An oomycete parasitizing algae occurring on dorsal shells of turtles

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ABSTRACT

This paper reports the identity of a parasite (belonging to the Oomycota—and more broadly, the Straminipila), found in cladophoraceous algae (*Basicladia*) growing in tufts or more extensive coverings on the external surface of the carapace of freshwater turtles collected in Alabama and Mississippi. Whereas this Oomycete might have been placed in genus *Lagenidium* (historically a large group of plant and animal parasites), investigation by various workers has shown *Lagenidium* to be nomenclaturally (and systematically) questionable—its identity interwoven with a related (and also problematic) genus *Myzocytium*. We do not attempt to resolve the nomenclatural origins of *Lagenidium* and *Myzocytium*, but rather, after discussion of taxonomic problems, focus on placement of this Oomycete within current segregate genera (of *Lagenidium*)—established by Dick (1997, 2001), partly in response to taxonomic confusion. The best candidate for assignment of this Oomycete is Dick's genus *Syzygangia*. Since there is also some question about the particular species of the algal host (genus *Basicladia*), occurring on turtles we examined, the specific identity of the alga is given consideration herein as well. Published on-line: **www.phytologia.org** *Phytologia 95(1): 34-41(Feb. 1, 2013).*

KEY WORDS: *Basicladia*, *Lagenidium*, *Myzocytium*, nomenclature, *Pythium*, *Sternotherus*, *Syzygangia*, *Trachemys*, type, zoosporogenesis.

A number of relatively inconspicuous, oomycetous pseudofungi—internal parasites of plants, other Oomycota, various invertebrates, etc.—are interesting subjects of study. Some are highly significant, well-studied plant pathogens, including potentially devastating forms such as late-blight of potato (*Phytophthora infestans*). Others, especially those with holocarpic life cycles (in which the "vegetative" thallus is converted, at a certain stage, entirely to reproductive structures), have often remained more poorly known (cf. Blackwell, 2011). The putatively holocarpic, Oomycete parasite considered here was found in algae (WB#86, October, 1998) attached to the external surface of the dorsal part (carapace) of the shell of turtles (inhabiting sloughs, creeks, or lake margins) in central Alabama—Stink-pot turtles (genus *Sternotherus*), Tuscaloosa Co., collected by Dr. Gordon Ultsch—and, in western Mississippi—harvested from "slider" turtles (genus *Trachemys*) collected in an area of catfish-farm-ponds near Itta Bena, MS, May 5, 2012, by Mr. Andrew DeSantis. The algal host (of the Oomycete) in both cases proved to be the green alga *Basicladia* (Cladophoraceae). This alga, though the subject of study, is incompletely known; we devote space to its history and species—as such relates to specimens in our study. The species identity of these algal specimens proved somewhat debatable. The Oomycete would formerly have been placed in the well-known (if not well-understood) genus *Lagenidium*. However, *Lagenidium* is fraught with nomenclatural problems, stemming from an imprecise original description and inadequate initial species designations under this generic name. Further problems arose from early confusion of *Lagenidium* and another lagenidiaceous genus, *Myzocytium*. Morphological intergradation was noted between *Lagenidium* and *Myzocytium* (Barron, 1976), and questions of relationship of included taxa persist. It became necessary to seek further generic placement for the Oomycete in our study.

HISTORICAL BACKGROUND OF *LAGENIDIUM*

The name *Lagenidium* was proposed by Schenk; however, the exact date of Schenk's publication (usually cited as 1859, cf. Sparrow, 1960) is debatable, since one or more parts of the publication may have appeared prior to 1859 (personal communication, Dr. Scott Redhead, Curator of the National

Mycology Herbarium, Canada), viz. 1858 (or possibly 1857). Schenk did not formally include species (binomials) in *Lagenidium*, though he suggested two very similar species, "*Pythium proliferum*" and "*Pythium globosum*"—names apparently coined by Schenk (cf. Matthews, 1931)—for possible inclusion. Only vegetative and asexual reproductive features of these two "taxa" were observed by Schenk. In complication, *P. proliferum* was also proposed by Schenk (1858), in a different publication, as the basis of his genus *Myzocytium*, but no nomenclatural combination was provided there either. It is unclear if *Myzocytium* was published before or after *Lagenidium* (given the uncertainty of the publication date of *Lagenidium*).

