Mycologia, 102(1), 2010, pp. 93–107. DOI: 10.3852/07-190

Aquatic gilled mushrooms: *Psathyrella* fruiting in the Rogue River in southern Oregon

Jonathan L. Frank

Department of Biology, Southern Oregon University, Ashland, Oregon 97520

Robert A. Coffan

Department of Environmental Studies, Southern Oregon University, Ashland, Oregon 97520

Darlene Southworth[1]

Department of Biology, Southern Oregon University, Ashland, Oregon 97520

Abstract: A species of *Psathyrella* (Basidiomycota) with true gills has been observed fruiting underwater in the clear, cold, flowing waters of the upper Rogue River in Oregon. Fruiting bodies develop and mature in the main channel, where they are constantly submerged, and were observed fruiting over 11 wk. These mushrooms develop underwater, not on wood recently washed into the river. Substrates include water-logged wood, gravel and the silty riverbed. DNA sequences of the ITS region and a portion of the ribosomal large subunit gene place this fungus in *Psathyrella sensu stricto* near *P. atomata*, *P. fontinalis* and *P. superiorensis*. Morphological characters distinguish the underwater mushroom from previously described species. Fruiting bodies have long fibrillose stipes with small diameter caps. Immature stages have a thin veil that is soon lost. Gills lack reddish edges. Cystidia are ventricose with subacute apices. Spores were observed as wedge-shape rafts released into gas pockets below the caps. Underwater gills and ballistospores indicate a recent adaptation to the stream environment. This particular river habitat combines the characteristics of spring-fed flows and cold, aerated water with woody debris in shallow depths on a fine volcanic substrate. Based on molecular and morphological evidence we conclude that the underwater mushrooms are a new species, *Psathyrella aquatica*. This report adds to the biodiversity of stream fungi that degrade woody substrates. The underwater environment is a new habitat for gilled mushrooms.

Key words: Agaricales, aquatic fungi, ballistospores, Psathyrellaceae, psychrophilic fungi, stream fungi

Submitted 8 Nov 2007; accepted for publication 11 Jun 2009.

[1] Corresponding author. E-mail: southworth@sou.edu

INTRODUCTION

Mushrooms with true gills have been observed fruiting underwater in the clear, cold, flowing waters of the upper Rogue River on the western flanks of Crater Lake in Oregon. Aquatic mushrooms first were observed (by R.A. Coffan) in the North Fork of the Rogue River in Jul 2005. Specimens were collected Jul–Sep 2005, 2007 and 2008. These are truly underwater mushrooms and not mushrooms fruiting on wood recently washed into the river. They grow in the main channel, where they are constantly submerged at depths up to 0.5 m. Stipes are erect and attached to substrates including alluvial gravel, silt and woody debris. The Rogue River does not run dry; the habitat is continuously inundated. At the time of year when these aquatic fungi fruit, the land nearby is dry and no similar fruiting bodies occur.

Aquatic fungi in freshwater commonly include members of the Oomycota and Chytridiomycota, as well as aquatic hyphomycetes that are anamorphic stages of Ascomycota and Basidiomycota (Shearer et al 2004, Shearer et al 2007). The ascomycete *Vibrissea truncorum* (Alb. & Schwein.) Fr. fruits submerged on wood in cold running water; its spores are thread-like and dispersed underwater (Mains 1956, Tylutki 1979). Other Ascomycota fruit on submerged wood in lakes in Japan, Thailand and Costa Rica (Minoura and Muroi 1978, Pinruan et al 2004, Ferrer et al 2008). A basidiomycete with a smooth hymenium, *Gloiocephala aquatica* Desjardin, Martinez-Peck & Rajchenberg, that forms submerged basidiocarps has been reported from lakes and ponds in southern Argentina (Desjardin et al 1995). Basidiocarps of 11 species of homobasidiomycetes occur in marine ecosystems (Hibbett and Binder 2001). These basidiocarps are cyphelloid, minute enclosed cups or spheroids. None are gilled mushrooms.

The aquatic gilled mushrooms from southern Oregon appear to represent a novel taxon within the Psathyrellaceae in the large polyphyletic genus *Psathyrella* (Smith 1972, Padamsee 2008). Based on morphological characters and DNA sequences we propose it as a new species in *Psathyrella*.

MATERIALS AND METHODS

Site.—Submerged basidiocarps were collected underwater in the Rogue River at 42°51′42″N, 122°30′28″W, 900 m elevatioin, approximately 45 km downstream from Boundary Springs, the predominant source of water for the upper reaches of the Rogue River in the Rogue River-Siskiyou National Forest (CES 2006). At this site base flow is relatively high and constant during the summer. Streamflow data (1930–1952) from the nearest USGS gauging station (14327500) 6 km downstream from the site indicate a mean monthly flow in September of 8.4 (SD, 1.6) cubic meters per second (cms) (OWRD 2007). The lowest flow rate recorded 1930–1952 was 5.1 cms, an order of magnitude greater than in nearby streams not fed by springs. Mean monthly high flow for this gauging station was 25.3 cms. Real-time streamflow data from gauging station USGS 14330000, 20 km downstream on the main stem of the Rogue River, showed annual water at 2–14 C with diurnal fluctuations of 1.5 C in winter and 3 C in summer (OWRD 2007). Stream water samples, collected 21 Aug 2007, were analyzed for nitrate, total phosphorus and total organic carbon at Neilson Research Corp., Medford, Oregon (www.nrclabs.com).

Collection.—Basidiocarps were photographed in the river, collected and measured in 2005, 2007 and 2008. Some were photographed in situ; others were collected and transported to the lab without exposure to air. Observations and measurements were made on fresh specimens. Pilei were placed over paper to capture spore prints. Specimens were observed with a Leica MZ75 dissecting microscope and Leica DMLB compound microscope. Images were captured with SPOT-RT digital cameras and software. Gill tissue was stained with Melzer's reagent and treated with 5% KOH and H_2SO_4. Specimens were compared to descriptions in Smith (1972), Kits van Waveren (1985), Hansen and Knudsen (1992), Breitenbach and Kränzlin (1995), Gibson (2007) and Larsson and Örstadius (2008). Terminology of fruitbody characters follows Largent et al (1977). Nonstandardized color names in lowercase are followed by parenthesized Munsell (1976) alphanumeric color references. Herbaria abbreviations follow Holmgren and Holmgren (1998).

We used the following general procedure to classify the underwater mushrooms within genus *Psathyrella*, which currently includes 414 species known from North America (Smith 1972). We described our collections on the basis of macromorphology, micromorphology, habit, habitat and DNA sequences. We used the keys of Smith (1972) and Breitenbach and Kränzlin (1995) and molecular phylogeny of Padamsee et al (2008) to identify a group of species most similar to our specimens that we then used for more detailed morphological comparisons. We also selected additional species for DNA sequence comparisons on the basis of morphological characteristics. This iterative approach was employed due to the publication of two large datasets of *Psathyrella* DNA during the preparation of this manuscript (Padamsee et al 2008, Vašutová et al 2008). Here we use the provisional name, *Psathyrella aquatica*, to refer to our collections of underwater mushrooms.

Herbarium specimens were obtained from the University of Michigan Fungus Collection (MICH) http://www.herb.lsa.umich.edu/Bioinformatics.htm, from the Oregon State University Mycological Collection (OSC) http://ocid.nacse.org/research/herbarium/myco/databases.html, and from M. Padamsee, University of Minnesota (MIN) (TABLE I). In addition to *P. aquatica* a total of 89 collections in 33 species were examined.

Molecular methods.—DNA was extracted from 12 fresh pileus or stipe tissues of *Psathyrella aquatica* and from herbarium specimens of 28 other *Psathyrella* species that were related morphologically (subgenus *Psathyrella* section *Psathyrella*) or by DNA sequences, as compared to the phylogenetic tree in Padamsee et al (2008) or that were distributed in Oregon and Washington (TABLE I). Tissue samples were stored in buffer (0.1 M Tris, 0.3 M NaCl, 0.04 M EDTA) at 4 C, extracted in 2% cetyltrimethyl ammonium bromide (CTAB) with chloroform. In addition lyophilized CTAB phenol-chloroform extracts of three species, *P.* aff. *brooksii* (initially identified as *P. brooksii*), *P. atomata* and *P. ramicola*, were provided by M. Padamsee. Because the ITS region of the specimen originally identified as *P. brooksii* by Padamsee et al (2008) was found to differ by more than 5% from the *P. brooksii* holotype we use the nomenclature *P.* aff. *brooksii* to refer to specimen Padamsee 098 (MIN) at the recommendation of M. Padamsee (pers comm).

