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New Approach to the Ultrastructure of the Capillitium in the Order Trichiales (Myxomycetes, Amoebozoa) and its Phylogenetic Implications

Iván García-Cunchillos^{a,1}, Belén Estébanez^b, and Carlos Lado^a

^aReal Jardín Botánico, CSIC, Plaza de Murillo 2, 28014 Madrid, Spain

^bDepartamento de Biología (Botánica), Facultad de Ciencias, Universidad Autónoma de Madrid, Campus de Cantoblanco, Darwin 2, 28049 Madrid, Spain

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¹Corresponding author; e-mail igcun@rjb.csic.es (I. García-Cunchillos).

Myxomycetes constitute one of the major lineages within the supergroup Amoebozoa. At the end of their life cycles, most myxomycetes produce spore-bearing fruiting bodies, in which additional structures develop, like the capillitium, a system of sterile filaments intermingled with the spores. The capillitium is a relevant structure in the taxonomy of the order Trichiales, the target group in this study. However, the introduction of molecular phylogenies in Myxomycetes systematics is challenging our comprehension of this structure. We studied the capillitium of 25 species representing nine Trichiales genera, with both scanning and transmission electron microscopy. In this order, the capillitium showed higher diversity than so far recognized. Thus, we distinguished and described five capillitium types and two subtypes based on the presence or absence of a lumen and the wall ultrastructure. These types followed the evolutionary history reported in recent phylogenies, although not all of them defined monophyletic groups. Besides, the spiral ornamentation, which most taxonomists considered to have appeared once, occurred in three different capillitium types. The ultrastructural approaches in Myxomycetes systematics enable the reconsideration of their morphological features in the new phylogenetic scenario.

Key words: Amoebozoa; microscopy; SEM; systematics; TEM; phylogeny.

Introduction

Myxomycetes, also known as Myxogastria or acellular slime molds, constitute one of the major lineages within the supergroup Amoebozoa (Baldauf et al. 2000; Fiore-Donno et al. 2010) and currently has more than 1000 species (Lado 2005-2020). These organisms present a distinctive life cycle, alternating between assimilative and motile stages (myxamoebae, flagellated cells, and plasmodia) and a static phase, in which spores develop inside fruiting bodies (de Bary 1859; Kang et al. 2017). These fruiting bodies (Fig. 1A, C, E) consist of a mass of spores (black arrows in Fig. 1) and a system of sterile filaments intermingled with the spores, the capillitium (white arrows in Fig. 1), surrounded and protected by a dehiscent peridium.

Generally, the assimilative stages do not present variations to distinguish among species, and thus, Myxomycetes systematics relies on the morphological structures of the fruiting bodies (Martin and Alexopoulos 1969). The order Trichiales encompasses species with bright-colored spores and a flexuous and ornamented capillitium (Macbride 1922). Trichiales taxonomy considers the distinct capillitium features are fundamental characters to construct a hierarchical classification (Lado and Eliasson 2017). A solid capillitium defined the family Dianemataceae (Fig. 1A, B), in contrast to the hollow one in the remaining families (Fig. 1G). Within the latter, the capillitium ornamental elements (Lado and Pando 1997) served to differentiate the family Trichiaceae, with spirals (Fig. 1C, D), and the family Arcyriaceae, with cogs, rings, half-rings, spines, verrucae, or a combination of them (Fig. 1E, F, G). Furthermore, some authors considered a fourth, monospecific family (Minakatellaceae), with a hollow capillitium, showing no spiral ornamentation (Keller et al. 1973).

Despite the long-standing tradition in the study of Myxomycetes (Stephenson et al. 2008), the introduction of molecular tools and phylogenetic analyses is challenging its systematics (e.g., Fiore-Donno et al. 2005; Leontyev et al. 2019). Thus, the incongruences reported between the phylogenies and the morphology-based classifications question the traditional interpretation of how morphological characters evolved, like the capillitium in Trichiales (Fiore-Donno et al. 2013). For example, most taxonomists considered the capillitium spiral ornamentation as an exclusive characteristic of the family Trichiaceae (Lado and Pando 1997; Martin and Alexopoulos 1969). However, molecular phylogenies have pointed out distinct origins of these ornamental elements (Fiore-Donno et al. 2013).

The different capillitium features, especially the ornamental elements, are usually at the light microscopy resolution limit (Fig. 1B, D, F). Therefore, scanning electron microscopy (SEM) has become a suitable technique for taxonomic purposes (Lizárraga et al. 1999; Ronikier et al. 2020).

