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Baetimyces, a new genus of Harpellales, and first report of *Legeriomyces ramosus* from the northeastern Iberian Peninsula

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Abstract: *Baetimyces*, belonging to the Legeriomycetaceae, is described here as a new genus. Its single species, *B. ancorae*, has been found growing in the hindgut of mayfly nymphs of the genus *Baetis* (Ephemeroptera, Baetidae) inhabiting a Pyrenean Mountains canal-stream from the northeastern region of the Iberian Peninsula. The new genus is characterized by having trichospores with two thick appendages of unequal length, and by zygospores perpendicularly and medially attached to the zygosporophore. The new genus may be related to *Glotzia*, *Legeriomyes*, and *Zygopolaris*, and similarities with these and other genera are compared and discussed. The new species often coexists in the same gut with *Legeriomyces ramosus*, which is reported for the first time in Spain.

Key Words: Baetidae, gut fungi, Legeriomycetaceae, mayfly nymphs, taxonomy, Trichomycetes

INTRODUCTION

Trichomycetes (Zygomycota), growing within the digestive tracts of several orders of insects and other arthropods, have been reported from five continents. Current knowledge of Trichomycetes from the Iberian Peninsula is scant, with only twelve species previously reported (Santamaria 1997, Santamaria and Girbal 1997, 1998, Girbal and Santamaria 1998). We intensively surveyed the Iberian Peninsula to better understand its Trichomycete mycobiota. This research began in 2000 and is integrated with the project ''Flora Mycologica Iberica.''

Trichomycetes include three orders: Harpellales, Asellariales, and Eccrinales. The order Amoebidiales has been excluded from the class because molecular studies demonstrated its phylogenetic affinities are with protozoans (Benny and O'Donnell 2000). Harpellales, which is the most intensively studied order, includes two families: Harpellaceae and Legeriomycetaceae, the former for species with unbranched thalli living in the midgut, the latter for species with branched thalli living in the hindgut (Lichtwardt 1986).

Baetimyces, a new Trichomycete genus, is described from the hindgut of mayfly nymphs (Baetidae, Ephemeroptera). The single species, *B. ancorae*, was collected from a canal-stream of the northeastern Spain Pyrenean Mountains, during summertime. The presence of medially attached zygospores, and especially the peculiar trichospores bearing two thick appendages of unequal length, characterize the genus and represent a novel addition to the taxonomy of Legeriomycetaceae. *Baetimyces* is compared with *Glotzia* M. Gauthier ex Manier & Lichtw., *Legeriomyces* Pouzar and *Zygopolaris* S. T. Moss, Lichtw. & Manier on the basis of the ramification pattern of hyphae, on the shape of the holdfast apparatus, and on trichospore features.

MATERIALS AND METHODS

The description of the new taxon is based on material found in an unnamed artificial channel stream in Guils de Cerdanya (Lleida Province, Catalonia, Spain) in July 2000. Collections of insect nymphs were made by hand picking from rocks, wood, and leaves removed from streams and also by dragging aquatic nets. The insects were dissected to extract and clean the guts under a stereomicroscope, and the fungi were placed on a slide with water as a mounting medium and photographed using phase contrast or interference contrast optics. Afterwards, slides were fixed with lactophenol cotton-blue to be preserved and deposited in the herbarium BCB-Mycotheca, at the institutional address of the authors.

TAXONOMY

Baetimyces L.G. Valle et Santam., gen. nov.

Trichosporae subcylindroides, cum duabus latus appendicibus, quarum prima elongata, secunda brevis. Appendices helicte dispositae intra cellulam genitalem ante trichosporarum liberationem. Zygosporae biconicae, zygosporophoro ad perpendiculum et in medio affixae. Thalli inaequaliter ramificantes. In unoquoque thallo duo magisve pedes laterales vel terminales. Ad cuticulam proctodaei nympharum Baetis affixi.

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Trichospores subcylindrical, bearing two thick appendages, one elongated and the other short. Appendages helically oriented within the generative cell before trichospore release. Zygospores biconical, perpendicularly and medially attached to zygosporophore. Thalli irregularly branched. More than one holdfast per thallus, in lateral and terminal positions. Attached to hindgut cuticle of *Baetis* (Ephemeroptera: Baetidae) nymphs.

Etymology. Latin, *Baetimyces*, fungus growing in *Baetis*, the host genus.

TYPUS generis. *Baetimyces ancorae* L.G. Valle et Santam.

