



First Record of *Psorospermium* sp. (Class: Mesomycetozoea) in Northern Clearwater crayfish *Faxonius propinquus* Girard 1852 (Decapoda: Cambaridae) from Michigan, U.S.A.

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Psorospermium cf. *haeckeli* Hilgendorf 1883 is a unicellular, eukaryotic protozoan within the class Mesomycetozoea, phylogenetically situated near the animal-fungal divergence (Cavalier-Smith 1998; Ragan *et al.* 1998). Although only one species has been identified, there have been four morphotypes described in 17 species of crayfish from the Holarctic, Neotropical, and Australasian regions (Herbert 1987; Henttonen *et al.* 1992, 1994; Rug & Vogt 1994). However, molecular analyses of the internal transcribed spacer DNA suggest morphotypes may represent distinct species (Bangyeekhun *et al.* 2001).

The life cycle of *P.* cf. *haeckeli* has a free-living amoeboid stage and a host-dependent sporocyst stage (Vogt & Rug 1999). After the crayfish host dies, sporocysts are released into the environment where a spore receptacle emerges from the sporocyst. The spore receptacle then releases free-living amoeboids. After entering a crayfish, each amoeboid forms a sporocyst with a thin wall that thickens over time (Vogt & Rug 1999). Sporocysts have been found within the subepidermal connective tissue (Henttonen *et al.* 1992; Rug & Vogt 1994), ovarian epithelial tissue (Rudolph *et al.* 2007), and thoracic arteries (Rug & Vogt 1994). *Psorospermium* has been reported to affect the immune system of its crayfish host and has been shown to lead to mortality in aquaculture systems and therefore may represent a threat to crayfish populations (Söderhäll, 1988). Despite this knowledge, *Psorospermium* has only been described from a few locations in each of the regions it is found. For example, in the U.S., *Psorospermium* has been documented in Florida, Louisiana, Minnesota, Mississippi, Texas, and Wisconsin (Overstreet 1983; Lee *et al.* 1985; Henttonen *et al.* 1992, 1994). But because few locations have been sampled in these states, we may not know the full range of *Psorospermium* in the U.S.

Faxonius propinquus Girard 1852 is native to the Great Lakes region. Its range includes the lakes and their drainages, but extends south to Indiana, Illinois and into south-eastern Minnesota, and north-eastern Iowa and to the east its range extends to eastern Vermont (Fitzpatrick 1967). However, *F. propinquus* infections with *Psorospermium* have only been identified in Wisconsin (Henttonen *et al.* 1994). One reason for this limited association appears to be a lack of investigation into the range of *Psorospermium*. Here I describe, for the first time the presence of *Psorospermium* in *F. propinquus* from two river systems in the Upper Peninsula of Michigan.

In September of 2019, eight adult crayfish were submitted to the Parasitology Class at Northern Michigan University by local fishermen. Three came from the Au Train River (46.423363, -86.836181) in Alger County and 5 crayfish from the Chocolay river (46.50125, -87.35775) in Marquette County, both located in the Upper Peninsula of Michigan within 1.6 km from Lake Superior. Crayfish were collected by hand, stored in river water, and frozen in the laboratory at Northern Michigan University until subsequent dissections. Crayfish were identified to species according to Hobbs (1972).

To prepare for dissections, crayfish were set at room temperature to defrost. Because a majority of spores are found in the connective tissue beneath the carapace (Vogt and Rug, 1995), we focused on this region. The posterior end of the carapace was cut with scissors on the left side up to the anterior end. The carapace was then carefully peeled back to expose connective tissues and the organs of the cephalothorax. This cut was repeated on the right side. Pieces of the connective tissue, digestive gland, and heart were placed on a microscope slide and examined at 10 ×, 40 ×, and 100 × using an Olympus CH-30 compound microscope (Olympus, Tokyo, Japan). Forms of *P.* cf. *haeckeli* sporocysts were identified using Henttonen *et al.* (1997) and all terminology is according to Henttonen *et al.* (1994). Digital images of *P.* cf. *haeckeli* were taken with an iPhone 8S and the length, width, and cell wall width were obtained using ImageJ software and calibrated with the known ocular lens field of distance (Schneider *et al.* 2012). All morphologically variable forms were photographed.

