THE OCCURRENCE OF *BLYTTIOMYCES SPINULOSUS* IN ALABAMA AND ARGENTINA, AND COMMENTS ON THE GENUS *BLYTTIOMYCES* (CHYTRIDIOMYCOTA)

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ABSTRACT

Our study documents the occurrence of *Blyttiomyces spinulosus* (Chytridiomycetes) in Alabama and Argentina. Argentine *B. spinulosus* specimens infected zygospores of *Spirogyra*. The find of *B. spinulosus* on zygospores of *Sirogonium* in Alabama records parasitism of a new generic host. *Blyttiomyces*, though originating as a generic segregate of the operculate genus *Chytridium*, was thought to be inoperculate. However, an operculum, a structure often difficult to observe but of significance in assessing generic affinities, is here demonstrated in specimens identifiable as *B. spinulosus*. The genus *Blyttiomyces* and taxonomic problems involving several species are discussed. *Phytologia 93(3): 304-315 (December 1, 2011)*

KEY WORDS: Apophysis, chytrid, discharge pore, operculum, parasitism, resting spore, sporangium, thallus, zoospores, zygospore.

Since its inception (Bartsch, 1939) *Blyttiomyces* has seemed distinct; yet, similarities to several genera have been noted. Generic delimitation, and the number of species included, should be reinvestigated. The most recent revisionary treatment was that of Dogma (1979), a study not all-inclusive, containing several unnamed taxa. *Blyttiomyces* is now in need of taxonomic revision, but such an undertaking is premature; more collection and observation (as presented here) should be done, and more information (molecular data,

e.g., James et al., 2006) gathered. Although we do not here attempt a revision, we consider taxonomic questions concerning the genus and its species.

THE OCCURRENCE OF *BLYTTIOMYCES SPINULOSUS*

Blyttiomyces spinulosus is known from wide-spread locations: sites in Europe (including parts of Scandinavia), China, Japan, the Philippines, Cuba, and the central northern United States (Wisconsin and Michigan). We here report *B. spinulosus* from the southeastern United States. Specifically, we found this chytrid (collections WB124, WB128), March-April 2000, in central West Alabama, in standing water of a road-side ditch along Hwy 17 in northern Sumter County (approximately 1 mi. south of Geiger, AL). This is a new geographic record for *B. spinulosus*, and its occurrence on zygospores of *Sirogonium* (Zygnemataceae) documents a new algal host. This chytrid appears limited to parasitism (and possibly saprophytism, cf. Bartsch, 1939) of members of the Zygnemataceae. Previous reports noted the occurrence of *B. spinulosus* on zygospores of *Mougeotia*, *Zygnema*, *Zygnomopsis*, and particularly *Spirogyra* (cf. Sparrow, 1960; Dogma, 1979). *Sirogonium* is similar to *Spirogyra* but is distinguished by essentially parallel (not spiraled) plastid bands in the cell, and by shorter, broader conjugation connections (cf. Smith, 1950, fig. 213). We also observed one instance of infection of zygospores of *Spirogyra* by *B. spinulosus* at the Alabama locality.

Argentine specimens of *Blyttiomyces spinulosus* are from "Pozo Verde" path, Villa General Belgrano, Córdoba Province; collection by C. Vélez, on 7-15-2008. This first, formal report of *B. spinulosus* from Argentina considerably expands the distribution record of this species. However, since *B. spinulosus* is geographically widespread, and habitats in which it may occur are common, future collecting will likely fill in gaps of an apparently disjunct distribution. Whereas Alabama collections involve a new host, *Sirogonium*, the Argentine specimens further confirm the occurrence of *B. spinulosus* on what is perhaps its main host worldwide, *Spirogyra*. The Argentine and Alabama specimens, and those recorded in the literature, are generally similar in appearance—not suggestive of distinct morphological varieties.

