# **Underwater spore dispersal:** a preliminary report of aquatic invertebrates associated with *Psathyrella aquatica*

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In 2005 Robert Coffan, a hydrologist in southern Oregon, made what is believed to be the first discovery of an underwater mushroom. While several ascomycetes are known to grow and fruit underwater, for example *Vibrissea truncorum*, *V. filisporia*, *Cudoniella clavus*, no gilled mushroom had previously been known to do so. Consequently, this report was met with skepticism in the mycological community. Someone commented in an online forum that "it makes about as much sense as opening an umbrella underwater." It took Coffan two years of trying to contact mycologists to confirm his observation, before he convinced Darlene Southworth and me to take a look. Dr. Southworth and I had been studying truffles and ectomycorrhizal fungi associated with oaks and conifers in Oregon; when Robert contacted us, our field sites were bone dry, and we enthusiastically welcomed the opportunity to take a field trip in August. As unlikely as it may still sound (one reviewer for this article felt inclined to write, "I still have some reservation on the phenomenon of the underwater mushroom"), we arrived at the site and observed little brown mushrooms growing underwater. A few years later, based on morphological and molecular study, the underwater little brown mushroom was described as a new species, *Psathyrella aquatica*, and the phenomenon was reported in Frank et al. (2010). However, while this report contained abundant details and information, no spore dispersal mechanism was observed.

Figure 1. Mayfly collected on *P. aquatica*.

Figure 2. Caddisfly on *P. aquatica* with mirror reflection in situ.

Insects are known to consume a wide range of basidiomycete fungi and have been implicated as spore dispersal vectors (Fogel, 1975; Lilleskov and Bruns, 2005). As with truffles that fruit underground and require animals to dig them up and eat them to spread their spores, some dispersal vector is likely required to counter the constant flow of water downstream. Aquatic insects might be involved in this process as underwater mycophagists and dispersal vectors. To investigate this, I collected a few mushrooms to inspect for evidence of insects. Also, I used an underwater video camera to capture images that could be reviewed one frame at a time. Eventually, I donned neoprene gloves because the water is cold (7-10º C), making it difficult to hold the camera underwater for more than a few seconds. Even so, it was challenging to hold the camera steady in the current and to aim it in focus at the elusive little mushrooms. Despite these challenges, underwater videography enhanced my ability to observe the mushrooms and minimized the need for sporocarp collections.


Figure 3. Black fly larva on stipe of *P. aquatica*.

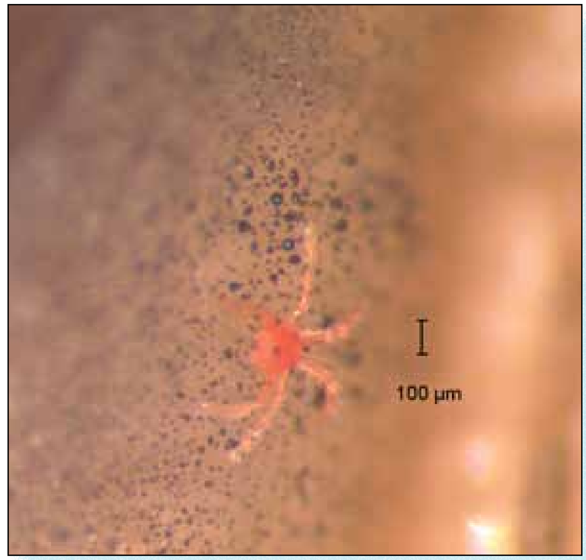


Figure 4. Red spider mite among gills of *P. aquatica*.



Each year since 2007, I have revisited the main fruiting site and observed fruiting bodies (Table 1). The underwater mushroom has been observed from early July to early October. I have also visited streams and rivers in southern and central Oregon, to look for new sites. However, lack of funding limits these surveys to only a few days per year. The Rogue River upstream from Prospect, and Union Creek Oregon in Jackson Co. (approximately 20 km) as well as the Breitenbush River and French Creek in Marion Co. (approximately 20 km) have been surveyed repeatedly. The underwater mushroom is now listed as a sensitive species by the Oregon Biodiversity Information Center (ORBIC 2010), however survey protocols have yet to be established or tested. My survey method included targeting sections of rivers and streams with *Alnus rubra* (red alder) growing nearby. I have also responded to several reports of underwater mushrooms from amateurs, finding them to be either gilled mushrooms that have been inundated by rising waters or one of the ascomycetes mentioned above.

