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Echinostelium australiense (Myxomycetes: Echinosteliaceae), a new species of slime mould described from Western Australia

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Abstract

Knight, K.J., Stephenson, S.L. & Novozhilov, Y.K. *Echinostelium australiense* (Myxomycetes: Echinosteliaceae), a new species of slime mould described from Western Australia. *Nuytsia* 34: 179–186 (2023). A new and unusual species of *Echinostelium* de Bary appearing on samples of bark collected in Western Australia and placed in moist chamber cultures is described and illustrated. This new species is clearly distinct from all other species of *Echinostelium* as it differs in a number of morphological and colour characters. The dark colouration of the sporotheca, stalk and spores with distinct patches of closely arranged warts arranged in a loosely circular pattern, and a large bi-coloured petaloid collar are unusual for the genus.

Introduction

Echinostelium is a genus of myxomycetes (slime moulds) in which the inconspicuous sporocarps generally occur on the bark of living trees, with occasional occurrences on vines or various types of dead plant material (Keller & Brooks 1976; Whitney 1980; Haskins & Clark 2016) and very rarely on the weathered dung of herbivorous animals (Novozhilov & Schnittler 2008). Due to a short-lived life cycle strategy and the diminutive size of sporocarps, mostly less than 0.5 mm in height with the exception of *E. novozhilovii* A.Vlasenko, which can reach a height of 2.5 mm (Vlasenko *et al.* 2018), new records and new species are usually observed from material recovered using the moist chamber technique and examination of substrate samples under a dissecting microscope.

An unusual new myxomycete was first discovered by the late English myxomycologist David W. Mitchell. Specimens were harvested from moist chamber cultures in late 2002 (pers. correspondence Margaret H. Brims) on bark samples of *Dryandra sessilis* (Knight) Domin, currently *Banksia sessilis* (Knight) A.R.Mast & K.R.Thiele, collected from Stoneville, *c*. 30 km east of Perth, Western Australia (*Mitchell* 6749). Mitchell regarded this as a potential new species of *Echinostelium* and provided a short description, diagnosis, and image in a paper published shortly after its discovery (McHugh *et al.* 2003). The following year, one of the authors of the aforementioned paper (the late Margaret H. Brims), harvested the collection of the specimen listed below in a moist chamber culture prepared

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with bark samples from *Eucalyptus todtiana* F.Muell. from Hi Vallee farm near Badgingarra, *c.* 250 km north-north-west of Perth, Western Australia (*Brims* 685). At the time, the significance of the find was overlooked, and the specimen was deposited in the Western Australian Herbarium. Subsequent research has shown that these unusual specimens represent a new taxon, described here, thus confirming Mitchell's original idea.

Echinostelium is a relatively small genus with only 16 species (Lado 2005–2023) but with a worldwide distribution (GBIF Secretariat 2001–2023). Some species (e.g. *E. colliculosum* K.D.Whitney & H.W.Keller), are common in arid regions (Novozhilov *et al*. 2006; Novozhilov & Schnittler 2008) but it seems are rare or absent in humid tropics (Schnittler & Stephenson 2000; Novozhilov *et al*. 2017). The 11 species recorded for Australia are *E. apitectum* K.D.Whitney, *E. arboreum* H.W.Keller & T.E.Brooks, *E. bisporum* (L.S.Olive & Stoian.) K.D.Whitney & L.S.Olive, *E. brooksii* K.D.Whitney, *E. coelocephalum* T.E.Brooks & H.W.Keller, *E. colliculosum*, *E. corynophorum* K.D.Whitney, *E. elachiston* Alexop., *E. fragile* Nann.-Bremek., *E. minutum* de Bary and *E. paucifilum* K.D.Whitney; of these, *E. colliculosum* is the only species not known from Western Australia. Stephenson (2021) states 6 species are not known from Australia (*E. cribrarioides* Alexop., *E. ladoi* Pando, *E. lunatum* L.S.Olive & Stoian., *E. microsporum* A.Vlasenko, *E. novozhilovii* A.Vlasenko and *E. paucifilum*), however *E. paucifilum* was recently recorded from Western Australia (GBIF Secretariat 2001–2023). The description of the new species *E. australiense* K.J.Knight, S.L.Stephenson & Novozh. brings the Australian species to 12 (nine in Western Australia) and the world total to 17 species.