Walz (1870) treated *Pythium globosum* as a synonym of *P. proliferum*. Walz, however, appeared to be dealing with a mixed collection (cf. his figs. 13-19), i.e., what probably constituted *Pythium proliferum* (*sensu* Schenk) and *Lagenidium rabenhorstii* (Zopf, 1878)—as noted by Matthews (1931) and Dr. Readhead (personal communication). Regardless, the two "Pythiums" of Schenk (1859) are probably the same species; they do not in any case belong to *Pythium* (Matthews, 1931), but would be morphologically associated with either *Myzocytium* or *Lagenidium*—depending on which generic name might have priority and whether either holds up as a legitimate name. Additionally complicating is that *L. rabenhorstii* Zopf, a different organism from the two "Pythiums," has been considered (Scherffel, 1925) the type of *Lagenidium*—a typification which Dick (2001) opposed, because *L. rabenhorstii* was not part of the concept of *Lagenidium* "established" by Schenk (1859).

Lagenidium taxonomy, already confusing, became complex, with a large (and diverse) number of species described—some, morphologically, seeming less closely related than others (cf. Sparrow, 1960; Karling, 1981). For a recent confirmation of "differential relatedness" of taxa (at one time or another placed in *Lagenidium*)—this involving molecular analysis—see Beakes and Sekimoto (2009).

"SOLUTION" OF THE "*LAGENIDIUM* PROBLEM"

No doubt in response to the frustrating state of *Lagenidium* systematics, Dick (2001), in a drastic step, excluded all species but one from the genus. More than 50 taxa were excluded by Dick and assigned to other genera, as species or synonyms. Approximately another 15 putative taxa of *Lagenidium* were considered "unidentifiable." The only species retained by Dick (2001) in *Lagenidium* was *L. giganteum* Couch (1935)—a large and partly extramatrical form occurring on mosquito larvae. Initially (Couch, 1935), only asexual development was known for *L. giganteum*; a number years later, details of sexual development were described (Couch and Romney, 1973) but not illustrated. In any case, the morphological features of *L. giganteum* apparently suited Dick's concept of what *Lagenidium* should represent. Dick (2001) proposed *L. giganteum* as "lectotype" of the genus. In seeming anticipation of excluding a number of species from *Lagenidium*, and some from *Myzocytium*, Dick (1997) established several segregate genera, e.g., *Chlamydomyzium*, *Myzocytiopsis*, and *Syzygangia*. Regardless of his questionable typification of *Lagenidium* (not based on earlier material, such as in Zopf, 1878), and his severe alteration of *Lagenidium* taxonomy (and to an extent *Myzocytium*), Dick's segregate genera—in conjunction with lagenidiaceous genera still available (e.g., *Aphanomycopsis* Scherffel), and the few taxa retained in *Myzocytium*—can be viewed as at least a workable solution to difficult nomenclatural problems. We note, however, that some authors (e.g., Kiziewicz, 2004; Beakes and Sekimoto, 2009) continued to recognize certain species (though excluded by Dick, 2001) under the name *Lagenidium*.

At a minimum, Dick's revised system (1997, 2001) has the benefit of allowing resolution of certain problems of relationship of organisms in the former Lagenidiaceae (cf. Karling, 1981). For example, Dick's (1997) system served to clarify that traditional *Myzocytium* housed, perhaps unnaturally, taxa which were either algal or animal (nematode and rotifer) parasites. This enabled Pereira and Vélez (2004) to properly assess that the algal parasite they observed (possessing extrasporangial zoosporogenesis, and catenulate thallus morphology)—described initially as *Myzocytium megastomum* by

Wildeman (1893)—should be retained in *Myzocytium*, and not placed in *Myzocytiopsis* (designated by Dick for animal parasites, and exhibiting intrasporangial zoosporogenesis). Conversely, recognition of Dick's (1997) genus *Myzocytiopsis* permitted proper referral of *Myzocytium vermicolum* to *Myzocytiopis*—in an ultrastructural study of this nematode parasite by Glockling and Beakes (2006).