However, the use of transmission electron microscopy (TEM) and the study of the ultrastructural features remained rather unusual in Myxomycetes (e.g., Charvat et al. 1973; McHugh et al. 2000; Mims 1969). Based on capillitium TEM observations in Trichiales, Ellis et al. (1973) described five capillitium types, refuting the previously prevailing dichotomy between solid versus hollow filaments. Moreover, these authors hypothesized that a continuum gradient from solid to hollow filaments could emerge with the study of more specimens and species. Unfortunately, there have been no other comparative studies testing this hypothesis.

In this study, we explore with TEM and SEM the diversity of ultrastructural capillitium features in Trichiales. We include specimens from species already study in the literature to ascertain the stability of these features at the species level. With a broader taxa sampling, we evaluate whether the description of discrete capillitium types or the continuum gradient hypothesis better reflects the capillitium ultrastructural variability in Trichiales. Last, we analyze these results in the current Trichiales phylogenetic framework.

Results

The capillitium morphogenesis in Trichiales species is a critical process to analyze its ultrastructure. Briefly, Strasburger (1884) and Harper and Dodge (1914) described this process as starting with the appearance of multiple vacuoles, which then arrange in rows, acquiring the final disposition of the capillitium in the sporotheca. Posteriorly, the wall formation implies the deposition of the component material underneath the vacuoles membranes (Mims 1969). Thus, the basic structure of a capillitium filament resembles a hollow tubule, in which a more or less dense wall surrounds a central space, termed lumen by Ellis et al. (1973). We can then define a *lumen* as a unique, open, and shape-defined space that occupies a variable portion of the filament (Figs 10, 19, 26, 29). Despite this hollowness, the lumen may contain small fragments of material from the wall, and other trapped material, such as bacteria or protoplasmic remnants (e.g., Figs 11, 15, 20). However, some species do not show such a lumen and present almost solid filaments, occasionally interrupted by open, small, and irregularly-shaped spaces (Fig. 2; b). We termed the central part with these spaces as *core* to differentiate it from the lumen. To further emphasize this difference, we reserve the terms *tubule* and *thread* to refer to those filaments presenting a lumen (type A) and a core (types B-E), respectively.

In the wall descriptions as seen with TEM, we differentiated between sections (Fig. 2; a, b) rather than layers because there were no sharp-defined frontiers between them. Besides, some of

these sections presented regions with a different electron density. For the description of ornamental elements, as seen with SEM, the terminology followed Rammeloo (1983; 1984a; 1984b; 1986).

In the nine genera and 25 species studied, we distinguished five ultrastructural capillitium types and two subtypes, considering the presence of a lumen versus a core and the organization of the material forming the wall.

Capillitium type A consists of nearly solid filaments, with occasional open spaces in the central zone, referred to as a core. We distinguished between two subtypes (A1, A2) according to the extension of this core.

In *capillitium subtype A1* (Fig. 2), the wall occupies almost the entire thread diameter. The presence of a core is unusual and, when it is present, it consists of small, irregular, open spaces (arrows in Figs 2, 3, 5). This subtype appeared in the monospecific genus *Prototrichia* and some species of *Dianema* (Table 1).

As seen with TEM, the wall structure varies among the species studied. In *Dianema depressum* (Fig. 3) and *D. corticatum* (not shown), the wall is composed of two sections that are differentiated by their thicknesses and electron densities, as depicted in Fig. 2 (a, b). The inner section (Figs 2 (b), 3) is the thickest and more electron-dense, and it occupies most of the thread diameter. The wall in *Prototrichia metallica* is composed of two sections (Fig. 5), although their characteristics differ from those of *Dianema*. In this species, the outer section is compressed and electron-opaque, while the inner one has less electron density, highlighting the fibrillar nature of the wall component. These fibrils are arranged concentrically around the open spaces of the core (Fig. 5).

The ornamental elements in this subtype, as seen with SEM, are also diverse. They consist of small, irregularly distributed verrucae in *D. corticatum*, a meshed reticulum in *D. depressum* (Fig. 4), and smooth spirals in *P. metallica* (Fig. 6).

Threads in *capillitium subtype A2* are also nearly solid but, when a core is present, it occupies most of the thread diameter (Figs 7, 8), unlike in subtype A1. This subtype was solely present in the species *Dianema succulenticola*.

The wall in this species, as seen with TEM, also includes two sections (Fig. 8). The outer one is composed of concentric fibrillar 'layers' with different electron densities (Figs 7 (a), 32 (a)), while the inner section is less compacted and more electron-translucent (Figs 7 (b), 32 (b)). There are cytoplasmic remnants trapped inside of these filaments (arrows in Figs 7, 8).