Methods

The moist chamber culture technique used for the substrate samples collected by the first author took into consideration the morphology and hygrophobic nature of the bark of *Eucalyptus todtiana*. The bark of *E. todtiana* is longitudinally fibrous, and large samples were required to maintain their integrity in the moist chamber. The samples were thick, up to 1.5 cm, and relatively long, up to 10 cm. Substrate samples were submerged in distilled water for about 10 seconds to help break the surface tension on the upper surface, and left to soak for 24 or 36 hrs. The substrate samples soaked for 36 hrs were more productive than those soaked for 24 hrs; however, it is unknown whether the longer soak period caused the difference. The pH of the soak water was recorded with an EcoTestr pH2 handheld meter, and after excess soak water was removed the substrate samples were placed in lidded plastic containers, the base lined with paper towel and maintained at room temperature in diffuse light following ambient night/day conditions. Microscopic examination was carried out on dried specimens rehydrated and mounted in Hoyer's solution. Scanning electron micrographs by Novozhilov were obtained with a JSM-6390 LA scanning electron microscope (SEM) at 10–15 kV using cryo-dried specimens and those by Dillon with a Neoscope JCM-5000 SEM at 10 kV. All mounts were on copper stubs via double-sided sticky film and sputter-coated with gold.

Taxonomy

Echinostelium australiense K.J.Knight, S.L.Stephenson & Novozh., *sp. nov.* (MB 838442).

Type: Hi Vallee Farm, 5 km east of Brand Highway on Tootbardi Road, *c.* 250 km north-north-west of Perth, Western Australia, 22 August 2020, *K.J. Knight* MC 284 [from a moist chamber culture of the bark of living *Eucalyptus todtiana*, 7 September 2020] (*holo*: PERTH 09260307, *iso*: MA).

Echinostelium sp. (DWM 6749), D.W. Mitchell in McHugh *et al.* (2003).

Sporocarps scattered to gregarious, stalked, 60–180 µm high. *Hypothallus* bulbous and/or minutely disc-like, often inconspicuous, fuscous-black or reddish-black. *Stalk* 100–180 µm long, curved or inclined, frequently nodding, cylindrical, tapering up from an expanded base 20–35 µm diam. to a narrow apex 3–4 µm diam., bicoloured, lower part (1/4–3/4) dull black, upper part more or less shiny, pale yellow or colourless, stalk walls colourless, grading to a translucent grey-brown for about 10 µm just below the apex, stuffed with dark, granular refuse giving a roughened appearance on the outer surface, refuse variably less dense distally, sometimes almost completely filling the stalk. *Sporotheca* essentially globose with a somewhat flat base, 32–55 µm diam., dark grey to black when freshly mature, fuscous black when dry, robust, clinging to the spore-like body, or when dislodged, often dropping as a cohesive unit. *Peridium* membranous, early fugacious except for a large persistent collar attached to the lower half of the spore-like body, widely petaloid, minutely striate, divided into two distinct portions, the inner basal portion flat or widely saucer shaped, well defined, small, about 10 µm diam., almost stellate with distinct striations, brown or violaceous-brown, the outer portion hyaline, faintly violaceous, striations less distinct and interrupted, appearing as lines of warts, edge of peridium minutely irregularly stellate or roughened, somewhat thickened and recurved. *Columella* absent. *Spore-like body* present, usually single, rarely twinned, globose or more usually subglobose with a flat base, 13–20 µm wide, 12–17 µm high, often with an attached spore, but more usually sandwiched between two or more spores, the spores adhering to the peridial collar, colour and ornamentation same as the spores. *Capillitium* absent. *Spores* by transmitted light grey-brown or grey-lilac, mostly globose, sometimes subglobose or ovoid or appearing very slightly angular, (12–)13–18(–19) µm diam., the spore wall of uniform thickness, verruculose, with conspicuous, minute, variably sized and unevenly distributed warts which are often very scattered, with rather discreet, approximately circular darker patching formed by closely arranged warts, sometimes forming clusters of 2–5 warts, when viewed by SEM the warts of the dark patching are arranged in small arcs, lines, groups or joined to form short ridges, sometimes warts are also arranged on the edge of the patch in a circular pattern, warts overall are taller than wide and flat- or round-topped, smooth or minutely ridged. *Plasmodium* not observed. (Figures 1, 2)

