

Taxonomy and morphology of *Macrochytrium* (Chytridiomycota)

Will H. Blackwell and Martha J. Powell

Biological Sciences, The University of Alabama, Tuscaloosa AL, USA, 35487

ABSTRACT

Macrochytrium is a potentially large, monocentric chytrid, and has attracted taxonomic attention by virtue of its size and aspects of morphology. A single species, *M. botrydioides*, was initially described (Minden, 1902)—for many years the only published taxon. Eventually, two additional taxa (one species and one variety) were proposed, both proving to be nomenclaturally invalid. Irrespective of nomenclature, the rather considerable variation described for *M. botrydioides* does not appear to represent distinct, additional taxa. *Macrochytrium* is morphologically interesting in that the sporangium, in most cases, does not arise directly from the apex of the primary thallus-axis, but forms subapically (laterally) from this structure—the original apex often remaining as a small protuberance or bulge below the sporangium. A difficulty in assessing the morphology of *Macrochytrium* is that its earliest stages of development (germination of the zoospore-cyst) remain unknown, leading to difficulties in attempting to assign a particular ‘thallus-type’ to this genus. The general systematic relationships of *Macrochytrium*, as a member of the Chytridiomycota, seem reasonably clear, based in part on the perceived form and structure of the zoospore. However, its ordinal/familial relationships within Chytridiomycetes remain uncertain, even though the sporangium is distinctly operculate. Knowledge of morphology (especially early development) of this saprotrophic (probably cellulosic) genus—occurring on decaying fruits and twigs—could benefit from further collection, culturing, and life-cycle observations. Ultrastructural and molecular analyses would surely clarify its relationships. *Published on-line www.phytologia.org Phytologia 102(2): 75-82 (June 24, 2020). ISSN 030319430.*

KEY WORDS: Chytrid, cyst, monocentric, operculum, rhizoids, sporangium, thallus, zoospores.

Macrochytrium is not common, but is widely distributed (Europe, including parts of Scandinavia; North America; India). There is a superficial resemblance to the bulbous alga, *Botrydium*; hence the epithet of the species name, *M. botrydioides* (Minden, 1902). Interest in this saprotrophic genus has arisen in part because, in robust examples, it is the largest known monocentric chytrid (Karling, 1977; Das-Gupta and John, 1988), and can be visible to the naked eye. Maximum sporangial lengths of approximately a millimeter have been reported, although sporangia are often smaller than this. It has also proved of interest because of an unusual sporangiate-thallus development (Bessey, 1950; Karling, 1977); ontogeny should be further investigated since earliest developmental stages (zoospore-cyst germination, potential nuclear migration?) remain unknown. In spite of interest in *Macrochytrium*, and informative summaries (e.g., Sparrow, 1960; Karling, 1977), there is no current, detailed taxonomic/nomenclatural review of the genus—this, a goal of our investigation. Considered a member of the Chytridiomycota, relationships within this phylum have continued to be uncertain—though some ideas concerning relationship are more plausible than others (discussed herein). Interestingly, a form similar to *Macrochytrium* is demonstrably ancient; Krings et al. (2016) described fossil forms resembling *Macrochytrium* and *Blastocladiella* from shale or soil-like layers preserved within the 410-Ma-old Rhynie-chert (Scotland); certain of their illustrations indicate the presence of a sporangial operculum, an operculum being suggestive of *Macrochytrium* not *Blastocladiella*.

TAXONOMIC HISTORY OF GENUS *MACROCHYTRIUM*

Minden (1916) provided an extensive description and discussion of *Macrochytrium*, including a detailed diagnosis of this genus and, following, a diagnosis of species *M. botrydioides*. Minden (1916) clearly indicated these taxa as being, respectively, a new genus and new (the only) species. However, Minden (1911), in more summary form, had already published these names, with descriptive material and illustrations. Even more relevant to nomenclature is that Minden (1902) *first* published the name *Macrochytrium botrydioides* in a still-earlier publication, with descriptive information of the thallus, sporangium, and limited but critical information on the zoospore. Thus, the original publication of *Macrochytrium botrydioides* should be accepted (as it has usually been) as 1902, not 1911 or 1916. There is no doubt that Minden (1902) was discussing a new genus and species (viz., use of the name *Macrochytrium botrydioides*, with descriptive information). This ‘combined’ description, in fact, serves to validate both genus and species (cf. Article 38.5, 38.6 of ICNAFP).