The ornamental elements in this species, as seen with SEM, include abundant and uniformly distributed verrucae (Fig. 9).

In **capillitium type B**, a two-sectioned wall surrounds a usually large lumen, and the material of the inner section presents a loose structure (Fig. 10). This tubules type was present in all species studied of the genera *Arcyodes*, *Arcyria*, and *Calonema*, and in some species of *Perichaena*, *Hemitrichia*, and *Trichia* (Table 1).

As seen with TEM, the outer section of the wall (Fig. 10; a) is usually compacted, although minute gaps or holes are present in the species *Arcyria denudata* and *Arcyodes incarnata* (arrow in Fig. 33). Different species show different thicknesses in this section (Figs 11, 15). The fibrillar component of the wall becomes less compacted in the inner section (Fig. 10; b). The extension of this section also varies among the species. It barely invades the lumen in *Arcyria ferruginea* (Fig. 13), while its fibrils spread throughout the whole lumen in *Calonema foliicola* (Fig. 11), *Hemitrichia calyculata* (Fig. 15), and *Hemitrichia leiocarpa* (Fig. 34). The transition between both sections can be gradual, as in *A. ferruginea* (Fig. 13) or *H. leiocarpa* (Fig. 34), or abrupt, as in *H. calyculata* (Fig. 15) or *Arcyria versicolor* (not shown). Some material embedded in the lumen, such as undigested bacteria and protoplasmic remnants (Figs 11, 15, 17), may appear. TEM images showed that the ornamentation always corresponds with the outer section of the wall.

The diverse ornamental elements in capillitium type B, as seen with SEM, can be classified into three groups. First, rings, half-rings, verrucae, and small projections (Fig. 14), sometimes forming a reticulum through their connection by short ridges, as in *Arcyodes incarnata*, *Arcyria globosa*, *A. versicolor*, and *A. ferruginea*. The species *A. denudata* and *A. insignis* present longitudinal striae between these elements (not shown). TEM images showed that some of these elements are not solid (arrow in Fig. 13). In *Perichaena corticalis* (Fig. 18), there are numerous holes on the capillitium surface, which do not reach the lumen, besides the irregular projections (Fig. 17).

The second group includes spiral elements present in the species *Hemitrichia calyculata* (Fig. 16), *H. clavata*, and *H. abietina* (not shown). These spirals are single- or double-crested (seen as Y-shaped in TEM cross-sections, Fig. 15). In *H. calyculata*, these elements appear connected by characteristic bridges (arrows in Figs 15, 16). The spirals in *H. leiocarpa* are irregularly shaped and usually bifurcated (not shown).

The third group, only observed in species *Calonema foliicola*, consists of subparallel bands along the capillitium surface, with occasional bifurcations (Fig. 12).

Capillitium type C also shows a two-sectioned wall surrounding a lumen (Fig. 19). However, the inner section in these tubules presents a fractured pattern (Fig. 19; b). This type appeared in the genera *Metatrichia*, *Trichia* and some *Hemitrichia* species (Table 1).

As seen with TEM, the outer section of the wall consists of a material with high electron density (Fig. 19; a), including the ornamentation. The inner section shows the same characteristics as the outer one, yet with a polygonal organization (Figs 19 (b), 35). Lumen in capillitium type C includes protoplasmic remnants and other particles (arrows in Figs 19, 20). A slightly different variant of this general model occurs in the species *Trichia persimilis* (Fig. 22), *T. scabra* (Fig. 35), and *T. varia* (not shown). The outer section of the wall in these species presents two distinct regions: an external, thinner, less electron-dense region (Figs 22 (d), 35 (d)) and an internal, thicker, and electron-opaques region. However, these two regions are not constant over even a single capillitium cross-section (Fig. 22).

As seen with SEM, the ornamentation in this capillitium subtype includes spirals, which can besides bear secondary ornamental elements. In the species *Trichia persimilis* (Fig. 23), *T. affinis*, and *T. scabra* (not shown), these secondary elements consist of short and irregular spines. *Metatrichia horrida* shows abundant, long, and somewhat tortuous spines (Figs 24, 25). Species *Hemitrichia leiotricha* (Fig. 21) and *T. varia* does not present secondary ornamental elements.

Tubules in **capillitium type D** consist of a three-sectioned wall surrounding a large lumen (Fig. 26). It was only present in the species *Perichaena chrysosperma*.