Diagnostic characters. This species is readily distinguished from all other species of *Echinostelium* by the following combination of characters: a fuscous black sporotheca with the spores joined together in a cohesive unit; distinctly bicoloured stalk, with the lower part black and the apical region by transmitted light grey-brown; peridial collar widely petaloid, divided into two distinctly coloured areas, brown in lower half and faintly violaceous and hyaline in upper part, the edge somewhat thickened and recurved; spores and spore-like body relatively dark, grey-brown or grey-lilac by transmitted light, the ornamentation distinct, with patches of closely arranged warts arranged in a loosely circular pattern.

Other specimens examined. WESTERN AUSTRALIA: Hi Vallee Farm, Badgingarra, 2 Apr. 2004, *M.H. Brims* 685 (PERTH 07213840); Hi Vallee Farm, 5 km E of Brand Hwy on Tootbardi Road, *c*. 250 km NNW of Perth, 9 Feb. 2020, *K.J. Knight* MC 281 (PERTH 09260277), *K.J. Knight* MC 282 (PERTH 09260285), *K.J. Knight* MC 283 (PERTH 09260293); Hi Vallee Farm, 5 km E of Brand Hwy on Tootbardi Road, *c*. 250 km NNW of Perth, 22 Aug. 2020, *K.J. Knight* MC 285 (PERTH 09260315), *K.J. Knight* MC 286 (PERTH 09260854), *K.J. Knight* MC 287 (PERTH 09362185); Stoneville, date unknown, *D.W. Mitchell* 6749 (K).

Ecology, distribution, and habitat. Associated with the acidic bark of living *Eucalyptus todtiana* (pH of soak water 3.4 – *K.J. Knight* MC 281, MC 282, MC 283, and pH 4.0 – *K.J. Knight* MC 284, MC 285, MC 286, MC 287) and *Banksia sessilis*. The most productive moist chambers were those prepared with the substrate samples collected at the end of a winter-wet period and may have been more favourable for this species versus the substrate samples collected after a hot dry summer. The

