Synchytrium microbalum sp. nov. is a saprobic species in a lineage of parasites

Joyce E. Longcore*

School of Biology and Ecology

University of Maine

Orono, Maine 04469

D. Rabern Simmons

School of Forest Resources and Conservation

University of Florida

Gainesville, Florida 32611

Peter M. Letcher

Department of Biological Sciences

The University of Alabama

Tuscaloosa, Alabama 35487

Keywords:

JEL517

Micromyces

Molecular phylogeny

Nowakowskia

Synchytriales

Transmission Electron Microscopy

ABSTRACT

The diversity of the *Chytridiomycota* is poorly known and sequence information is not well represented in databases, often preventing identification of chytrid sequences retrieved from environmental samples. We found an unknown, saprobic chytrid, related to *Synchytrium*, which heretofore has been considered a lineage of parasites. Because of its phylogenetic relationship, and ecological dissimilarity to other *Synchytrium* species, we considered this fungus of scientific

* Corresponding author. 5722 Deering Hall, Orono, Maine, 04469-5722, USA
e-mail address: longcore@maine.edu; telephone: 1-207-581-4396; Fax: 1-207-581-2969

interest and describe it herein. We based our study on an analysis of 18S rDNA, light microscopic morphology, and ultrastructural characters of the zoospores. The fungus is tiny and produces filamentous projections 4–5 times as long as the diameter of the sporangium. The small zoospores are longer than wide and their ultrastructure is similar to that of *Synchytrium endobioticum*, except that they lack a microtubule root. Our molecular analysis placed the organism within a clade of *Synchytrium* species and we name the new species *S. microbalum*.

Introduction

The fungal genus *Synchytrium* De Bary and Woronin (*Chytridiomycetes*, *Chytridiomycota*) contains over 200 species (<http://www.mycobank.org/>), all of which are parasites of terrestrial, vascular plants. *Synchytrium endobioticum* (Schilb.) Percival, the cause of black wart of potato, is the best known species. Although *Synchytrium* is the largest and best known genus of the *Synchytriaceae* Schröter, the family also contains *Micromyces* Dangeard and *Endodesmidium* Canter (Sparrow 1960, 1973). Species in these aquatic genera, however, instead of being parasites of vascular plants, are parasites of green algae (Canter 1949). Karling's (1953) placement of *Micromyces* spp. into *Synchytrium*, for the most part, has not been followed (Miller 1955; Sparrow 1960, 1973; Rieth 1962).

Since Canter's (1949) work on the aquatic *Synchytriaceae* and the descriptions of *M. grandis* Miller and *M. furcata* Rieth, aquatic *Synchytriaceae* have received scant research attention; consequently, DNA sequence information is unavailable for them. In contrast, some *Synchytrium* species are represented in molecular phylogenies. In the James *et al.* (2006) analysis of the 18S, 28S and 5.8 subunits of rDNA, *Synchytrium* species grouped together and were labeled as the “*Synchytrium* clade.” More recently, Smith *et al.* (2014) presented an 18S rDNA phylogeny of 14 *Synchytrium* species representing Karling's (1953) six subgenera. In this

phylogeny, the species of *Synchytrium* were monophyletic, but the analysis did not support as monophyletic all of Karling's subgenera, which were based on stages of the life cycle.

Concurrently with a small, probable member of the *Rhizophydiales* (*Chytridiomycota*), we isolated a tiny, spherical organism from pollen bait placed with a collection from a small, acidic lake. We, at first, considered the new organism as a possible member of the *Labyrinthulomycota* because colonies on agar were surrounded by fine projections similar to the ectoplasmic net extensions formed by some species in this stramenopile phylum. Analysis of 18S rDNA sequence, however, revealed that this chytrid grouped with *Synchytrium* species. Herein we present molecular, morphological, and TEM features as a basis for describing *Synchytrium microbalum* sp. nov., the first member of the genus in pure culture.