DNA was amplified in polymerase chain reactions (PCR) with fungal primer ITS1F (5′-ggtcatttagaggaagtaa-3′) and universal eukaryote primer TW14 (5′-gctatcctgagggaaacttc-3′) (White et al 1990; Gardes and Bruns 1993, 1996). PCR reactions (20 μL) were performed with 0.6 units GoTaq and 4 μL 5× colorless buffer (Promega), 200 μM each dNTP, 0.3 μM each primer, 2.5 mM $MgCl_2$, and 2 μL undiluted DNA template. An initial 3 min at 93 C was followed by 30 cycles of 30 s at 95 C, 2 min at 54 C, and 3.5 min at 72 C, with a final cycle 10 min at 72 C. When necessary shorter fragments from older herbarium specimens were amplified with fungal primer pairs ITS1F and ITS4 (5′-tcctccgcttattgatatgc-3′) for the ITS and ITS4r (5′-gcaatatcaataagcggagga-3′) and TW14 for the 28S region; 20 μL PCR reactions were amplified as above with the annealing temperature reduced to 51 C and the extension time reduced to 2 min. The primer ITS4r was designed as the reverse complement of ITS4. PCR products were electrophoresced on 1.5% agarose gels, stained with ethidium bromide (1 mg/mL) and viewed under a Kodak EDAS 290 UV transilluminator.

PCR products were purified with QIAquick PCR Purification kits (QIAGEN, Valencia, California), prepared with BigDye Terminator Ready Reaction Mix 3.1 and sequenced in an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, California) in the Biotechnology Center at Southern Oregon University. Molecular data were obtained by sequencing the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene and ITS2, and part of the 28S ribosomal gene, with forward primers ITS1F, ITS1 (5′-tccgtaggtgaacctgcgg-3′), ITS3 (5′-gcatcgatgaagaacgcagc-3′) and ITS4r, and reverse primers ITS4, TW13 (5′-ggtccgtgtttcaagacg-3′) and TW14.

Sequences were edited with Chromas 1.45 (McCarthy 1998); contigs were assembled in Sequencher 4.7 (Gene Codes Corp. Ann Arbor, Michigan) and compared to other fungal ITS and 28S sequences in GenBank with BLAST (Altschul et al 1990). Clustal X was used to generate

TABLE I. Collections of *Psathyrella* species examined, with collector, number, herbarium, collection date, state in which collected (USA), habitat and GenBank numbers for ITS and 28S regions

Psathyrella species	Collector No./ herbarium	Date	State	Habitat	GenBank ITS	GenBank 28S
P. aquatica	R.A. Coffan (D. Southworth 1086)/SOC	4 Jul 2005	OR	Underwater in the Rogue River		
P. aquatica	R.A. Coffan (D. Southworth 1087/SFSU	4 Jul 2005	OR	Underwater in the Rogue River	EU664989	
P. aquatica	R.A. Coffan (D. Southworth 1088)/OSC	4 Jul 2005	OR	Underwater in the Rogue River		
P. aquatica	R.A. Coffan (D. Southworth 1089)/OSC	4 Jul 2005	OR	Underwater in the Rogue River	EU664990	
P. aquatica	R.A. Coffan (D. Southworth 1090)/MICH	4 Jul 2005	OR	Underwater in the Rogue River	EU664991	EU664994
P. aquatica	R.A. Coffan (D. Southworth 1091)/MICH	4 Jul 2005	OR	Underwater in the Rogue River		
P. aquatica	R.A. Coffan (D. Southworth 1092)/MICH	4 Jul 2005	OR	Underwater in the Rogue River		
P. aquatica	R.A. Coffan (D. Southworth 1093)/MICH	4 Jul 2005	OR	Underwater in the Rogue River		
P. aquatica	J.L. Frank 1334/MICH	14 Aug 2007	OR	Underwater in the Rogue River	EU259194	EU259195
P. aquatica	J.L. Frank 1335/MICH	14 Aug 2007	OR	Underwater in the Rogue River	EU259196	
P. aquatica	R.A. Coffan (D. Southworth 1096)/MICH	14 Aug 2007	OR	Underwater in the Rogue River		
P. aquatica	R.A. Coffan (D. Southworth 1097)/MICH[a]	14 Aug 2007	OR	Underwater in the Rogue River	EU259192	EU259193
P. aquatica	J.L. Frank 1336/MICH	21 Aug 2007	OR	Underwater in the Rogue River		
P. aquatica	J.L. Frank 1337/SFSU	21 Aug 2007	OR	Underwater in the Rogue River		
P. aquatica	R.A. Coffan (D. Southworth 1100)/MICH	21 Aug 2007	OR	Underwater in the Rogue River		
P. aquatica	D. Southworth 1101, /MICH	21 Aug 2007	OR	Underwater in the Rogue River		
P. aquatica	J.L. Frank 1347/OSC	21 Sep 2007	OR	Underwater in the Rogue River		
P. aquatica	J.L. Frank 1348/OSC	21 Sep 2007	OR	Underwater in the Rogue River		
P. aquatica	D. Southworth 1261/MICH	4 Sep 2008	OR	Underwater in the Rogue River		
P. alluviana A.H. Sm.	A.H. Smith 19272/MICH[a]	30 Sep 1944	OR			
P. alluviana A.H. Sm.	A.H. Smith 28232/MICH	25 Oct 1947	OR	Meadow		
P. alluviana A.H. Sm.	A.H. Smith 23782/MICH	27 Sep 1946	OR	On debris, vine maple forest		
P. alluviana A.H. Sm.	A.H. Smith 30217/MICH	17 Aug 1948	WA	On debris of *Betula papyrifera*	FJ899609	FJ899627
P. alnicola A.H. Sm.	A.H. Smith 70222/MICH[a]	6 Sep 1964	ID	Under *Alnus*		
P. alnicola A.H. Sm.	A.H. Smith 70223/MICH	6 Sep 1964	ID	Under *Alnus*		
P. alnicola A.H. Sm.	E. Trueblood 162/MICH	7 May 1957	ID			
P. alnicola A.H. Sm.	E. Trueblood 2280/MICH	19 Sep 1963	ID	Coniferous forest near rotting *Populus*		

TABLE I. Continued

Psathyrella species	Collector No./ herbarium	Date	State	Habitat	GenBank ITS	GenBank 28S
P. alnicola A.H. Sm.	E. Trueblood 2680/MICH	16 Jun 1967	ID	*Juniperus*		
P. alnicola A.H. Sm.	E. Trueblood 1439/MICH	5 Jun 1961	ID	Coniferous forest		
P. alnicola A.H. Sm.	E. Trueblood 1458/MICH	5 Jun 1961	ID	Debris along creek, *Salix* and *Alnus* leaves		
P. atomata (Fr.) Quel.	J.S. Hopple 139/DUKE		NC		FJ899610	
P. atomata (Fr.) Quel.	N.S. Weber 2949/MICH	12 Jun 1972	ID	On damp soil under *Salix* along creek		
P. atomata (Fr.) Quel.	N.S. Weber 2948/MICH	12 Jun 1972	ID	On dirt under *Salix* and low herbs along small stream		
P. atomata (Fr.) Quel.	A.H. Smith 8565/MICH	22 Jun 1938	MI	On wet soil		
P. atomata (Fr.) Quel.	A.H. Smith 78106/MICH	15 Sep 1969	MI	On soil under weeds		
P. atomata (Fr.) Quel.	A.H. Smith 43070/MICH	23 Sep 1953	MI	Grass		
P. atomata (Fr.) Quel.	A.H. Smith 74433/MICH	4 Jul 1967	MI	On wet soil		
P. atomata (Fr.) Quel.	A.H. Smith 74436/MICH	4 Jul 1967	MI	On wet soil		
P. atomata (Fr.) Quel.	C.H. Kauffman/MICH 47963	22 Jul 1912	MI	Lawn		
P. atomata (Fr.) Quel.	K. McKnight F1009/MICH	30 Jul 1955	UT	Soil in *Populus-Abies* forest		
P. atomata (Fr.) Quel.	K. McKnight F1565/MICH	21 Aug 1956	UT			
P. brachycystis A.H.Sm.	T. E. Brooks 1605/MICH[a]	1 Sep 1946	KS	Terricolous		
P. aff. *brooksii*	M. Padamsee 098/MIN	3 Jan 2003	WA	On wood chips under *Cornus*	EU664992	
P. brooksii A.H. Sm.	T. E. Brooks 1594/MICH[a]	2 Sep 1946	KS	Terricolous next to pile of corn cobs	EU664993	EU664995
P. calvinii A.H. Sm.	C.H. Kaufman/MICH 11891[a]	9 Sep 1923	WY			
P. calvinii A.H. Sm.	A.H. Smith 34788/MICH	13 Jul 1950	WY	On soil by road	FJ899611	
P. calvinii A.H. Sm.	R. Leach 6/MICH	Jan 1944	CA	On sandy soil covered by grass along creek		
P. candolleana (Fr.) Maire.	J.M. Trappe 19657/OSC	17 Apr 1997	OR		FJ899612	
P. carbonicola A.H.Sm.	S. Carpenter CH-186/OSC	22 Sep 1980	WA	Blow-down area near dead forest		
P. caudata (Fr.) Quel.	Smith A.H. 35985/MICH	19 Sep 1950	MI			
P. caudata (Fr.) Quel.	C.H. Kauffman/MICH 32909	28 Sep 1922	OR	On dung and debris near barn		
P. caudata (Fr.) Quel.	S. Lundell 1770/MICH	29 Sep 1940	Sweden	On grassy slope close to farmyard		