Baetimyces ancorae L.G. Valle et Santam., sp. nov. FIGS. 1–8, 10–17

Cellulae hypharum 3.5–5 μm in latitudine. Trichosporae (40–)50(–58.5) × (3.5–)4.5(–5.5) μm, 3–5 in unoquoque ramo fertili ortae. Zygosporae (34–)45(–54) × 7–9 μm. Pedes cupulati. Zygosporophorae cum anchorali delineatione ubi conservatae et tinctae sunt.

Thalli sparsely branched, attached to the hindgut lining of *Baetis* nymphs by laterally secreted, cup-like holdfasts (FIGS. 7, 11), one of them in terminal position at the end of a sinuous basal cell. The presence of a main axis is not always evident due to the variable direction of projecting branches (FIG. 1). Hyphal cells 3.5–5 μm diam. Fertile branches bearing 3–5 trichospores each (FIGS. 1, 3–6, 8). Trichospores subcylindrical, (40–)50(–58.5) × (3.5–)4.5(–5.5) μm, straight to slightly arcuate (FIGS. 1–6, 8, 15–17). Generative cells 12–18 × 5–7 μm, distally swollen at the trichospore-formation area (FIGS. 3–6, 8). After release, the trichospores show two thick appendages: an elongated appendage, slightly wider at the proximal end, and a small, short appendage which contacts at the basal area and continues with the longest appendage (FIGS. 2, 15–17). A very small and inconspicuous collar can be seen in trichospore base with an accurate observation at high microscope magnification (FIG. 15). Both appendages are helically oriented within the generative cell before the trichospore release, appearing as broad oblique bands (FIGS. 3–6). Zygospores biconical, (34–)45(–54) × 7–9 μm, perpendicularly and medially attached to the zygosporophore (Type I; Moss et al 1975). The zygosporophore subtending a mature zygospore, when preserved and stained with lactophenol cotton-blue, contains cytoplasmic material restricted to a well-defined central zone showing cytoplasmic contractions with lateral expansions in the overall shape of an anchor (FIGS. 10, 12–13). Conjugation scalariform, with zygospores arising from the distal end of cells of either conjugating branches. After release, zygospores have an empty collar which is the zygosporophore remnant, and no appendages (FIG. 14).

Etymology. Latin, *ancorae*, referring to the anchor-like contraction of the cytoplasm in the zygosporophore when preserved and stained with lactophenol cotton-blue.

Specimens examined. SPAIN. CATALONIA: Prov. of Lleida, in an unnamed canal-stream of Guils de Cerdanya, on the way to Meranges lake, alt. 1700 m., UTM 31T DH0601, in the hindgut of *Baetis* sp. (Ephemeroptera, Baetidae), 13 Jul 2000, *L. G. Valle* [Tr0152, BCB (HOLOTYPE) that includes zygospores and trichospores] [Tr0153, Tr0158, Tr0159, Tr0160, Tr0161, Tr0162, Tr0163, Tr0164, Tr0168, Tr0171, Tr0172, Tr0176, Tr0178, Tr0179; BCB (ISOTYPES)].

DISCUSSION

Among the examined nymphs of *Baetis*, a high percentage (nearly 70%) were infested with *Baetimyces ancorae*. *Legeriomyces ramosus* Pouzar was also present in 20% of these nymphs. When both species were present in the same gut, thalli grew in a dense net-like mass of hyphae, and were yet more compact when hyphae conjugated and zygospores of both species were fully developed (see FIG. 12).

Baetimyces ancorae resembles *Legeriomyces ramosus* in holdfast and thallus characteristics (lateral and broad holdfast, irregular branching without a clearly defined main axis and overall habit). However, the