Materials and methods

Isolation, culture and morphology

We isolated JEL517 from pollen bait placed with a sample from Mud Pond, a small, acidic lake in Hancock Co., Maine, USA. Stock cultures are maintained in PmTG liquid medium (Barr 1986), refrigerated after evident growth, and transferred after ~100 d. For light microscopy, we grew JEL517 on modified PmTG (0.04 % peptonized milk, 0.04 % tryptone, 0.20 % glucose, 1.0 % agar in distilled water) and in autoclaved pond water with added spruce pollen, both at room temperature (~ 23 °C) and ambient light. We photographed growth stages with a Spot RT3 (Diagnostic Instruments, Inc. Sterling Heights, Michigan, USA) digital camera attached to a Nikon Eclipse E400 compound microscope and measured morphological features on enlarged digital photographs with Spot RT3 imaging software. To test the upper temperature limit for

growth, we grew JEL517 in 75 ml of PmTG liquid medium in 3 flasks each at 27, 28-29, 30, and 31 °C and compared growth visually with that at 23 °C.

Ultrastructure

We grew strain JEL517 in 50 ml of PmTG liquid medium in 125 ml flasks on a VWR (VWR International, www.us.vwr.com) Incubating Mini Shaker at 24 °C and 140 rpm for approximately two weeks. After we observed sufficient growth, we transferred ~ 3 ml of inoculant to Cd plates (0.02 % peptonized milk, 0.04 % tryptone, 0.2 % cellobiose, 0.2% soluble starch, 0.8 % agar in distilled water) and spread it uniformly over the plate surface. Plates were incubated at 24 °C for 3 days, at which time we observed zoospores releasing from sporangia. We then flooded plates with 3 ml of sterile distilled water, which stimulated additional zoospore release. We collected zoospores and thalli in a 15-ml centrifuge tube and fixed them for 1 h in 2.5% glutaraldehyde (Sigma Life Sciences, St. Louis, Missouri, USA) in 0.1 M sym-collidine buffer at pH 7.4 (Electron Microscopy Sciences, Hatfield, Pennsylvania, USA). Following fixation, we filter separated the suspension of thalli and zoospores with a 5 µm isopore membrane (EMD Millipore, Darmstadt, Germany). The two components (i.e., predominantly thalli or predominantly zoospores) were washed in buffer, post fixed in osmium tetroxide, embedded in 2% agar, cut into 1 mm³ pieces, en bloc stained with uranyl acetate, and infiltrated with Epon Araldite as in Letcher and Powell (2005). We cut random sections with a diamond knife (Diatome-US, Hatfield, Pennsylvania, USA ultra 35°) and a Leica (www.leica-microsystems.com) Ultracut UCT ultramicrotome, collected them on nickel grids (Ted Pella, Inc, Redding, California, USA), post-stained for 10 min in saturated uranyl acetate in 70 % ethanol, and examined stained sections on a Hitachi 7650 transmission electron microscope.

98

99 ***DNA and phylogenetic analyses***

100 We extracted, amplified, and sequenced 18S rDNA for JEL517 as in Simmons (2011) and
101 downloaded sequences for other *Synchytrium* species from GenBank (James *et al.* 2006, Smith *et*
102 *al.* 2014). Although the 18S rDNA sequences for *S. taraxaci*, *S. australe*, and *S. plantigeum* were
103 much shorter (~200 bp; 3' end of 18S rDNA) than those of other species in our analysis (~1200–
104 1700 bp), we included them. We used members of the *Lobulomycetales* (Simmons *et al.* 2009) as
105 outgroup taxa because, in a previous analysis (James *et al.* 2006) they were the nearest neighbor
106 to the *Synchytrium* clade. We aligned sequences in Geneious (Geneious version 9.0.5) and used
107 the Akaike information criterion in jModeltest 0.1.1 (Guindon and Gascuel 2003, Posada 2008)
108 to select the nucleotide substitution model. Maximum likelihood (ML) phylogenetic analyses
109 were conducted in GARLI 2.01 (Zwickl 2006) with the recommended parameters to determine
110 the best tree topology (Fig 1) and bootstrap support values from 500 search replicates, which
111 were summarized in SumTrees (Sukumaran and Holder 2010). We estimated Bayesian posterior
112 probabilities (BPP) with the same partition parameters in an analysis conducted in MrBayes
113 3.1.2 (Ronquist and Huelsenbeck 2003), in which two runs of four chains each were executed
114 simultaneously for 5 000 000 generations, with sampling every 500 generations. SumTrees was
115 used to compute BPP from a summary of 7501 trees retained after a burn-in of the first 2500
116 trees collected.