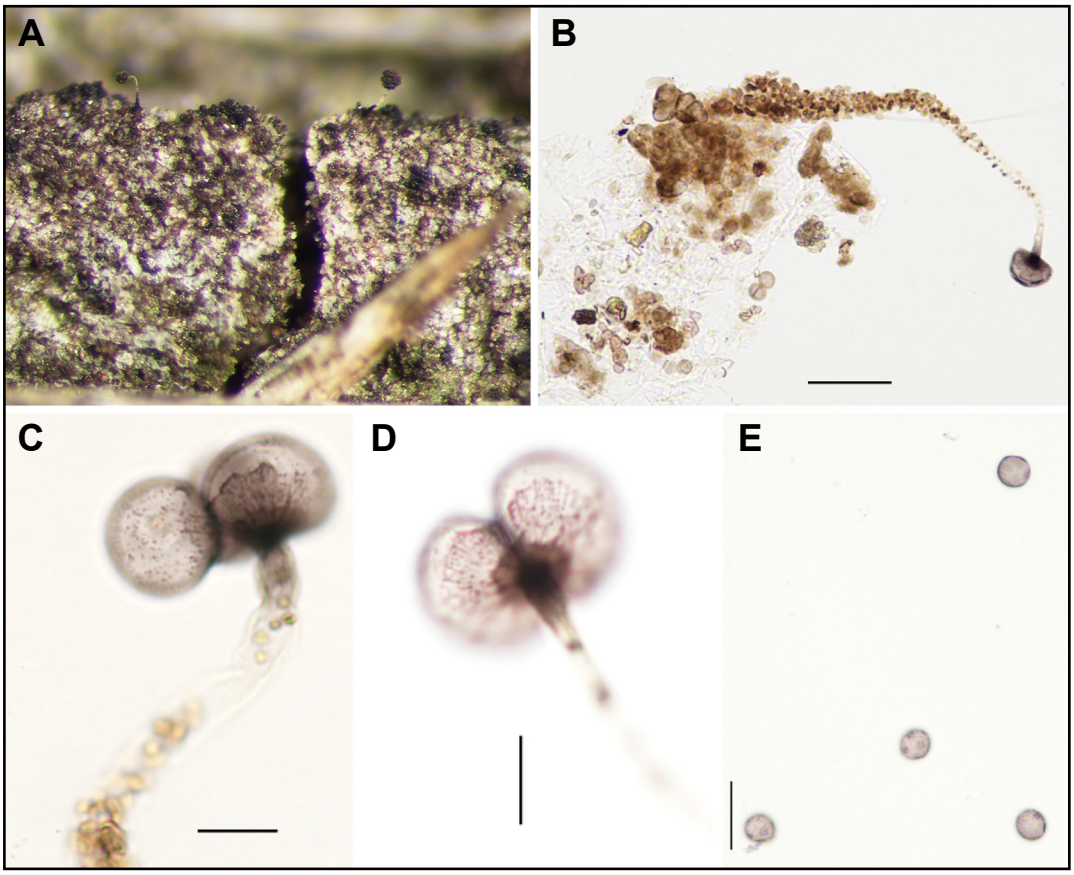

Figure 1. *Echinostelium australiense*. A – two sporocarps *in situ* showing the habit with nodding or inclined stalks, black lower stalk, and black sporotheca; B – sporocarp by transmitted light showing the granular stalk with a coloured grey-brown apex just below the subglobose spore-like body; C – sporocarp by transmitted light showing spore-like body draped with a large bi-coloured, minutely striate peridial collar along with a spore adhering to the spore-like body and peridial collar; D – sporocarp by transmitted light showing a twinned spore-like body with a large bicoloured, minutely striate and petaloid peridial collar; E – spores by transmitted light showing the rather discreet, approximately circular areas of darker patching. Images from *K.J. Knight* MC 281 (B); MC 284 (A, D); MC 285 (C, E). Photographs by J.M. Huisman & K.J. Knight. Scale bars: 100 µm (A); 30 µm (B, E); 10 µm (C, D).

two localities the species is recorded from are disjunct in Western Australia, occurring in semi-arid and warm areas in the Geraldton Sandplains and Jarrah Forest bioregions (Department of Environment 2013). The *K.J. Knight* and *M.H. Brims* collections are from proteaceous scrub-heath in an open eucalypt woodland, while the *D.W. Mitchell* collection is from a region characterised by a shrubland associated with a Jarrah-Marri forest.

The *D.W. Mitchell* collection (DWM 6749) indicates that the substrate was the bark of *Banksia sessilis* (McHugh *et al.* 2003); at the time the bark was collected there was some doubt about this. From personal correspondence between Mitchell and Brims in early 2003, the description of the plant from which the substrate was collected was described as 'needle-like, grey-green leaves, bark bare of epiphytes, flaked in strips, leaves looked quite xerophytic'. Brims questioned the taxon name as the leaf description did not match *Banksia sessilis*. However, Mitchell further confirmed the plant's identity with the comment 'the specimen did have small holly-like leaves with tiny irregularly distributed black dots on the pale blue-green leaves'. The bark of living plants of *Banksia* typically decorticate