TABLE I. Continued

Psathyrella species	Collector No./ herbarium	Date	State	Habitat	GenBank ITS	GenBank 28S
P. coloradensis A.H. Sm.	A.H. Smith 51659/MICH[a]	3 Aug 1956	CO	On debris		FJ899628
P. conopilea (Fr.) Pearson & Dennis	T. O'Dell 174/OSC	1 May 1990	OR		FJ899613	
P. filamentosa A.H. Sm.	A.H. Smith 78074/MICH[a]	13 Sep 1969	MI	On mud flats		
P. fontinalis A.H. Sm.	A.H. Smith 25644/MICH[a]	12 Jul 1947	MI	On black muck in low area among elm and ash		
P. fontinalis A.H. Sm.	A.H. Smith 25652/MICH	12 Jul 1947	MI	Muck	FJ899614	FJ899629
P. fontinalis A.H. Sm.	A.H. Smith 28751/MICH	15 Jun 1948	MI	Muck		
P. fontinalis A.H. Sm.	A.H. Smith 28753/MICH	15 Jun 1948	MI	Muck		
P. gracilis (Fr.) Quel.	S. Pittam 170/OSC	5 Jun 2000	OR			
P. cf. *gracilis*	J.L. Frank 1307/SOC	27 Apr 2007	OR	Terrestrial in oak woodland	FJ235146	
P. hydrophila (Fr.) Maire	S.M. Zeller/OSC 5951	16 Nov 1921	OR	Rotting wood		
P. hydrophila (Fr.) Maire	J.M. Trappe 22479/OSC	3 Dec 1997	OR	Under *Pseudotsuga menziesii*	FJ899615	
P. intermedia (Pk.) A.H. Sm.	A.H. Smith 3563/MICH	12 Aug 1950	WY	On moss in spring		FJ899630
P. intermedia (Pk.) A.H. Sm.	W. Gruber 5/MICH	Jan 1944	CA	Growing from rotting wood	FJ899616	
P. marcescibilis (Britz.) Singer	J.M. Trappe 19674/OSC	4 Dec 1997	OR		FJ899617	
P. nitens A.H. Sm.	A.H. Smith 30388/MICH[a]	21 Aug 1948	WA	On debris	FJ968757	FJ899631
P. nitens A.H. Sm.	A.H Smith. 29526/MICH	28 Jul 1948	WA			
P. nitens A.H. Sm.	A.H. Smith 29602/MICH	29 Jul 1948	WA	On debris		
P. nitens A.H. Sm.	A.H. Smith 29642/MICH	30 Jul 1948	WA			
P. nitens A.H. Sm.	A.H. Smith 30175/MICH	16 Aug 1948	WA	On humus		
P. nitens A.H. Sm.	A.H. Smith 30239/MICH	18 Aug 1948	WA	On debris		
P. nitens A.H. Sm.	A.H. Smith 30241/MICH	18 Aug 1948	WA	On *Alnus* debris	FJ968757	
P. opacipes A.H. Sm.	A.H. Smith 4990/MICH[a]	2 Jul 1936	MI			FJ899632
P. opacipes A.H. Sm.	A.H. Smith 25280/MICH	23 Jul 1947	MI			
P. oregonensis A.H. Sm.	A.H. Smith 28182/MICH[a]	24 Oct 1947	OR	On rotten conifer wood		
P. parvicystis A.H. Sm.	A.H. Smith 18305/MICH[a]	02 Jun 1942	MI	On muck under aspen		

TABLE I. Continued

Psathyrella species	Collector No./ herbarium	Date	State	Habitat	GenBank ITS	GenBank 28S
P. parvicystis A.H. Sm.	A.H. Smith 26004/MICH	27 Jul 1947	MI	On mud in roadway		
P. parvicystis A.H. Sm.	V. Potter 4834/MICH	16 Jun 1948	MI	On & along side of hardwood limb		
P. parvicystis A.H. Sm.	E.B. Mains 6134/MICH	21 Aug 1941	MT	On wet soil		
P. praeatomata A.H. Sm.	A.H. Smith 9-28-69/MICH[a]	28 Sep 1969	MI	On mud		
P. prona (Fr.) Gillet	J.M. Trappe 19678/OSC	17 Apr 1997	OR		FJ899618	
P. prona (Fr.) Gillet	L.R. Hesler 19032/MICH	9 Jul 1949	NC	Horse dung and rich soil		
P. prona (Fr.) Gillet	H.C. Beardslee Jr. 683/MICH	19 Jun 1901	OH			
P. prona (Fr.) Gillet	A.H. Smith 13859/MICH	29 May 1939	WA	On manure pile and soil in farm yard		
P. prona (Fr.) Gillet	A.H. Smith 14080/MICH	5 Jun 1939	MI	On straw and dung pile		
P. prona (Fr.) Gillet	A.H. Smith 14193/MICH	9 Jun 1939	MI	On dung and soil in farmyard		
P. prona (Fr.) Gillet	A.H. Smith 14782/MICH	5 Jul 1939	MI	On dung and straw pile		
P. prona (Fr.) Gillet	J.B. Flett/MICH 33314	8 Apr 1941	MI	On grassy area by road		
P. prona (Fr.) Gillet	W.B. Cooke 19881/MICH	9 Jun 1947	MI	On manure in apple orchard		
P. prona (Fr.) Gillet	W.B. Cooke 19882/MICH	9 Jun 1947	MI	On manure in apple orchard		
P. prona (Fr.) Gillet	A.H. Smith 35271/MICH	29 Jul 1950	WY			
P. quercicola A.H. Sm.	A.H. Smith 55689/MICH[a]	15 Nov 1956	OR	On *Quercus* stump		
P. quercicola A.H. Sm.	A.H. Smith 55690/MICH	15 Nov 1956	OR	On *Quercus* log		
P. quercicola A.H. Sm.	A.H. Smith 55377/MICH	10 Nov 1956	OR	On mossy *Quercus* log		
P. rainierensis A.H. Sm.	A.H. Smith 30929/MICH[a]	5 Sep 1948	WA		FJ899619	
P. ramicola A.H. Sm.	P.B. Matheny 871/WTU	1 Oct 1946	WA		FJ899620	
P. rogueiana A.H. Sm.	A.H. Smith 55708/MICH[a]	16 Nov 1956	OR	On clay soil along logging road		
P. subincarnata A.H. Sm.	A.H. Smith 63594/MICH[a]	21 Jul 1961	MI	On mud under *Pinus*	FJ899621	FJ899633
P. subolivacea A.H. Sm.	A.J. Smith 11042/MICH[a]	23 Sep 1938	MI			
P. subolivacea A.H. Sm.	A.J. Smith 4991/MICH	2 Oct 1936	MI	Scattered, on *Quercus* leaves		
P. subolivacea A.H. Sm.	C. Nimke 150/MICH	11 Oct 1971	MI	On chip dirt		
P. superiorensis A.H. Sm.	J.F. Ammirati 2251/MICH[a]	14 Aug 1968	MI	Scattered in drying drainage pond	FJ899623	FJ899634
P. superiorensis A.H. Sm.	A.H. Smith 32107/MICH	24 May 1949	MI	On humus and sawdust	FJ899622	