Blyttiomyces spinulosus is distinctive, though not unique among chytrids, in often forming an elongate, proximal, rhizoidal ("haustorial") tube or "stalk," cf. Figs. 1 and 3, developed from the germ-tube of the encysted zoospore. This stalk-like tube connects the sporangium (usually epibiotic, on the surface of the host algal cell) with an apophysis (two or three apophyses may occur in tandem). The apophyseal system is typically intramatrical (in the host zygospore); however, an apophysis may occur external to the zygospore, i.e., between the parent algal cell wall and the contained zygospore wall. In some cases, particularly when the zygospore is more or less appressed to the parent algal wall, the "rhizoidal stalk" can be abbreviated or essentially lacking (cf. Fig. 5). Both elongate and "reduced" rhizoidal tubes were illustrated by Bartsch (1939) for *B. spinulosus*.

In *Blyttiomyces*, this (sometimes elongate) proximal rhizoidal tube appears, in part, to serve to position the endobiotic system (apophysis and rhizoids) within the host zygospore. A comparable stalked structure (if somewhat more inflated or sac-like in appearance) is found in *Chytridium olla*, parasitic on oogonial contents of *Oedogonium* (cf. Denis, 1926; Sparrow, 1960, 1973; Vélez et al., 2011). Stalked, subsporangial structures may be seen in other chytrids as well. The often elongate sporangium of the saprophytic genus *Cylindrochytridium* (cf. Karling, 1977, pl. 85) may adjoin the rhizoidal system, or be subtended by an intervening cylindrical stalk. The stalk of *Cylindrochytridium*, possibly of nutritive (initially) and supportive function, can resemble the sporangium in size and shape, becoming separated from it by a transverse wall; it is thus plausible to think of this stalk as derived from a structure which is fundamentally sporangial in nature. The somewhat thickened, cup- or stalk-like base (not always evident) of the often aculeate sporangium of *Obelidium* appears to have a supportive function (cf. Karling, 1977, pl. 43, fig. 10). Such haustorial, positioning, or supporting structures (considering a number of examples) apparently have not been accorded a special name. Since it is not clear that all these structures are morphologically (or functionally) comparable, it is perhaps still ill-advised to coin such a name.

Although drawings of *Blyttiomyces spinulosus* are available (Bartsch, 1939; Sparrow, 1960; Karling, 1977; Dogma, 1979), the

photographic record is sparse; hence, we present our photographs: Figs. 1-4, for Alabama specimens, and Figs. 5-6, for Argentina specimens. Note, in Argentine material, the documentation of zoospore discharge (Fig. 5). The observation of a true sporangial operculum ("lid," initially present over the discharge pore of the zoosporangium, Figs. 2, 6) is here reported in *B. spinulosus* (Blytt's, 1882, report of an operculum apparently involved a case of mistaken morphological identity, as subsequently discussed). Our find of an authentic operculum is taxonomically significant, supporting speculations (Bartsch, 1939; Dogma, 1979)—even though *Blyttiomyces* was thought to be inoperculate—of similarity to the operculate genus *Chytridium*. An apophysis may be seen (Figs. 2, 4, 5), generally resembling that of *Chytridium lagenaria* (cf. Karling, 1936; Blackwell et al., 2002). The intramatrical resting spore (Figs. 3, 4) likewise suggests similarity to *Chytridium*. The apical portion of the future sporangium (developing zoospore cyst) exhibits a thickening (cf. Fig. 4) which will become, or contribute to, the apiculus (a cap-like excrescence, generally at the apex of the sporangium). The apiculus (Figs. 2, 3, 5), discharge pore (Figs. 3, 6), and spiny sporangial wall (e.g., Fig. 6) are also photographically illustrated. Sporangial spines can be distinctive or quite small (cf. Dogma, 1979). Consistent with our observations, drawings of spines of *B. spinulosus* by Bartsch (1939) show them to be distributed over the sporangial surface, possibly excepting the apiculus. The spines in some cases appear to occupy ridge-like areas on the surface (cf. Fig. 6).

