Questions regarding genus *Myzocytium* (Oomycota, Straminipila) and its species: Variation and identity of specimens in west-central Alabama

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ABSTRACT

Myzocytium is a poorly known genus of aquatic Oomycetes, formerly containing taxa parasitizing algae (mainly freshwater forms) or kinds of aquatic invertebrates. Recently, the genus has been restricted to species inhabiting algal hosts. *Myzocytium proliferum*, *M. megastomum*, *M. netrii*, and *M. rabenhorstii* (a form of debatable placement, resembling *Lagenidium*) occur in Zygnemataceae, Desmidiaceae, or Cladophoraceae. *Myzocytium* and *Lagenidium,* both traditionally placed in the Lagenidiaceae, can be morphologically and ecologically similar, and questions persist as to their morphological distinction and initial nomenclatural recognition; several later described genera must also be considered. Populations of *Myzocytium* (found in *Spirogyra*) in western Alabama exhibited features, such as the form of the sporangial discharge tube, of either *M. proliferum* or *M. megastomum,* or were intermediate between them; these observations (and a common host occupied) challenge distinction of these species, and invoke questions of the role of genetics (vs. conditions encountered in host cells) in aspects of morphology. Objectives of our investigation included confirmation of specimens collected as belonging to genus *Myzocytium*, as well as determination of species identity (or intermediacy). A key to species is presented.
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The holocarpic Oomycete genus *Myzocytium* Schenk (1858) has been historically placed in the Lagenidiales (e.g., Sparrow, 1960), more recently in the Pythiales (cf. Dick, 2001). *Myzocytium* has been confused since its inception with *Lagenidium*—both genera based initially on similar (identical?) species, considered then to belong to genus *Pythium*. The date of publication of *Lagenidium* (usually credited as Schenk, 1859; e.g., Sparrow, 1960) may have been published in 1858 (or 1857) depending on when separates of Schenk's "1859" publication were issued (S. Redhead, personal communication). It remains uncertain which generic name (*Myzocytium* or *Lagenidium*) appeared first—of concern if these genera are synonymous. Further nomenclatural complication is that neither name was formally combined with a specific epithet in initial publication. Morphological intermediacy has been observed between *Lagenidium* and *Myzocytium* (Barron, 1976); distinction of these genera and priority of their names must be further sorted (Dick, 2001; Redhead, personal communication)—involving many species, and several segregate genera (e.g., *Syzygangia*)—not goals of our investigation, other than discussion. Our study deals with organisms (algal parasites) placed in *Myzocytium*, as currently understood (cf. Dick, 2001). *Myzocytium* may usually be distinguished from *Lagenidium* (as traditionally recognized) by a more regular, catenulate thallus (individual cells often developing a sub-spherical shape, and sometimes separated by distinct partitions) and by less differentiated gametangia (often without obvious fertilization tubes). The taxonomy of *Lagenidium*—considered (Karling, 1981) the largest genus of Lagenidiales—was drastically altered (taxa greatly reduced) by Dick (2001), and remains controversial (Blackwell et al., 2013).

TAXONOMIC SUMMARY AND BIOLOGICAL OCCURRENCE

The genus *Myzocytium*—originally monotypic, based on an organism called "*Pythium proliferum*" by Schenk (1858)—underwent taxonomic expansion, followed by reduction of taxa. *Myzocytium* was considered by Sparrow (1960) to have five species, and by Karling (1981) as many as 16. The genus was traditionally viewed as containing parasites of both green algae and animals (nematodes and rotifers).

More recently—in connection with recognition of several new genera (Dick, 1997; Dick, 2001)—*Myzocytium* was restricted to algal parasites. The four species of *Myzocytium* still recognized (Dick, 2001), all algal-inhabiting, are: *M. proliferum* Schenk (the type), *M. megastomum* De Wildeman, *M. rabenhorstii* (Zopf) Dick, and *M. netrii* (Miller) Dick. As for other possible taxa: *Myzocytium irregulare* Petersen was thought to be synonymous with *M. megastomum* (Dick, 2001; Canter, 1947), or possibly to belong to *Lagenidium* (Fitzpatrick, 1930); *Myzocytium lineare* Cornu is too poorly known for generic placement; and *M. proliferum* forma *marinum* Kobayashi & Ookubo (an apparent instance of marine occurrence) is doubtfully distinct from typical *M. proliferum* (cf. Johnson and Sparrow, 1961). *Myzocytium globosum* Schenk and *M. anomalum* Dasgupta & John (possibly an illegitimate name) are considered synonyms of *M. proliferum* Schenk; see Dick (2001) and *Index of Fungi* (current) for lists of synonyms and excluded/doubtful names. No new species were found under *Myzocytium* since Dick's (2001) coverage.