TABLE I. Continued

Psathyrella species	Collector No./ herbarium	Date	State	Habitat	GenBank ITS	GenBank 28S
P. tenera Peck	A.H. Smith 65853/MICH	19 Aug 1962	ID	On mud	FJ899624	FJ899635
P. tenera Peck	A.H. Smith 29601/MICH	29 Jul 1948	WA	On wet earth		
P. uskensis A.H.Sm.	A.H. Smith 73377/MICH	14 Sep 1966	WA	On mud	FJ899625	FJ899636
P. velutina (Fr.) Singer	S. Carpenter CH-186/OSC	22 Sep 1982	WA	Mount St Helens	FJ899626	FJ899637

[a] Holotype.

alignments of the 28S region. Alignments were edited manually with BioEdit (Thompson et al 1997, Hall 1999). Sequences generated in this study have been deposited in GenBank (TABLE I). A total of 27 sequences were aligned, 12 that we generated and 15 from GenBank. All were in *Psathyrella sensu stricto* Clade A v, except *Psathyrella melleipallida* and *P. tephrophylla* from Clade A iv, which were used as outgroup (Padamsee et al 2008). All but one were from North America; the European taxon (an unidentified species) closest to *P. aquatica* was included (Vašutová et al 2008).

Phylogenetic trees built with parsimony and maximum likelihood with 1000 bootstrap replicates and 1000 jackknife replicates were generated from 28S sequences using PAUP 4.10b10 (Swofford 2002). Consensus trees with 50% majority rule were generated with a tree-bisection-reconnection branch swapping algorithm. All characters were given equal weight; gaps were treated as missing. Concensus trees were examined to confirm branch positions.

TAXONOMY

Psathyrella aquatica J.L. Frank, Coffan, & Southworth, sp. nov. FIGS. 1–10
Mycobank: MB511824

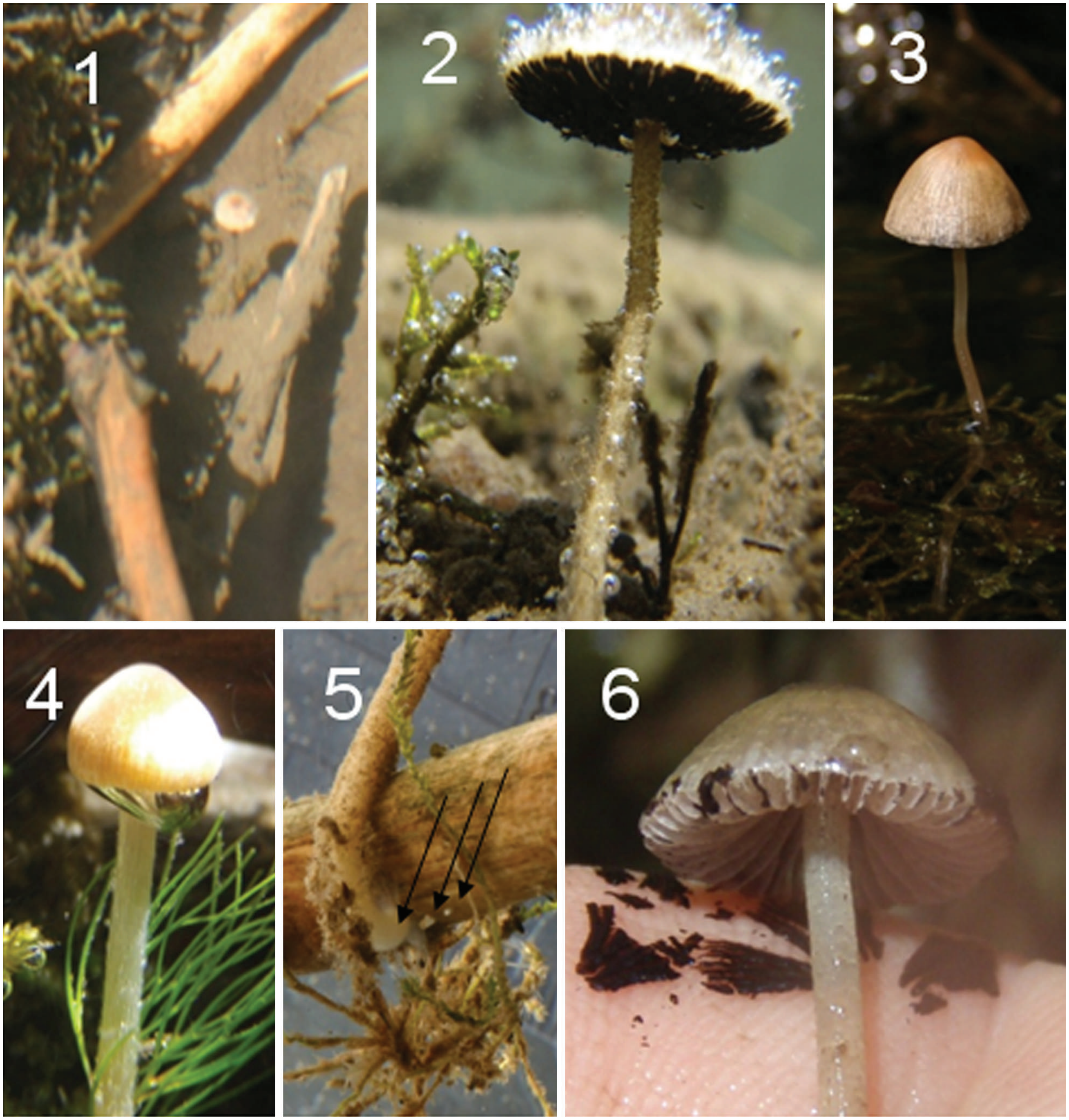
Basidiomata 4.5–10 cm alta, immersa. Pileus 0.8–1.5 cm latus, brunneolus vel brunneigriseus. Basidioporae ellipsoideae, leves, brunneae, 10–14 × 6–8 μm, poro germinali. Cystidia hymeniales: cheilocystidia pleurocystidiaque similaria, ventricosa, 25–45 × 10–18 μm. Lamellae adnatae. Stipes textura porrecta.

Macromorphology. *Basidiomata* (FIGS. 1–4, 6) immersed, 4.5–10 cm tall; *pileus* 0.8–1.5 cm diam, broadly parabolic to campanulate, light brown to brownish gray (10YR 7/2–6/1), sometimes with central orange-brown (10YR 5/4) disk, sometimes mottled or striate, smooth, hygrophanous; *pileus context* thin above gills, light tan to orange-brown; *odor* not distinctive; *lamellae* adnate, thin, light tan, densely speckled with dark brown spores, extending to pileus margin, lamellulae in two ranks and extending from one-half to one-fourth of the radius; *stipe* 4.0–9.5 cm long, diameter expanding from 1.0–2.2 mm at apex to 1.8–3.2 mm at base, white to pale yellow, hollow, lacking annulus, fibrous, surface fibrillose covered with wefty white to gray-white mycelium, and with cottony rhizomorphs and mycelial tomentum emanating from base.

Micromorphogy. *Basidiospores* (FIG. 7) 10–14 × 6–8 μm, ave. 12.3 × 6.9 μm, elliptical with a germ pore, smooth, dark reddish brown in water and in Melzer's, fading to gray-brown in KOH and to lilac in H_2SO_4, spore print purple-black; *basidia* (FIG. 8) 4-spored, clavate, 32–40 × 10–13 μm, hyaline; *cheilocystidia* (FIG. 10) 25–45 × 10–18 μm ventricose, apex subacute to elongate, thin walled, colorless, hyaline; *pleurocystidia* (FIG. 8, 9) 25–40 × 10–13 μm, ventricose, apex subacute, scattered, thin-walled, colorless, hyaline; *caulocystidia* 32–40 × 10–13 μm, cylindrical to ventricose, in fascicles, apex obtuse; *pileipellis* cellular, *suprapellis* a single layer of spherical to isodiametric, inflated cells, 25–35 μm diam, on 30–50 × 3–5 μm peduncles that extend into the pileus trama, clamp connections absent; *pileus trama* thin-walled hyphae 8–15 μm diam, interwoven; *stipe* hyphae 35–70 × 8–14 μm, parallel; *clamp connections* present in mycelium at stipe base, absent elsewhere.