As seen with TEM, the outer (Figs 26 (a), 36 (a)) and middle (Figs 26 (b), 36 (b)) sections are similar regarding the compaction of the fibrillar component, although the middle one is thicker and more electron-dense (Fig. 36). In this species, the wall presents a highly electron-opaques border, different from the lower electron-dense outer section (Figs 26, 27). The innermost section is the less electron-dense, with a looser fibrillar component occupying a large portion of the lumen (Figs 26 (c), 36 (c)). Again, the lumen can sometimes contain remnants of protoplasmic materials (Fig. 36).

The ornamental elements in this species, as seen with SEM, consist of long and tortuous spine-like projections (Fig. 28).

Capillitium type E also consists of a three-section wall surrounding a large lumen (Fig. 29). This type was only present in the species *Perichaena quadrata*.

The outer section of the wall, as seen with TEM, is made of compact fibrils, concentrically arranged (Fig. 29 (a), 37 (a)). The innermost section is similar to the outer one but with a lower electron density (Figs 29 (c), 37 (c)). This section projects into the lumen as loose fibrillar masses (arrows in Figs 29, 37). Between both, there is a middle and narrow one with higher electron

density (Figs 29 (b), 37 (b)). The lumen can contain protoplasmic remnants and other trapped material (Fig. 30).

As seen with SEM, this capillitium presents an irregular outline and some projections as ornamental elements, like verrucae or irregular cogs (arrow in Fig. 31).

Discussion

Most capillitium types described here can be associated with those recognized by Ellis et al. (1973). However, our interpretation differed from their definitions, and, therefore, we propose a new classification of the capillitium types in Trichiales (Fig. 38). Besides, we explore how these new capillitium types fit the current phylogenetic of the order (Fig. 39).

Capillitium Types in the Order Tricales

Capillitium type A features corresponded to those of type II (Fig. 38), defined by Ellis et al. (1973) based on the species *Dianema corticatum*. Moreover, these characteristics were constant in the specimen studied here and in other related species (Table 1). Ellis et al. (1973) proposed the existence of a continuum gradient between solid and hollow filaments in Trichiales. This gradient occurred in every single filament studied in capillitium type A. However, the remaining types never showed this variation.

The capillitium in *Prototrichia metallica* fitted capillitium type A (Fig. 5). However, Ellis et al. (1973) reported a variable structure of the capillitium in this species, usually consisting of a two-sectioned wall with a core either solid or traversed by fibrous elements (type III, Fig. 38). A similar variation was present in all species studied within capillitium type A, so we consider that establishing a new class to accommodate this species is not justified.

Besides, Ellis et al. (1973) analyzed the species *Calomyxa metallica*, not included here, and established a unique capillitium type I, consisting of solid filaments (Fig. 38). On the contrary, Locquin (1948) described both solid and partially hollow segments in every single filament in this species. These results may suggest the occurrence of capillitium type A in *C. metallica*, yet further examination is necessary to confirm this circumscription.

Remarkably, we detected protoplasmic remnants inside most filaments in the different capillitium types (e.g., Figs 8, 11, 20). As previously stated, the capillitium wall development implies the external deposition of the component material beneath the vacuoles membranes (Harper

and Dodge 1914; Mims 1969). Thus, it remains unknown whether these cytoplasmic remnants inside the filaments indicate an additional internal supply of material to the wall formation.

The capillitium type we designed as B corresponds with Ellis type IV (Fig. 38). These authors described it as tubules with a single-sectioned wall, while we distinguished two sections with a looser structure of the inner one. While they did not notice this section, it becomes evident in their images, although with a lesser extension. These differences could be ascribed to the age of the specimens (older in this study) or the sample processing since we used prolonged vacuum conditions and an extended embedding protocol, probably assuring a better resin infiltration.

Different specimens of the same species, e.g., *Arcyodes incarnata*, *Arcyria denudata*, or *Perichaena corticalis* (Table 1), showed identical ultrastructural capillitium features, pointing out the stability of type B at the species level (Table 1). Moreover, it seems to be constant in the genus *Arcyria*, as all species so far studied showed this capillitium type. The opposite applies to the genera *Calonema*, *Hemitrichia*, and *Perichaena*, which presented other capillitium types besides this type B (Table 1). Here, we first describe the occurrence of capillitium type B in the genus *Trichia*.

Capillitium type C characteristics matched those of Ellis type V. The description of this type V included two sections differentiated by their electron-densities (Fig. 38). However, this difference is not always present, and it usually affects only a portion of the wall diameter (Fig. 22; d). We consider them as a single section in which regions with different electron densities may appear. Ellis et al. (1973) described structures, referred to as globular projections, lining the lumen boundary. These projections have the same characteristics as the wall component, and both are continuous (Fig. 35). Thus, we consider them a second inner section in capillitium type C. This type seems to be the most usual, yet not the only one, in the genera of the family Trichiaceae (Table 1).

