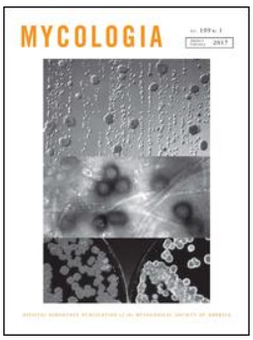

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Fireworks under the microscope: a spectacular new species of *Zodiomyces* from the Thaxter collection

Walter Rossi
Sect. Environmental Sciences, Dept. MeSVA, University of L'Aquila, 67100 Coppito (AQ), Italy

Danny Haelewaters
Donald H. Pfister[1]
Farlow Reference Library and Herbarium of Cryptogamic Botany, Harvard University, 22 Divinity Avenue, Cambridge Massachusetts 02138

Abstract: A new species of *Zodiomyces* (Ascomycota, Laboulbeniales) is described, *Z. rhizophorus*, parasitic on a hydrophilid beetle (Coleoptera, Hydrophilidae) from Trinidad. This species was discovered during the examination of the slides of Laboulbeniales made by Roland Thaxter. It is characterized by numerous long, slender, multicellular and multiseriate outgrowths at the base of the receptacle. Thaxter's outstanding illustrations have set a standard in the field of mycology; we provide a review of the methods he employed in the preparation of these illustrations.

Key words: Hydrophilidae, Laboulbeniales, parasitic fungi, taxonomy

Introduction

Representatives of the order Laboulbeniales (Ascomycota, Laboulbeniomycetes) grow as microscopic multicellular thalli on the exoskeleton of invertebrates. More than 2000 species in 140 genera are described (Rossi and Santamaría 2012). The hosts are primarily beetles, but millipedes, mites and a variety of insects (ants, flies, cockroaches) also are infected. Unlike other multicellular fungi Laboulbeniales exhibit determinate growth; that is the fungus develops from a two-celled ascospore by regulated mitotic divisions in several planes that result in a single thallus with a defined number of cells and distinctive three-dimensional cell arrangements. The genera *Columnomyces* R.K. Benj., *Euzodiomyces* Thaxt., *Kainomyces* Thaxt., *Scepastocarpus* Santam. and *Zodiomyces* Thaxt. are exceptional among the Laboulbeniomycetes in forming relatively large thalli with many-celled, pseudoparenchymatous receptacles.

Although the earliest observations on these parasites was in the 1840s (Rouget 1850), it was not until Roland Thaxter's research that the Laboulbeniales were studied in depth and their enormous diversity was realized. In 1890 he published the first in a series of 21 descriptive papers. These papers, in which hundreds of species were described and nomenclatural priority was established, were unillustrated. His illustrated five-volume monograph, published 1896–1931, provides the foundation for all subsequent studies of the group. Thaxter died shortly after the fifth volume was published. Thaxter (1931) suggested that a sixth volume would be published to treat the large genus *Laboulbenia* and also to give "a general review, classification and host-index". This volume was never prepared, and he left no manuscript (Benjamin 1971). However, the Farlow Reference Library holds preliminary drawings and notes related to both previously described but unillustrated species and also to undescribed species, some of which were intended to be part of this sixth volume. These archival materials are valuable in interpreting Thaxter's unfinished work and to authenticate taxa that were not fully documented. To both document little known taxa and to understand Thaxter's modus operandi these documents recently were examined in conjunction with a study of some of the Thaxter's microscope slides.

Thaxter's (1896, 1908, 1924, 1926, 1931) monographs are illustrated with superb line and stipple drawings. The original inked illustrations are in the Farlow Reference Library. In addition, Farlow archives have a number of camera lucida drawings. These were made with pencil on tracing paper and include notes and observations on species, some of which were not illustrated when originally described (Fig. 1 of *Zodiomyces subseriatus* Thaxt. is one example). His method seems to have been to make these rough camera lucida drawings, accumulate them, then select those with which he was satisfied and use them to assemble the final densely organized plates. The sketches were trimmed and arranged on illustration board, then they were coated on the reverse with graphite. The sketches were repositioned on the illustration board and the drawings were traced, thus transferring the outline to the board. Having completed a full set of transfers, the images were inked, stippled and labeled with his distinctive monogram. The final plates were approximately 25 × 34 cm. *Zodiomyces vorticellarius* from the original (Thaxter 1896, plate XXIII) is provided herein (Fig. 2). The detail of Thaxter's illustrations is evident particularly in the enlarged view of the perithecium (Fig. 2D).