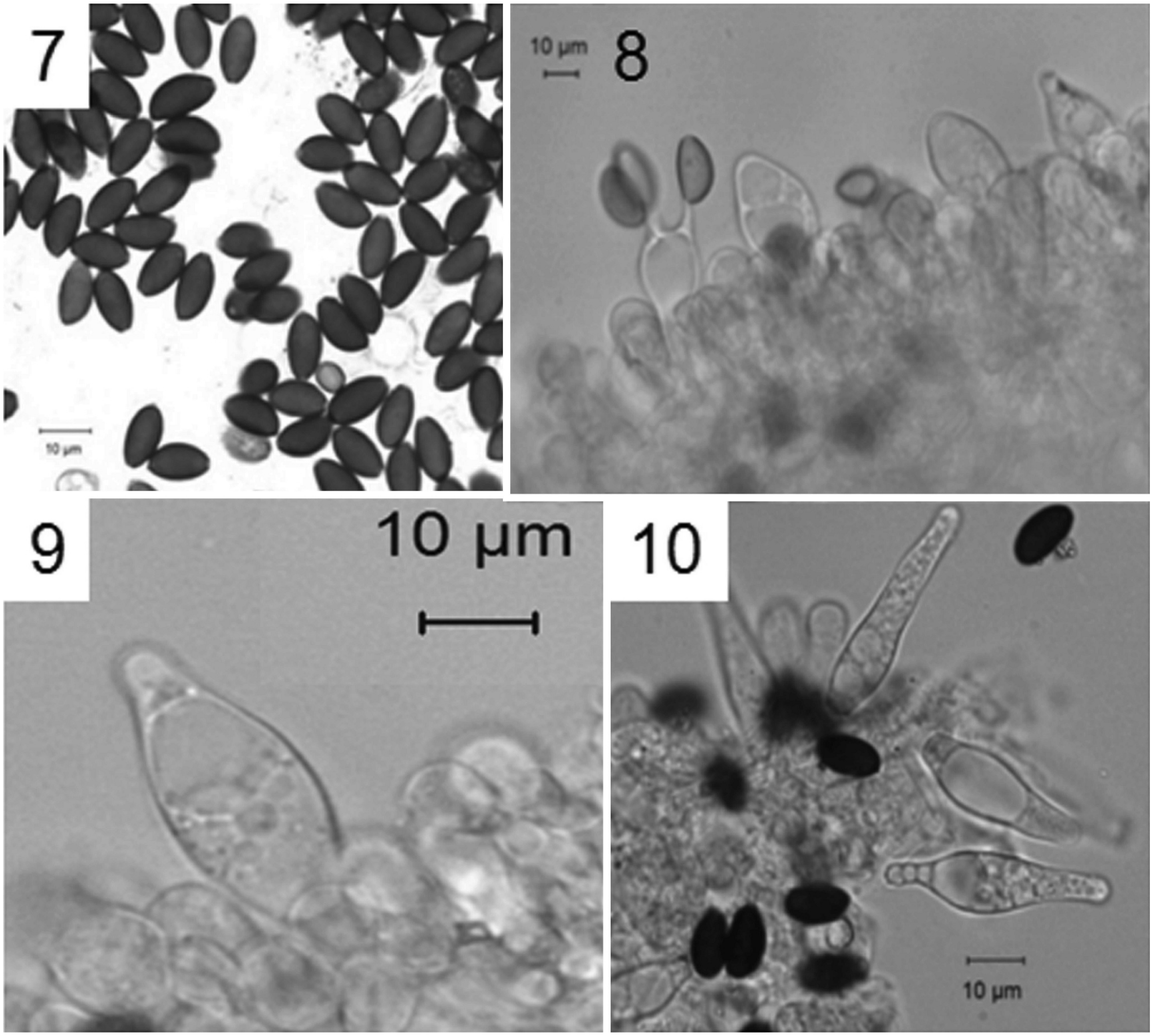
Habit. Basidiomes were observed in below rapids and areas of turbulence. Specimens were anchored at depths up to 0.5 m, most over an area of approximately 200 m^2, with two specimens collected 1 km upstream. The pileus of one specimen was above water; all others were submerged. One specimen was growing in an eddy behind woody debris in the main river channel; all others were in moving water. No basidiomata were observed in slack water. The lateral distance from submerged basidiomata to the nearest stream bank or gravel bar was 20–340 cm. In Aug and Sep 2007 epigeous fungi, including *Collybia* sp., *Russula* spp., *Alnicola* sp. and *Lycoperdon* sp., were observed in adjacent terrestrial areas.

Specimens were attached to a substrate of sticks (FIG. 5) or gravel or embedded in silt (FIG. 1). Basidiomata often grew out of, or close to, aquatic mosses (*Scleropodium obtusifolium* [Jaeg.] Kindb. in Mac. & Kindb.) and cyanobacteria (*Anabaena*) (FIG. 3). The stipe bases of several specimens origi-



FIGS. 1–6. *Psathyrella aquatica*. 1. Underwater mushroom fruiting in silt near waterlogged wood. 2. Underwater mushroom with dark spores on gills and gas bubbles on cap and stipe. 3. Mushroom growing with aquatic moss (*Scleropodium obtusifolium*) emerging from water. 4. Mushroom with undulating gas bubble under pileus. 5. Stipe initiating growth on underside of submerged twig. Arrows point to base of developed stipe and to two primordia. 6. Cap lifted above water showing rafts of spores from burst gas pocket; gills are white after spore discharge.

nated from the underside of a piece of gravel or stick (FIG. 5, arrow) before curling around to elongate upward. Gas bubbles were observed on stipes and pilei (FIG. 2), and gas pockets were trapped beneath the pilei (FIG. 4). Some underwater mushrooms with white gills apparently already had shed spores (FIG. 6), but most retained spores on the gills (FIG. 2). Basidiospores collected at the water-gas interface beneath the pileus in some specimens. When specimens were lifted gently from the water



FIGS. 7–10. *Psathyrella aquatica*. 7. Basidiospores. 8. Basidium with spores attached, also pleurocystidia. 9. Pleurocystidium. 10. Cheilocystidia. Bars = 10 µm.

the gas pocket remained intact for a few seconds and undulated from the movement (FIG. 4). Then the gas pocket burst open, releasing wedge-shape rafts of spores that adhered to each other, the stipe and our fingers (FIG. 6).

Habitat. Aquatic vegetation near the attachment of the underwater mushrooms included dense beds of *Scleropodium obtusifolium* and abundant gelatinous masses of cyanobacteria, *Anabaena* sp., with heterocysts. Terrestrial vegetation on surrounding stream banks was dominated by *Pseudotsuga menziesii* with *Pinus monticola* in the canopy and understory trees, *Alnus rubra*, *Acer circinatum* and *Cornus nuttallii*. Upland vegetation and riparian vegetation reached the water's edge at all times of the year, including the late summer during the period of lowest flow.

Organic carbon in the water sample totaled 0.52 mg/L, nitrate measured less than the testing equipment limit of 0.2 mg/L and total phosphorus less than 0.05 mg/L. Water temperature during field observations was 7–13.1 C; ambient air was 23–32.8 C.

Known distribution. Oregon.

Etymology. In reference to the aquatic habitat.

HOLOTYPE: USA. OREGON: Jackson County. North of Prospect (42°51′42″N, 122°30′28″W), underwater in the Rogue River on wood, 8 Aug 2007, R.A. Coffan (*D. Southworth 1097* MICH).

Other specimens examined. See TABLE I.