Macrochytrium continued to be monotypic (cf. Sparrow, 1960; Karling, 1977), containing only *M. botrydioides* (misspelled ‘*botryoides*’ in Clements and Shear, 1931), until Das-Gupta (1982) listed a ‘new species’ *Macrochytrium botrydiella*—this name not accompanied by a description—thus a *nomen nudum* (Articles 38.1, 38.2, 50B and Glossary, ICNAFP) and therefore illegitimate (Art. 6.5). From the name ‘*M. botrydiella*,’ one could speculate Das-Gupta may have observed a small specimen(s) of *M. botrydioides*. Subsequently, Das-Gupta and John (1988) indeed described a small form of *Macrochytrium botrydioides*—as a new variety, *M. botrydioides* var. *minutum*. In addition to smaller size, they considered aspects of its morphology and development (later here discussed) to not be identical to typical *M. botrydioides*. Although a description of this ‘new variety’ was given (Das-Gupta and John, 1988), no Latin diagnosis was provided (required at the time; ICNAFP, Article 39.1). Thus, this varietal name is not valid. Das-Gupta and John (1988) gave no indication if this ‘variety’ was comparable to the ‘species’ Das-Gupta proposed in 1982; both names are accounted for in Longcore (1996); only the variety is listed in *Index Fungorum* (in addition to ‘typical’ *M. botrydioides*). No other names have been introduced, and *Macrochytrium* remains (nomenclaturally) monotypic.

HOW MANY ‘TAXA’ SHOULD BE RECOGNIZED IN THE GENUS?

Regardless of nomenclatural irregularities, invalid names, etc., *should* a taxon (perhaps better said, ‘biological entity’) additional to *Macrochytrium botrydioides* be recognized?—i.e., based on smaller size (and associated morphology). This question was addressed (see below) by Johnson (1968) regarding a collection (Lake Itasca, Minnesota) of small specimens of *Macrochytrium* (maximum sporangial length = 46 μm). Sporangial sizes (of ca. 40 μm) reported by Das-Gupta and John (1988) are similar, but they did not mention Johnson’s work in this regard. Minden (1911) reported sporangial lengths up to 900 μm (enormous, among monocentric chytrids). A range of sporangial lengths of 300-800 μm was subsequently noted (Minden, 1916)—even 300 μm is very large for chytridiomycetous organisms. Lund (1934) reported sporangial lengths of 45-284 μm , thus of apparent intermediate size. Lund’s account is confusing, since he reported a ‘plant-length’ (including sporangium, “basal cell,” and rhizoidal axis?) of 100-558 μm . It is difficult to tell, in some publications, how much of the actual sporangium was measured, since a transverse wall often comes to delimit a smallish, lower (non-fertile) portion—only somewhat distinct from the thallus-branch axis bearing it (Minden, 1916, figs. 79, 82).

Johnson (1968) concluded there was insufficient basis for describing a new taxon based on smaller size, and one questionable morphological difference (later discussed); we tentatively agree, though the matter is not resolved. Morphological differences observed by Das-Gupta and John (1988) in their smallish specimens are perhaps explainable (they allowed as an ‘alternative explanation’ to taxonomic differences) by the perhaps young stages of sporangiate-thalli available to them; yet, they favored the idea that reliable taxonomic differences were present in this ‘small variety’—hence, its formal

description. Morphological matters are further discussed in the following section. We, presently, consider *Macrochytrium* monotypic (without clearly separable entities). Nonetheless, if a smaller variant of *M. botrydioides* (Johnson, 1968; Das-Gupta and John, 1988) is found to exhibit reliable morphological differences, associated with distinctly smaller size, then ‘var. *minutum*’ (Das-Gupta and John, 1988) could be validated by an additional diagnosis—or, a new variety or species proposed.

MORPHOLOGY AND DEVELOPMENT (See Figures 1-4)

Though gaps in knowledge remain, the morphology and development of *Macrochytrium botrydioides* was described in detail in three publications by Minden (1902, 1911, 1916). Minden’s work on *Macrochytrium*—particularly that of 1916—was succinctly summarized (and illustrated) by Gäumann and Dodge (1928) and Gwynne-Vaughan and Barnes (1937), among others. Relatively early-on, thus, *Macrochytrium* was a well-known and carefully (if incompletely) studied organism among researchers investigating ‘phycomycetes’ (former category for ‘algal-like fungi’).