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[1] Corresponding author. E-mail: dpfister@oeb.harvard.edu

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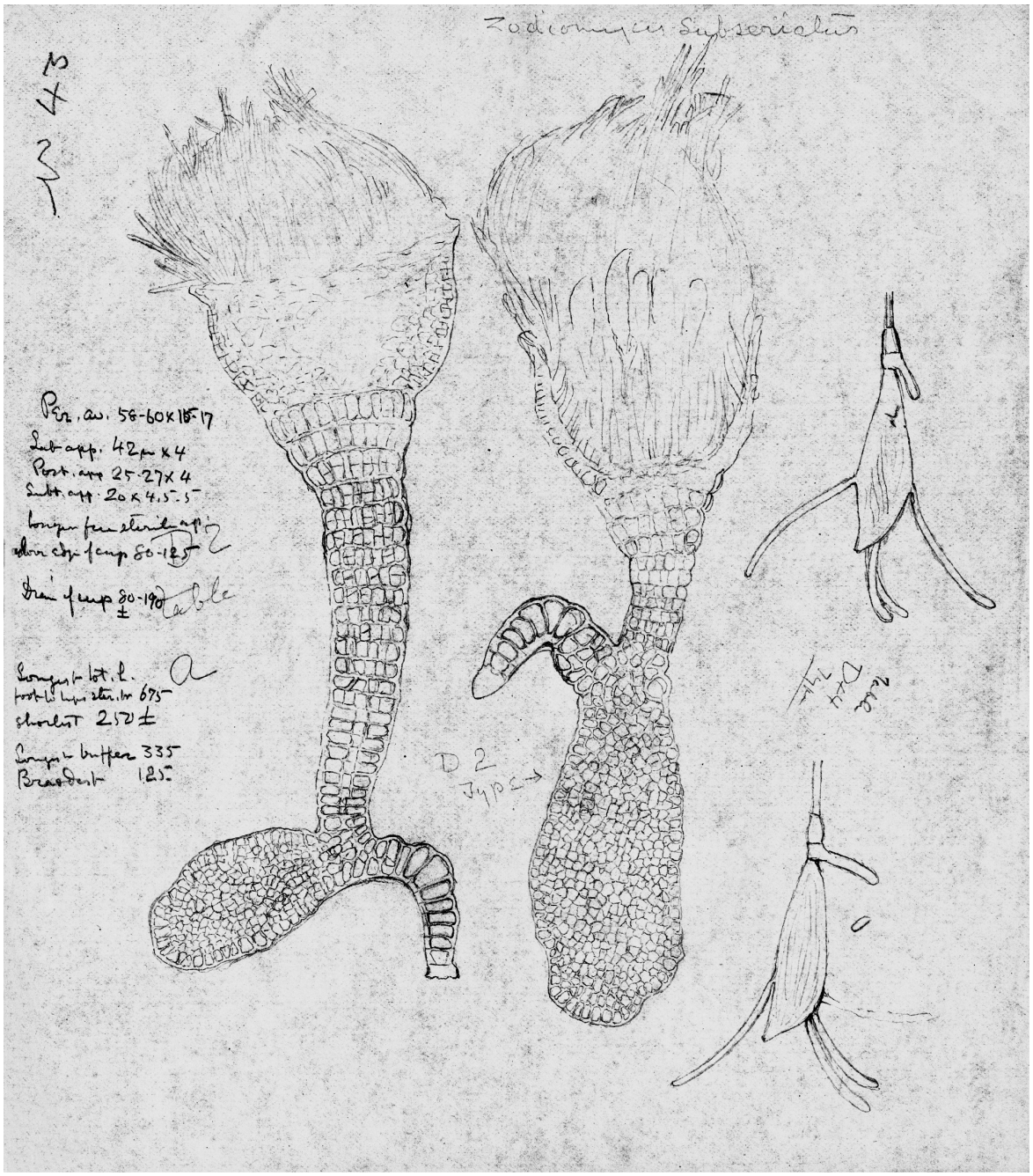