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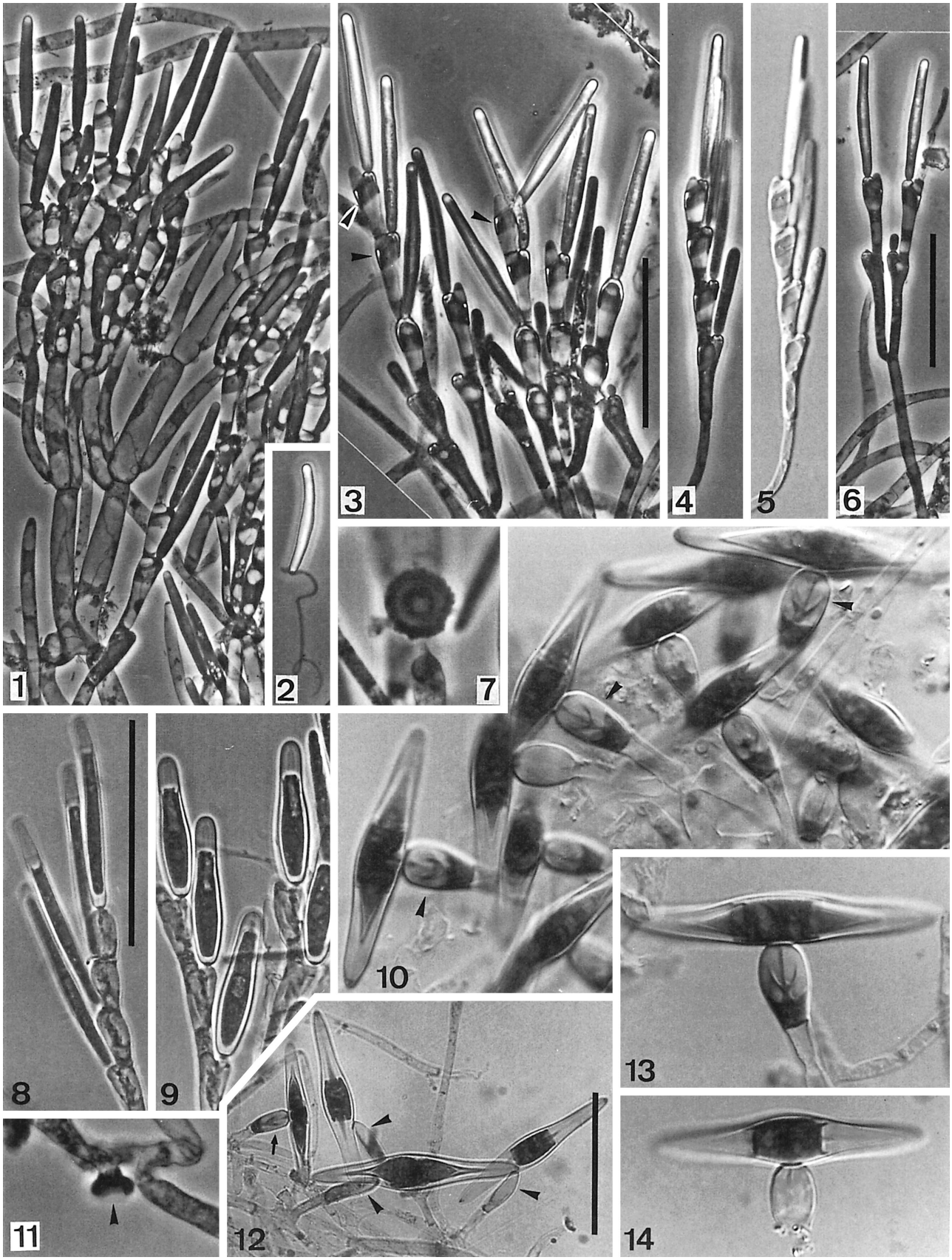
FIGS. 1–14. *Baetimyces ancorae* [1–6, BCB-Tr0172; 7, 11, BCB-Tr0158; 8, BCB-Tr0161; 10, 13–14, BCB-Tr0152; 12, BCB-Tr0160]. FIGS. 9, 12. *Legeriomyces ramosus* [9, BCB-Tr0161; 12, BCB-Tr0160]. 1. Overall view of a branching and sporulating thallus. 2. Released trichospore showing the two appendages. 3. Fertile branches showing helically oriented appendages inside the generative cell (arrowheads). 4–6. Fertile branches. 7. Lateral holdfast in frontal view. 8–9. Fertile branches of *B. ancorae* and *L. ramosus*, respectively, both from the same slide. 10. Zygospores showing the ''anchor-like'' pattern in the zygosporophore (arrowheads). 11. Lateral holdfast in side view. 12. Zygospores of *B. ancorae* (arrow) and *L. ramosus* (arrowheads). Note the different attachment to zygosporophores. 13. Zygospore showing the ''anchor-like'' structure in zygosporophore. 14. Released zygospore with an empty collar. [FIGS. 1–6 in water-mounted slides; remaining FIGS. in lactophenol cotton-blue slides] [FIGS. 1–4, 6–9, 11 photographed with phase contrast optics; FIGS. 5, 10, 13–14 photographed with interference contrast optics; FIG. 12 photographed with light transmission optics] [Scale bar 3 = 50 μm for 3–5, or 25 μm for 7, 10–11, 13–14; Scale bar 6 = 50 μm for 1, 2, 6; Scale bar 8 = 50 μm for 8–9; Scale bar 12 = 50 μm for 12].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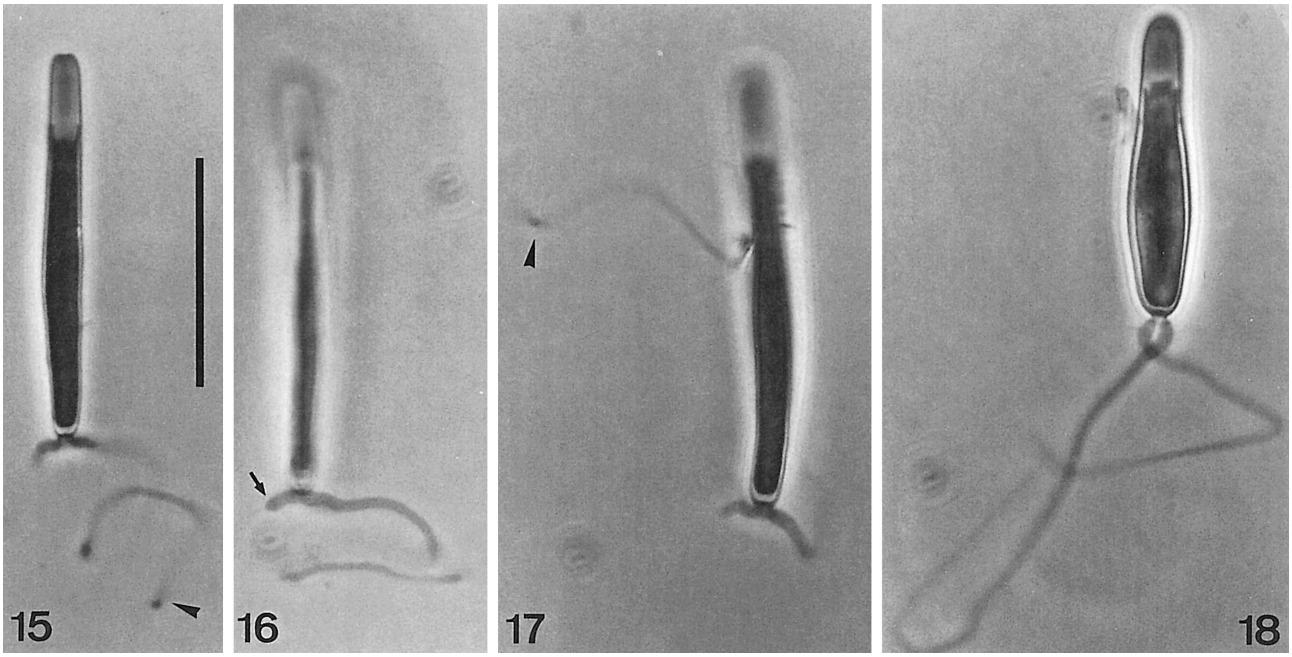