In typical morphology, the thallus of *M. botrydioides* consists of a substantial cylindrical axis (deriving *ultimately* from the zoospore, early details lacking) which develops stout, sometimes coarse, rhizoids at the base. Just below the apex of the main axis, a lateral branch typically forms which swells into a clavate structure, soon becoming dominant, in size and position, over the true apex—the latter, in effect, “pushed aside” (Gäumann and Dodge, 1928, p. 45). The dominating lateral-branch continues growth, forming a (potentially large) sporangium. The original apical-axis grows little more (if any) after sporangial establishment, usually remaining as a protuberance below the sporangial base on one side. The spheroidal to ellipsoidal (sometimes obpyriform or almost cylindrical) sporangium continues enlarging, and often eventually becomes delimited (by a transverse wall), toward its base, from a lower sterile portion of the sporangium more or less continuous with the thallus-branch axis. A large mass of smallish zoospores (2.5-3.5 μm each) is formed within the (larger) fertile portion of the sporangium. The sporangium develops a sizeable operculum (‘lid’) on its apex. Zoospores are released (after opercular dehiscence), often first as a mass surrounded by a membrane (vesicle), the upper part of this mass pressing out through the single pore (where the operculum was). In *Macrochytrium*, the emergence of zoospores within a vesicle seems similar to that of species of *Chytriumyces* (cf. Karling, 1977, p. 130); but a vesicle may not always be present in *Macrochytrium* (Johnson, 1968). However, when a vesicle ruptures, the (posteriorly uniflagellate) zoospores swim individually (as typical chytrid zoospores). When zoospores settle on a substrate (rotting fruit, decaying twigs) they are capable of amoeboid movement (a pliable, more elongate shape) over the substrate-surface; the amoeboid ability of zoospores (which can also occur at an earlier stage) is advantageous (Gäumann and Dodge, 1928) if a substrate-surface is populated by other ‘phycomycetes’ or bacterial growth. Reports of resting-spores are questionable (Karling, 1977)—probably representing thick-walled sporangia.

Any consideration of the development and life-cycle in *Macrochytrium* should be tempered by admission that the earliest stages of thallus-development in this genus—i.e., details of the germination of the zoospore-cyst—have still not been observed. If it is not known, for example, whether the cyst simply enlarges (elongates) to become the young thallus (and subsequently the sporangium) or whether a germ-tube is formed to accomplish this—and whether the nucleus of the cyst migrates, or remains in the cyst—it is then difficult to place pursuant development in context. Critical is the ability (or maybe better said, inability) to ascertain where remnants (whether readily visible or not) of the zoospore-cyst wind up (i.e., which eventual structure subsumes or perhaps still bears evidence of the original cyst). The fate of the ‘upper’ cyst-wall can be significant in interpreting the location of the original apex of the thallus. It is not pertinent here to review all developmental thallus-types of Chytridiomycetes; such discussions are found in Whiffen (1944), Roane and Paterson (1974), and Blackwell et al. (2006).

Directly pertinent though is Whiffen's (1944) conclusion, regarding *Macrochytrium*, that [lacking knowledge of earliest developmental stages, i.e., zoospore-cyst germination] it was impossible to be certain of its type of development (she thus omitted the genus from her classification of thallus-types). Incomplete knowledge of thallus-development has indeed hampered systematic understanding of *Macrochytrium*. However, useful comments are possible. The youngest stages actually observed are of the relatively young thallus, which has become elongate and slightly clavate ('baseball-bat' shaped), cf. Karling (1977, p. 277, his figs. 7, 8). The form of this young thallus is unusual; but in the next stage, something more unusual may be observed—in that a lateral (subapical) bud occurs, just below (behind) the thallus-apex; this lateral branch produces the sporangium, and leaves the thallus-apex behind as a bump- or knee-like process (not developing appreciably further). Though unusual among chytrids, this pattern is perhaps not unique. In genus *Scherffeliomyces* Sparrow, the zoospore-cyst (and a portion of the discharge-tube) persists apically on the thallus, as a vestige—the sporangium (and its discharge-papilla) developing, at an angle, subapically below (see Johns, 1956). But, without early developmental stages of *Macrochytrium*, one cannot be confident these developments are truly comparable.