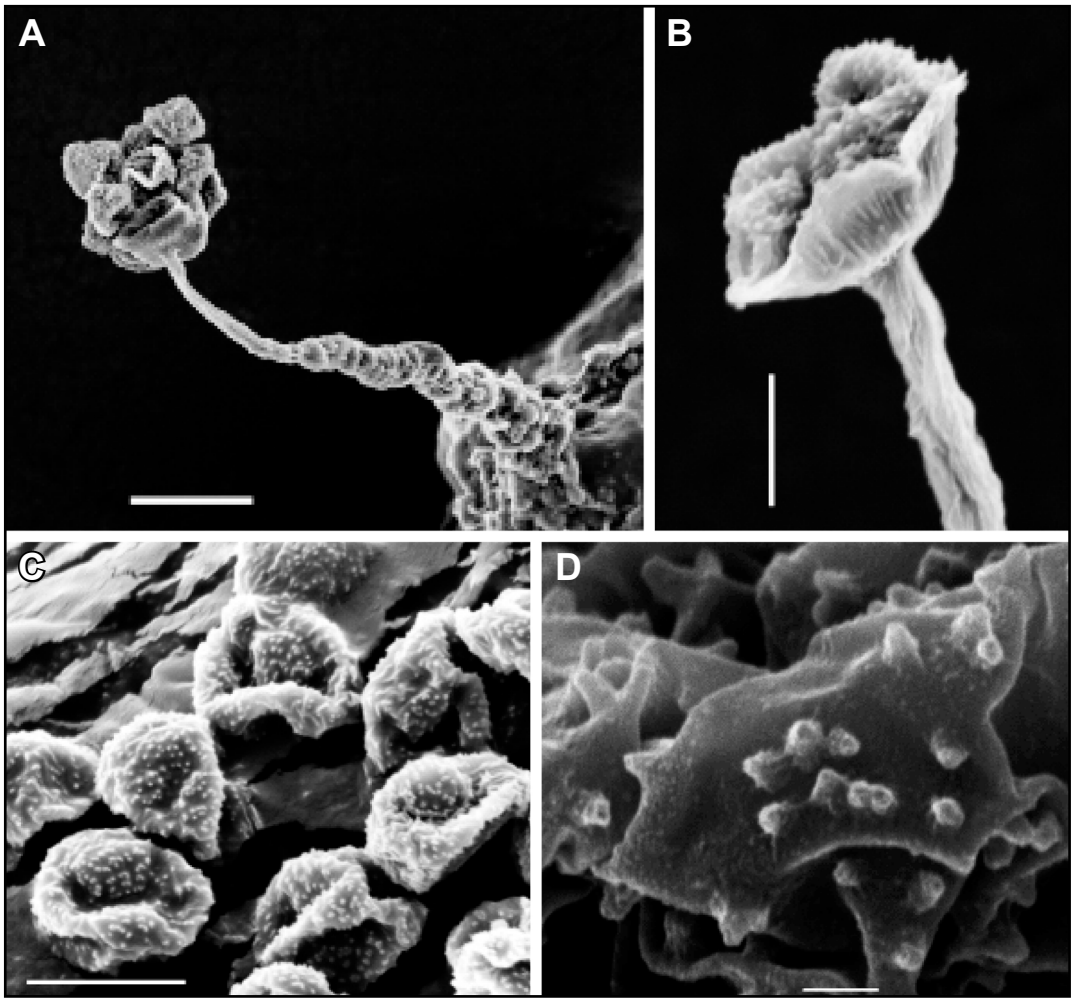

Figure 2. *Echinostelium australiense* as viewed with scanning electron microscopy. A – sporocarp showing the tapering stalk, rough below and a large peridial collar; B – large minutely striate petaloid collar showing the minutely widely stellate or roughened, somewhat thickened and recurved edge; C – collapsed spores showing sparsely warted ornamentation between the rather discreet, approximately circular darker patching with the warts arranged in clusters, lines or joined; D – spore ornamentation formed by rounded or flat topped warts and when clustered with minute ridges between the warts. Images from *K.J. Knight* MC 284 (B); MC 285 (A, C); MC 286 (D). Photographs by Steve Dillon (A-C) and Yuri Novozhilov (D). Scale bars: 20 µm (A); 5 µm (B); 10 µm (C); 0.5 µm (D).