TAXONOMIC PLACEMENT OF THE OOMYCETE PARASITE

Based on thallus morphology and nature of parasitism, the oomycetous algal parasite in our study (Figs. 1-5) would have been placed in *Lagenidium*—had problems with the nomenclature and taxonomy of this genus not been detected. Examination of classical treatments, e.g., Cook (1935), Sparrow (1960) and Karling (1981), reveals a range of algal-inhabiting *Lagenidium* species. In comparing traditional *Lagenidium* taxa with the Oomycete observed in *Basicladia* (the first report of an Oomycete parasitizing this genus that we are aware of) there are approximations, but no precise match. *Lagenidium marchalianum* Wildeman, occurring in the green alga *Oedogonium*, bears some resemblance, except that the tubes of its thallus are more slender and straight. *Lagenidium marchalianum* was transferred by Dick (1997) to *Syzygangia*, as *S. marchaliana*. The thallus of *Lagenidium oedogonii* Scherffel, a taxon also transferred by Dick to *Syzygangia*, is more delicate than in our parasite, and its "hyphae" pass readily from cell to cell of the host. Our parasite tends to be confined to individual host cells (though sometimes extensively developed within, cf. Figs. 2-4, even completely filling, the cell); it does not appear to as readily traverse the thick and often lamellated cross-walls (Figs. 6, 9) of *Basicladia*, although we have observed adjacent cell infection, and possible penetration of the end-wall (Fig. 4). *Lagenidium closterii* Wildeman, found in desmids, also somewhat resembles our parasite, but its thallal tubes are more slender, and sometimes—in addition to the usual intramatrical growth—develop extramatrically. *Lagenidium destruens* Sparrow, occurring as a parasite in the Saprolegniaceous genus, *Achlya*, can rather strongly resemble our organism, especially in potentially filling (and destroying the contents of) a host cell; however, the short, stubby, lobed (often "single-celled") branches of *L. destruens* do not usually exhibit the more extensive, almost "mycelial," development sometimes observed in the *Basicladia* parasite (Fig. 4); also, the (asexual) resting spores of *L. destruens* are more squarrose or rectangular than the more spherical spores (Fig. 5) observed in the *Basicladia*-inhabiting organism. Zoospores were not observed, but germinating zoospore cysts were (Fig. 1). Though we found no precise comparison for the Oomycete in *Basicladia*, only asexual stages were seen. Nonetheless, an algal parasite such as this is best placed in genus *Syzygangia* (cf. Dick, 1997, 2001)—described for endoparasites of plants, especially algae. Generic referral seems clear, but this possibly undescribed taxon must be left for now as *Syzygangia* sp.

IDENTITY OF THE ALGAL HOST OF THIS PARASITE

Algae attached to dorsal shells of freshwater turtles are usually members of the Cladophoraceae (cf. Skinner et al., 2008), the primary such epizoic genus being *Basicladia*. The algal host in our study (Figs. 6-10) is identified as *Basicladia* (cf. Smith, 1950), reaffirmed as a genus distinct from *Cladophora* (see Garbary, 2010)—based not only on unusual habitats occupied, but on differentiation of the thallus into a branched prostrate-system and an upright-system of mostly unbranched filaments. Van den Hoek (1963), in his revision of European species of *Cladophora*—and with only brief reference to North American taxa—had tentatively viewed *Basicladia* as a Section of *Cladophora* (the grounds for such inclusion not being entirely clear). *Basicladia* in North America, as typically seen in freshwater environments, is unusual (among any kinds of algae) in preferential occurrence on the carapace of turtles (Fig. 10)—though it may found, or cultured, on other hard (even inorganic) substrates (cf. Prescott, 1962; Graham et al., 2009); one species occurs on shells of snails (Normandin and Taft, 1959).