FIGS. 15–17. *Baetimyces ancorae* [15, BCB-Tr0163; 16–17, BCB-Tr0153]. FIG. 18. *Legeriomyces ramosus* [BCB-Tr0161]. 15–17. Released trichospores showing both appendages: the longer (arrowheads in 15 and 17) and the shorter which is entirely focused in 16 (arrow). 18. Released trichospore showing both appendages. [All from lactophenol cotton-blue slides and photographed with phase contrast optics] [Scale bar 15 = 50 μm for all FIGS.]

presence of medially attached zygospores (Type I) and the appendaged trichospores bearing two appendages of unequal length, make *Baetimyces* a distinct genus among the family Legeriomycetaceae. In earlier examinations it was thought that the short appendage was a normal long appendage broken during the manipulation process, or that it was due to a premature release of trichospores from the generative cell. However, the persistence of this character in successive preparations makes it a diagnostic characteristic of the new genus.

Trichospores of *Legeriomyces ramosus* differ from those of *Baetimyces ancorae* not only by the presence of a second well-developed, elongate appendage, but also by its size (27–31 × 6.5–7.5 μm in our samples), being smaller, broader and more elliptical than those of *B. ancorae* (FIGS. 9, 18). Moreover, appendages of *L. ramosus* appear evidently enlarged near the base without any trace of a collar in the trichospore base (FIG. 18). Differences between the length of both appendages were reported in *L. ramosus* (as *Genistella ramosa* L. Léger & M. Gauthier) by Moss (1979), but this asymmetry was not so extreme as in *B. ancorae* and the shape of these trichospores follow *Legeriomyces* characteristics. *Legeriomyces aenigmaticus* Lichtw. & M. C. Williams produces three ranges of trichospore size (Lichtwardt and Williams 1983), while in the species here described the size is invariable. *Legeriomyces rarus* Lichtw. & M. C. Williams has long-obpyriform to almost ellipsoidal trichospores (25–31 × 5.5–8 μm in Williams and Lichtwardt 1993) with 2 long appendages, thus differing from those of *B. ancorae*. Additionally, the genus *Legeriomyces* has thalli with a bulbous basal cell and type II zygospores (submedially and obliquely attached to the zygosporophore) (FIG. 12).