FIG. 1. *Zodiomyces subseriatus*. Original camera lucida sketch, drawn by Roland Thaxter. Image courtesy of the Archives of the Farlow Herbarium of Cryptogamic Botany.

Recent study of Thaxter's collection, consisting of 10 036 slides (T.W. Wang pers comm), has resulted in the description of several new species of *Laboulbenia* on carabid (*L. poplitea* Haelew.), erotylid (*L. erotylaria* Haelew.) and chrysomelid hosts (*L. bilobata* Haelew. & W. Rossi, *L. longipilis* Haelew. & W. Rossi, *L. pfisteri* Haelew. & W. Rossi) (Haelewaters and Yaakop 2014, Haelewaters and Rossi 2015) and the designation of lectotypes for *Cantharomyces denigratus* Thaxt., *Laboulbenia philonthi* Thaxt., *Peyritschiella protea* Thaxt., *Stichomyces conosomatis* Thaxt. and *Teratomyces actobii* Thaxt. (De Kesel and Haelewaters 2014, Haelewaters et al. 2015). Because Thaxter collected widely and had correspondents worldwide we expect that many of his preparations will yield additional important information about distributions, host ranges and diversity of these fungi. The study of Thaxter's collection proceeds slowly because some of the slides require restoration.

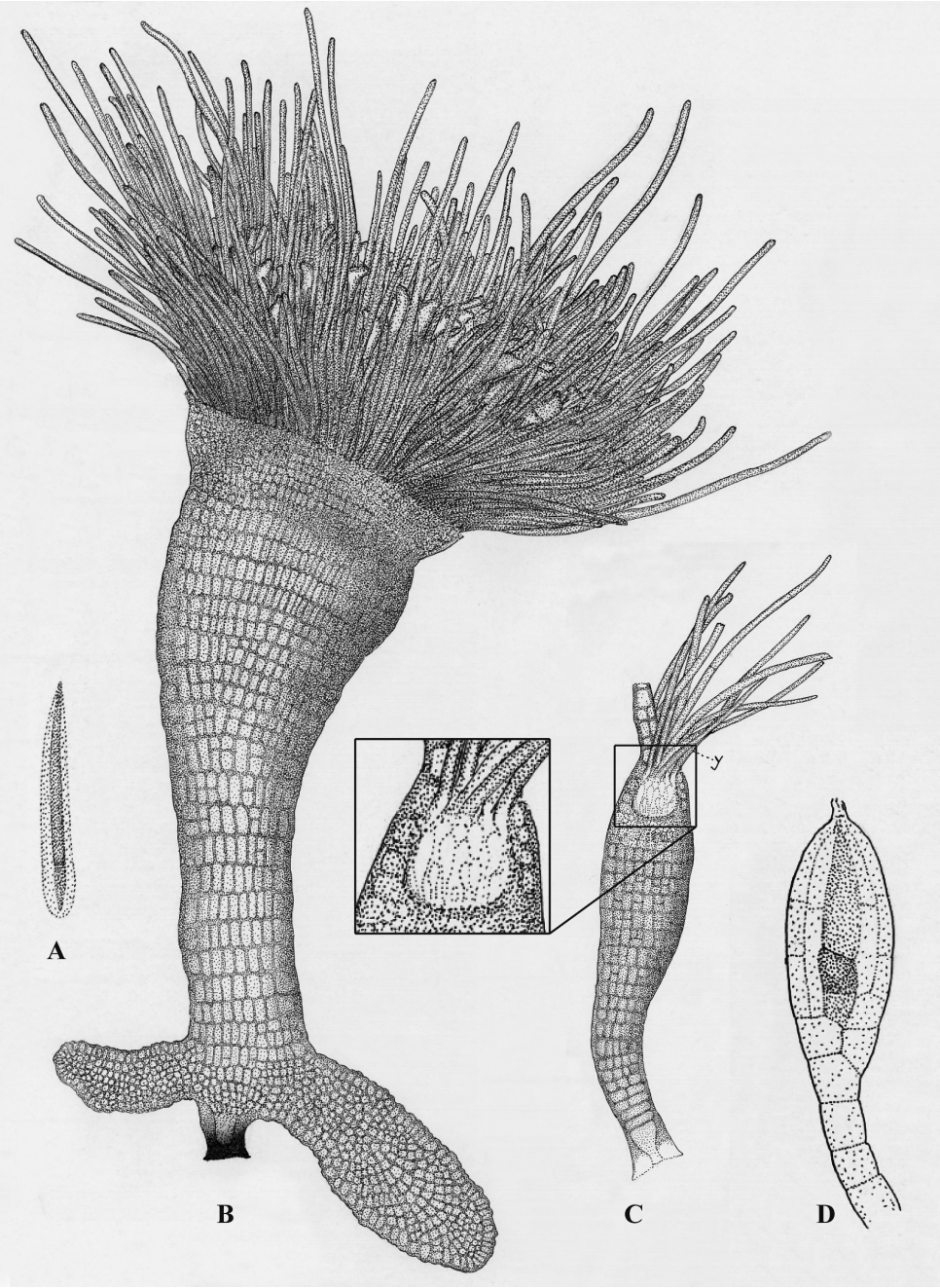

FIG. 2. *Zodiomyces vorticellarius*. An enlargement of Thaxter's (1896, p 425) finished plate XXIII, showing his skillful technique. A. Two-celled ascospore, with gelatinous sheath. B. Mature thallus. C. Immature thallus; the end of the primary appendage at the left is broken, the secondary appendages have burst through the apical-most cells of the receptacle, and the perithecia are in early stages of development (enlarged to show stippling details). D. Developing perithecium in which the ascogenic cell has divided, with only a remnant of the broken trichogyne visible at the perithecial tip. Image courtesy of the Archives of the Farlow Herbarium of Cryptogamic Botany.

In this paper we provide the description of a new species in the morphologically highly elaborate genus *Zodiomyces*. It is characterized by a crown of long appendages that resemble, when stained with eosin, the colorful and exuberant trailing of fireworks. This species is represented by five slides prepared by Thaxter.

The genus *Zodiomyces* was erected by Thaxter (1891). Three species are currently described. Santamaría (2004) provided a key and distributional records. *Zodiomyces vorticellarius* Thaxt., the type species, is distributed worldwide (Thaxter 1931, Santamaria 2004, Huggert and Eriksson 2010, Haelewaters et al. 2012). *Zodiomyces*