A total of seven species of *Basicladia* are known worldwide (Garbary, 2010; and *AlgaeBase*, see Guiry, 2012), but only three are native to the United States (two of these, *B. crassa* and *B. chelonum*, are found attached to turtle shells). *Basicladia*, as seen on turtles, is coarsely filamentous, and the basal-

system may have attachment or "holdfast" cells (Hoffman and Tilden, 1930). The often substantial algal covering on the dorsal shell has led to the nickname "moss back" for such inhabited turtles (Hoffmann and Tilden, 1930). Hoffmann and Tilden expressed surprise that algae as distinct as *Basicladia* were undescribed (as a genus) prior to 1930; though known earlier, they were misplaced in genus *Chaetomorpha* (noted by Normandin and Taft, 1959). *Basicladia* remained poorly known, and is not mentioned in every phycology text—e.g., Bold and Wynne (1985), even though they provided a treatment of Cladophoraceae. The genus, however, received recent recognition in Graham et al. (2009).

Turtle-inhabiting species of *Basicladia* in the United States occur mainly east of the Rocky Mountains—*B. crassa* is a northern species, and *B. chelonum* is more wide-spread (cf. Hoffmann and Tilden, 1930; Smith, 1950; Prescott, 1962). These species are distinguished by dimensions of vegetative cells—larger in *B. crassa*. Both were reported mainly on snapping turtles (genus *Chelydra*), cf. Prescott (1962) and Graham et al. (2009), but other turtle hosts were noted (Hoffmann and Tilden, 1930). Anderson and Sinclair (1966) studied *Basicladia crassa* collected from "western painted turtle," "*Chrysemys picta bellii*," in Illinois. *Basicladia* has been examined infrequently in the southeastern US; *B. chelonum* was reported in North Carolina (Whitford and Schumacher, 1969) "on several species of turtles, especially the common mud turtle" (identities now difficult to determine). In our study—central Alabama, western Mississippi—the alga was found on turtles identified, respectively, as "stink-pots" (*Sternotherus odoratus*) and "red-eared slider" (*Trachemys scripta elegans*). It is not surprising to find *Basicladia* on these turtles, given habits of shallow submersion. Neill and Allen (1954) noted that a number of kinds of turtles (including *Sternotherus odoratus*, and *Macrochelys temmincki*, the alligator snapping turtle) may be "epizoized" by algae, presumably *Basicladia*, in Florida. The closest fit for the *Basicladia* we found (similar in Alabama and Mississippi) is *B. chelonum*. But, cell-widths of our specimens (distal filaments)—typically in the 30 to 60µm range—overlap measurements for *B. chelonum* and *B. crassa* (Hoffmann and Tilden, 1930). Also, pre-zoosporogenesis germination papillae (Figs. 7-8) are more prominent than illustrated for either species. Thus, the alga (as well as the Oomycete) here considered could bear future scrutiny.

ACKNOWLEDGMENTS

We thank Drs. Robert Roberson (Arizona State University) and Sonali Roychoudhury (Patent Agent, New York) for manuscript review—and Dr. Stephen Secor (U. Alabama) for help with the project.