Then, there is the matter of phenotypic plasticity in chytrids (Miller, 1968). There can be variation within species regarding: sporangial shape, presence of an apophysis, extent of rhizoidal system, etc. *Macrochytrium*, indeed, exhibits variation. The tremendous range in sporangial size (even shape), especially the occurrence of small sporangia, may have to do with degree of crowding on a substrate (cf. Johnson, 1968) or age of specimens (small sporangia more common in young specimens; cf. Das-Gupta and John, 1988). There is even variation in developmental pattern; Das-Gupta and John did not observe a lateral-branch displacing the young thallus-apex; apparently, the thallus-apex was observed to produce the sporangium; Johnson (1968), though, did observe evidence of a lateral-branch forming the sporangium, i.e., a remnant where the original thallus-apex terminated. Additionally, Johnson observed a circumferential 'flange' at the base of the fertile sporangium (not related to the 'apical remnant'); this small 'collar' was not evident in illustrations by Das-Gupta and John, but there is a hint of such in illustrations by Lund (1934). Das-Gupta and John did not observe a transverse-wall toward the sporangial base; this was though seen in Johnson's specimens. Again, some observations (e.g., presence of this transverse-wall) may relate to specimen age, not 'taxonomic difference.'

SYSTEMATICS: POTENTIAL RELATIONSHIPS OF *MACROCHYTRIUM*

As indicated above, earliest stages of development remain unknown in *Macrochytrium*. However, life-cycle stages that are known, including the apparent form and structure of the zoospore, suggest this genus should (as most have concluded) be placed in the Chytridiomycota, as opposed to other fungal or 'pseudofungal' phyla, and suggest that some variation observed in the genus is age- or environment-related—arguing, for now, against recognition of taxa additional to the original species, *M. botrydioides*. Familial (even ordinal) relationships of *Macrochytrium* are unclear; a review of suggested relationships is useful, though, some suggestions being more instructive than others.

Minden (1902, p. 824) considered *Macrochytrium* to have "*Chytridineencharakter*" (chytridiaceous features), its relationships thus sought among this general group. Within "Reihe: Chytridiineae," Minden (1911) classified *Macrochytrium* in family Hyphochytriaceae; this presumed familial relationship—based on perceptions of general morphology—was echoed by Gäumann and Dodge (1928) and Gwynne-Vaughan and Barnes (1937). Minden's choice of putative relationship of *Macrochytrium botrydioides* was, doubtless, based specifically (at least in part) on the single flagellum of the zoospore. Whereas it is true that zoospores of members of the Hyphochytriaceae have a single flagellum, the flagellum in this family is anterior, and of the tinsel type (having lateral, tubular 'hairs'). The single flagellum of the zoospore of *Macrochytrium* is now known to be posterior, and of the whiplash (non-tinseled) type—hence, 'chytridiaceous.' As knowledge of structure and ultrastructure of such 'chytrid-like' (more broadly, 'phycomycetous') organisms progressed, it became clear that