We established a new capillitium type D (Fig. 38) based on the unique ultrastructural features of *Perichaena chrysosperma* (Fig. 27). Ellis et al. (1973) also studied this species and assigned it to their type IV (type B, according to our classification). However, the images they provided are overexposed, hindering the analyses of the target features. Besides, the capillitium of this species always shows numerous spine-like projections decorating its surface. Instead, these authors reported "oddly scarce" spines in their studied specimen, questioning its identity. The capillitium type E (Fig. 38) also constitutes a genuinely new type, based on the species *Perichaena quadrata*, analyzed with TEM for the first time.

Capillitium Types in the Phylogenetic Context

As depicted in Figure 39, the available phylogenies in Trichiales revealed unexpected phylogenetic relationships within the order (Fiore-Donno et al. 2013). First, some *Hemitrichia* and *Trichia*

species formed a clade (*Hemitrichia* 1 and *Trichia* 1 in Fig. 39), here represented by species *H. abietina*, *H. calyculata*, and *T. decipiens*. However, other species within these genera branched separately (e.g., *T. persimilis*, *T. scabra*, and *T. varia*), more closely related to genera *Cornuvia*, *Metatrichia*, and *Oligonema*. Besides, Walker et al. (2015) reported a paraphyletic origin of the genus *Perichaena*, here represented by the species *P. corticalis* (*Perichaena* 1) and *P. chrysosperma* (*Perichaena* 2). Fiore-Donno et al. (2013) compared the different capillitium types proposed by Ellis et al. (1973) with the phylogenetic clades they recovered, although both studies shared a limited number of species. We re-examined the potential use of capillitium types to delimit monophyletic clades with our broader taxa sampling.

The capillitium type A is exclusively present in species of the genera *Dianema*, *Calomyxa*, and *Prototrichia* (Fig. 39). The different phylogenies always retrieved these close relationships (Fiore-Donno et al. 2013; Leontyev et al. 2019; Ronikier et al. 2020). These genera differ in the capillitium ornamentation, consisting of spirals in *Prototrichia* (Rammeloo 1983), verrucae, spirally arranged, in *Calomyxa* (Lado and Pando 1997), and either verrucae, reticula, or faint spiral elements *Dianema* (Rammeloo 1983). Taxonomists traditionally classified *P. metallica* within the family Trichiaceae, based on hollow capillitium with spiral ornamentation (Kowalski 1967). However, our results showed that its capillitium structure is not different from that of *Dianema*, supporting its classification within the family Dianemataceae. While the phylogenetic affinity of *Calomyxa* with *Dianema* and *Prototrichia* is well known (Fiore-Donno et al. 2013; Leontyev et al. 2019), it remains unknown whether the former has a capillitium type A (see above). The distinction between subtypes, A1 and A2, cannot be evaluated from a phylogenetic perspective since no molecular information is available from the only species with a subtype A2 (Table 1).

The capillitium type B does not define a monophyletic group of species. Instead, it is present in three different clades (Fig. 39). First, it appears in a clade comprising some *Hemitrichia* and *Trichia* species (Table 1, *Hemitrichia* 1 and *Trichia* 1 in Fig. 39). The capillitium of these species shows spiral ornamentation. Remarkably, the type species of the genus *Hemitrichia* (Table 1) present capillitium type B, but no phylogenetic information is yet available. Second, this capillitium type occurs in the closely related genera *Arcyria* and *Arcyodes*. The ornamental elements of their capillitium are diverse (see Results), but none of the species studied included spirals. Third, capillitium type B is present in one of the two clades known to comprise *Perichaena* species (*Perichaena* 1 in Fig. 39). The type species of this genus, *P. corticalis*, branched within this clade (Walker et al. 2015). Besides, the only representative of the family Minakatellaceae, *M. longifila*, showed the same features that capillitium type B, pointing out a close relationship with these clades, as Keller et al. (1973) stated.

Only one species presented capillitium type E, *Perichaena quadrata*, with no molecular information available. This species is highly similar to *Perichaena depressa* (Keller and Eliasson 1992), not studied here. Several phylogenies have pointed out a close relationship between *P. depressa* and *P. corticalis* (Fiore-Donno et al. 2013; Leontyev et al. 2019). Therefore, it remains unknown if *P. quadrata* also shows the same affinities with *P. corticalis*, and so, different capillitium types could then occur within that clade.