Two of the 3 (67%) crayfish from the Au Train river site and 3 of 5 (60%) crayfish from the Chocolay river were infected with sporocysts of *P. cf. haeckeli*. Infections were only found in the connective tissue below the carapace. *P. cf. haeckeli* forms included a single, short American round pointed (herein “short form”), short American curved (herein “curved form”), and a long American round pointed (herein “long form”) (Fig. 1A). The short form was $107.3 \pm 6.4 \mu\text{m}$ ($102.8\text{-}111.8$) long and $44.7 \pm 2.5 \mu\text{m}$ ($42.9\text{-}46.5$) wide ($N = 4$) (Fig 1B). The cell wall was $10.1 \pm 0.9 \mu\text{m}$ ($9.4\text{-}10.7$). The curved form had an average length of $92.3 \pm 8.9 \mu\text{m}$ ($73.2\text{-}106.1$) and were $39.6 \pm 3.0 \mu\text{m}$ ($36.3\text{-}46.1$) with wall widths of $6.45 \pm 1.6 \mu\text{m}$ ($4.1\text{-}8.5$) ($N = 12$) (Fig. 1B). The long form length was $133.9 \pm 6.2 \mu\text{m}$ ($124.6\text{-}143.9$) and $44.1 \pm 2.6 \mu\text{m}$ ($39.9\text{-}51.3$) in width ($N = 25$). The cell wall was $10.9 \pm 1.4 \mu\text{m}$ ($8\text{-}13.5$). All measurements reported include standard deviation. All forms contained a spore receptacle containing lipid globules and non-lipid globules (Fig. 1C) however some of the long forms were undifferentiated and without a spore receptacle (Fig. 1D). Scrapings of connective tissue collected from beneath the carapace from a single infected crayfish from each site were deposited in the collection of the Harold W. Mater Laboratory of Parasitology (HWML), University of Nebraska State Museum, accession numbers HWML-112228 and HWML-112229.