Myzocytium proliferum, *M. megastomum*, *M. netrii*, and *M. rabenhorstii* parasitize freshwater algae, mostly "Conjugatae" (*Spirogyra*, *Mougeotia*, *Zygnema*, and certain desmids). The first two of these (*Myzocytium*) species may also be found in Cladophoraceae (*Cladophora* or *Rhizoclonium*). *Myzocytium rabenhorstii* (occurring in *Spirogyra*) and *M. netrii* (found in the "saccoderm desmid," *Netrium*) were both originally described as species of *Lagenidium*. Placement of *Lagenidium netrii* Miller (1965) in *Myzocytium* (Dick, 2001) seems morphologically appropriate. However, *Myzocytium rabenhorstii* (Zopf) Dick (2001)—based on *Lagenidium rabenhorstii* Zopf (1878)—exhibits traits of traditional *Lagenidium* (e.g., an obvious fertilization tube) or of the segregate genus, *Syzygangia* (Dick, 1997). The generic border between *Lagenidium* and *Myzocytium* being indistinct, their further evaluation—and reappraisal of related genera (*Syzygangia*, *Chlamydomyzium*, *Myzocytiopsis* and *Aphanomycopsis*)—is obviously advisable. Molecular-genetic study of these genera has been limited (Beakes and Sekimoto, 2009). *Myzocytium*, if restricted to algal parasites (Dick, 2001), is yet to be analyzed in such investigations (difficult in obligate endo-parasites); as evident below, a range of thallus types is represented.

Sparrow's (1960) key to *Myzocytium* taxa included parasites of plants or animals; Dick's (2001) treatment recognized only algal parasites, but offered no key—hence, our key to species below.

Key to species of *Myzocytium* presently recognized

1. Thallus typically remaining one-celled……*M. netrii*

1. Typically becoming multi-celled, the thallus often with a chain-like appearance.

2. Thallus often more or less linear (sometimes tiered or irregular in part), catenulate; partitions between cells sometimes becoming plate-like; cells prone to attain a spheroidal shape.

3. Sporangial discharge tube bulbous prior to exit from the cell, usually not especially elongate……..…….…..*M. megastomum*

3. Discharge tube more uniformly cylindrical, sometimes elongate and extending well beyond the host matrix…….……...…...*M. proliferum*

2. Thallus typically irregular, not necessarily catenulate or with apparent plate-like partitions between cells; cells often more elongate or variable in form…….…*M. rabenhorstii*

OUR COLLECTIONS AND OBSERVATIONS

(See Figs. 1-5, and Figs. 6-7 mentioned in Discussion)

Although ours is apparently the first report of *Myzocytium* from Alabama, this finding is not surprising, given a broad distribution of the genus (cf. Sparrow, 1960). Our study (in two counties of west-central Alabama—Tuscaloosa Co. and Choctaw Co.) focused on what we came to refer to as the *M. proliferum*/*M. megastomum* complex. Specimens—collected in late spring at locations in Northport, Tuscaloosa, and Jachin, AL (collections: WB68,70,71,133)—were obtained from *Spirogyra* occurring in modest accumulations (in shallow pond margins, stagnant creeks, ditches, etc., Figs. 1a,b) around submerged bases of cattails (*Typha* sp.) or other aquatic angiosperms. These *Myzocytium* specimens were parasitic within *Spirogyra* vegetative cells (Figs. 2a,b,c); variously, they could exhibit features of both *M. proliferum* and *M. megastomum* (as subsequently discussed). Special emphasis has been given to the morphology of the zoosporangial discharge tube (Wildeman, 1893; Canter, 1947; Sparrow, 1960; Karling, 1981). Although *M. proliferum* is envisioned to possess a uniformly cylindrical sporangial discharge tube, the acceptance of some variation in morphology (i.e., the amount of tube swelling) is evident (cf., Martin, 1927; Sparrow, 1960; Karling, 1981); Canter (1947) thought that the somewhat swollen discharge tube of specimens recognized by Martin (1927) as *M. proliferum* might in fact be more indicative of *M. megastomum*. A distinct swelling of the discharge tube, internal to its exit from the host cell, has indeed been considered a diagnostic feature of *M. megastomum* (Wildeman, 1893; Canter, 1947). Alabama specimens exhibited discharge tubes representative of either species (Figs. 3a,b,c), but were often varyingly intermediate (Figs. 4a,b,c). It remains for a future study to decipher the extent to which the "environment" encountered inside the host-cell (e.g., the path to, and the thickness/resistance of, the host cell-wall) determines the bulge occurring in the discharge tube, as opposed to the role in this morphology played by genetics. In any case, our observations led us to question the morphological distinction of these species in our study area. Delimitation by host occurrence may also be questionable since specimens identifiable as *M. megastomum*—reported (Wildeman, 1893; Canter, 1947) in desmids—were found in *Spirogyra* (Figs. 3b,c), as were (more expectedly) forms identifiable as *M. proliferum* (Fig. 3a; 5). Sparrow's (1960) report of *M. megastomum* from *Cladophora* was based on Martin's (1927) report of "M. proliferum."