According to the branching pattern, the next capillitium type in the phylogeny is type D, solely present in *Perichaena chrysosperma*. Walker et al. (2015) reported a paraphyletic origin of the genus *Perichaena* based on this species (*Perichaena* 2 in Fig. 39). Thus, this species constitutes a new phylogenetic clade, more closely related to the genera of the family Trichiaceae (e.g., *Trichia*, *Oligonema*) than to clade *Perichaena* 1.

The capillitium type C is exclusively present in a clade encompassing most genera in the family Trichiaceae (Fig. 39), including the type species of some genera (Table 1). While the phylogenetic studies always retrieved this clade (Fiore-Donno et al. 2013; Walker et al. 2015; Leontyev et al. 2019), the relationships among these taxa remain unresolved (Ronikier et al. 2020). The ornamentation of the capillitium within these taxa always consists of spirals.

Conclusions

The study of Myxomycetes systematics with phylogenetic approaches challenged our knowledge of the distinct structures in the fruiting bodies. The study of the broad diversity of capillitium ultrastructural features in Trichiales recognized five discrete capillitium types, in contrast to the traditional solid versus hollow filaments dichotomy. The spiral capillitium ornamentation traditionally defined the family Trichiaceae, considering it to have appeared once in the Trichiales evolutionary history. However, we described three capillitium types bearing these ornamental elements, directly related to three independent phylogenetic clades in the order. Thus, the value of this character in Trichiales merits a re-examination in their taxonomy. The study of morphological structures in Myxomycetes fruiting bodies with ultrastructural approaches, such as TEM and SEM, enables the reconsideration of their basic features in the light of the recently discovered evolutionary scenarios. Ultrastructural approaches, such as TEM and SEM, enable the reinterpretation of these morphological characters in the light of a still cryptic evolutionary scenario.

Methods

Samples: A total of 25 species of 9 genera (Table 1) were selected to include the broad diversity of capillitium features in the order Trichiales. We first verified the correct development of the capillitium features in the 28 specimens studied (Supplementary Material) with light microscopy and, posteriorly, with SEM, before processing them for TEM observations. TEM and SEM studies were always performed on the same specimen, except in *Prototrichia metallica*, with scarce fruiting bodies. In this species, we selected two identical samples to conduct each technique (Supplementary material). All samples are kept in the Myxomycetes collection of the herbarium MA-Fungi Herbarium (Real Jardín Botánico, CSIC).

Transmission electron microscopy (TEM): One or two fruiting bodies were collected from each sample in 1.5 ml safe-lock Eppendorf tubes with distilled water. Samples were subjected to vacuum conditions for 5-10 minutes to eliminate possible trapped air bubbles in the capillitium, which might interfere with the fixing process. The inclusion of the samples followed the protocol of Ligrone and Duckett (1994) with minor modifications. Briefly, the samples were fixed in 3% glutaraldehyde in a 0.1 M cacodylate buffer (pH=7.4) in two successive steps, one hour each, and rinsed three times in the buffer. The samples were then post-fixed in 1% osmium tetroxide, 2 hours at room temperature, and subsequently rinsed twice with distilled water (30 minutes each). Species with fragile capillitium filaments (e.g., *Dianema* and *Prototrichia*) were embedded in 1% agarose after the post-fixation. All samples were dehydrated in an ethanol series, transferred into propylene oxide (4° C) in two steps (15 minutes each), and embedded in the modified Spurr low-viscosity embedding medium (Spurr 1969), yet using the resin component ERL4221 instead of the cancerigenous ERL4206 of the original formula. This process was accomplished in dark conditions (4 °C), in seven steps, once every 24 hours, without shaking. Ultrathin sections were obtained with a diamond knife on a Leica Ultracut S ultramicrotome. At least ten capillitium sections, often more than 50, were studied in each specimen. We analyzed both cross and longitudinal sections, although we only illustrate the former, as they better show the characteristics described. Besides, we obtained sections at different depths in the resin block containing each sample to ensure the study of distinct filaments. The preparations were contrasted with lead citrate and uranyl acetate and observed with a JEOL JEM1010 (100 kV) transmission electron microscope coupled to a Gatan Orius 200 SC camera.

Scanning electron microscopy (SEM): Samples were dehydrated in a series of acetone (15 minutes per step). Then they were subjected to critical point drying with a CPD 7501 Polaron Quorum Technologies equipment. Later they were sputtered with gold (with an SCD 004 Sputter Coater Balzers, Leica) and observed in a Hitachi S3000N scanning electron microscope operating at 10-15 kV.

