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The mushroom family Psathyrellaceae: Evidence for large-scale polyphyly of the genus *Psathyrella*

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Abstract

Psathyrella is the archetypal little brown mushroom genus with few easily discernable characters causing it to be considered a "clean-up" genus for other small brown-spored saprotrophic species found worldwide. While molecular studies have demonstrated that mushroom genera based on homoplastic morphological characters are artificial, the degree of phylogenetic heterogeneity contained within *Psathyrella* and Psathyrellaceae has never been appropriately addressed. For this study, 132 ribosomal sequences from approximately one-tenth of the known *Psathyrella* species worldwide, including representatives of most subgeneric subdivisions, and three closely related coprinoid genera (*Parasola*, *Coprinopsis*, *Coprinellus*) were evaluated using multiple phylogenetic methods, including likelihood, with Agaricaceae as the outgroup. Our results indicated that *Psathyrella* was polyphyletic. Conservatively, the genus can be separated into 11 clades of which five can be raised to generic status. Most species of *Psathyrella*, including its type species *P. gracilis*, formed a large clade with *Coprinellus*, which appeared to be derived from within *Psathyrella*. Generic limits of *Parasola*, *Lacrymaria*, and *Coprinopsis* should be reevaluated. Several taxa previously synonymized based on morphological features were phylogenetically distinct. Morphological features traditionally used to subdivide *Psathyrella* appeared to be mostly convergent (homoplasious) when traced upon the resulting phylogenies, although several had high RI values. These results were interpreted in light of the two major taxonomic treatments of *Psathyrella* and revealed substantial inconsistencies between the molecular- and morphology-derived inferences of relationships.
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1. Introduction

The genus *Psathyrella* (Fries) Quélet consists of 400–600 species of mushrooms (Hawksworth et al., 1995; Kirk et al., 2001; Vašutová, 2006). Species of *Psathyrella* are cosmopolitan (Hawksworth et al., 1995); most have been described from either Europe or North America but many have been reported from Africa, Australia, China, India, Japan, Mexico, and Sri Lanka (Bi, 1991; Dennis, 1955; Guzmán et al., 1988; Kits van Waveren, 1985; Natrajan,

1978; Pegler, 1965, 1966; Petch, 1917; Singer et al., 1958; Smith, 1972; Takahashi, 2000). The overwhelming majority of *Psathyrella* species is saprotrophic and serves as either primary or secondary decomposers in terrestrial ecosystems (Smith, 1972). Species of *Psathyrella* are often found on decaying logs, woody debris, humus, or soil, in woodlands, lawns, or bogs and can have either broad or specific substrate relationships (Smith, 1972). For example, the generic type *Psathyrella gracilis* can be found on soil, humus, or wood debris whereas *P. epimyces* is a specialized parasite on the Shaggy Mane mushroom, *Coprinus comatus*. Some *Psathyrella* species are consumed for food and several show antimicrobial activity or produce other medically important

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compounds (Nieves-Rivera, 2001; Singer, 1986; Stadler et al., 2005; Suay et al., 2000; Ueda et al., 2002).

Psathyrella is distinguished from other genera of Agaricales by spore deposit color in mass (dark brown to brownish-black or medium reddish-brown or pinkish-gray), spores that discolor in concentrated sulfuric acid, and typically, a fragile pileus (cap). The generic name is based on this characteristic pileus (*Psathyros*, Greek = fragile), which is composed of ellipsoid or more or less round cells (cellular pileipellis). The lack of striking features such as deliquescent (auto-digesting) gills at maturity created a fluid generic concept for *Psathyrella* until Singer (1949, 1962) recognized it as a major genus within the Agaricales. There are two major monographic treatments for *Psathyrella*: Smith (1972), which contains over 400 species, most of which are only reported from North America, and Kits van Waveren (1985), which focuses on the European flora and unites species into large complexes thereby recognizing 123 taxa. Additional *Psathyrella* species are not placed into either of these classification systems or are poorly integrated (Dennis, 1955; Singer, 1986). Kits van Waveren (1985) synonymized many species in Smith (1972), making a case for employing a broad morphological species concept. On the other hand, Smith (1972) used a narrow species concept; he argued that as the genus has been poorly sampled all over the world, comparisons between different continents could not be conducted. There is little congruence between the subgeneric taxonomy of Smith (1972) and that of Kits van Waveren (1985) (Table 1). Kits van Waveren (1985) divides the genus into two subgenera while Smith's system (1972) employs 11 subgenera. The subgenera are further subdivided into sections (Table 1). One of the subgenera recognized by Smith (1972) is *Lacrymaria*, which is treated as a genus by Kits van Waveren (1985) and others (Watling, 1979; Watling and Gregory, 1987). Phylogenetic studies of the genus have been impeded by challenges in species identification, divergent species concepts, and the ephemeral nature of *Psathyrella* fruit bodies with phenotypically plastic morphologies.