DISCUSSION

Among organisms formerly placed in the Lagenidiaceae (see Sparrow, 1960, for traditional classification, and Dick, 2001, for review of revisions of classification), there is considered to be generic variation not only in the category of host occupied but in the position (relative to asexual reproductive structures produced) of occurrence of zoosporogenesis. *Myzocytium* is now circumscribed (Dick, 2001) to encompass only algal parasites; these possess extra-sporangial sporogenesis, cleavage of zoospores (or completion of same) occurring in a thin, external vesicle at the distal end of a discharge tube (see interpretation of *Myzocytium* by Pereira and Vélez, 2004, in light of Dick's 1997 paper on Myzocytiopsidaceae). The genus *Myzocytiopsis* was established (Dick, 1997) for organisms, similar to *Myzocytium*, which are invertebrate parasites—of nematodes, rotifers and amphipods (see Kiziewicz and Nalepa, 2008, re: amphipod parasitism)—and which exhibit intra-sporangial sporogenesis (no external vesicle produced, though a discharge tube may be present). Traditional *Lagenidium* (cf. Sparrow, 1960)—most species now dispersed between several genera (Dick, 2001)—contained organisms exhibiting both modes of zoospore development, although external development was more common; Karling (1981) noted that both modes can occur in one species, *L. oedogonii*. Zoospore formation in all genera discussed should be reinvestigated. In specimens of putative *Myzocytium* we observed (algal parasites) zoospores completed development in an external cluster (Figs. 5,6)—probably vesiculate (based on the compact, rounded grouping at the tip of the discharge tube)—reinforcing placement in genus *Myzocytium* (*sensu* Dick, 2001). While some authors (e.g., Glockling and Beakes, 2006) followed Dick's (1997, 2001) recognition of *Myzocytiopsis* for *Myzocytium*-like organisms infecting invertebrates, others (e.g., Kiziewicz and Nalepa, 2008) did not, interpreting *Myzocytium* broadly. As stated, the genetic integrity (inclusiveness) of *Myzocytium* requires confirmation. The kinds of host organisms invaded should be reinvestigated—not only because of our finding of *M. megastomum* in *Spirogyra* (rather than desmids),

but because Czeczuga and Muszyńska (2004) reported *Myzocytiopsis microspora* (a rotifer parasite) from plant spores. Developmental studies would also prove interesting. As mentioned, cells of *Myzocytium* are prone to be spheroidal (in contrast to the often more elongate or variable cells of *Lagenidum*). In apparent contradiction to information in Karling (1981, p. 91, last paragraph), this more spherical shape (in *Myzocytium*) appears to "evolve" during development (Figs.7a,b; 2b)—the ellipsoid shape of young cells of *Myzocytium* being reminiscent of mature cells of *Lagenidium*. Again, in seeming contrast to what Karling indicated, cells of *Myzocytium* may be distinctly constricted and appear septate or "partitioned" at an early stage (7a).