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Tables

Table 1. Capillitium types in the order Trichiales according to our classification (*New types*) and the one proposed by Ellis et al. (1973). Species in bold correspond with those here studied. Shaded in grey, the type species of each genus. Capillitium types in bold (third and fourth columns) reflect the original assignment of the authors, including every available study: ¹Mims (1969), ²Keller et al. (1973), and ³Ellis et al. (1973). Otherwise, we indicate the correspondence among both classifications, according to the descriptions or images. In the *N specimens* column, we summarize the total number of specimens for which capillitium ultrastructural information has been reported in each species (both here and in previous studies). An asterisk points out a conflict of results (see Discussion). Phylogeny indicates if there is molecular information for the species.

FAMILY	GENUS	SPECIES	CAPILLITIUM		N SPECIMENS	PHYLOGENY
			NEW TYPES	ELLIS TYPES		
Arcyriaceae	<i>Arcyodes</i>	<i>incarnata</i> ³	B	IV	2	YES
	<i>Arcyria</i>	<i>cinerea</i> ¹	B	IV	1	YES
	<i>Arcyria</i>	<i>denudata</i> ³	B	IV	3	YES
	<i>Arcyria</i>	<i>ferruginea</i>	B	IV	1	YES
	<i>Arcyria</i>	<i>globosa</i>	B	IV	1	YES
	<i>Arcyria</i>	<i>insignis</i>	B	IV	1	-
	<i>Arcyria</i>	<i>versicolor</i>	B	IV	1	-
	<i>Perichaena</i>	<i>chrysosperma</i>	D	IV	2*	YES
	<i>Perichaena</i>	<i>corticalis</i> ³	B	IV	3	YES
	<i>Perichaena</i>	<i>microspora</i> ³	B	IV	1	-
	<i>Perichaena</i>	<i>quadrata</i>	E	-	1	-
Dianemataceae	<i>Calomyxa</i>	<i>metallica</i> ³	-	I	1	YES
	<i>Dianema</i>	<i>corticatum</i> ³	A1	II	2	YES
	<i>Dianema</i>	<i>depressum</i>	A1	-	1	-
	<i>Dianema</i>	<i>succulenticola</i>	A2	-	1	-
Minakatellaceae	<i>Minakatella</i>	<i>longifila</i> ²	B	IV	1	-
Trichiaceae	<i>Calonema</i>	<i>aureum</i> ³	C	V	1	-
	<i>Calonema</i>	<i>foliicola</i>	B	IV	1	-
	<i>Hemitrichia</i>	<i>abietina</i>	B	IV	1	YES
	<i>Hemitrichia</i>	<i>calyculata</i>	B	IV	1	YES

<i>Hemitrichia</i>	<i>clavata</i>	B	IV	1	YES
<i>Hemitrichia</i>	<i>leiocarpa</i>	B	IV	1	YES
<i>Hemitrichia</i>	<i>leiotricha</i>	C	V	1	-
<i>Hemitrichia</i>	<i>montana</i> ³	B	IV	1	-
<i>Hemitrichia</i>	<i>serpula</i> ³	C	V	1	YES
<i>Metatrichia</i>	<i>floriformis</i> ³	C	V	1	YES
<i>Metatrichia</i>	<i>horrida</i>	C	V	1	-
<i>Metatrichia</i>	<i>vesparia</i>	C	V	1	YES
<i>Oligonema</i>	<i>flavidum</i>	C	V	1	YES
<i>Prototrichia</i>	<i>metallica</i>	A1	III	2	YES
<i>Trichia</i>	<i>affinis</i>	C	V	1	-
<i>Trichia</i>	<i>decipiens</i>	B	IV	1	YES
<i>Trichia</i>	<i>persimilis</i>	C	V	1	YES
<i>Trichia</i>	<i>scabra</i>	C	V	1	YES
<i>Trichia</i>	<i>varia</i>	C	V	1	YES

Figure Legends

Figure 1. Representative fruiting bodies of the three families of the order Trichiales studied (**A, C, E**), details of the capillitium filaments (white arrows) and spores (black arrows), as seen with light microscopy (**B, D, F**), and detail of a hollow capillitial filament, as seen with SEM (**G**). **A-B:** family Dianemataceae (*Dianema succulenticola*). **C-D:** family Trichiaceae (*Trichia varia*). **E-G:** family Arcyriaceae (*Arcyria insignis*, **E-F**; *A. nigella*, **G**).