Hyphochytriomycetes are unrelated to Chytridiomycetes—or to true fungi in general—and are related instead to ‘pseudofungi,’ i.e., ‘straminipilous fungi’ (see Fuller, 1989; Blackwell and Powell, 2000; Kendrick, 2000; Dick, 2001 and Blackwell, 2009 for changing concepts of ‘hyphochytrids’). Because of incomplete data available, lack of clear understanding of distinctions among groups of the catch-all category ‘phycomycetes’ led some authors (e.g., Wolf and Wolf, 1947) to suggest (on general appearance) a link between certain Chytridiales and the Leptomitales [forms such as *Rhipidium* and *Mindeniella*, with which *Macrochytrium* is sometimes found associated in nature—growing on rotting fruit or twigs]. The Leptomitales, however, have been recognized for some time (see Alexopoulos, 1962) to belong to the Oomycetes, which are unrelated to Chytridiomycetes, but belong (as do hyphochytrids) to group Straminipila (Dick, 2001). A resemblance of *Macrochytrium*, in form and development, to *Blastocladiella* was also suggested (Sparrow, 1943, 1960); indeed, superficial resemblance of these genera can be striking. However, zoospore-structure of the Blastocladiomycota (James et al., 2014; Powell, 2016) is distinct from Chytridiomycota; *Macrochytrium* appears to have chytridiaceous zoospores; also, no members of Blastocladiomycota (known) have *operculate* sporangia (Karling, 1977, dismissed the idea of relationship of *Macrochytrium* to *Blastocladiella*). Members of Blastocladiaceae exhibit bipolar development of the zoospore-cyst (Powell, 2016), whereas chytrids have unipolar development. Since earliest stages of development of *Macrochytrium* are unknown, one cannot be certain it has unipolar development; but this can be inferred, since it is unlikely a sporangial septum would develop *belatedly* (or not at all) if *Macrochytrium* possessed bipolar development; relatedly, the rhizoidal system of *Macrochytrium* forms from thallus already present beneath the developing sporangium, not from a distinct basal-cell as in many Blastocladiomycetes.

Fitzpatrick (1930) suggested that *Macrochytrium* (except for being larger and coarser) resembled the genus *Rhizophyidium* (Rhizophydiaceae, Chytridiales). *Rhizophyidium* is a true chytrid, with a life-cycle and probable development similar to *Macrochytrium*; however, in addition to looking little like *Macrochytrium*, the sporangium of *Rhizophyidium* does not possess an operculum (present in *Macrochytrium*), this structure being a major distinguishing character within Chytridiomycetes (Sparrow, 1960). And, the usually delicate rhizoids of *Rhizophyidium*, in effect, originate directly from the base of the entirely sporogenous, often delicate, sporangium. Perhaps confusingly, Cox (1939) and Wolf and Wolf (1947) hinted at relationship of *Macrochytrium* to the chytrid family Rhizidiaceae (not the Rhizophydiaceae), possibly based in some genera of this family (e.g., *Siphonaria*) on the occurrence of a single thallus-axis giving rise to the sporangium. There is some similarity of *Macrochytrium* to *Siphonaria*; but, in *Siphonaria* the thallus is truly interbiotic, the sporangium inoperculate, zoospore-discharge can be other than apical, and there is no walled-off ‘lower-part’ to the sporangium.

Classification of *Macrochytrium* has, thus, been uncertain. Sparrow (1943) placed this operculate genus in order Chytridiales, family Chytridiaceae; however, it was distinct enough that he accorded it its own subfamily, Macrochytrioideae, containing no other taxa (it had earlier been placed in its own family, Macrochytriaceae; Lund, 1934). Sparrow (1960) placed *Macrochytrium* in subfamily Chytridioideae, next to genus *Karlingiomyces*—to which it bears little resemblance. Blackwell et al. (2004) challenged the inclusiveness and relationships of *Karlingiomyces*, considering a possible connection to the ‘*Lacustromyces* clade’ (Longcore, 1993; James et al., 2000). *Karlingiomyces* (*pro parte*) was placed by Longcore and Simmons (2012) in a new, predominantly chitinophilic, chytrid order, Polychytriales (including *Lacustromyces hiemalis*, *Polychytrium aggregatum* and *Neokarlingia chitinophila*)—a further indication that *Karlingiomyces* is not closely related to *Macrochytrium*.

Sparrow (1973) retained *Macrochytrium* in subfamily Chytridioideae (family Chytridiaceae, order Chytridiales), not indicating other possible relationships. Bessey (1950) and Karling (1977), on the other hand, suggested a possible relationship of *Macrochytrium* to the family Entophlyctaceae (Chytridiales); this suggestion at first seemed unlikely because of endobiotic thallus development within this family; however, the genus *Endochytrium* (of family Entophlyctaceae)—based on rhizoidal system, form of the