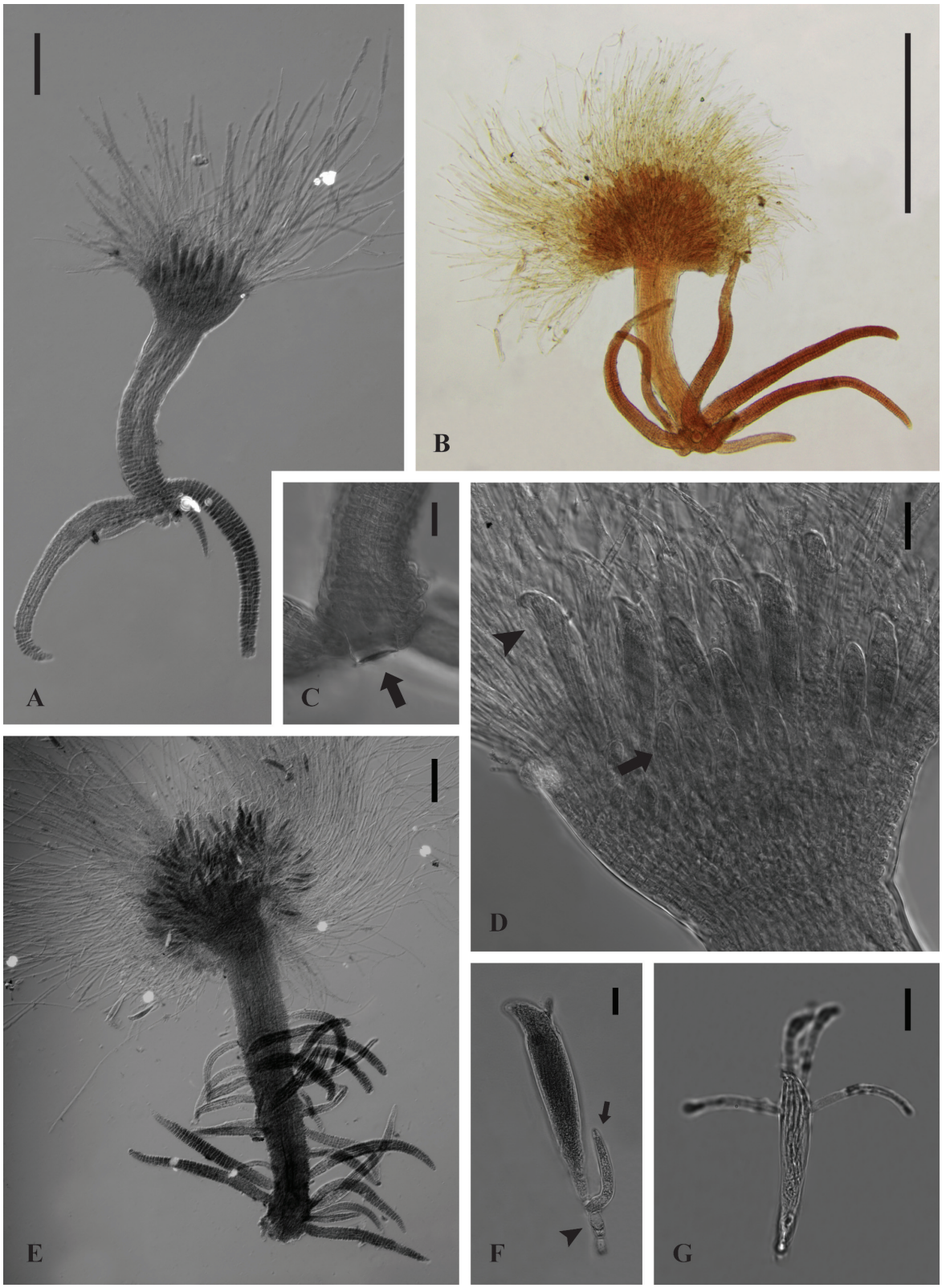

FIG. 3. *Zodiomyces rhizophorus*. A. Young thallus with two well developed and two developing multicellular outgrowths around the basal-most part of the receptacle (FH 00313537). B. Mature thallus with the usual reddish color caused by the eosin stain (FH 00313538). C. Detail of receptacle; arrow points to the basal cell with the sucker-like structure that is the single point of attachment to the host (HOLOTYPE, FH 00313536). D. Detail of upper part of the receptacle, on top of which multiple

odae T. Majewski & K. Sugiy. is known only from Iriomote Island, Japan, (Majewski and Sugiyama 1989) and *Z. subseriatus* Thaxt. has been found in Asia (China, peninsular Malaysia, the Philippines, South Korea, Taiwan), Poland and Sudan (Santamaria 2004). All three species of *Zodiomyces* grow on aquatic beetles in the family Hydrophilidae (Coleoptera). Species distinctions are based on the presence/absence and number of buffer projections and the number of cells at the base of the receptacle (Santamaría 2004).