I first observed aquatic insect larvae associating with *P. aquatica* in 2009 when I collected three fruiting bodies and later inspected them under a microscope. To my surprise, two black fly larvae emerged from the cap of one specimen, and a mayfly naiad from another. After visiting the Rogue River site six times in September and October of 2011, I obtained video images of aquatic invertebrates associating with the underwater mushroom. These video data were first presented at the Ashland Independent Film Festival in the short documentary, *AQUATICA: The Underwater Mushroom* (Frank, 2013). Mayflies, with their distinctive caudal filaments, were stationed on the upper stipe of several mushrooms (Figure 1). I also captured images of a caddisfly, replete with casing, crawling along the upper stipe of one mushroom (Figure 2). I brought specimens of the mayfly, caddisfly, black fly larvae (Figure 3) and a red spider mite (Figure 4), back to the laboratory to examine for the presence of any *P. aquatica* spores within or adhering to the exterior of their bodies. I made slides of the caddisfly and mayfly guts, and slides of entire black flies and red spider mites, as they were too small to dissect. Distinctive ellipsoid smooth brown spores of *P. aquatica* were observed from slides made of the guts of the caddisfly and the mayfly, and from mounts of the entire black flies (Figure 5). The red spider mite, though collected from spore-covered gills, did not have spores adhering to its exoskeleton.

These observations suggest that aquatic insects are involved in spore dispersal, either as mycophagists, grazers, or filter feeders collecting spores as they move along the mushrooms underwater. While more data will be needed to confirm the roles of these invertebrates, aquatic insects

| year | # fb observed | # stems w/o caps | # fb collected | aquatic invertebrates | *Psathyrella* spores |
|---|---|---|---|---|---|
| 2005 | 20 | NA | 8 | NA | NA |
| 2006 | NA | NA | 0 | NA | NA |
| 2007 | 28 | NA | 8 | NA | NA |
| 2008 | 2 | NA | 0 | NA | NA |
| 2009 | 24 | NA | 3 | black fly (Diptera)<br>mayfly (Ephemeroptera) | Y<br>N |
| 2010 | 18 | 3 | 3 | NA | NA |
| 2011 | 21 | 5 | 3 | caddisfly (Tricoptera)<br>mayfly (Ephemeroptera)<br>red spider mite (Arthropoda) | Y<br>Y<br>N |
| 2012 | 16 | 4 | 0 | NA | NA |

Table 1. Number of fruiting bodies observed *in situ* in upper Rogue River 2005-2012. Stems without caps were first observed in 2010. Aquatic insects were observed in 2009 and 2011. Data from 2005 were reported by R. A. Coffan. No data were collected in 2006.

have the ability to counter the flow of water downstream and potentially move spores upstream to new suitable habitats. Additionally, the insects can be consumed by fish or birds and these may distribute spores even further.

In addition to collecting and videographing aquatic invertebrates associated with *P. aquatica*, I observed another physical phenomenon that may pertain to the ability of this underwater mushroom to stay in one place. These mushrooms did not fall over and decompose quickly. Near mature fruiting bodies, older mushrooms had lost their caps, and the mushrooms had been reduced to softening stipes undulating in the current, trapping debris and spores among the abundant stipe fibrils. Not far from these undulating structures, detached caps were lodged in the vegetative debris and sediments of the river bottom. This pattern of caps detaching and the slow physical deterioration of stipes may provide a redundant mechanism for maintaining inoculum in suitable habitats. Figure 6 illustrates the life cycle of *P. aquatica*.

While these observations suggest viable mechanisms for spore dispersal for the new underwater mushroom, *P. aquatica*, the functional roles of this underwater mushroom in aquatic ecosystems remain to be more thoroughly investigated. Since *P. aquatica* has thus far only been observed in the upper Rogue River, this underwater fruiting ability may be obligately associated with the cold, clear oxygenated section of the upper Rogue River with its volcanic sediments and small diameter alder debris or with river systems with similar conditions. Further surveys to establish the range of this species will be essential for determining the relevant conditions for this unusual fruiting phenomenon.