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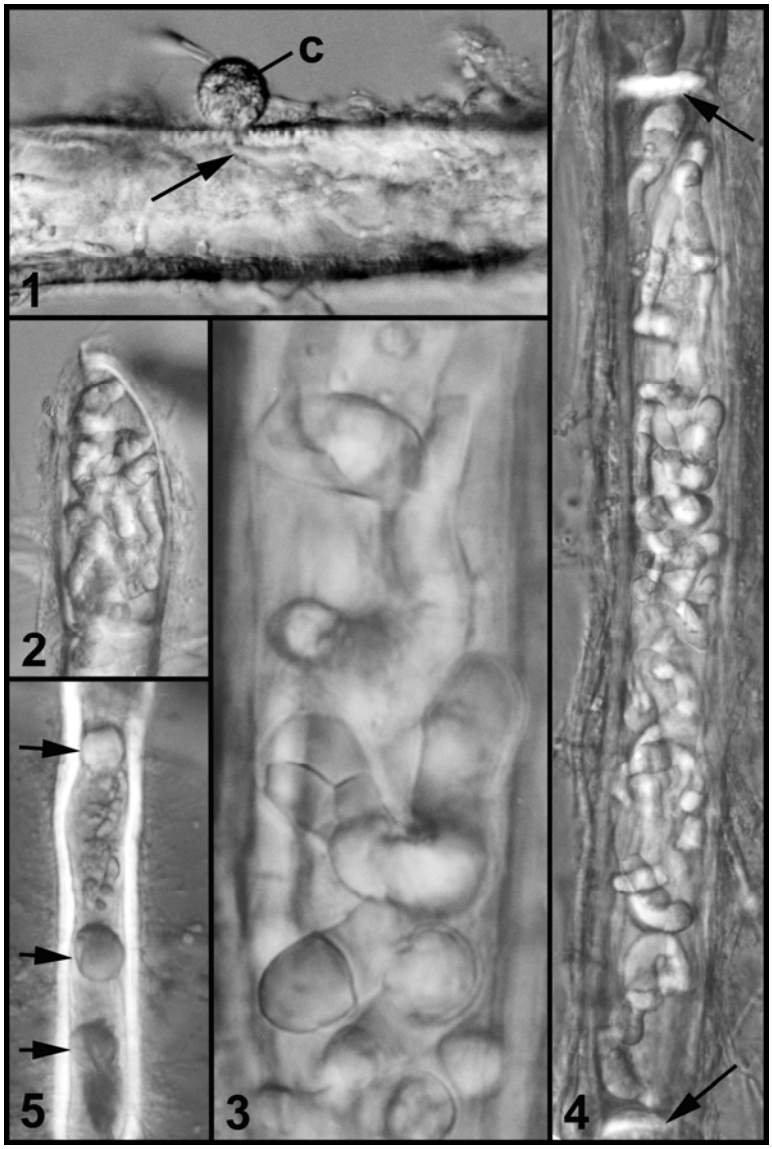
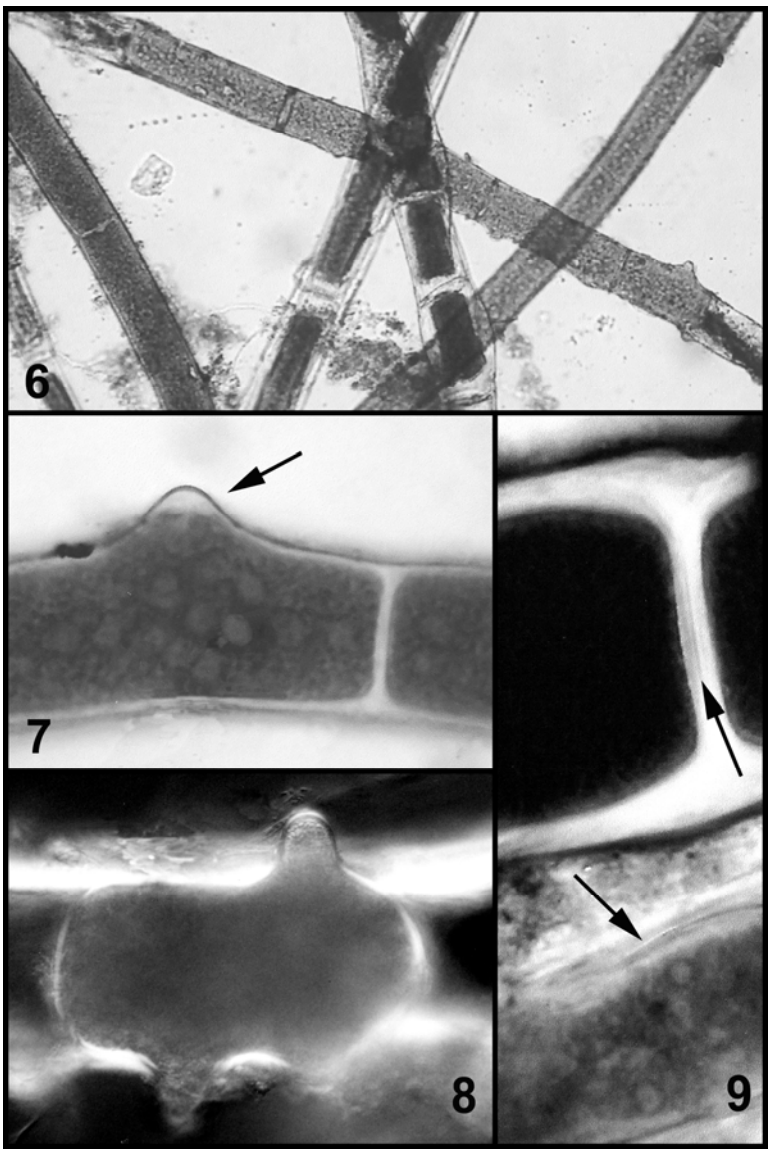