only in very mature individuals and then in small rectangular chips that adhere to the tree trunk (pers. obs. KJK and pers. comm. A.S. George) vs 'flaked in strips' as described by Mitchell. After viewing the Mitchell collection, it is clear the substrate is not as indicated *Banksia sessilis* but is likely to be a species of *Eucalyptus* with fibrous bark. It should also be noted that the geocode provided for the Mitchell collection in McHugh *et al.* (2003) is incorrect since it places the collection *c*. 53 km west-south-west of Stoneville (in the ocean just west of Fremantle).

Conservation status. Myxomycetes in Western Australia are poorly known and until recently were rarely collected. Although *E. australiense* is known from just two disjunct localities approximately 250 km apart, it is not considered to be under conservation threat since its occurrence is likely to be more common and widespread.

Etymology. The epithet is from the Latin *australiense*, the name Mitchell coined (as '*australis'*) in correspondence with Brims in early 2003 (15/1/2003) and refers to its discovery in Australia.

Vernacular name. Black Echinostelium.

Affinities. *Echinostelium australiense* is clearly distinct and could not be confused with other species in the genus since it differs in a number of readily apparent morphological and colour characters. The dark colouration of the sporotheca, stalk, and spores in *E. australiense* is unusual for the genus, which more typically has a pale sporotheca, a hyaline to pale brown stalk of uniform colour, and pale or hyaline spores (Haskins & Clark 2016). It is easily distinguished from the other species with spore-like bodies (*E. apitectum*, *E. coelocephalum*, *E. colliculosum*, *E. ladoi*, and *E. novozhilovii*) by the diagnostic characters listed above, which do not occur in combination within this grouping. The spores of *E. elachiston* have a similar distribution of warts as *E. australiense*, described by Pando and Oltra (2000) as warted, sometimes coarsely, the warts irregularly distributed or more characteristically grouped in clusters giving the impression of forming pads. *Echinostelium australiense* is readily distinguished from *E. elachiston* by the presence of a spore-like body (vs a rudimentary capillitium), the larger sporotheca 32–55 μm (vs 30–40 μm), dark coloured sporotheca and stalk (vs pale colouring) and size of spores 12–19 μm diam. (vs 6–8 μm).

The stalks of the genus *Echinostelium* are typically cylindrical tubes with a small, solid, translucent, colourless apical region (Haskins & Clark 2016). The apical region in *E. australiense* is typical except for the grey-brown colouring for the uppermost 10 μm. This type of stalk and colouring is characteristic of both its sister genus *Barbeyella* Meyl., where the stalk is black at the apex (Kowalski & Hinchee 1971), and the genus *Clastoderma* A.Blytt, where it may be brown as in *C. debaryanum* A.Blytt, reddish-brown as in *C. microcarpum* (Meyl.) Kowalski and *C. pachypus* Nann.-Bremek. (Haskins & Clark 2016) or black as in *C. confusum* K.J.Knight & Lado (Knight & Lado 2020). *Echinostelium australiense* is readily distinguished from the genus *Barbeyella* by the presence of a spore-like body (vs a cylindric columella dividing at the middle of the sporotheca into rudimentary capillitium) and an early fugacious peridium with remnant collar (vs persistent lobes); and distinguished from the genus *Clastoderma* by the presence of a spore-like body (vs columella present or absent and capillitium always present).

The peridium of *Echinostelium* is typically fugacious and hyaline, remaining only as a small basal collar attached to the stalk, although it may be somewhat persistent in a few species before it is reduced to a collar or breaks into peridial fragments that remain attached to the capillitial tips as in *E. arboreum* (Haskins & Clark 2016). The peridium of *E. australiense* is typical for the genus only in its fugacious nature; otherwise, it is uniquely petaloid, variously ornamented with striations and with two distinct portions, an inner section conspicuously coloured brown and the outer portion faintly violaceous.