117

118 **Results**119 ***Molecular phylogeny***

120

Based on our analysis of 18S rDNA sequences, isolate JEL517 lies within the genus *Synchytrium*, with the type species of the genus, *S. taraxaci*, branching basally within the generic clade (Fig 1). The sequence for *S. taraxaci*, however, was short (~200 bp), and the species' position could change with a longer, more informative read. Isolate JEL517 is sister to *S. cupulatum* (95% BPP, 75 % ML bootstrap), and this lineage forms a monophyly (93 % BPP, 63 % ML bootstrap) with the remainder of the *Synchytrium* species (Fig 1, arrow). The 18S rDNA alignment was deposited in TreeBASE (submission 18855).

Morphology and development

We followed the life cycle of JEL517 growing on the surface of pond water that also contained spruce pollen grains. Development in pond water took between 2 and 3 days at ~23 °C. In PmTG liquid nutrient medium, growth was abundant at 23 °C, 27, 28-29 but absent at 30 and 31 °C. Zoospores (Fig 2 a–c, f) were frequently elongate (2.5–3.5 X 2.0 µm) and often had amoeboid, apical extensions (Fig 2 a, f). Flagella averaged ~10 µm long with the longest being 11 µm. Encysted zoospores were round or slightly ovate (Fig 2 d) and soon produced projections. Projections were branched (2 or 3 branches) or unbranched, with branching occurring only near the base (Fig 2 e, arrows). These flexuous projections, which appear to arise from 6–8 sites around the equator of the sporangium, were about the same diameter as the flagella of zoospores, and increased in length as the sporangia grew (longest measured = 39 µm, usually 30 µm or less). Projections also formed on nutrient agar (Fig 2 i) but not as consistently as on pond water; they did not extend into the nutrient medium as would be expected for rhizoids. Mature sporangia achieved diameters up to 7 µm on pond water and up to 11 µm on modified PmTG agar (Fig 2 i, j). When the organism grew on the surface of pond water, zoospores escaped

through a pore at the side of the zoosporangium (Fig 2 g). Nutrients leach from the spruce pollen and thus most of the thalli grew on the surface of the water, however, some thalli developed on the isthmus between the wings of the spruce pollen grains where the wall is thinner (Fig 2 h) but we could not determine the position of the projections on these thalli. Almost all thalli, when developing on pond water, became zoosporangia and released motile zoospores. In contrast, when developing on nutrient agar or in liquid media, a second generation of thalli often developed within the parent thalli (Fig 2j; 3n).

TEM of thalli and zoospores

Our fixation methods provided a mix of thalli at various stages of development. On TEM photos, thalli appeared spherical to sub-spherical, ~3–8 μm diam, with thin, elongate projections from the sporangial wall (Fig 3 k). Cytoplasm seems to be continuous between the sporangium and the projection (Fig 3 l), but organelles (nuclei or mitochondria) were not seen within the projection cytoplasm. Early stage sporangia contained a single, large nucleus, multiple mitochondria, and vacuoles (Fig 3 k). Development culminated in fully formed zoospores, which contained kinetosomes (Fig 3 m). Commonly, zoospores encysted (formed walls) and began enlarging (Fig 3 n) within the parent sporangium and ultimately disrupted the parent wall.

Most of the fixed zoospores (Fig 3 a, b) were elongate in longitudinal section, ~1.5 μm wide and 3 μm in length, although some were pleomorphic (Fig 4 c, d). An electron-opaque plug occupies the flagellar base; the portion of the plug within the axoneme was ~ 70 nm thick, with electron-opaque extensions both anterior and posterior around the axoneme (Fig 3 g–i). A thin terminal plate lies anterior to the flagellar plug at the base of the kinetosome (Fig 2 i); flagellar props (Fig 3 h–j) connect the base of the kinetosome to the plasma membrane. A non-flagellated

centriole is attached to the kinetosome, at an angle of $\sim 30^\circ$ by a fibrillar bridge (Fig 3 g). A Golgi apparatus (Fig 3 f) is located lateral to the kinetosome on the side opposite the non-flagellated centriole.