TABLE II. Maximum identity match to the ITS region of *Psathyrella aquatica* and classification, according to Smith (1972), of *Psathyrella* collections examined

Psathyrella species	Subgenus/section/subsection/series	Length (b.p.)	Query coverage (%)	Max ident (%)
P. fontinalis FJ899614	*Psathyrella/Psathyrella/Psathyrellae/Psathyrellae*	556	94	99
P. aff. *brooksii* EU664994	*Pannucia/Pannucia/Mixtae*	564	90	99
P. superiorensis FJ899623	*Psathyrella/Psathyrella/Psathyrellae/Psathyrellae*	402	98	98
P. atomata FJ899610	*Psathyrella/Psathyrella/Psathyrellae/Psathyrellae*	666	76	98
P. superiorensis FJ899622	*Psathyrella/Psathyrella/Psathyrellae/Psathyrellae*	531	94	96
P. prona FJ899618	*Atomatae/*	455	100	95
P. brooksii EU664993	*Pannucia/Pannucia/Mixtae*	453	99	95
P. subincarnata FJ899621	*Psathyrella/Psathyrella/Psathyrellae/Tenerae*	628	81	95
P. alluviana FJ899609	*Psathyrella/Psathyrella/Psathyrellae/Tenerae*	507	89	94
P. ramicola FJ899620	*Psathyrella/Umbonatae*	593	83	94
P. hydrophila FJ899615	*Pannucia/Appendiculatae/Hydrophilae*	268	100	93
P. marcescibilis FJ899617	*Candolleana/*	445	99	93
P. rainierensis FJ899619	*Psathyrella/Psathyrella/Mesosporae*	213	98	93
P. uskensis FJ899625	*Psathyrella/Psathyrella/Psathyrellae/Tenerae*	514	98	93
P. nitens FJ968757	*Psathyrella/Psathyrella/Mesosporae*	540	91	93
P. intermedia FJ899616	*Psathyrella/Psathyrella/Psathyrellae/Tenerae*	330	100	92
P. calvinii FJ899611	*Psathyrella/Psathyrella/Psathyrellae/Tenerae*	437	100	92
P. tenera FJ899624	*Psathyrella/Psathyrella/Psathyrellae/Tenerae*	491	99	92
P. cf. *gracilis* FJ235146	*Psathyrella/Psathyrella/Psathyrellae/Psathyrellae*	528	93	92
P. candolleana FJ899612	*Candolleana/*	551	84	91
P. conopilea FJ899613	*Psathyrella/Subatratae*	414	78	87
P. velutina FJ899626	*Lacrymaria/*	467	94	85

Molecular analyses.—DNA sequences from eight specimens of *P. aquatica* confirmed that only one species was present; six ITS sequences and three 28S sequences of *P. aquatica* specimens were identical. Sequences of the ITS1 and ITS2 regions, the 5.8S ribosomal gene and the 28S ribosomal gene from six specimens of *P. aquatica* have been deposited in GenBank (TABLE I).

ITS sequences were obtained from 18 other species of *Psathyrella* from 19 herbarium collections (two collections of *P. superiorensis* yielded distinct ITS sequences) and from three lyophilized extracts (TABLE II). Sequences of the 28S region were generated from 12 species.

Of 528 total characters in the phylogenetic alignment of 28S sequences, 490 were constant, 24 were considered parsimony informative and 14 variable characters were considered parsimony uninformative. Both the parsimony and the maximum likelihood consensus trees generated from our 28S alignment placed *P. aquatica* near *P. superiorensis* (Ammirati 2251, holotype), *P.* aff. *brooksii* (Padamsee 098, but not Brooks 1594, holotype) and *P. fontinalis* with *P. superiorensis* and *P. aff. brooksii* (Padamsee 098) closer than *P. fontinalis* (FIG. 11, TABLE II). Pairwise analysis of ITS sequences show *P. aquatica* to be closer to *P. fontinalis* and *P. aff. brooksii* than to *P. superiorensis* (holotype) (TABLE II). That two collections identified by A.H. Smith as *P. superiorensis* both from Michigan differed by more than 2% in the ITS sequence highlights the problems of species identification in *Psathyrella*. Molecular data show that *P. aquatica* is close to several species in series *Psathyrellae* and aligns into Clade A v *Psathyrella sensu stricto* (Padamsee et al 2008).

Commentary.—*Psathyrella aquatica* belongs to subgenus *Psathyrella* based on these characteristics: smooth unornamented spores; absence of fasciculate hymenial cystidia; not parasitic on *Coprinus*; glabrous pileus; absence of granulose veil; pleurocystidia with wall in neck up to 0.5 μm, apex smooth or with only finely granular incrustations; cheilocystidia not lecythiform; and veil thin to rudimentary or absent, not well developed (Smith 1972). Among the 177 species in subgenus *Psathyrella*, *P. aquatica* belongs to section *Psathyrella* based on these characters: not coprophilous, subacute to obtuse pleurocystidia and with spores at least 9–12.5 μm long. Distinguishing between subsections *Mesosporae* and *Psathyrellae* depends on basidiospore dimensions in which there is a slight overlap, with spore length in subsection *Mesosporae* 9–12.5 μm and in *Psathyrellae* 11–17 μm. A mean basidiospore length of 12.3 μm and extreme lengths of 10–14 μm place *P. aquatica* among the 27 species in subsection *Psathyrellae*. Within subsection

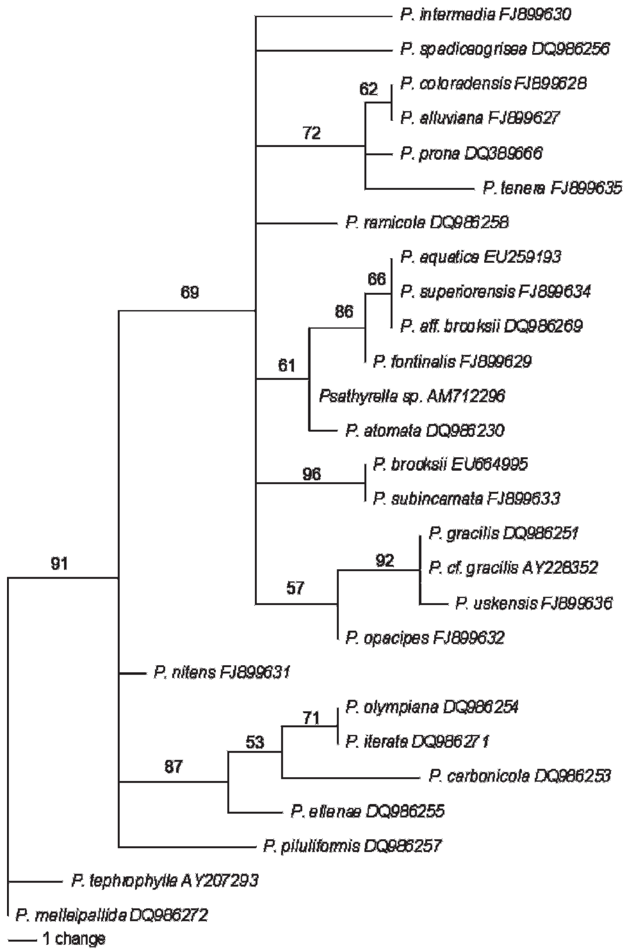


FIG. 11. Phylogenetic tree using parsimony for 28S data (with GenBank numbers) showing the position of *P. aquatica* in *Psathyrella sensu stricto*, with 1000 bootstrap replicates; bootstrap numbers greater than 50% are included above branches.

Psathyrellae two series, *Psathyrellae* and *Tenerae*, are distinguished by the color of the pileus margin and gill edges. Species in series *Psathyrellae* have pink gill edges, lacking in series *Tenerae*. Pink tints were not observed on any specimens of *P. aquatica* at any stage from immature with veil still attached to post spore discharge. Morphology places *P. aquatica* in series *Tenerae*; DNA sequences place *P. aquatica* in series *Psathyrellae* (TABLE II, FIG. 11).

Morphological comparison with closely related species.— Morphological traits differentiate *P. aquatica* from described species in series *Psathyrellae* (TABLE III). Among species most closely related based on molecular phylogenetic analysis, *P. fontinalis* has a glabrescent stipe, longer spore maximum length (16 μm), longer pleurocystidia, cylindric to clavate caulocystidia and lighter spore color in KOH. *Psathyrella superiorensis* has longer pleurocystidia, smaller spores and shorter stipe. Neither *P. fontinalis* nor *P. superiorensis* have been reported outside Michigan. *Psathyrella* aff. *brooksii* differs in having a thick white fibrillose veil when young and shorter yellow-brown stipe (M. Padamsee pers comm). *Psathyrella atomata* lacks pleurocystidia; *P. preatomata* has hyaline cells among the cheilocystidia; and *P. gracilis* and *P. opacipes* have longer, more acute pleurocystidia. Among species for which no DNA sequences were obtained, *P. filamentosa* has clavate to vesiculose cells along the gill margin with cheilocystidia.

Species in series *Tenerae*, which are less close to *P. aquatica* based on ITS and 28S sequences, similarly lack pink tints (Smith 1972). In addition there are other morphological differences. *Psathyrella calvinii* has incrustations on stipe hyphae, *P. subincarnata* has vinaceous lamellae when young, *P. tenera* has brachybasidioles and *P. alluviana* has a rugulose pileus. Pleurocystidia in *P. intermedia* are small and infrequent. Both *P. uskensis* and *P. coloradensis* are notably small and fragile, even for this genus.