The black sporotheca and relatively dark, grey-brown or grey-lilac spores with conspicuous warts as observed under transmitted light in *E. australiense* are unique for the genus. Typically, the spores of *Echinostelium* by transmitted light are hyaline, with pale yellowish or lilac hues and the ornamentation described as smooth or minutely roughened, although by SEM some ornamentation is usually present in the smooth state (Haskins & Clark 2016). An exception to this is the recently described *E. microsporum*, where although the colour is typically pale and hyaline, the spores are described as ornamented with distinct large warts (Vlasenko *et al.* 2019). In the recent phylogeny of the class Myxomycetes (Leontyev *et al.* 2019), the presence of dark (melanised) spores separates the pale-spored genus *Echinostelium* from its sister genus *Barbeyella*. The presence of dark spores in *E. australiense*

suggests that the melanisation of the spores is possibly less diagnostic than previously accepted and the generic limits of *Echinostelium* may eventually be broadened. Doing so would result in the primary criterion separating the genus *Barbeyella* from *Echinostelium* being the presence of a tough persistent peridium (vs fugacious, or as membranous remnants as in *E. arboreum*).

Echinostelium australiense retains its spores on the collar at maturity, or when the spores are dislodged, they tend to drop as a cohesive unit. This contrasts with most other species of *Echinostelium*, also without a capillitium, in which the spores readily disperse and are usually lacking on herbarium specimens (Whitney 1980). The rather discreet, approximately circular darker patching of the spore formed by closely arranged warts may play a role in this, the warts interlocking to some extent (Figure 2D). The areas of patching on the spores of *E. australiense* as observed by transmitted light do not appear conspicuously thickened, but may be somewhat flattened, giving the spore a slightly angular outline (Figure 1E). The rather discreet, approximately circular area of dark patching appears more rigid than the surrounding spore wall as it retains an almost flat, plate-like circular shape when the remaining spore wall is desiccated and collapsed inwards (Figure 2C). The presence of the lower spores adhering to the large peridial collar may also enhance the cohesiveness of the sporotheca (Figures 1C, 2C). It is interesting to note the likely presence of a second species of dark-spored *Echinostelium*. According to the third author, one of his colleagues (Konstantin Fefelov, from Ekaterinburg, Russia) recovered a single collection of what was clearly a species of *Echinostelium* with a dark sporotheca and dark spores from a moist chamber culture. However, it can be readily distinguished from *E. australiense* by its hyaline stalk throughout (vs bicoloured with a very dark base) and seemingly smooth spores (vs distinctly warted).

As stated above, *E. australiense* has similarities with both *Clastoderma* (Clastodermataceae) and *Barbeyella* (Echinosteliaceae). The relationship between *Clastoderma*, *Echinostelium* and *Barbeyella* has been variously interpreted by those who study myxomycetes. The families have long been regarded as closely allied, with Martin and Alexopolous (1969) considering within the order Echinosteliales, *Barbeyella* and *Clastoderma* as sister genera within the Clastodermataceae, and *Echinostelium* assigned to the Echinosteliaceae. However, Kretzschmar *et al.* (2016) proposed a new phylogeny within the Echinosteliales, based on SSU 18S rRNA gene sequences, and showed the polyphyly of the family Clastodermataceae, as *Barbeyella* was more closely related to *E. arboreum* than to *Clastoderma*, while *C. debaryanum* was the earliest branching clade in Echinosteliales. More recently, Clastodermataceae was raised to ordinal level as the Clastodermatales by Leontyev *et al.* (2019) within the superorder Stemonitiidia (vs Echinosteliales in superorder Echinosteliidia), based on gene sequences. With the emergence of an uncharacteristically dark-coloured *Echinostelium*, molecular analysis of this species may help resolve the still uncertain relationships among these groups.

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