sporangium, and release of many, small zoospores (contained initially in a membrane)—is indeed a plausible connection. Critical in establishing this relationship would be the observation of early development-stages of *Macrochytrium* to assess potential similarity to *Endochytrium*. *Endochytrium* was eventually determined to belong to the order Cladochytriales (Mozley-Standridge et al., 2009). Intriguing, as well, is genus *Cylindrochytridium*, which Karling (1977) classified near *Endochytrium*. *Cylindrochytridium* has since also been determined to belong to the Cladochytriales (Steiger et al., 2011). The presumed mode of thallus development in *Macrochytrium* resembles that of *Cylindrochytridium* (the similarity of *Macrochytrium* and *Cylindrochytrium* was initially pointed out by Whiffen, 1944). This similarity [in some ways striking] between *Macrochytrium* and *Cylindrochytridium* has even caused confusion; in Crasemann's (1954) detailed physiological study, involving a presumed *Macrochytrium* isolate, she was in fact dealing with a culture of *Cylindrochytridium* (cf. Sparrow, 1960). In spite of informed guesses as to relationship, systematic placement of *Macrochytrium* remains undetermined (cf. Karling, 1977). Molecular studies could resolve taxonomic relationships, and morphological studies could clarify early development.

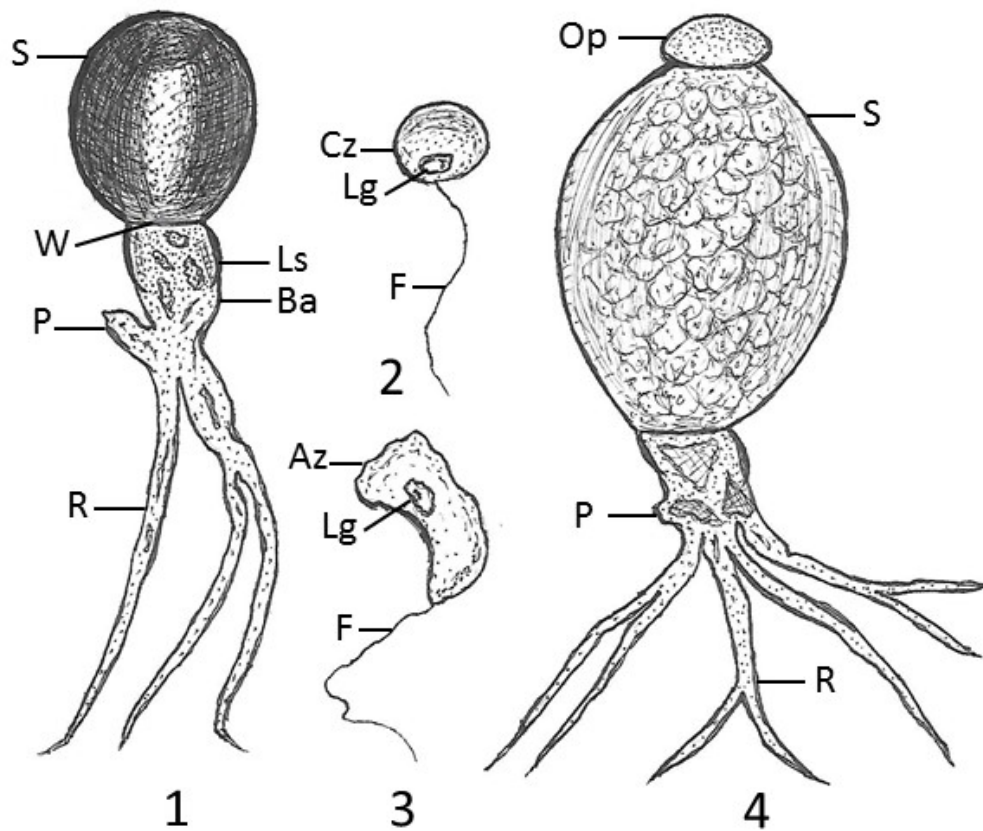
ACKNOWLEDGEMENTS

We thank Dr. William J. Davis and Dr. Robert W. Roberson for review of this manuscript.