Centrally in the zoospore body, the nucleus lies either anterior (Fig 3 a) or posterior to the lipid globule (Fig 3 b). A simple MLC cisterna abuts the single lipid globule. The mitochondrion appears to be a single, large, lobed entity, in close association with the lipid globule, nucleus, and microbody (Fig 3 d). Strands of endoplasmic reticulum (ER) are near the nucleus (Fig 3 a, c), lipid globule (Fig 3 d, e), and mitochondrion (Fig 3 d). The anterior zoospore cytoplasm contains mostly ribosomes, which also occupy the cytoplasm around the organelles in the central and posterior part of the spore. Vacuoles occur throughout the cytoplasm and cored vesicles occur, but are not abundant. No microtubule roots were observed in the zoospore..

Taxonomy

Synchytriales Doweld AB, 2014. *Index Fungorum* no **92**, 2014; MycoBank No.: 550404.

Emended by Longcore, DR Simmons and Letcher to be the least inclusive clade of the *Chytridiomycetes* that includes *Synchytrium taraxaci* and *Synchytrium* species included in James *et al.* (2006; as the *Synchytrium* clade), Smith *et al.* (2014) and Longcore *et al.* (this paper).

Synchytriaceae J Schröt., in Engler & Prantl, *Nat. Pflanzenfam.*, Teil. I (Leipzig) 1: **71**, 1892; MycoBank No.: 81448.

Emended by Longcore, DR Simmons and Letcher to be the least inclusive clade of the *Chytridiomycetes* that includes *Synchytrium taraxaci* and the *Synchytrium* species included in

James *et al.* (2006; as the *Synchytrium* clade), Smith *et al.* (2014) and Longcore *et al.* (this paper).

Synchytrium microbalum Longcore, DR Simmons & Letcher **sp. nov.**

Mycobank No.: MB817291; Fig 2, 3

Etym.: Micro (Greek μικρο) = tiny; bála (Greek μπάλα) = ball; refers to the small, spherical zoosporangia.

Saprobic sporangia small (diam usually 10 µm or less), monocentric, with multiple, isodiametric, 0.3–0.4 µm diam, up to 39 µm long, flexuous projections that may branch once or twice, only at the base. Zoospores small, elongate (~ 3 x 2 µm), often changing shape; released from zoosporangium through a pore. Flagellum ~10 µm long. Zoospore ultrastructure similar to that of *S. endobioticum* but lacking microtubules. On nutrient agar and in nutrient liquid medium sporangia often develop within parent sporangia. 18S rDNA GenBank # KU672728.

Specimen examined. Isolate JEL517 collected from **United States**, MAINE, Hancock County, 44.633, -68.087. Baited from spruce pollen placed with an aquatic sample collected 14 May 2006 from Mud Pond, a small (1.6 ha), acidic (pH 4.5) lake (Hunter *et al.* 1986) located in the Donnell Pond Public Reserved Land. **Type** is Fig 2 in this publication. Ex type culture JEL517 is cryopreserved in the JEL culture collection and deposited in CBS-KNAW.

Notes: As allowed by ICN, we have declared Fig 2 as the type, because a slide or fixed material is relatively uninformative and deep freezing zoosporic fungi, in our experience, is not always a permanent form of preservation.

Discussion

Effect of Synchytrium microbalum on Synchytrium taxonomy

Descriptions of *Synchytrium* and *Synchytriaceae sensu* Sparrow (1960) stress parasitism. The finding of *S. microbalum* in a parasitic clade means that relationship with hosts, as a defining character for the Family and Genus, needs to be augmented or replaced. Although the name *Synchytriales* has been in use at least since 1948 (Schussnig 1948), Doweld (2014) published the name as a new order, with his description based on the endobiotic nature of the thalli, presence of a prosorus, and zoospore ultrastructure. Development of sporangia external to a substrate (epibiotic) and the lack of a prosorus stage in *S. microbalum* contradict the first two characters. The description of zoospore ultrastructure for the order was based on only two (*S. endobioticum* and *S. macrosporum*) of the > 200 species in the order, neither of which is the type. Moreover, the description of the zoospore ultrastructure for the order is confusing, stating, in part (Doweld 2014), “kinetosomes two” and also “flagellar apparatus consists of kinetosome and associated secondary centriole.” Also, the flagellar plug was referred to as a “flagellar terminal plate.” Because of the variation in zoospore ultrastructural in the three examples of the *Synchytriales* studied so far, plus the variation in sporangial morphology that has been added by the discovery of *S. microbalum*, we have amended the order and the family but leave the amendment of the genus *Synchytrium* until additional species of it and *Micromyces* are studied.

