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Proposed Changes to the Nomenclature of *Ichthyophonus* sp. Life Stages and Structures

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ABSTRACT: Much of the terminology describing *Ichthyophonus* sp. life stages and structures can be traced to the mistaken classification of this organism as a fungus. This misidentification led early investigators to use mycological terms for the structures they observed; while some terminology is not so easily explained, it appears to have been co-opted from the fields of botany and bacteriology. The purpose of this exercise is to attempt to standardize the terminology associated with *Ichthyophonus* and to bring it into agreement with terminology currently used to define similar life stages of other protists. The proposed changes are (1) spore/macrospore/mother spore to "schizont," (2) microspore/endospore to "merozoite," and (3) pseudohyphae to "hyphae" or "germ tube."

Ichthyophonus sp. is a pathogen of fresh and saltwater fishes with a worldwide distribution (reviewed in McVicar, 2011). The organism was first described by Hofer (1893) and subsequently classified as a protozoan, *Ichthyosporidium hoferi* (Caullery and Mesnil, 1905). Several years later it was reclassified as a fungus, *Ichthyophonus hoferi* (Plehn and Mulsow, 1911), where it remained until it was recently placed into the mesomycetozoa, a group of organisms near the animal-fungal boundary (Spanggaard et al., 1996; Mendoza et al., 2002; Ragan et al., 2003). Although the organism is often referred to as *I. hoferi*, the original species description was incomplete (McVicar, 2011), and no type specimen exists for comparison. Since no definitive criteria for assigning species within this genus are currently available, the generic term *Ichthyophonus* will be used hereafter.

In a recent review McVicar (2011) pointed out that because *Ichthyophonus* was for many decades perceived to be a fungus, that terminology used to describe the various stages of *Ichthyophonus* "closely followed that of the mycologist," and anyone familiar with the biology of fungi can see the similarities; however, some terminology was also co-opted from the fields of botany and bacteriology. Because the long-standing misuse of these terms in scholarly articles has perpetuated the errors, McVicar (2011) also proposed "some reappraisal would now seem appropriate." Science is a self-correcting enterprise, and it seemed timely to update the descriptive terminology to make it consistent with the organism's correct classification as a protist.

The most common morphological form of *Ichthyophonus* seen in the living fish host has been variously identified as a "spore," "macrospore," "mother spore," or "resting spore." This is a large multinucleate spherical cell ranging from ~10 to >200 μm in diameter (growth progression), which is surrounded by a PAS-positive non-cellular laminate membrane (Fig. 1). Although there is no all-inclusive definition of the term "spore," it is generally defined as a structure produced by many prokaryotes and fungi that is adapted for dispersal and survival for extended periods of time under unfavorable environmental conditions, such as desiccation, heat, freezing, disinfectants, and UV radiation (Madigan et al., 2000; Stedman's Medical Dictionary, 2012). These specialized cells (i.e., spores) are produced by most fungi, certain genera of bacteria such as *Clostridia* and *Bacillus* (Smith and Conant, 1960), microsporidians and myxosporidians (Roberts and Janovy, 2005), as well as algae and non-flowering plants (Bold, 1957). Additionally, a true spore is a single cell capable of developing into a new individual, either directly or after fusing with another spore, while the multinucleate *Ichthyophonus* cell is a reproductive structure that undergoes asexual endogenous cell division entirely within the fish host (Okamoto et al., 1985). Unlike true spores, the large multinucleate *Ichthyophonus* cell occurs only in the living host, not free in the environment, and is viable only within the environmental tolerance limits of the host. The cell is non-viable in vitro at temperatures above 25 C (Spanggaard and Huss, 1996), is killed at –8 to –10 C (Slocombe, 1980; Athanassopoulou, 1992), and is inactivated by antifungal agents (Hontoria et al., 2009) and disinfectants (Hershbergeret al., 2008).

The term "macrospore" (aka "megaspore") is also used interchangeably with "spore" in the *Ichthyophonus* literature. "Macrospore" is defined as "The larger of 2 types of spore produced in heterosporous plants, that develops into a female gametophyte" (Bold, 1957; Abercrombie et al., 1962). Therefore, the terms "spore" and "macrospore," derived from different fields of biology, are not synonymous and do not describe the large multinucleate cell seen in *Ichthyophonus* infections.