THE GENUS *BLYTTIOMYCES*: HISTORY AND TAXONOMIC COMMENTARY

Blyttiomyces presents unresolved questions, including generic relationships and the number of recognized species. Similarities of this genus have been suggested to *Chytridium*, *Phlyctochytrium*, *Obelidium*, *Catenochytridium*, *Polyphlyctis* and *Canteria* (cf. Bartsch, 1939; Karling, 1977; Dogma, 1979). No single feature will always distinguish *Blyttiomyces* from other chytrid genera; however, a combination of features typically present in *Blyttiomyces* will usually serve to do so (listed as follows): a distinct apiculus; typically non-apical sporangial discharge, with one to several (variously placed) discharge pores; significant epibiotic and endobiotic (but usually not distinctly interbiotic) thallus development; one or more, often intramatrical

apophyses; in some cases a "haustorial" tube connecting the sporangium with the apophyseal system; branched rhizoids extending (further into the host substrate) from the "innermost" (or only) apophysis; and an intramatrical resting spore. Dogma (1979, p. 245) viewed *Blyttiomyces* as "one of the inoperculate segregates of [the typically operculate genus] *Chytridium*;" *Blyttiomyces* has in fact traditionally been considered inoperculate (Sparrow, 1960, may simply have accepted Bartsch's, 1939, interpretation of *B. spinulosus* as inoperculate). But, as noted, we observed an operculum in specimens of *B. spinulosus*. It is uncertain if any other species of *Blyttiomyces* possesses an operculum. Nor, given a body of literature indicating *B. spinulosus* to be inoperculate, is it certain that all isolates of *B. spinulosus* will be found to possess an operculum. In further potential complication, it is possible, if improbable, that operculate and inoperculate taxa are masquerading under what otherwise seems to be a morphologically defined species. Future morphological and molecular work will be invaluable if any such determinations come to bear.

Bartsch (1939) based the new genus *Blyttiomyces* and the type species, *B. spinulosus* (Blytt) Bartsch, on *Chytridium spinulosum* Blytt (1882), from Norway, and specimens Bartsch observed from Wisconsin—emphasizing is his description the presence of an apiculus and a subapical mode of spore discharge (involving a single discharge pore). Bartsch (1939) believed *Blyttiomyces* to be inoperculate, concluding that Blytt (1882) had mistaken the apiculus for an operculum (Blytt, thus, describing the new species as a *Chytridium*); Bartsch noted that Blytt did not observe zoospore discharge. Sparrow (1952) continued work on *Blyttiomyces* in the United States, recording *B. spinulosus* from northern Michigan. Sparrow (1952) also described *Blyttiomyces laevis*, from a Michigan bog, a species (parasitic on *Zygnema*) with smooth sporangial walls (lacking the small spines of *B. spinulosus*) and a small, immediately subsporangial apophysis (apparently consistently placed). Sparrow noted that *B. laevis* sporangia could possess more than one subapical discharge pore. Sparrow and Barr (1955) described *Blyttiomyces helicus*, also from Michigan, a taxon (on pine pollen, sometimes associated with *Sphagnum* debris) with distinct helical bands on the sporangium, and one or two basal or subbasal discharge pores. Sparrow (1960) emended *Blyttiomyces* to include forms with more than one discharge pore, but did not mention

the observation of basal discharge. In discussing *Blyttiomyces rhizophlyctidis* Dogma, a parasite on the chytrid *Rhizophlyctis rosea*, Dogma and Sparrow (1969) noted that (1-12) sporangial discharge pores (often on raised papillae) could occur at various positions on the sporangium. In this paper, Dogma transferred *Phlyctochytrium vaucheriae* (found on *Vaucheria*) into *Blyttiomyces*. Dogma (1979) further emended *Blyttiomyces*, noting that discharge pores could be subapical, subbasal, or at other locations on the sporangium—discounting the exact number and position of these pores, and the precise location of the apiculus, as necessarily generically meaningful.

Dogma (1979, p. 247), in consideration of species and specimens on a world-wide basis, "redefined" *Blyttiomyces* to include these traits: "(a) posteriorly uniflagellate zoospores; (b) endo-exogenous thallus development; (c) epibiotic, inoperculate zoosporangium formed from the expanded portion of a functional spore cyst; (d) persistence of the unexpanded portion of the spore cyst in the form of a thickened appendage, the apiculus, on the zoosporangium; (e) apophysate endobiotic system; and (f) asexual formation of endobiotic resting spore by encystment of an apophysis." Dogma's redefinition, though useful, requires comment. While "(a)" is a true statement, this is not generically defining, since virtually all chytrids possess posteriorly uniflagellate zoospores. Concerning "(b)," whereas endogenous and exogenous development of the thallus occur in *Blyttiomyces*, these do not appear to be of the alternating "endo-exogenous" type—described by Karling (1936) for *Chytridium lagenaria*—in which an apophysis may function as a prosporangium and contribute directly to sporangial generation or regeneration, including "internal proliferation of sporangia" (cf. Blackwell et al., 2002, 2006). As for "c," as noted herein, at least one species of *Blyttiomyces* can be operculate, a topic deserving of further study. Finally, in regard to "(f)," although one to several apophyses form, and at least one of these may develop into a resting spore (involving a thickening of the wall, Fig. 4), there is no actual encystment—i.e., in the sense of an encysting zoospore.