In our phylogeny (Fig 1), *Synchytrium microbalum* groups with *S. cupulatum*, which Karling (1964) placed within the subgenus *Pycnochytrium*. More recent studies (Smith *et al.* 2014), however, do not support the monophyly of *Synchytrium* subgenera and they also will need to be revised pending future studies. Our support values (93% BPP; 63% ML bootstrap) for the clade containing all *Synchytrium* species, except *S. taraxaci*, were below our cutoff levels for optimum support, most likely because of a collapsed trichotomy or shuffling of the *S.*

microbalum-*S. cupulatum* lineage, the type species *S. taraxaci*, and the remaining *Synchytrium* species. The 18S rDNA sequences used for *S. taraxaci*, *S. australe*, and *S. plantigeum* were much shorter (~200 bp; 3' end of 18S rDNA) than those of other species in our analysis (~1200–1700 bp), and though Smith *et al.* (2014) reported these sequences, they were not included in their phylogenetic analysis. Though *S. australe* and *S. plantigeum* are deeper within the *Synchytrium* clade than *S. taraxaci*, we might expect a longer sequence from any of these species to lead to a more highly supported phylogeny with a possibly different topology; our analysis, however, is well-resolved and is otherwise similar to that of Smith *et al.* (2014).

Ultrastructural features of zoospores have been used to help define genera in the *Chytridiomycota* (e.g., Barr 1980; Longcore 1993; Letcher and Powell 2005; Simmons *et al.* 2009). The ultrastructure of the *S. microbalum* zoospore is similar to that of *S. endobioticum* (Lange and Olson 1978). The major difference is the lack of a microtubule root in *S. microbalum* zoospores; this lack of a microtubule root, which, in many genera, anchors the lipid globule or other organelles to the kinetosome, may account for the changeable location of the nucleus and lipid globule that we observed in TEM photos. The zoospore of *S. macrosporum* (Montecillo *et al.* 1980) differs from those of *S. endobioticum* and *S. microbalum* in lacking a plug in the base of the flagellum and in possessing a rumposome (= fenestrated cisterna of Letcher *et al.* 2005). Whether these differences in zoospore characters will, along with differences in DNA markers, eventually result in revising *Synchytriaceae* genera and subgenera awaits studies of additional species.

Morphological comparisons with other species of Synchytriaceae

Synchytrium microbalum differs from all other species of the *Synchytriaceae* in that it is saprobic and epi-or interbiotic, whereas other species grow within their hosts. During observations of *S. microbalum* sporangia, we found that when this fungus grows on nutrient agar, often a new generation of sporangia formed within the parent wall instead of zoospores being released from sporangia. It is tempting to identify this internal development as formation of a sorus, a distinct part of the life cycle found in other species of *Synchytrium* (Karling 1964). The typical *Synchytrium* sorus, however, is preceded by a prosorus. We believe that, because nearly all sporangia released zoospores when in pond water, the internal development of a second generation when on nutrient agar resulted from non-release and internal encystment of zoospores. Internal development of a second generation may be caused by excess nutrition and little free water; however, other chytrids in pure culture do not develop a second generation within the parent sporangium to this extent and zoospore development *in situ* may involve similar pathways as sorus formation.

The projections on the *S. microbalum* sporangium do not enter nutrient agar but float free of the agar surface when liquid is added; consequently, we do not refer to them as rhizoids. Whether they are analogous to the long, but more robust spines of *Micromyces grandis* (Miller 1955), also an aquatic species in the *Synchytriaceae*, is impossible to determine. The spiny, internal stage of the algal parasite *M. grandis* is a prosperangium, however, and not a sporangium. The presence of sporangia on the isthmus of spruce pollen grains suggests that the projections may help to anchor sporangia to a substrate as in *Ubelmesseromyces harderi* (Powell *et al.* 2015), but their small size prohibited determining how, even at high magnification. The flexuous projections, several times the length of the sporangium diameter, also may function to entangle the organism in detritus.