Because the large multinucleate *Ichthyophonus* cells display none of the characteristics associated with true spores, megaspores, or macrospores, these terms should be abandoned. The term that most closely describes the multinucleate *Ichthyophonus* cell is "schizont," defined as "A cell undergoing schizogony, a form of asexual reproduction in which multiple mitoses take place, followed by cytokinesis, resulting in many daughter cells," i.e., merozoites (Roberts and Janovy, 2005; Stedman's Medical Dictionary, 2012). Although the term "schizont" is most frequently associated with the apicomplexans, it is a descriptive term that can be applied to any cell undergoing similar asexual reproduction.

Over the years, a number of authors have described small uninucleate cells produced by tissue schizonts as well as in the apical tips of growing hyphae of *Ichthyophonus* (Dorier and Degrange, 1960; Paperna, 1986; Spanggaard et al., 1995; Rahimian, 1998; Franco-Sierra and Alvarez-Pellitero, 1999; McVicar, 2011). Similar cells, released through epidermal ulcers of infected herring, were also reported (Kocan et al., 2010). The terms "microspores" or "endospores" have been applied to these cells presumably because they are small and/or contained within what was erroneously believed to be a "spore" (Fig. 2).

Mycologists identify an "endospore" as an asexual spore formed within a cell (not a macrospore), such as the spherule of the fungal pathogen, *Coccidioides immitis* (Kwon-Chung and Bennett, 1992), while bacteriologists define "endospores" as being produced by several genera of Gram-positive bacteria (*Clostridium* and *Bacillus*) under conditions of nutrient deprivation. In this case a single endospore is produced within a single vegetative bacterial cell and is capable of surviving for long periods of time until environmental conditions become favorable for growth (Smith and Conant, 1960). Botanists define "microspore" as "the smaller of 2 types of spores that give rise to a male gametophyte in all seed plants" (Bold, 1957; Abercrombie et al., 1962).

Clearly, these definitions do not describe the mono-nucleate cells produced by *Ichthyophonus* schizonts, and therefore it is proposed that they be replaced with the term "merozoite," which is defined as a daughter cell arising from schizogony (Roberts and Janovy, 2005; Stedman's Medical Dictionary, 2012).

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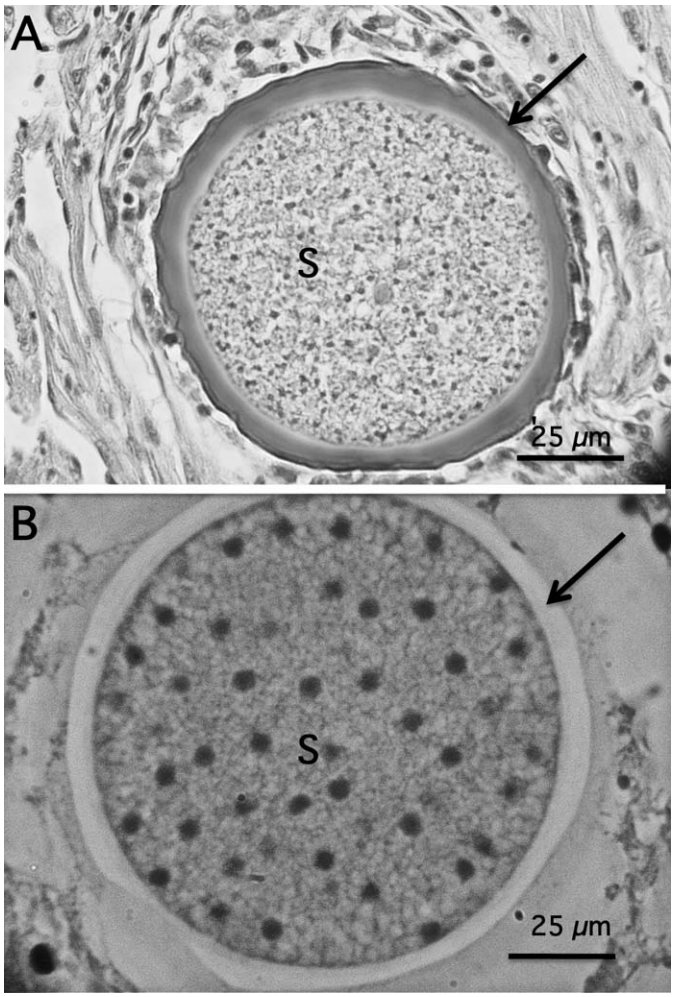

FIGURE 1. Multinucleate *Ichthyophonus* schizonts (S) surrounded by a non-cellular multilaminate membrane (arrows). (**A**) Histologic section of *Ichthyophonus*-positive cardiac muscle (PAS stain). (**B**) Histologic section of *Ichthyophonus*-positive kidney tissue (H&E stain), host: Chinook salmon (*Oncorhynchus tshawytscha*).