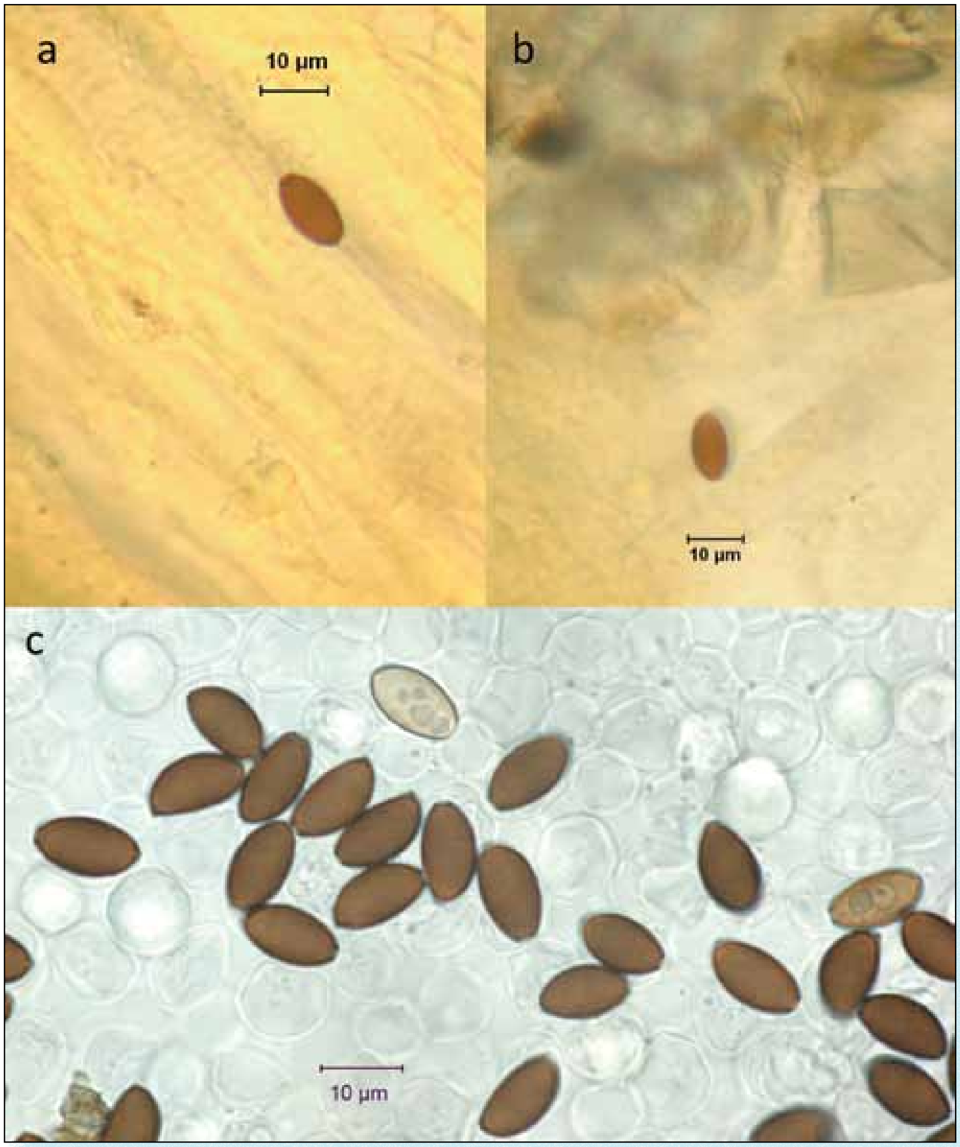


Figure 5. Spores of *P. aquatica* a) from gut of caddisfly, b) from gut of mayfly and c) from fruiting body.

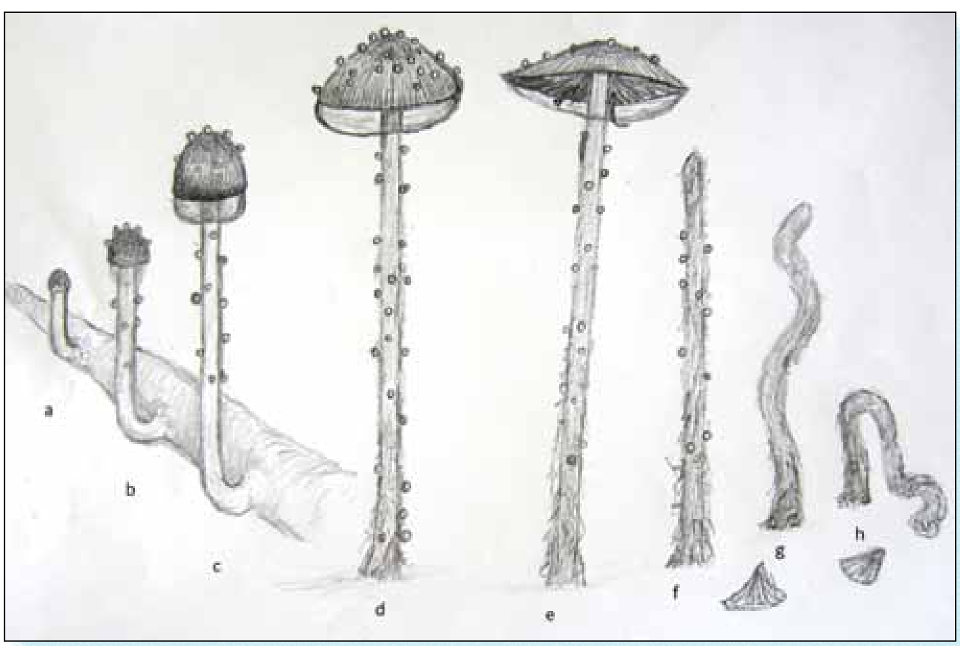


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Figure 6 (lower left). Basidiocarp development in *Psathyrella aquatica*: a-b) young basidiocarp with pileus slightly wider than stipe and cottony evanescent partial veil, c) veil is lost; cap is incurved and narrowly convex; gas collects beneath pileus; small gas bubbles adhere to hyphae, d) pileus enlarges, convex to campanulate; gas bubble beneath pileus enlarges and undulates in current; smaller gas bubbles continue to adhere to pileus and stipe; fibrils of lower stipe trap suspended sediments, e) cap broadens; spores mature; aquatic insect larvae and naiads present, some in the gills and on upper stipe f) stipe standing in current after cap detaches and falls into tangle of vegetation and debris below, g) stipe softens and undulates in current and h) falls back into substrate.