Another aquatic species with flexuous projections is *Nowakowskia hormothecae* Borzi. Although it is pictured in Sparrow (1960), Karling (1977), and in earlier chytrid compendiums, we can find no reports of its sighting since it was described 131 years ago (Borzi 1885). This organism parasitized encysted zoospores of what was identified as the xanthophyte alga *Hormotheca sicula*, later known as *Bumilleria sicula* Borzi. Similarities between *S. microbalum* and *N. hormothecae* include the small size of sporangia, the fine, scarcely branched projections (labeled rhizoids and some reaching to the algal host in *N. hormothecae*) and the size and shape of the zoospores. Morphology, however, is an untrustworthy character as is evident by the difference between historical taxonomy based on morphology and more recent taxonomy based on zoospore ultrastructure and DNA sequences. Zoospore shape and size, however, seems to be an exception and, in our experience, is consistent within most orders. Considering that Borzi (1885) worked at less magnification and without phase optics, his drawings and our photos have remarkable similarities in zoospore size, shape and presence of a single lipid globule. We have found no species other than *N. hormothecae* that is similar to *S. microbalum*. Because of a swarming zoospore stage in *N. hormothecae* (Borzi 1885) and its status as a pathogen, we do not consider the two organisms conspecific, however, we do suggest that *N. hormothecae* also may be a member of the *Synchytriales*.

Implications

The availability of a pure culture of *S. microbalum* is important because this species grows saprobically but is a member of a heretofore exclusively plant (including green algae) parasitic clade. It can be used in studies of comparative DNA content, especially with the agriculturally important pathogen *S. endobioticum*, providing an opportunity to identify genes associated with plant parasitism. With a sporangium of less than 10 μm diam, *Synchytrium microbalum* is truly a

microbe; it is near the size of some bacteria and unlikely to be found by microscopy of natural substrates, however, its 18S rDNA sequence information in GenBank will now allow identification of this and closely related species in environmental DNA studies. Whether this chytrid is common or belongs to a molecularly unexplored branch of the *Synchytriales*, such as the aquatic *Micromyces* spp. and *Nowakowskia hormothecae*, awaits finding and sequencing these organisms.

Acknowledgments

Portions of this study were supported by the JEL culture collection at the University of Maine and the National Science Foundation through MRI DEB-0500766 and DEB-1455611. We thank T. James for initial molecular placement of JEL517, M. Powell for helpful criticism of the manuscript and S. Pennycook for verifying the Latin species ending.

REFERENCES

- Barr DJS, 1980. An outline for the reclassification of the *Chytridiales*, and for a new order, the *Spizellomycetales*. *Canadian Journal of Botany* **58**: 2380–2394.
- Barr DJS, 1986. *Allochytridium expandens* rediscovered: morphology, physiology and zoospore ultrastructure. *Mycologia* **78**: 439–448.
- Borzi A, 1885. *Nowakowskia*, eine neue Chytridiee. *Botanisches Centralblatt* **22**: 23–26, Tafel 1.
- Canter H M, 1949. Studies on British Chytrids. VI Aquatic *Synchytriaceae*. *Transactions of the British Mycological Society* **56**: 69–94, Plates VII–XI.