CONSIDERATION OF SPECIES OF *BLYTTIOMYCES*

We do not here attempt to account for all possible taxa of *Blyttiomyces*. Nonetheless, some comments on various species seem in

order. In a revision of *Blyttiomyces*, Dogma (1979) described *B. verrucosus*, a new species from the Philippines. In all, eight formally named species of the genus were recognized by Dogma, and presented in his key. Four more potential taxa (given as *Blyttiomyces* sp.—no binomials applied) were mentioned, but not individually distinguished in his key. The eight named species of *Blyttiomyces* included by Dogma are broadly separated as follows (after Dogma, 1979):

Zoosporangial wall smooth
- Zoospores usually uniguttulate (with one main lipid globule)
  - *B. vaucheriae*, *B. laevis*, *B. aureus*
- Zoospores multiguttulate (with several lipid globules)
  - *B. harderi*, *B. rhizophlyctidis*

Zoosporangial wall ornamented
- Zoospores uniguttulate
  - *B. spinulosus*
  - *B. helicus*
- Zoospores multiguttulate
  - *B. verrucosus*

Johnson (1977) reported two species of *Blyttiomyces* from southern Scandinavia, from aquatic habitats containing *Sphagnum*—both species of *Blyttiomyces* being cultured on pine pollen bait. One of the species found by Johnson (1977) was Sparrow's (1952) *B. laevis*. Johnson (1977) placed *B. aureus* Booth (1969) in synonymy of *B. laevis*, based on what he considered to be taxonomically inconsequential differences in pigmentation, sporangial wall thickness, shape of the apiculus, and zoospore size and shape. To the contrary, Dogma (1979) saw these differences as grounds for distinguishing *B. laevis* and *B. aureus*. Unless such differences are shown to not be systematically meaningful—especially in the absence of molecular data—the merging of *B. aureus* and *B. laevis* does not seem warranted. Isolation of *B. laevis* on pine pollen supplements knowledge of its occurrence on *Zygnema* (cf. Sparrow, 1960).

The second species in Johnson's (1977) study, *Blyttiomyces conicus*, was described as new. This species has conical sporangia with subbasal (often somewhat papillate) discharge pores, and which also often possess subbasal (rounded or papillate) wall ornamentations. The

subbasal discharge of zoospores in *B. conicus* distinguishes it from the subapical to lateral discharge in *B. spinulosus* (cf. Dogma, 1979), but not necessarily from the generally basal (if less papillate) discharge of *B. helicus* (cf. Sparrow and Barr, 1955). Nonetheless, the raised, subbasal ornamentations and unusual sporangial shape of *B. conicus* seem unique within the genus, and there are no spiral sporangial-wall bands as in *B. helicus*. Dogma (1979) apparently became aware of Johnson's (1977) *B. conicus* only in time to mention it in a note added in proof, not in time to include it in the body of his systematic treatment of *Blyttiomyces*. However, Dogma (1979) did not question recognition of *B. conicus*, other than mentioning that its apiculus is not always strongly demarcated (cf. Johnson, 1977, p. 84). Karling (1977) discussed most of the recognized species of *Blyttiomyces*, but did not mention *B. conicus* (doubtless due to timing of publication). Regardless of scant attention since Johnson (1977), *B. conicus* appears distinct.