In subsection *Mesosporae*, *P. nitens* and *P. rainierensis* not only differ by DNA but also have smaller spores; *P. subhepatica* has longer pleurocystidia (44–70 μm). In subgenus *Atomatae* pleurocystidia are rare in *P. prona*. In subgenus *Pannucia P. hydrophila* has much smaller spores. Spores of *P. brooksii* are slightly larger (12–15 × 7–9 μm) than those of *P. aquatica* (10–14 × 6–8 μm); cystidia are subclavate to broadly ventricose, and the veil of *P. brooksii* is more or less well developed and remains attached to the cap. Pleurocystidia in *P. ramicola*, subgenus *Umbonatae*, are utriform.

Psathyrella aquatica is distinct from the two *Psathyrella* species that occur in terrestrial sites along the Rogue River in southern Oregon (TABLE III). *Psathyrella quercicola* in section *Fatuae*, collected 34 km downriver, has smaller basidiospores; *P. rogueiana* in subgenus *Candolleana*, collected 84 km downriver, has smaller basidiospores, rare pleurocystidia and clavate to utriform cheilocystidia.

DISCUSSION

In a genus as large as *Psathyrella*, determining a new species is complex because species are similar in both morphology and DNA sequences. Macromorphological characters can be insufficient to identify specimens to species, and micromorphological characters, especially cystidial shape and the color of gill edges, are variable. Kits van Waveren (1985) considered the character of red underlining of gill edges to be unreliable, although Smith (1972) and Breitenbach and Kränzlin (1995) used red underlining as a key character. Vašutová et al (2008) considered cystidial

TABLE III. Diagnostic characters among described species of *Psathyrella* in series *Psathyrellae* including those with close DNA matches to *P. aquatica*. Data from species other than *P. aquatica* from Smith (1972). Spore and cystidia dimensions in micrometers; clamp connections in pileus

Psathyrella species	Spore size	Cystidia	Clamps	Stipe	Suprapellis cuticle	Gill edge color	Height/cap diam (cm)
aquatica	10–14 × 6–8 av. 12.3 × 6.9	Pl: ventricose, subacute 25–40 × 10–13 Ch: ventricose, subacute 25–40 × 10–18 Ca: +	−	fibrillose, wefty at base	1 layer, pedicellate	white	4.5–10/0.8–1.5
atomata	11–13(–15) × 6–7	Pl: absent to rare Ch: fusoid-ventricose 35–48 × 9–14, acute to subacute, long neck Ca: ?	+	fragile, pulverulent to glabrous	2–3 layers, vesiculose	pink	3–5/1–2.5
filamentosa	11–14 × 5.5–7	Pl: fusoid ventricose, apex obtuse to subacute with adhering granules, 43–58 × 10–17 Ch: fusoid ventricose with vesiculose cells Ca: ?	+	naked, pruinose near apex	1–2 layers, inflated cells	white or pink	2–5/0.5–1.2
fontinalis	11–14(–16) × 6–7.5 (–8.5)	Pl: Ventricose-elongate, short neck, obtuse apex to fusoid-ventricose, subacute apex 38–65 × 10–16 Ch: similar or ventricose to clavate 15–26 × 8–12 Ca: +	+	glabrescent	palisade, clavate pedicellate	pink	(3–)5–10/1–3
gracilis	(10–)11–14 (–15) × 6.5–8	Pl: subaciculate to fusoid-ventricose, acute to subacute, neck flexuous 54–75 × 10–16 Ch: Shorter, more obtuse Ca: +	+	fragile, fibrillose above glabrous below	palisade, pyriform and vesiculose to elliptic	pink	6–12/1.5–3.5
opacipes	(12–)13–16.5 × 6–8	Pl: fusoid-ventricose, acute to subacute 38–70 × 9–16 Ch: saccate to clavate 18–26 × 10–15 Ca: +	+	glabrescent pruinose	1 layer, vesiculose or pedicellate	white or vinaceous	3–7/1–3.5
praeatomata	11–14 (–15) × 5.5–7	Pl: Ventricose with neck, subacute, 36–48 × 10–15 Ch: Subfusoid to clavate to fusoid-ventricose 8–12 wide Ca: ?	−	naked above, scattered fibrils below	2–3 layers, pedicellate and vesiculose	pink	2–5/0.8–1.5
superiorensis	11–13 × 5.5–6.5	Pl: Fusoid-ventricose 50–85 × 10–15 long neck, obtuse Ch: smaller or clavate Ca: ?	+	fibrillose, pallid above, brown below	1–2 layers, vesiculose	pink	2–4/0.6–1.4

Pl, pleurocystidia; Ch, cheilocystidia; Ca, caulocystidia; +, present; −, absent; ?, not mentioned.

shapes as homoplasic and insufficient to determine phylogeny. In a test of sorting within the *P. gracilis* group Kemp (1985) sent six split collections of *P. gracilis* to A.H. Smith and E. Kits van Waveren; taxonomic agreement was reached on only one. Padamsee et al (2008) found disagreement between molecular and morphological information among several pairs of *Psathyrella* species.

Studies show the value of molecular data in determining phylogenetic relationships within genus *Psathyrella* (Padamsee et al 2008, Vašutová et al 2008, Larsson and Örstadius 2008). Our analysis of 28S DNA sequences placed *P. aquatica* into Clade A v. Taken together morphological and molecular evidence support the hypothesis that the underwater mushroom is a new species of *Psathyrella* most closely related to *P. fontinalis*, *P. superiorensis*, *P. atomata* and the *P. gracilis* group. *Psathyrella aquatica* is characterized by a relatively long fibrillose stipe that is not fragile, relatively small cap, a thin veil disappearing at maturity, nonpink gill edges and ventricose pleuro- and cheilocystidia with subacute apices.

The habitat of *P. aquatica* appears unique among species of *Psathyrella*, none of which have been reported in running water. Most species of *Psathyrella* occur in terrestrial habitats, often on dung. However some, including several in subgenus *Psathyrella* section *Psathyrellae*, are associated with damp or wet soil, wet or drying muck or the margins of wetlands where the mycelium might grow underwater (Smith 1972). For example *P. filamentosa* and *P. opacipes* were found in damp habitats or at the edges of marshes (Smith 1972). Of the six species closest to *P. aquatica*, three (*P. atomata*, *P. fontinalis* and *P. superiorensis*) have been collected from damp habitats (e.g. muck, drying drainage ponds and damp soil). The other three (*P.* aff. *brooksii*, *P. gracilis* and *P. ramicola*) occur in dry terrestrial habitats on soil or wood. Although 28s and ITS sequences failed to separate *P. aquatica* from *P.* aff. *Brooksii*, morphological differences do not justify considering *P.* aff *brooksii* a conspecific specimen. Species in other subgenera, for example *P. typhae* (subgenus *Pannucia*), also grow on wetland plant debris and silt and on floating matter (Redhead 1979, 1981; Schulz et al 2005). Other Psathyrellaceae, such as *Coprinopsis kubickiae* (reported as *Coprinus amphibius*) and *Coprinellus congregatus* (reported as *Coprinus alkalinus*), have been isolated from submerged wood, but fruiting bodies were not formed underwater (Anastasiou 1967, Redhead and Traquair 1981).

The spore discharge mechanism remains enigmatic in *Psathyrella aquatica*. Spore prints were obtained, suggesting that basidiospores are discharged as ballistospores when conditions are appropriate. Furthermore basidiospores show the asymmetrically positioned hilar appendix, a feature compatible with forcible discharge (McLaughlin et al 1985). The wedge-shape rafts of spores released by the bursting gas bubble resemble sections of a spore print deposited on the gas-water interface beneath the cap. The ballistospore discharge mechanism involving a water droplet and a water film could not occur if gills were totally in contact with water (Money 1998, Pringle et al 2005). Gas pockets were trapped under many *P. aquatica* pilei; a similar bubble was observed under the hymenophore of *Gloiocephala menierii* collected from *Carex* stems near the mud-air interface (Redhead 1981, Desjardin et al 1995, Redhead pers comm). Trapped gases might provide the atmosphere needed for ballistosporic discharge in underwater environments.