- 326 Doweld AB, 2014. *Synchytriales* Doweld, ord.nov. *Index Fungorum* no 92.
327 <http://www.indexfungorum.org/Publications/Index%20Fungorum%20no.92.pdf>
- 328 Guindon S, Gascuel O, 2003. A simple, fast, and accurate algorithm to estimate large
329 phylogenies by maximum likelihood. *Syst. Biol.* **52**: 696–704.
- 330 Hunter ML Jr, Jones JJ, Witham JW, Mingo TM, 1986. Biomass and species richness of aquatic
331 macrophytes in four Maine (U.S.A.) lakes of different acidity. *Aquatic Botany* **24**: 91–95.
- 332 James TY, Letcher P M, Longcore J E, Mozley-Standridge S E, Porter D, Powell M J, Griffith
333 GW, Vilgalys R, 2006. A molecular phylogeny of the flagellated fungi (*Chytridiomycota*)
334 and description of a new phylum (*Blastocladiomycota*). *Mycologia* **98**: 860–871.
- 335 Karling, JS, 1953. *Micromyces and Synchytrium*. *Mycologia* **45**: 276–287.
- 336 Karling JS, 1964. *Synchytrium*. Academic Press, New York. 470 pp.
- 337 Karling JS, 1977. *Chytridiomycetorum Iconographia*. J. Cramer, Monticello, New York. 413 pp.
- 338 Lange L, Olson LW, 1978. The zoospore of *Synchytrium endobioticum*. *Canadian Journal of*
339 *Botany* **56**: 1229–1239.
- 340 Letcher PM, Powell, 2005. *Kappamyces*, a new genus in the *Chytridiales* (*Chytridiomycota*).
341 *Nova Hedwigia* **80**: 115–133.
- 342 Letcher PM, Powell MJ, Chambers JG, Longcore JE, Churchill PF, Harris PM, 2005.
343 Ultrastructural and molecular delineation of the *Chytridiaceae* (*Chytridiales*). *Canadian*
344 *Journal of Botany* **83**: 1561–1573.

- 345 Longcore JE, 1993. Morphology and zoospore ultrastructure of *Lacustromyces hiemalis* gen. et
346 sp.nov. (Chytridiales). *Canadian Journal of Botany* **71**: 414–425.
- 347 Miller, CE, 1955. *Micromyces grandis*, a new member of the aquatic Synchytriaceae. *Journal of*
348 *the Elisha Mitchell Scientific Society* **71**: 247–255.
- 349 Montecillo CM, Bracker C E, Powell MJ, 1980. Ultrastructure of *Synchytrium macrosporum*
350 zoospores. *Canadian Journal of Botany* **58**: 1885–1897.
- 351 Powell MJ, Letcher PM, Chambers JG, Roychoudhury S, 2015. A new genus and family for the
352 misclassified chytrid, *Rhizophlyctis harderi*. *Mycologia* **107**: 419–431.
- 353 Posada D, 2008. JModeltest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**: 1253–1256.
- 354 Rieth A, 1962. *Micromyces furcata* n.sp. nebst einigen Bemerkungen über seinen Wirt. *Die*
355 *Kulturpflanze: Berichte und Mitteilung aus den Institut für Kulturpflanze.* **3**: 286–295.
- 356 Ronquist F, Huelsenbeck JP, 2003. Mr Bayes 3: Bayesian phylogenetic inference under mixed
357 models. *Bioinformatics* **19**: 1572–1574.
- 358 Schussnig, Von B, 1948. Stammengeschichtlicher Formenwandel und Gestaltungstypen im
359 Reich der Pilze. *Sydowia* **2**: 83–230.
- 360 Simmons DR, 2011. Phylogeny of *Powellomycetaceae* fam. nov. and description of
361 *Geranomyces variabilis* gen. et comb. nov. *Mycologia* **103**: 1411–1420.
- 362 Simmons DR, James TY, Meyer AF, Longcore JE, 2009. *Lobulomycetales*, a new order in the
363 *Chytridiomycota*. *Mycological Research* **113**: 450–460.

364 Smith, DS, Rocheleau H, Chapados JT, Abbott C, Ribero S, Redhead SA, Lévesque CA, De
 365 Boer SH, 2014. Phylogeny of the genus *Synchytrium* and the development of TaqMan PCR
 366 assay for sensitive detection of *Synchytrium endobioticum* in soil. *Phytopathology* **104**:
 367 422–432.

368 Sparrow, FK, 1960. *Aquatic Phycomycetes*, 2nd ed, University of Michigan Press, Ann Arbor,
 369 Michigan, 1187 pp.

370 Sparrow, FK, 1973. *Chytridiomycetes, Hyphochytridiomycetes*. In: Ainsworth GC, Sparrow FK,
 371 Sussman AS (eds), *The Fungi, An Advanced Treatise Volume IVB*. Academic Press, New
 372 York, pp 85–110.

373 Sukumaran J, Holder MT, 2010. DendroPy: a python library for phylogenetic computing.
 374 *Bioinformatics* **26**: 1569–1571.

375 Zwickl, DJ, 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological
 376 sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The
 377 University of Texas at Austin.