MATERIALS AND METHODS

Specimens are filed among preparations made by Roland Thaxter deposited at the Farlow Herbarium, Harvard University (FH). The methods used to prepare the slides are in Thaxter (1896: 249).

Observations, measurements and photographs were made with an Olympus BX40 light microscope with Olympus XC50 digital camera and MicroSuite Special Edition software 3.1 (Soft Imaging Solutions GmbH). Additional photography was done at the Harvard Center for Biological Imaging with a Zeiss Cell Observer microscope equipped with differential interference contrast optics and a Hamamatsu Flash 4.0 2 sCMOS camera, running on ZEN software (Carl Zeiss Microscopy). Illustrations were optimized (with LEVELS and BRIGHTNESS/CONTRAST tools) and cropped in Adobe Photoshop CS 8.0 (San Jose, California).

TAXONOMY

Zodiomyces rhizophorus W. Rossi, Haelew. & Pfister, sp. nov. FIGS. 2, 3

MycoBank MB812837

Typification: TRINIDAD, near "Mayaro" (Mayaro County), on the legs of a "hydrophilid", *P.J. Darlington Jr*, Apr 1924, Thaxter No. 3608 (**holotype** slide FH 00313536).

Etymology: rhizo- (Greek) = root; *-phorus* (Greek) = bearing, referring to the outgrowths borne on the receptacle. This is the name Thaxter assigned to this species.

Description: Thallus 545–1020 μm from foot to perithecial tips. Basal cell turbinate or obconical, surrounded by a thick, rounded sucker-like anchoring structure. Receptacle elongate funnel-shaped to almost cylindrical, consisting of many small, quadrangular cells. Between 3 and 30 lateral outgrowths arise from the base of the receptacle in mature thalli. These are long, up to 670 μm, slender, multicellular and multiseriate, rarely branched, and sometimes also produced on the upper half of the receptacle. Sterile appendages numerous and long, up to 1 mm, exceeding the entire thallus. Cell VI is distinguishable when perithecia are young, becoming obliterated on maturation. Perithecia 58–90 × 13–18 μm, each supported by a three-celled pedicel, the upper cell of which gives rise to a lateral projection directed upward, 24–37 μm long. Perithecia narrowly clavate, with an acute and slightly curved apex, bearing four distal projections, two of which are laterally oriented, 54–87 × 4.3–6.6 μm, gently arched, and two are slightly above these and are directed upward and are curved or bent at the tip in the same direction, 48–58 × 4.4–5.8 μm.

Other specimens examined: Same data as the holotype, permanent slides FH 00313537, FH 00313538, FH 00313539 and FH 00313540 (PARATYPES). One immature and 12 mature thalli were examined.

DISCUSSION

Morphology.—The number, shape and position of the lateral outgrowths of the receptacle distinguish *Z. rhizophorus* from any other species in the genus. The number of lateral outgrowths increases and their position around the receptacle ascends as the thallus matures. Thaxter (1931: 332) wrote concerning the projections in *Z. subseriatus* that "their size is indeterminate, increasing with age". This is a unique feature among the Laboulbeniales, which otherwise are known for their strict determinate growth and well-defined thallus formation. Other species in the genus *Zodiomyces* have lateral projections only at the base of the receptacle, hence the reference to these structures as "buffer projections" or "buffer organs" (Thaxter 1931, Santamaría 2004). The lateral outgrowths of this new species are usually numerous, more slender, and can be inserted far from the base of the thallus. In *Z. vorticellarius* there are usually two or more lateral outgrowths only at the base of the receptacle. Moreover, compared to *Z. vorticellarius*, the receptacle of *Z. rhizophorus* is longer and more slender on average and the sterile appendages are distinctly longer.