A fungus growing in a fast flowing stream would encounter spore dispersal problems because the current tends to wash spores downstream. *Psathyrella aquatica* fruitbodies retain released basidiospores at the air-water interface of the pileal gas pocket until disturbed. Even then the spores do not disperse individually but appear hydrophobic, attracted to each other or to other hydrophobic surfaces. Desjardin (1995) recognized the problem of underwater agaric spore dispersal and hypothesized that wind-induced water currents or aquatic animals might disperse spores to nearby vegetation within a lake. Dispersal by currents in a turbulent stream however are unidirectional downstream, transporting spores to different and possibly unsuitable habitats. Adherence of *Psathyrella* spores to stipe and gills might counteract currents that would wash the spores downstream. In addition aquatic invertebrates might graze these fungi, keeping spores in the same habitat and dispersing them nearby, even upstream. Retention of spores near fruitbodies would allow for reinoculation in suitable habitats.

The growth of this fungus on alluvial gravel as well as on submerged wood suggests that it might obtain carbon from the film of bacteria, algae and sediment that collects on the surface of submerged substrates as well as from decomposing wood. It might obtain nitrogen from cyanobacteria colonies. The relatively constant conditions of the aquatic environment might help to explain the exceptionally long fruiting period. Growth below 20 C classifies the species as psychrophilic (Kendrick 2000).

Reproductive isolation could account for speciation because underwater fruiting would limit opportunities for genetic exchange between aquatic and terrestrial individuals. Combining evidence from DNA sequences, morphology and habitat, we conclude that the underwater mushrooms are a new species, *P. aquatica*, in Clade A v *Psathyrella sensu stricto* (Padamsee et al 2008). The conditions of this stream are distinctive, although not unique. This particular river habitat combines the characteristics of steady spring-fed flows, clear, cold, aerated water with woody debris in shallow depths on fine volcanic substrate. This underwater environment is a new habitat for gilled mushrooms.

ACKNOWLEDGMENTS

This study was supported by National Science Foundation Grants DEB-0516229 through Research at Undergraduate Institutions and DBI-0115892 to the Biotechnology Center at Southern Oregon University. We thank Maj Padamsee for three lyophilized DNA extracts, a specimen of *P.* aff. *brooksii*, and prepublication access to sequence data; Steven Jessup for identification of the aquatic moss; David Oline for discussion of molecular data; Jim Trappe for help with the Latin description; Patricia Rogers for loan of specimens from the University of Michigan Herbarium Fungus Collection; and Lorelei Norvell, David McLaughlin and two anonymous reviewers for close readings.

LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Anastasiou CJ. 1967. Two species of *Coprinus* from alkaline lakes. Can J Bot 45:2213–2222.

Breitenbach J, Kränzlin F, eds. 1995. Fungi of Switzerland. Vol. 4. Lucerne: Mykologia. 368 p.

Cascade Earth Sciences. 2006. Upper Rogue Watershed Assessment. Upper Rogue Watershed Association, Shady Cove, Oregon. Online at http://www.upper-rogue.org. Accessed Oct 2007.

Desjardin DE, Martinez Peck L, Rajchenberg M. 1995. An unusual psychrophilic aquatic agaric from Argentina. Mycologia 87:547–550.

Ferrer A, Huzefa AR, Shearer CA. 2008. *Lucidascocarpa pulchella*, a new ascomycete genus and species from freshwater habitats in the American tropics. Mycologia 100:642–646.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizas and rusts. Mol Ecol 2:113–118.

———, ———. 1996. ITS–RFLP matching for identification of fungi. In: Clapp JP, ed. Methods in molecular biology, species diagnostics protocols: PCR and other nucleic acid Methods. Vol. 50. New Jersey: Humana Press. p 177–186.

Gibson I. 2007. *Psathyrella* in the Pacific Northwest. Pacific Northwest Key Council. Online at http://www.svims.ca/council/Psathy.htm. Accessed Sep 2007.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Hansen L, Knudsen H, eds. 1992. Nordic macromycetes. Vol 2. Copenhagen: Nordsvamp. 474 p.

Hibbett DS, Binder M. 2001. Evolution of marine mushrooms. Biol Bull 201:319–322.

Holmgren PK, Holmgren NH. 1998. Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available at http://sweetgum.nybg.org/ih/

Kemp RF. 1985. Do fungal species really exist? A study of Basidiomycete species with special reference to those in *Coprinus* section Lanatuli. Bull Brit Mycol Soc (Apr 1985): 34–39.

Kendrick B. 2000. The fifth kingdom. Newburyport, Massachusetts: Focus Publishing. 386 p.

Kits van Waveren E. 1985. The Dutch, French and British species of *Psathyrella*. Persoonia 2:1–300.

Largent D, Johnson D, Watling R. 1977. How to identify mushrooms to genus III: microscopic features. Eureka, California: Mad River Press. 148 p.

Larsson E, Örstadius L. 2008. Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. Mycol Res 112:1165–1185.

Mains EB. 1956. North American species of Geoglossaceae. Mycologia 48:694–710.

McCarthy C. 1998. Chromas 1.45. Southport, Queensland, Australia: Griffith University.

McLaughlin DJ, Becket A, Yoon KS. 1985. Ultrastructure and evolution of ballistosporic basidiospores. Bot J Linnean Soc 91:253–271.

Minoura K, Muroi T. 1978. Some freshwater Ascomycetes from Japan. Trans Mycol Soc Japan 19:129–134.

Money NP. 1998. More *g*s than the space shuttle: ballistospore discharge. Mycologia 90:547–558.

Munsell Color. 1976. Munsell book of color (matte finish collection). Baltimore: Macbeth. 40 pl.

Oregon Water Resources Department. 2007. Surface water data. Online at http://egov.oregon.gov/OWRD/SW/index.shtml#Surface_Water_Data. Accessed Aug 2007.

Padamsee M, Matheny PB, Dentinger BTM, McLaughlin DJ. 2008. The mushroom family Psathyrellaceae: evidence for large-scale polyphyly of the genus *Psathyrella*. Mol Phylogenet Evol 46:415–429.

Pinruan U, Sakayaroy J, Gareth Jones EB, Hyde KD. 2004. Aquatic fungi from peat swamp palms: *Phruensis brunneispora* gen. et sp. nov. and its hyphomycete anamorph. Mycologia 96:1163–1170.

Pringle A, Patek SN, Fischer M, Stolze J, Money NP. 2005. The captured launch of a ballistospore. Mycologia 97: 866–871.

Redhead SA. 1979. *Psathyrella typhae*. Fungi Canadenses 133, http://www.cbs.knaw.nl/mycoheritage/

———. 1981. Agaricales on wetland Monocotyledoneae in Canada. Can J Bot 59:574–589.

———, Traquair JA. 1981. *Coprinus* sect. Herbicolae from Canada: notes on extralimital taxa and the taxonomic position of a low temperature Basidiomycete forage crop pathogen from western Canada. Mycotaxon 13: 373–404.

———, Vilgalys R, Moncalvo JM, Johnson J, Hopple JS Jr.

2001. *Coprinus* Pers. and the disposition of *Coprinus* species sensu lato. Taxon 50:203–241.

Schulz MJ, Cahill JF Jr, Currah RS. 2005. In vitro measurements of the competitive interactions between two saprobic basidiomycetes on *Typha latifolia*. Can J Bot 83:1523–1527.

Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanová L, Padgett D, Porter D, Raja HA, Schmit JP, Thorton HA, Voglymayr H. 2007. Fungal biodiversity in aquatic habitats. Biodivers Conserv 16:49–67.

———, Langsam DM, Longcore JE. 2004. Fungi in freshwater habitats. In: Mueller GM, Bills GF, Foster MS, eds. Biodiversity of fungi. London: Elsevier Academic Press. p 513–531.

Smith AH. 1972. The North American species of *Psathyrella*. Mem NY Bot Gard 24:1–633.

Swofford DL. 2001. PAUP*: phylogenetic analysis using parsimony (*and other methods). 4.0. 10th ed. Sunderland, Massachusetts: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876–4882.

Tylutki EE. 1979. Mushrooms of Idaho and the Pacific Northwest. Vol. 1. Discomycetes. Moscow, Idaho: University of Idaho Press. 133 p.

Vašutová M, Antonín V, Urban A. 2008. Phylogenetic studies in *Psathyrella* focusing on sections *Pennatae* and *Spadiceae*—new evidence for the paraphyly of the genus. Mycol Res 112:1153–1164. doi: 10.1016j.mycres. 2008.04.005.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR orotocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.