In addition to species in the systematic treatment by Dogma (1979), *Blyttiomyces conicus* and two other species, *B. lenis* and *B. spinosus* (supposedly different from *B. spinulosus*), are listed in *Index Fungorum*. There is, of course, potential for confusion given the similarity of spelling of "*spinosus*" vs. "*spinulosus*"—"*B. spinosus*" being intended by Dasgupta and John (1988) to apply to a different species than "*B. spinulosus*" (Blytt) Bartsch. Whether such names are to be treated as homonyms, even if not spelled exactly the same, is not presently clear (see ICBN, 2006; compare especially Articles 53.1 and 53.3). If these names were so interpreted, then *B. spinosus* Dasgupta & John (1988) would have to be rejected, being in this case the "later homonym." But this may prove a moot point, because examination of the description and discussion by Dasgupta and John (1988) of *B. spinosus* leads one to question its separation from *B. spinulosus*, and certain other species of *Blyttiomyces*, by virtue (in *B. spinosus*) of a "constant position of the exit pore," and "its mode of zoospore discharge as a globular mass"—among other, perhaps less defining differences mentioned. Whereas Dasgupta and John (1988, p. 34) indicated in discussion a "constant position of the exit pore," they stated in formal description of *B. spinosus* (p. 32) that the exit pore may be "basal, lateral, subapical, apical"—not suggestive of constancy of position. As evident in our photograph (Fig. 5), zoospore release in *B. spinulosus* can occur in a globular cluster, not essentially different from

discharge described for *B. spinosus* by Dasgupta and John (1988, p. 34) as "a globular mass." Were it not for the smooth sporangial walls (indicated by Dasgupta and John, 1988), *B. lenis* would also resemble *B. spinulosus*, occurring, as might be expected, on zygospores of *Spirogyra*. *Blyttiomyces lenis* may represent specimens of *B. spinulosus* in which the characteristic small spines are reduced. Possible additional species are listed in Longcore (1996), two represented by names that may be *nomina nuda* (cf. Longcore's listing).

It is perhaps obvious from above discussion that a number of taxa, or supposed taxa, of *Blyttiomyces* are not particularly well known. We can remind in this regard that four unnamed taxa were discussed in Dogma (1979), the status of these requiring further investigation. *Blyttiomyces* is thus in need of taxonomic attention, even as simply regards additional collection, and traditional morphological observation and description. An even more pressing issue, though, concerns the dearth of molecular information on the genus. Molecular data is presently available for *B. helicus*, this obtained from rDNA from a pollen culture of this chytrid (cf. James et al., 2006). The results of the attempted systematic placement of this chytrid using this molecular information, however, could only be described as inconclusive—*B. helicus* occurring in phylogenetic analyses on a branch by itself sister to the clade including the Rhizophlyctidales and Spizellomycetales. Not only is this species unresolved in taxonomic placement, but molecular information is not at this time available for any other *Blyttiomyces* species. Given questions as to the operculate vs. non-operculate nature of species of *Blyttiomyces* (as has been discussed)—and the diversity of presently included taxa—it is not entirely certain that the genus will ultimately prove to be monophyletic. Again, no definitive revision of this genus is possible without the availability of substantially more data.

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only be resolved by additional collection and molecular information. This work was supported in part by NSF Grant # DEB-0949305. CGV was partially supported by funds from OATs 70-07 and 65/08, FCEN, University of Buenos Aires.

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Figures 1-6: ***Blyttiomyces spinulosus***. Fig. 1: Zoospore cyst and germination tube; zygospore of algal host (*Sirogonium*). Fig. 2: Apiculus and operculum of sporangium; apophysis visible below sporangium. Fig, 3: Apiculus and sporangial discharge pore; tube or "stalk" evident, extending into host zygospore; resting spore inside zygospore. Fig. 4: Older zoospore cyst (developing into a sporangium), apophysis below; intramatrical resting spores, the lower exhibiting defined wall. Fig. 5: Sporangial wall with small spines and apiculus; apophysis (below sporangium) in *Spirogyra* zygospore; zoospores discharged in temporarily globular cluster (to right). Fig. 6: Spiny sporangial wall (spines, in some cases, on ridge-like areas); discharge pore and operculum (previously covering pore) evident. **Abbreviations**: Ap (Apophysis), Apic (Apiculus), Op (operculum), P (discharge pore), R (ridge, bearing spines), RS (resting spore), s (small

spines on exterior of sporangial wall), t ("stalk" developed from germination tube), Z (zygospore), ZC (zoospore cyst), Zs (zoospores).