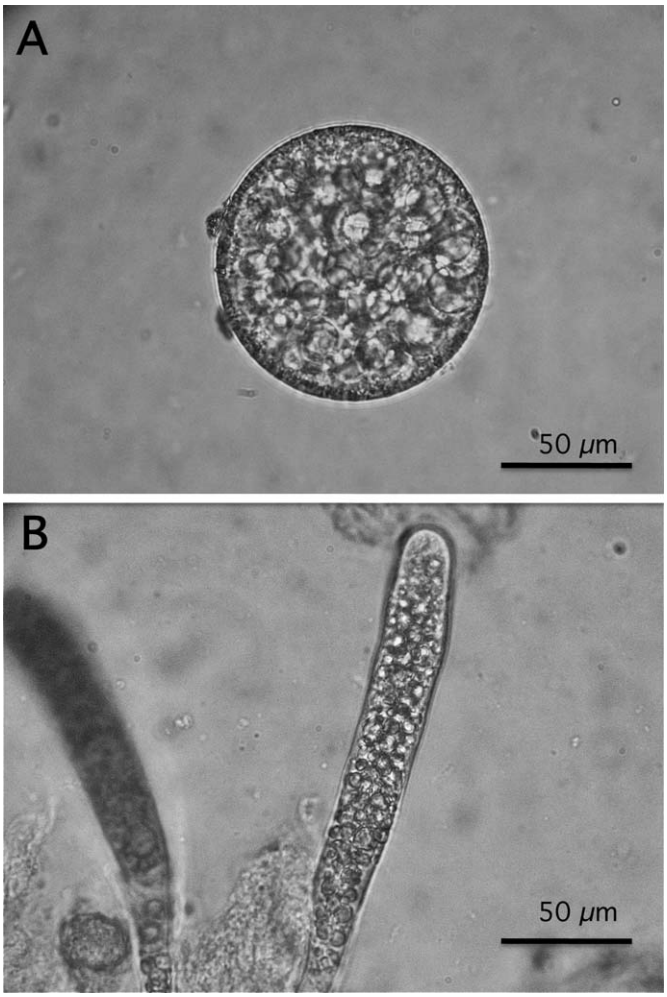

FIGURE 2. *Ichthyophonus* merozoites in (**A**) live *Ichthyophonus* schizont, host: Pacific herring (*Clupea pallasii*); and (**B**) apical tip of a hypha in vitro, host: rainbow trout (*Onchorynchus mykiss*).

Following the death of the host, *Ichthyophonus* schizonts are observed to either produce non-septate hyaline tubules or fragment into multi-nucleate motile cells that migrate away from the schizont through the dead tissue (Fig. 3). These multi-nucleate motile cells have been referred to as "amiboblastes" (Dorier and Degrange, 1960), "plasmodium-like bodies" (Okamoto et al., 1985), "plasmodio-spores" (Rahimian, 1998), and "multinucleate spores" (Franco-Sierra and Alvarez-Pellitero, 1999). These cells are believed to be the source of the infective amoeboid stage seen in the stomach and lamina propria of fish fed *Ichthyophonus*-infected tissue (Kocan et al., 2013). Since several authors have recognized the plasmodium-like features of this stage, and "plasmodium" is defined as a "multinucleate sheet of cytoplasm" (Abercrombie et al., 1962; Roberts and Janovy, 2005; Stedman's Medical Dictionary, 2012), the descriptive term "plasmodium" is proposed for this life stage of *Ichthyophonus*.

Non-septate hyaline tubules are produced by *Ichthyophonus* both in vivo and in vitro and have been referred to variously as "hyphae," "pseudo-hyphae," and germ tubes. Mycologists define "pseudohyphae" as "chains of successively budding yeast cells that have complete cell walls, but have not detached from one another" (Rippon, 1988; Kwon-Chung and Bennett, 1992). Because this is not an accurate description of the non-septate hyaline tubes produced by *Ichthyophonus*, the term "pseudohyphae" should be dropped.

Conversely, the term "hyphae" (singular hypha) refers to both septate and non-septate non-cellular tubules that occur primarily in the fungi (Madigan et al., 2000; Stedman's Medical Dictionary, 2012). Following the death of the fish host as well as in vitro, *Ichthyophonus* schizonts produce a structure morphologically indistinguishable from a non-septate fungal hypha (Fig. 4), but it does not contain chitin or cellulose (Rand, 1994). Because the term "hyphae" describes a structure, rather than a life stage, it can be applied to similar structures in other organisms. Therefore, the term "hyphae" would be appropriate for this structure produced by *Ichthyophonus*. Similarly, "germ tube" is defined as "The initial hyphal outgrowth of a germinating spore or yeast" and also describes a structure that would be synonymous with "hypha" (Rippon, 1988; Kwon-Chung and Bennett, 1992).