Figures 2-6. Capillitium subtype A1. **2.** Schematic representation of the sections of the wall (a, b) and the core (arrow) in a cross-sectioned capillitium. **3.** *Dianema depressum*, cross-section with a highly reduced core (arrow) (TEM). **4.** *D. depressum*, detail of the ornamentation (SEM). **5.** *Prototrichia metallica*, cross section with a highlight core (arrow) (TEM). **6.** *P. metallica*, spiral ornamentation (SEM). **Figures 7-9.** Capillitium subtype A2 **7.** Schematic representation of the sections of the wall (a, b) and protoplasmic remnants (arrow). **8.** *Dianema succulenticola*, cross-section with a large core with protoplasmic remnants (arrow) (TEM). **9.** *D. succulenticola*, detail of the verrucae ornamentation (SEM). Scale bar = 1 μm .

Figures 10-14. Capillitium type B. **10.** Schematic representation of the sections of the wall (a, b). **11.** *Calonema foliicola*, cross-sectioned capillitium (TEM). **12.** *C. foliicola*, detail of the ornamentation (SEM). **13.** *Arcyria ferruginea*, cross section pointing the not solid nature of some of the ornamental elements (arrow) (TEM). **14.** *A. ferruginea*, detail of the ornamentation (SEM).

Figures 15-18. Capillitium type B. **15.** *Hemitrichia calyculata*, cross section showing the links among the spiral ornamental elements (arrow) (TEM). **16.** *H. calyculata*, detail of the ornamentation showing the same links (arrow) (SEM). Scale bar = 1 μm . **17.** *Perichaena corticalis*, cross section (TEM). **18.** *P. corticalis*, detail of the ornamentation (SEM). Scale bar = 1 μm .

Figures 19-25. Capillitium type C. **19.** Schematic representation of the sections of the wall (a, b) and protoplasmic remnants (arrow). **20.** *Hemitrichia leiotricha*, cross-sectioned capillitium with protoplasmic remnants (arrow) (TEM). **21.** *H. leiotricha*, spiral ornamentation (SEM). **22.** *Trichia persimilis*, cross section with the presence of a less electron dense region (d) in the outer section of the wall (TEM). **23.** *T. persimilis*, spiral ornamental elements with detail of the short spines as secondary ornamentation (SEM). **24.** *Metatrichia horrida*, cross section (TEM). **25.** *M. horrida*,

spiral ornamental elements with a detail of the long, irregular spines as secondary ornamentation (SEM). Scale bar = 1 μm .

Figures 26-28. Capillitium type D. **26.** Schematic representation of the sections of the wall (a, b, c). **27.** *Perichaena chrysosperma*, cross-sectioned capillitium (TEM). **28.** *P. chrysosperma*, detail of the ornamentation (SEM). **Figures 29-31.** Capillitium type E. **29.** Schematic representation of the sections of the wall (a, b, c). **30.** *Perichaena quadrata*, cross section (TEM). **31.** *P. quadrata*, detail of the ornamentation (arrow) (SEM).

Figures 32-37. Details of the ultrastructural features of the different capillitium types (TEM). **32.** *Dianema succulenticola*, capillitium subtype A2, sections of the wall (a, b, c). **33.** *Arcyodes incarnata*, capillitium type B, characteristic minute gaps or holes (arrow) in the wall. **34.** *Hemitrichia leiocarpa*, capillitium type B, inner section of the wall extending throughout the whole lumen. **35.** *Trichia scabra*, capillitium type C, detail of a less electron dense region (d) within the outer section of the wall. **36.** *Perichaena chrysosperma*, capillitium type D, sections of the wall (a, b, c). **37.** *Perichaena quadrata*, capillitium type E, sections of the wall (a, b, c) and detail of the projections of the inner section into the lumen (arrow). Scale bar = 0.5 μm .

Figure 38. Schematic comparison and correspondence of the capillitium types defined by Ellis et al. (types I-V, left) and the ones here described (A-E, right).

Figure 39. Mapping of the ornamental elements and the capillitium types, as here described, on a schematic representation of the phylogenetic relationships of the main clades in the order Trichiales. Branch lengths indicate neither genetic distance nor time. The capillitium type E, present only in *Perichaena quadrata*, with no molecular information, is not represented here. * Some species can show smooth spirals or ornamental elements spirally arranged. ¹Capillitium type of species *Calomyxa metallica* remains unknown (see Discussion).

HIGHLIGHTS

Capillitium ultrastructure presents higher diversity than the previously recognized.

Five discrete capillitium types and two subtypes are distinguished.

Three distinct capillitium types show spiral ornamentation.

Ultrastructural features shed light into the cryptic phylogeny of Trichiales.

Declaration of interests

■ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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