Figures 1-5, ***Syzygangia* sp**. **(Oomycota) in the green alga *Basicladia***. Fig.1: Zoospore cyst (C) of *Syzygangia*; germination tube development (arrow). Fig. 2: Vegetative thallus of *Syzygangia* in terminal cell of an algal filament. Fig. 3: Closer view of thallus in intercalary algal cell. Fig. 4: Extensive development of *Syzygangia* thallus from septum to septum (arrows) of an algal cell; possible penetration of thallus into adjacent algal cell, above upper septum. Fig. 5: Rounded *Syzygangia* resting spores (arrows), probably asexually formed.



Figures 6-9, *Basicladia* (similar to *B. chelonum*), Cladophoraceae. Fig. 6: General view of representative, non-parasitized, rather coarse and unbranched algal filaments; thickened cross-walls and germination bumps occasionally evident. Fig. 7: Closer view of pre-zoosporogenesis germination papilla (arrow). Fig. 8: Multiple, germination papillae on one cell. Fig. 9: Thickened, lamellated cell-walls evident: cross-wall (upper arrow), lateral wall (lower arrow).

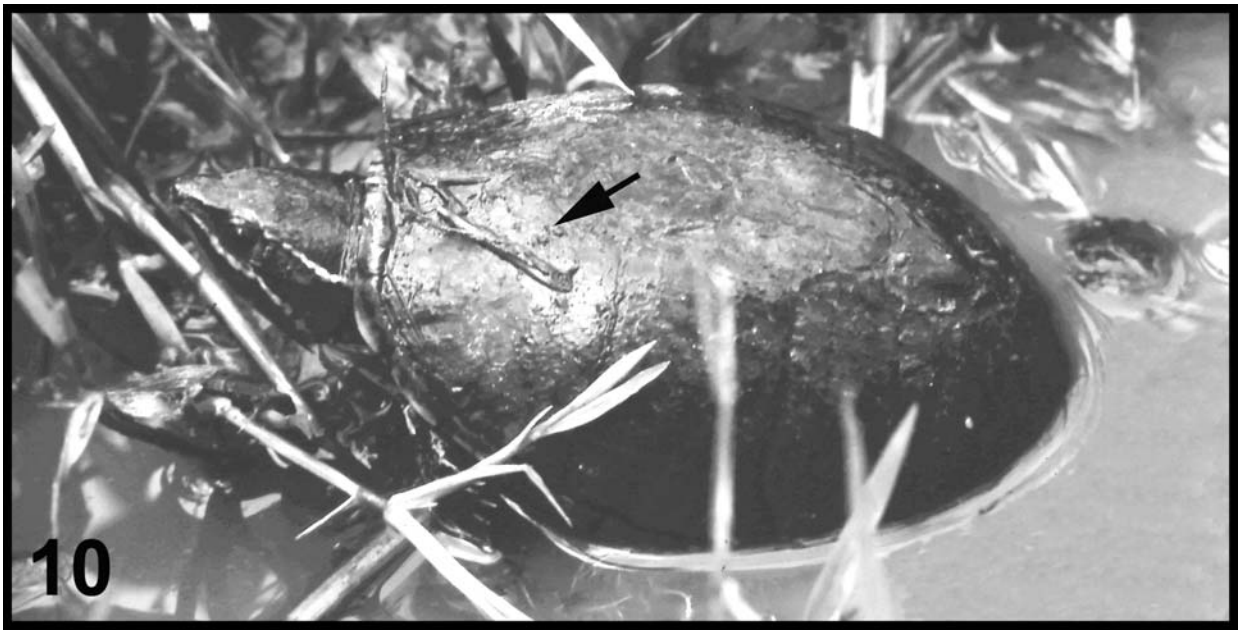


Figure 10, "Stink-pot" turtle (*Sternotherus odoratus*). Raised areas on carapace (arrow) are temporarily dried algae (***Basicladia***).