The other two described species in the genus, *Z. odae* and *Z. subseriatus*, are very different. The former among other characters lacks any basal outgrowth, and the latter has a series of undivided cells at the

←

perithecia are formed; a progression is seen from juvenile (simply oval, arrow) to mature perithecia (clavate, with curved apex, arrowhead) (FH 00313537). E. Mature thallus, with multicellular outgrowths from the base to the upper half of the receptacle (FH 00313537). F. Perithecium with three-celled stalk; arrowhead points to the lower two cells; the upper cell forms a continuous lateral, upward projection (indicated by arrow) (FH 00313539). G: Fully mature perithecium, descriptive for *Z. rhizophorus*, with two lateral, broadly arching projections and two upper projections bent at the tip (FH 00313540). Bars: A, E = 100 μm; B = 500 μm; C, D, F, G = 20 μm.

base of the thallus and bears a single outgrowth, which can be large, sometimes as large as the thallus (FIG. 1).

Santamaría (2004) studied all described species belonging to the subfamily Zodiomycetoideae (Thaxt.) I.I. Tav. and concluded that the perithecial characters are diagnostic for the group. In Zodiomycetoideae three genera have been described: *Capillistichus* Santam., characterized by a uniseriate receptacle; *Scepastocarpus* Santam., recognized by having abundant appendages and perithecia enclosed in a receptacle cavity; and *Zodiomyces*. Perithecia in all three genera are highly modified and share the presence of four projections (or "ligulae") (Tavares 1985, Santamaría 2004). The length and orientation of these projections differ among the genera. In *Capillistichus tenellus* Santam. all four projections are erect, while in *Scepastocarpus peritheciiformis* Santam. the lower (ventral) ones are usually downwardly oriented; in both these genera the two pairs are equally long. In the genus *Zodiomyces* the two pairs of projections are different in length, with the lower ones being longer, as also observed in *Z. rhizophorus*. In *Z. vorticellarius* the upper pair of projections is directed upward, ending in a broadly uncinate tip; the lower pair is oriented laterally and upward, arching and flexuous. In *Z. subseriatus* the upper projections are more upright, less curved; the lower ones again are oriented laterally and upward but are less flexuous. In *Z. rhizophorus* the upper projections are bent at the tip; the lower ones are directed strictly laterally, broadly arching. Also the upward projection arising from one cell below the perithecium is a shared character among *S. peritheciiformis*, *Z. rhizophorus*, *Z. subseriatus* and *Z. vorticellarius*. This feature is not present in *C. tenellus*, but apparently this species has a similar outgrowth above cell VII that prematurely breaks off (Santamaría 2004). No perithecial characters were described in *Z. odae* (Majewski and Sugiyama 1989).

Zodiomyces rhizophorus can reach up to 2 mm long from the foot to the tip of appendages. The size is unusual in the Laboulbeniales but not exceptional. Thaxter (1931, p 330) reports a thallus of *Z. vorticellarius* from Argentina of nearly 3 mm. We re-examined this material (slide FH 00313535) and measured the thallus as 2.75 mm long. To our knowledge the tallest species among the Laboulbeniales is *Laboulbenia kunckelii* (Giard) Thaxt., 2–4 mm long (Giard 1892, Sugiyama and Phanichapol 1984).

Ecology.—Species of *Zodiomyces* exclusively occur on Hydrophilidae, a family of water beetles. The thalli are found exclusively on the ventral parts of these beetles, including the legs. Most infected specimens have been collected from sieving in stagnant or slow-flowing waters, in leaves or among plant remains at the margins (Thaxter 1931, Majewski 1994, Haelewaters et al. 2012).

Questions remain about the manner in which ascospores are transmitted in the aquatic environments. Scheloske (1976) suggested in *Eusynaptomyces benjamini* Scheloske on *Enochrus testaceus* (Fabricius 1801) water beetles (Coleoptera, Hydrophilidae) that insect mating behavior determines the deposition of the sticky spores of the fungus to strict positions on the host body. More recently Goldman and Weir (2012) delivered observational and molecular data that support this statement in species of *Chitonomyces* Peyr. on *Laccophilus maculosus* (Dytiscidae). Whether this is accomplished in *Zodiomyces* by mating of the insects or some other passive means is not known.

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