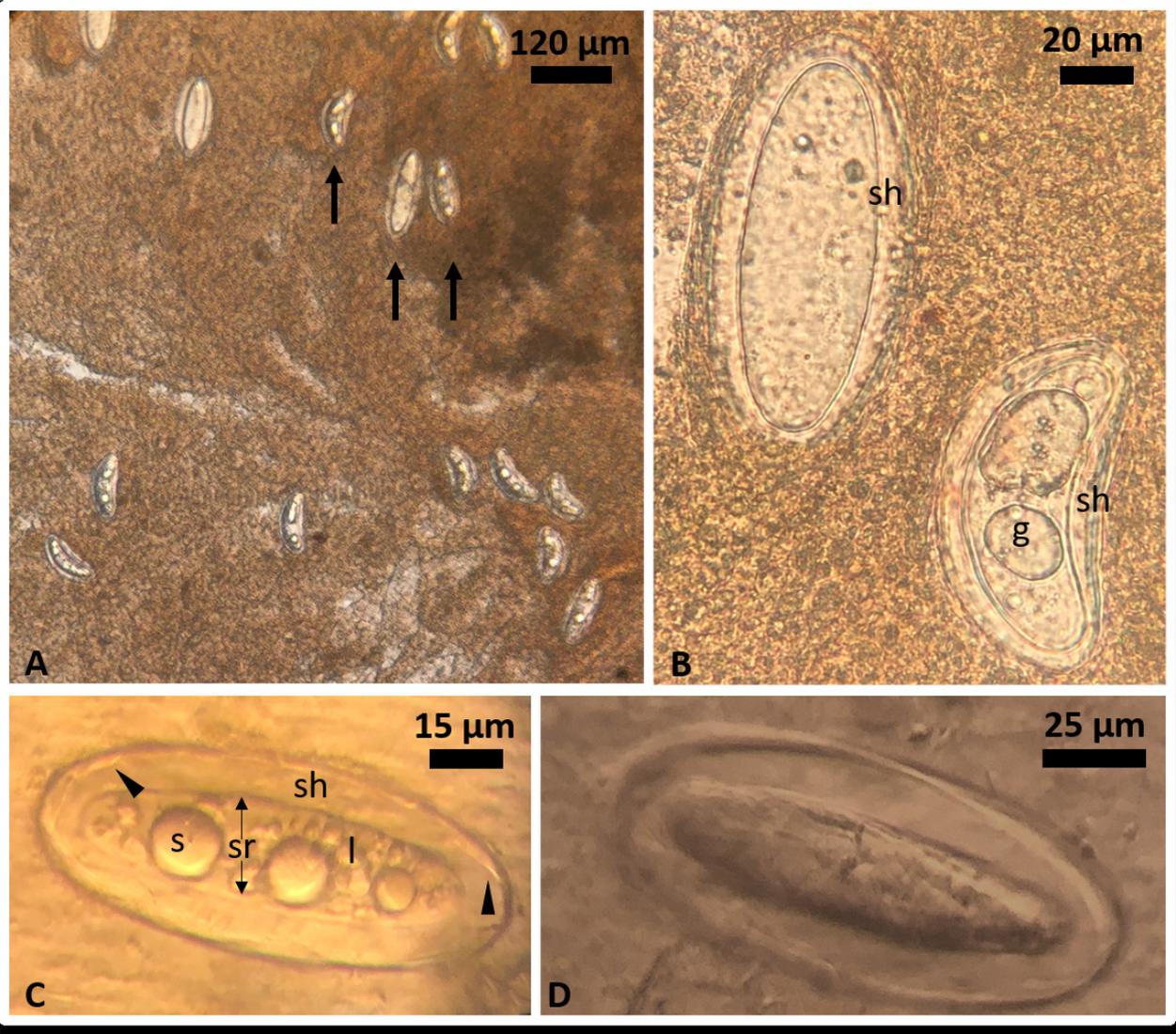


FIGURE 1. Connective tissue of *F. propinquus* with *Psorospermium cf. haeckeli* infection. (A) Photomicrograph showing density of *Psorospermium* spp. in tissue. Arrows, from left to right, represent curved form, long form, and short form. Magnification: 100 \times . (B) Unsporulated short form and curved form containing globules (g) covered by a cell wall (sh). Magnification: 400 \times . (C) Mature sporocyst (short form) with spore receptacle (sr) containing spores (S) and lipid globules (l). Arrowheads represent shell plates. Magnification: 400 \times . (D) Undifferentiated long form sporocyst viewed at 400 \times .

The most common forms of *P. cf. haeckeli* found are the long, short form and ovoid form (Henttonen *et al.* 1994; Vogt *et al.* 1995,1999; Henttonen *et al.* 1997), while the curved form has been found less commonly from Australia, S. America, and one location in the U.S. (Henttonen *et al.* 1994; Rudolph *et al.* 2007). Here I identify three forms (long, short, and curved) from a novel location in the Upper Peninsula of Michigan.

Morphotypes described by Henttonen *et al.* (1994) are based on several thousand specimens from North America that were identified by size. Our findings are consistent with the morphology and measurements obtained by Henttonen *et al.* (1994) but was not surprising since *P. cf. haeckeli* has been found in the rusty crayfish (*Orconectes rusticus*) in the neighboring state of Wisconsin, the only location in North America where the curved morphotype had been found (Henttonen *et al.* 1994). The less common curved form has been identified from crayfish in Australia and was previously known as the “Australian form.” However, since the curved form was previously found in Wisconsin and this study, it seems the curved form may be more common than previously thought.

It has been suggested that morphotypes of *P. cf. haeckeli* represent distinct species (Herbert, 1987; Henttonen *et al.* 1992, 1994; Rug and Vogt 1995). Rug and Vogt (1995) found two morphotypes of *P. cf. haeckeli* in crayfish found within two farmed populations of European crayfish. They suggested these morphotypes may represent distinct species based on observations of development and the presence of an envelope of host connective tissue around one morphotype but not the other. Rug and Vogt (1995) therefore acknowledged that further investigations into development stages are needed to determine if they are different species. In 2001, Bangyeekhun *et al.* found genetic divergence between two morphotypes from Europe and North America when the ribosomal internal transcribed spacer (ITS) 1 and ITS 2 regions were amplified but they also acknowledged that although they have distinct ribosomal ITS sequences, further studies on life cycle stages and morphology are needed. Using detailed morphological studies of life cycle stages combined with DNA barcoding, further studies can be done to determine if the morphotypes in this study and future studies are distinct species.

Future research of *Psorospermium* spp. would benefit from surveys across North America to gain an understanding of the full distribution of *P. cf. haeckeli* in crayfish hosts. Similarly, these investigations should include molecular barcoding methods from various locations to assess the genetic diversity as was found by Bangyeekhun *et al.* (2001). Because the highest diversity of crayfish occurs in North America, it is likely that *P. cf. haeckeli* is more common in N. America than previously described.

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