Much of the terminology relating to *Ichthyophonus* has appeared in the scientific literature for over a century, and most investigators working with the organism intuitively understand what is meant by each term, and through convention and convenience continue to use them (including this author); however, some terms are inherently incorrect and should be changed. The purpose of this exercise is to attempt to standardize the

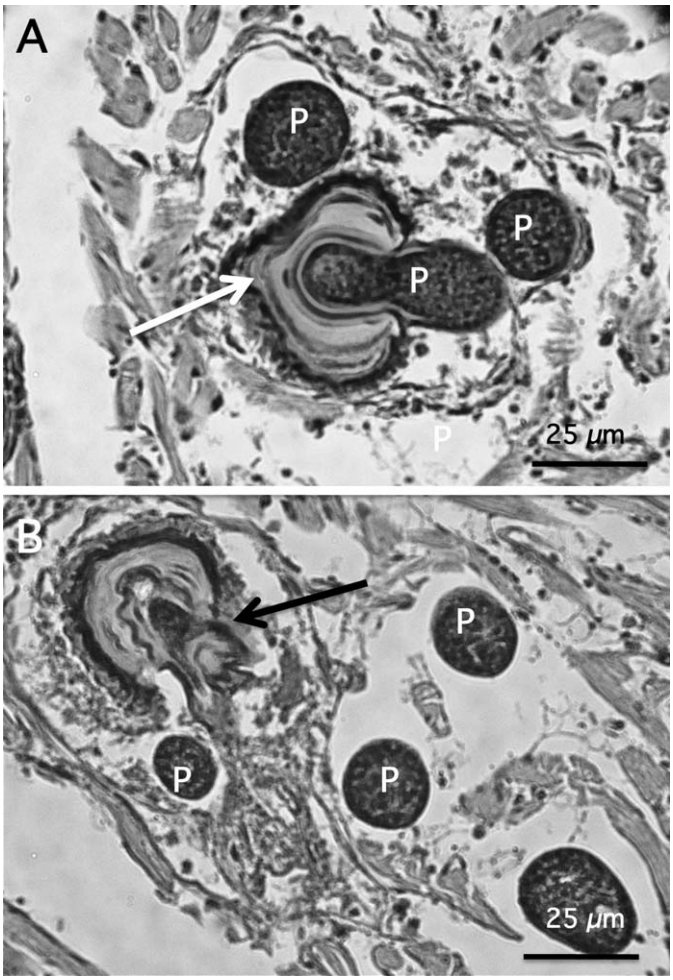

FIGURE 3. (**A**) Plasmodium (P) exiting a collapsed *Ichthyophonus* schizont (arrow) with two plasmodia free within the tissue. (**B**) Four plasmodia (P) migrating away from an empty schizont (arrow), host: Chinook salmon (*Oncorhynchus tshawytscha*).

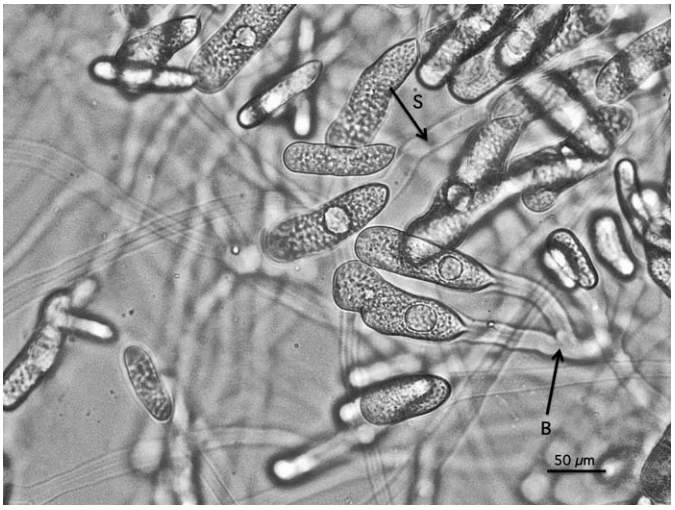

FIGURE 4. Single (S) and bifurcate (B) non-septate hyphae produced in *Ichthyophonus* explant culture. Multinucleate apical tips will detach, round-up, and become the next generation of schizonts; host: rainbow trout (*Onchorynchus mykiss*).

terminology associated with *Ichthyophonus* and to bring it into agreement with existing terminology used to define similar life stages of other protistan parasites.

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