LITERATURE CITED

- Alexopoulos, C. J. 1962. Introductory Mycology, 2nd edition. John Wiley & Sons; New York, London and Sydney.
- Bessey, E. A. 1950. Morphology and Taxonomy of Fungi. Blakiston Company; Philadelphia, Toronto.
- Blackwell, W. H. 2009. Chromista revisited: a dilemma of overlapping putative kingdoms, and the attempted application of the botanical code of nomenclature. *Phytologia* 91: 191-225.
- Blackwell, W. H., P. M. Letcher and M. J. Powell. 2004. Synopsis and systematic reconsideration of *Karlingiomyces* (Chytridiomycota). *Mycotaxon* 89: 259-276.
- Blackwell, W. H., P. M. Letcher and M. J. Powell. 2006. Thallus development and the systematics of Chytridiomycota: an additional developmental pattern represented by *Podochytrium*. *Mycotaxon* 97: 91-109.
- Blackwell, W. H. and M. J. Powell. 2000. A review of group filiation of Stramenopiles, additional approaches to the question. *Evolutionary Theory and Review* 12: 49-88.
- Clements, F. E. and C. L. Shear. 1931. The Genera of Fungi. Hafner Reprint, New York, 1973.
- Crasemann, J. M. 1954. The nutrition of *Chytridium* and *Macrochytrium*. *Amer. J. Bot.* 41: 302-310.
- Cox, H. T. 1939. A new genus of the Rhizidiaceae. *J. Elisha Mitchell Sci. Soc.* 55: 389-397.
- Das-Gupta, S. N. 1982. Discourse on aquatic Phycomycetes of India. *Indian Phytopath.* 35: 193-216.
- Das-Gupta, S. N. and R. John. 1988. A contribution to our knowledge of the zoosporic fungi. *Bull. Bot. Surv. India* 30 (1-4): 1-82 (delayed? date of publication listed as 1990 in some publications).
- Dick, M. W. 2001. Straminipilous Fungi. Kluwer Academic; Dordrecht, Boston and London.
- Fitzpatrick, H. M. 1930. The Lower Fungi. Phycomycetes. McGraw-Hill, New York and London.
- Fuller, M. S. 1989 (1990). Phylum Hyphochytriomycota. Pp. 380-387 in L. Margulies et al., Handbook of Protoctista. Jones and Bartlett, Boston. (different dates of publication appear in frontispiece)
- Gäumann, E. A. and C. W. Dodge. 1928. Comparative Morphology of Fungi. McGraw-Hill, New York and London.
- Gwynne-Vaughan, H. C. I. and B. Barnes. 1937. The Structure & Development of the Fungi, 2nd edition. Cambridge at the Press, Britain.
- IF = Index Fungorum—current online database of fungal names: indexfungorum.org.
- ICNAFP = International Code of Nomenclature of for Algae, Fungi, and Plants. 2018. Shenzhen Code.