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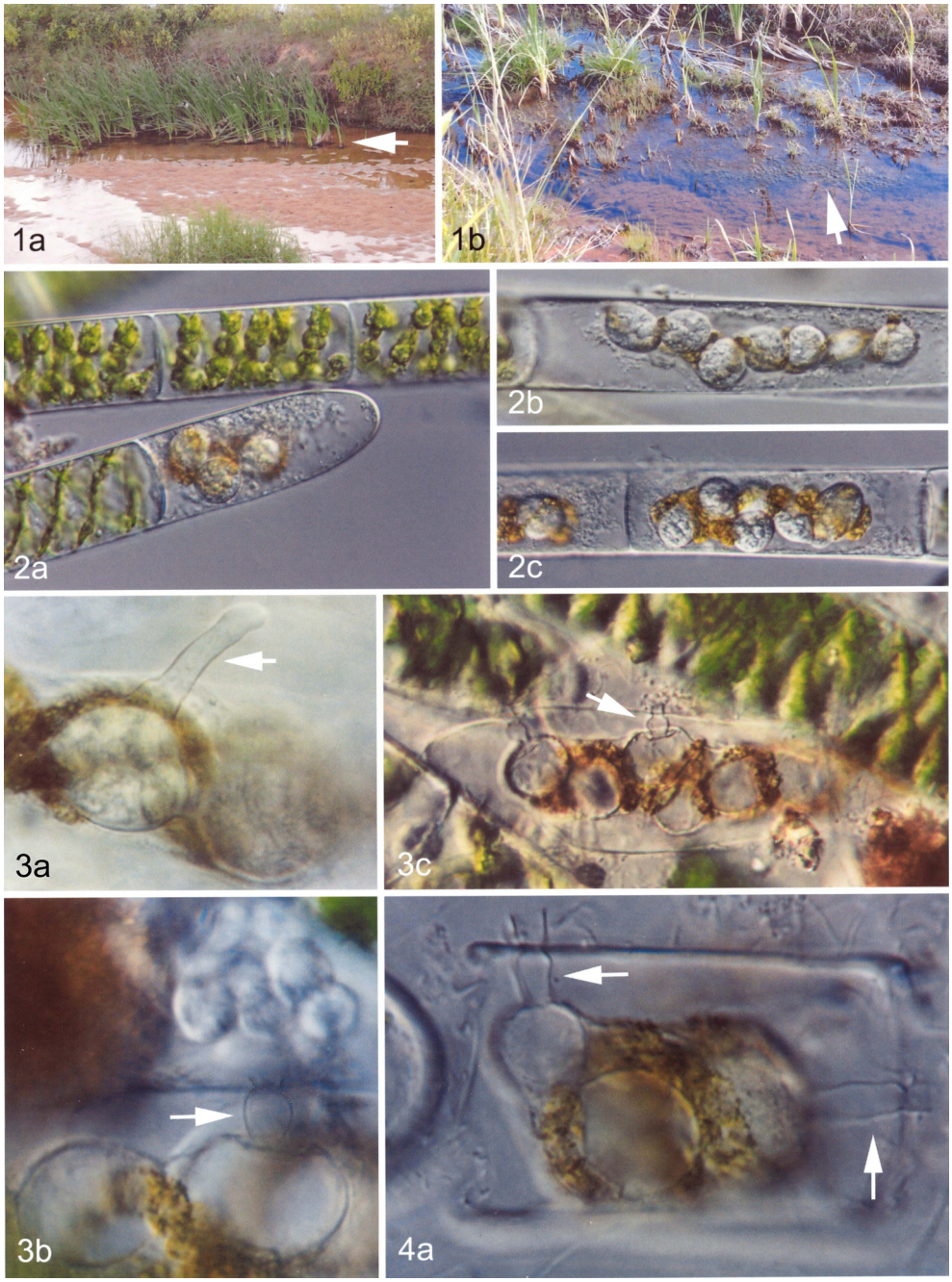

Figures 1-7: Myzocytium habitat, host and specimens from Alabama. Figs. 1a,b: Habitat: Specimens of *Myzocytium* may be found in stagnant creeks or ditches in *Spirogyra*, accumulating around bases of cattails (1a, arrow) or other "emergent" vegetation (1b, algae present, arrow). **Figs. 2a,b,c:** Relatively mature vegetative cells of *Myzocytium* within *Spirogyra* cells: (2a) parasitizing a terminal *Spirogyra* cell; (2b,c) in intercalary cells of *Spirogyra* filament; note chain-like (2b) and more tiered (2c) morphology of the *Myzocytium* thallus. **Figs. 3a,b,c:** Zoosporangial discharge tubes: characteristic of *Myzocytium proliferum* (3a, arrow), and of *M. megastomum* (3b,c; arrows); discharged zoospores are evident (3b, above arrow and host cell-wall). **Figs. 4a,b,c:** Intermediate forms of discharge tube: Discharge tube suggestive of *M. megastomum* (4a, left arrow) and of *M. proliferum* (4a, right arrow) present on the same thallus. Cells (as vegetative cells, or converted to zoosporangia) of *Myzocytium* thallus range in size between 12 and 50 µm.

