (Brolemann 1920) (order Stemmiulida, family Stemmiulidae) from Tanzania (Fig. 1E) are equally enigmatic. Brolemann (1920) thought that they might be Laboulbeniales, but this is more than doubtful (cf. Rossi & Balazuc 1977).

Surely, many more small fungi growing on millipede remain to be found. Our recent experience with Laboulbeniales (Santamaria et al. 2016) shows that close study of collections may reveal a high diversity of Laboulbeniales and most probably the non-laboulbenielean fungi are equally diverse. Unlike Laboulbeniales, which live on their host during their entire life cycle, amphoromorphs, ‘Thaxteriola’ etc. are stadia in more complicated life cycles involving different types of substrate and the isolated study of the phoretic stages can give only limited information.



**Fig. 4** – Fungi growing between cuticular microscutes of millipedes of the family Odontopygidae (order Spirostreptida, A–E and Dorypetalidae (order Callipodida, F). A from *Chaleponcus netus* Enghoff 2014. B from *Prionopetalum kraepelini* (Attems 1896). C–D from *Chaleponcus nikolajsharffi* Enghoff 2014, the arrow in Fig. 4C indicates an opened fungal body. E from *C. tintin* Enghoff 2014. F from *Lusitanipus alternans* (Verhoeff 1893). Scale bars: A, B, E, F = 2  $\mu\text{m}$ ; C = 1  $\mu\text{m}$ , D = 0.2  $\mu\text{m}$ . After Enghoff (2014, 2016) and Reboleira & Enghoff (2015) modified.

These small organisms are likely to be overlooked, as some of the examples mentioned above show. As a curiosity it can be mentioned that not only diplopodologists and arachnologists overlook small fungi. In a recent paper Solodovnikov & Shaw (2017) showed a SEM image of the tarsus of an Australian rove beetle (Coleoptera, family Staphylinidae). The beetle species in question is characterized by a peculiar comb which in Solodovnikov & Shaw's image is indicated with a red bar. Incidentally the red bar ends at two long structures that look very much like the fungus described above from Danish julid millipedes. We have been able to re-examine the SEM mount and confirm the similarity of the fungus with those found on *Xestoiulus* and *Ophiulus*.

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