- James, T. Y., D. Porter, C. A. Leander, R. Vilgalys and J.E. Longcore. 2000. Molecular phylogenetics of the Chytridiomycota supports the utility of ultrastructural data in chytrid systematics. *Can. J. Bot.* 78: 336-350.
- James, T. Y., T. M. Porter and W. W. Martin 2014. Blastocladiomycota. Pp. 177-207 in D. J. McLaughlin and J.W. Spatafora (Eds.), *The Mycota VIIA*, 2nd ed. Springer; Berlin and Heidelberg.
- Johns, R. M. 1956. Additions to the phycomycete flora of the Douglas Lake region. III. A new species of *Scherffeliomyces*. *Mycologia* 48: 433-438.
- Johnson, T. W. 1968. A note on *Macrochytrium botrydioides* Minden. *Arch. f. Mikrobiol.* 63: 292-294.
- Karling, J. S. 1977. *Chytridiomycetorum Iconographia*. J. Cramer; Vaduz, Liechtenstein; and Lubrecht & Cramer; Monticello, New York.
- Kendrick, B. 2000. *The Fifth Kingdom*, 3rd edition. Focus Publishing, R. Pullins Co., Newburyport, MA. (Mycologue Publications)
- Krings, M., T. N. Taylor and H. Martin. 2016. An enigmatic fossil fungus from the 410 Ma Rhynie chert that resembles *Macrochytrium* (Chytridiomycota) and *Blastocladiella* (Blastocladiomycota). *Mycologia* 108: 303-312.
- Longcore, J. E. 1993. Morphology and zoospore ultrastructure of *Lacustromyces hiemalis* gen. et sp. nov. (Chytridiales). *Can J. Bot.* 71: 414-425.
- Longcore, J. E. 1996. Chytridiomycete taxonomy since 1960. *Mycotaxon* 60: 149-174.
- Longcore, J. E. and D. R. Simmons. 2012. The Polychytriales ord. nov. contains chitinophilic members of the rhizophlyctoid alliance. *Mycologia* 104: 276-294.
- Lund, A. 1934. Studies on Danish freshwater Phycomycetes and notes on their occurrence particularly relative to the hydrogen ion concentration of the water. *Kgl. Danske Vidensk. Selsk. Skrift., Naturv. Math., Afd. 9* (6): 1-97.
- Miller, C. E. 1968. Observations concerning taxonomic characteristics in chytridiaceous fungi. *J. Elisha Mitchell Sci. Soc.* 84: 100-107.
- Minden, M. von. 1902. Über Saprolegniineen. *Centralblatt. für Bakteriol., Parasitenk. und Infektionskrankh., Abt. 2*(8): 805-810, 821-825.
- Minden, M. von. 1911. Chytridiineae, Ancylistineae, Monoblepharidineae, Saprolegniineae. *Kryptogamenfl. Mark Brandenburg* 5(3): 353-496. (all 4 parts of Vol. 5 collated in 1915)
- Minden, M. von. 1916. Beiträge zur Biologie und Systematik einheimischer submerser Phycomyceten. *In R. Falck, Mykolog. Untersuch. Berichte* 2(2): 146-255, 8 pls., 24 figs.
- Mozley-Standridge, S. E., P. M. Letcher, J. E. Longcore, D. Porter and D. R. Simmons, 2009. Cladochytriales—a new order in Chytridiomycota. *Mycol. Res.* 113: 498-507.
- Powell, J. J. 2016. Blastocladiomycota. Pp. 1-25 in J. M. Archibald et al. (Eds.). *Handbook of the Protists*; Springer International Publishing, Switzerland.
- Roane, M. K. and R. A. Paterson. 1974. Some aspects of morphology and development in the Chytridiales. *Mycologia* 46: 147-164
- Sparrow, F. K. 1943. *Aquatic Phycomycetes, Exclusive of the Saprolegniaceae and Pythium*. Univ. Michigan Press, Ann Arbor; Humphrey Milford, London; and Oxford Univ. Press.
- Sparrow, F. K. 1960. *Aquatic Phycomycetes*, 2nd revised edition. Univ. Michigan Press, Ann Arbor.
- Sparrow, F. K. 1973. Chytridiomycetes, Hyphochytridiomycetes. Pp. 85-110 in G. C. Ainsworth, F. K. Sparrow and A. S. Sussman. *The Fungi, and Advanced Treatise*. Academic Press; New York and London.
- Steiger, R. A., D. R. Simmons and J. E. Longcore. 2011. *Cylindrochytridium johnstonii* is a member of the Cladochytriales. *Mycotaxon* 118: 293-302.
- Whiffen, A. J. 1944. A discussion of taxonomic criteria in the Chytridiales. *Farlowia* 1: 583-597.
- Wolf, F. A. and F. T. Wolf. 1947. *The Fungi*, Volume 1. John Wiley & Sons, New York; and Chapman & Hall, London.



Figures 1-4. *Macrochytrium botrydioides*. **Fig. 1:** Young, darkish, sporogenous-portion of sporangium (S); transverse wall (W) delimits a lower, non-fertile portion of sporangium (Ls) which merges with the thallus-branch axis (Ba); prominence (P) at the base of the sporangial branch-axis represents the original, primary, vegetative thallus-apex which has been 'pushed aside' by growth of the sporangial branch; beneath this 'prominence,' coarse rhizoids (R) are evident. **Fig. 2:** Rounded 'chytrid'-form of zoospore (Cz); lipid globule (Lg) and single, posterior flagellum (F) evident. **Fig. 3:** 'Amoeboid'-form of zoospore (Az); lipid globule (Lg) and flagellum (F) still evident. **Fig. 4:** Mature, fertile sporangium (S), with distinct operculum (Op) beginning to be 'lifted' by pressure of zoospore-mass within sporangium; vestige of original thallus-apex (P) still evident on lower part of sporangial thallus-axis, rhizoids (R) below this. Figs. 1, 2, 4 after Minden, 1911; Fig. 3 after Minden, 1916. Additional useful illustrations, see Minden (1916), Tafel VIII, figs. 76-85; and Karling (1977), Plate 98, figs. 1-16.