378

379

380

Figure legends

Fig 1 – Best ML tree from GARLI analysis of 18S rDNA data matrix, with GenBank accessions after taxa. Values at nodes represent ML bootstrap percentages $\geq 70\%$ from a summary of 500 replicates, and branches in bold represent BPP $\geq 95\%$. Support values for node at arrow = 63 % ML bootstrap, 93 % BPP, which are near, but below, the cutoff values printed on the figure. T = type; *Synchytrium taraxaci* sequence data comprises only 201 bp.

Fig 2 – Morphology and development of *Synchytrium microbalum* in pond water plus spruce pollen (a–g, h) and on mPmTG agar (i, j). (a, b) motile zoospores; (c) flattened zoospore showing lipid globule; (d) young, ovate thallus with projections; (e) developing thalli with long projections, which may be bi-or trifurcate at the base (arrowheads); (f) empty zoosporangium (arrow) surrounded by released zoospores; (g) two empty zoosporangia, arrows indicate discharge pores; (h) zoosporangia (arrows) on isthmus of spruce pollen grain; (i) Developing sporangia on agar; (j); mature or nearly mature sporangia, arrows point to young sporangia developing within parent sporangia. Bar in e = 10 μm for all photos.

Fig 3 – Ultrastructural features of the zoospore and thallus of *Synchytrium microbalum*. (a) Longitudinal section (LS), in which the lipid is posterior to the nucleus; endoplasmic reticulum is adjacent to the nucleus, a lobe of the mitochondrion is lateral to the nucleus, and a simple microbody-lipid globule complex (MLC) cisterna is appressed to the lipid surface. (b) LS, in which the nucleus is posterior to the lipid globule, lobes of the mitochondrion and a microbody are adjacent to the lipid globule and nucleus, and a vacuole is present in the cytoplasm. (c) Transverse section (TS), with endoplasmic reticulum and lobes of the mitochondrion adjacent to

the nucleus. (d) LS of the MLC showing the intimate association between the nucleus, mitochondrion, microbody, and lipid globule. (e) Transverse section showing endoplasmic reticulum adjacent to the microbody and lipid globule. (f) LS through the Golgi apparatus, which is lateral to the kinetosome; arrows indicate bases of three flagellar props. (g) LS through the kinetosomal region, showing the non-flagellated centriole at an $\sim 30^\circ$ angle to the kinetosome, the fibrillar bridge connecting the two, and the electron-opaque flagellar plug in the base of the flagellum. (h) LS section illustrating the flagellar props, the flagellar plug, and extensions of the flagellar plug (arrows) that are both anterior and posterior to the body of the plug. (i) LS illustrating the fine terminal plate anterior to the flagellar plug and posterior to the kinetosome. (j) Grazing LS showing the base of three flagellar props. (k) Sporangium with a single nucleus and two sporangial projections (arrows). (l) LS through a sporangial appendage; arrow indicates cytoplasmic continuity between sporangium and projection. (m) Sporangium with cleaved zoospores; arrow indicates base of a flagellum with an electron-opaque plug. (n) Multiple walled encysted zoospores (arrows), each with a single nucleus. Bars in a–e = 0.5 μm ; f–j = 0.25 μm ; and k, m, n = 1.0 μm , l = 0.05 μm . Abbreviations for Figs 3 and 4: CF, cleavage furrow; CV, cored vesicle; ER, endoplasmic reticulum; F, flagellum; FB, fibrillary bridge; FP, flagellar plug; G, Golgi apparatus; K, kinetosome; L, lipid; M, mitochondrion; Mb, microbody; N, nucleus; NfC, non-flagellated centriole; P, flagellar prop; R, ribosomes; SC, simple MLC cisterna; Sp, sporangium; SpW, sporangium wall; TP, terminal plate; Vac, vacuole; Ves, cored vesicle.

Fig 4 – Schematic drawing of longitudinal sections through the zoospore of *Synchytrium microbalum*. (a) Ultrastructural features of the zoospore with its most typical morphology.

426 Dotted lines connecting mitochondrial profiles indicate that the single, large mitochondrion is
427 lobed. (b–d) Alternative and less typical zoospore morphologies.

Figure 1

Figure 2

Figure 3

Figure 4