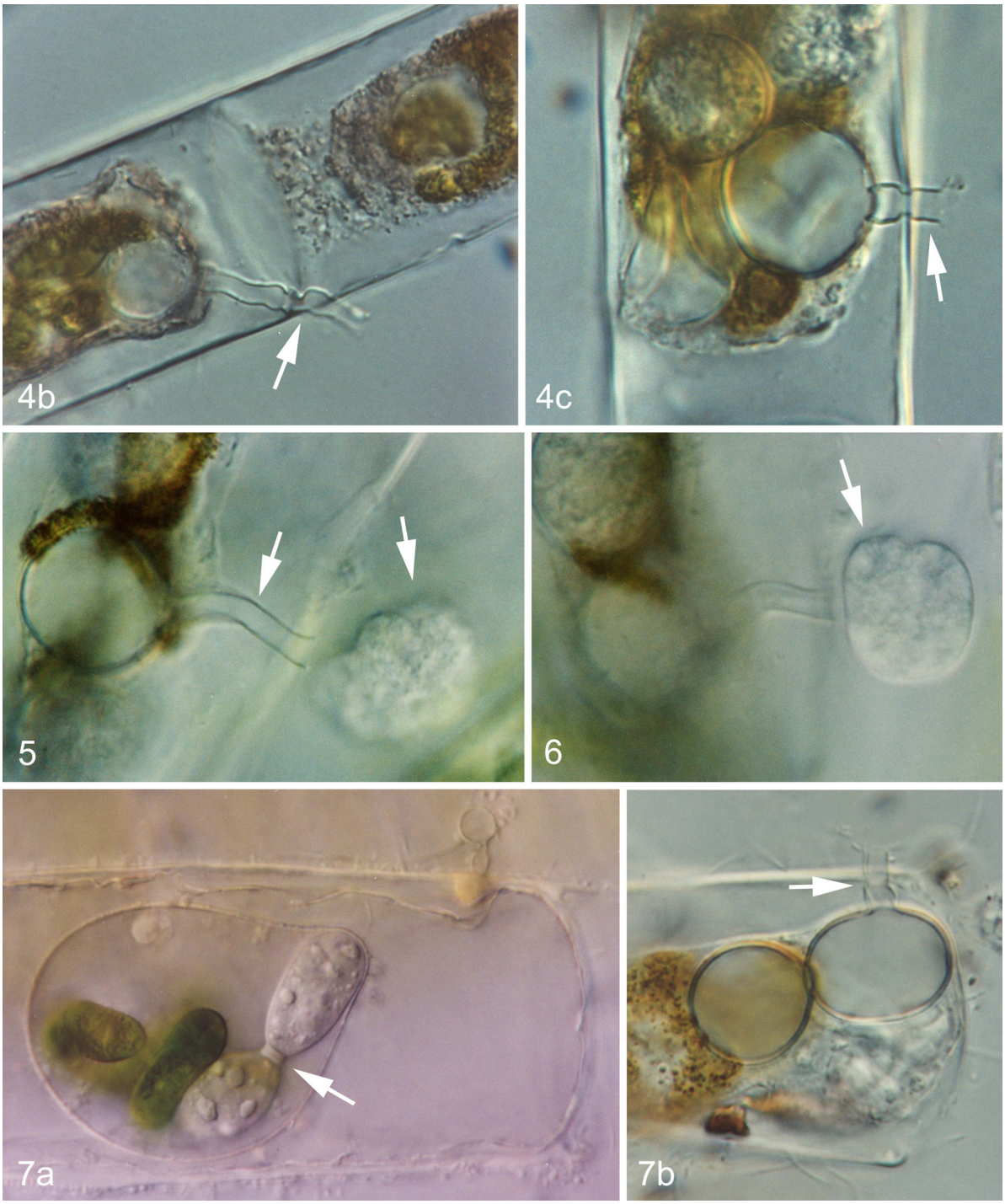

Fig. 4b: Discharge tube (arrow) resembling that of *M. proliferum*; 4c (arrow) discharge tube more like that of *M. megastomum*. **Figs. 5** and **6** (Zoospore discharge): (5)Zoospore mass (right arrow) which has been discharged from an evacuation tube (left arrow) with a morphology consistent with that of *M. proliferum*; the rounded, probably vesiculate, nature of the developing zoospore mass is evident in Fig. 6 (arrow). **Figs. 7a,b:** Developmental change in shape in cells of *Myzocytium*: Young cells (7a) are often ellipsoid (note plate-like partition between cells, arrow); mature cells are more spherical (7b, 2b), and can develop a generally thickened wall (7b, note also old discharge tube on upper part of cell to right, arrow). Thallus cells (zoosporangia) of *Myzocytium* are generally between 12 and 50 µm in diameter.