Glotzia M. Gauthier ex Manier & Lichtw., described (Gauthier 1936) from *Baetis* nymphs, possesses one long slender central appendage and two short broader divergent lateral appendages. Zygospores in *Glotzia* are of type II whereas in *Baetimyces* they are of type I. Trichospores of *Glotzia* and *Baetimyces* have some resemblance in shape, in both genera being subcylindrical, but sizes are rather different. Currently, the genus *Glotzia* includes four species: (1) *G. ephemeridiarum* Lichtw. has the longest trichospores (45–70 × 4.5–5 μm; Lichtwardt 1972); (2) *G. centroptili* M. Gauthier ex Manier & Lichtw. has smaller trichospores (40 × 4 μm; Gauthier 1936; Manier and Lichtwardt 1968) with the central appendage helically arranged around the two shorter, broader, somewhat rigid lateral appendages, and a lateral holdfast on a branched basal cell; (3) *G. coloradense* M. C. Williams & Lichtw. (Williams and Lichtwardt 1999) has longer trichospores than those of *B. ancorae*; and (4) *G. tasmaniensis* Lichtw. & M. C. Williams (Lichtwardt and Williams 1990) is sim-

ilar to *G. centroptili* and *G. ephemeridarum* but differs in holdfast structure.

Baetimyces ancorae shares characteristics with both species of the genus *Zygopolaris* S. T. Moss & Lichtw. that grow in Ephemeroptera: *Ephemerella* (Ephemerellidae), *Epeorus* (Heptageniidae) and *Baetis*. Zygospores in *Zygopolaris* are of type IV, being attached to one pole in the same zygosporophore axis. These zygospores have been considered as typical biconical zygospores of Harpellales modified by the peculiar polar attachment (Moss et al 1975, Moss and Lichtwardt 1977). Moss et al (1975) described *Zygopolaris ephemeridiarum* and stated that the released trichospores do not present defined appendages under the light microscope. Later ultrastructural studies on this species demonstrated that early in trichospore development an electron-opaque material accumulates between the cytoplasmic membrane and the wall of the generative cell, just below the trichospore (Moss and Lichtwardt 1977). Nevertheless, no further appendage differentiation has been observed.

The zygosporophore of *Zygopolaris* is separated from its conjugate by a septum, as in *Baetimyces*. The material contained inside the mature *Baetimyces* zygosporophore tends to remain in a rather constant position and in the shape of an anchor when treated with lactophenol cotton-blue. In *Zygopolaris*, the zygosporophores contain fibrous material that restricts the cytoplasm to the central region (Moss and Lichtwardt 1977). We have contrasted this character by studying two slides of *Z. ephemeridarum* borrowed from Lichtwardt's collection, and with several digitized images of this species as well as *Z. borealis* Lichtw. & M. C. Williams (Lichtwardt and Williams 1975). No structure similar to *B. ancorae* has been observed in this stained material. Also remarkable is the presence of both lateral and terminal types of holdfasts in *Baetimyces*, with the terminal one situated at the base of a sinuous cell such as that described for *Z. borealis*. *Zygopolaris ephemeridiarum* has only lateral holdfasts. Despite these similarities, species of *Zygopolaris* can be easily distinguished from *B. ancorae* by zygospore and trichospore characters.

Another genus inhabiting Baetidae guts that has zygospores of type I is *Spartiella* Tuzet & Manier ex Manier, but trichospores bear one appendage attached medially to the proximal end. Also, the presence of a lobulate terminal holdfast cell is characteristic of this genus. Trichospores of *Spartiella barbata* Tuzet & Manier ex Manier measure 22–27 × 7.5–10 μm (Tuzet and Manier 1950), shorter and broader than the new species. Similarly, *Spartiella animae* Lichtw. differs in trichospore size, appendage formation and holdfast structure.

Lastly, we wish to promote the lactophenol cotton-blue staining treatment in mounting slides for the observation of certain characters of taxonomic interest that otherwise are overlooked in water mounts. In *B. ancorae*, the distinctive structure seen in the zygosporophore is only observed in thalli preserved and stained with lactophenol cotton-blue (we have not studied the effect of other mounting media). However, *in vivo* studies with water mounts are required to observe some structures such as appendage arrangement inside generative cells and other fragile structures that are lost with fixation.

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