Prior phylogenetic studies that have incorporated species of *Psathyrella* indicated that the genus might not be monophyletic. Hopple and Vilgalys (1999), which included three species, suggested that *Psathyrella* is paraphyletic and derived within the genus *Coprinus* Pers. More critically, this study revealed that a character that distinguished *Coprinus sensu lato*, i.e., deliquescent gills, has evolved more than once. However, another study with close to 900 agaricoid taxa, which included three additional *Psathyrella* species (six total), suggested that certain coprinoid (*Coprinus*-like species with deliquescent gills now in the genus *Coprinellus* P. Karst) arose from a clade of psathyrelloid (fragile pileus, dark spores, non-deliquescent gills) taxa (Moncalvo et al., 2002). In a more recent study of the order Agaricales, Walther et al. (2005) reiterated the suggestion that *Psathyrella* was not monophyletic and that a comprehensive sampling of *Psathyrella* was needed to clarify relationships in its family. All three studies utilized a region of the nuclear

ribosomal large subunit gene (LSU), which was shown to be useful to understand relationships between Fungi at the genus and species level (Moncalvo et al., 2000, 2002; Vilgalys and Hester, 1990).

In this study, we reexamined phylogenetic relationships within *Psathyrella* and the recently erected family Psathyrellaceae (*Psathyrella*, *Coprinopsis*, *Coprinellus*, and *Parasola*) (Redhead et al., 2001). To utilize existing sequence data, the identical LSU region was sequenced and *Psathyrella* was examined within the context of prior studies (Hopple and Vilgalys, 1999; Moncalvo et al., 2002; Walther et al., 2005). The classification systems of Smith (1972) and Kits van Waveren (1985) were used as a guide to sample *Psathyrella* and were also evaluated. Before this study, the widespread convergence of certain morphological features used to characterize Smith's (1972) and Kits van Waveren's (1985) subgenera and sections was unknown. This study is the first extensive examination of *Psathyrella* and increases the sampling from six to multiple populations of 57 species or approximately 10% of the known species (600, Hawksworth et al., 1995). Thus, we wish to ask: (1) Is *Psathyrella* polyphyletic? (2) Are the taxonomic treatments of Smith and Kits van Waveren indicative of evolutionary groups? (3) Are traditional or other morphological features (e.g., spore color, deliquescence) indicative of phylogenetic relationships?

2. Materials and methods

2.1. Taxon sampling

This study was based on 120 species with two to five representatives of 11 taxa from different geographic locations (Table 1). The specimens of *Psathyrella* were collected fresh or acquired from the University of Minnesota Herbarium (MIN), University of Michigan Herbarium (MICH), Duke University Herbarium (DUKE), Field Museum of Natural History (F), and University of Washington Herbarium (WTU) (Table 1). *Psathyrella* specimens were identified or the identifications were checked using Smith (1972) and Kits van Waveren (1985); in the case of N. American collections, Smith's species concepts (1972) were employed to apply names. Moser (1983) and Kühner and Romagnesi (1953) were also consulted. Species within *Psathyrella* used in this study were chosen to represent the subgenera and sections of Smith (1972) and Kits van Waveren (1985) (Table 1). Smith (1972) treated the genus *Lacrymaria* as a subgenus within *Psathyrella* and representatives of this group were included. Each section was represented by at least two species except in the case of monotypic sections or subgenera. Species not classified by either taxonomist were placed into these classification systems based upon the criteria the authors used to diagnose units (or higher taxa). Subgenera *Conocybella* and *Panaeolina* of Smith (1972) and sections *Cystopsathyra* and *Bipelles* of Kits van Waveren (1985) were not represented. Sequences from all of the coprinoid taxa in the Hopple and Vilgalys (1999)

Table 1
Psathyrella sensu lato collections used in this study, their collection number, geographical location, and subgeneric and sectional taxonomy

Species[a]	Collector No./Herb.[b]/ GenBank Voucher ID	Collection location	Subgenera/ sections (Smith)	Subgenera/sections (Kits van Waveren)	GenBank ID
Lacrymaria lacrymabunda (Bull.) Pat.	GLM 45943	Europe	Lacrymaria/-	Psathyra/ Pseudostropharia	**AY207218**
L. velutina[1] (Pers.) Konrad & Maubl.	J.S. Hopple 100/ DUKE	N. America	Lacrymaria/-	Psathyra/ Pseudostropharia	**AF041534**
L. velutina[2] (Pers.) Konrad & Maubl.	G. Thorn 930923/09	Wisconsin, USA	Lacrymaria/-	Psathyra/ Pseudostropharia	**AF139972**
L. velutina[3] (Pers.) Konrad & Maubl.	M.G. Weaver 2089/ MIN	Minnesota, USA	Homophron/ Homophron	Psathyra/ Spadiceae	**DQ986247**
Psathyrella ammophila (Durieu & Lév.) P.D. Orton	J.F. Ammirati 426/ WTU	N. America	Psathyrella/ Psathyrella	Psathyrella/ Ammophilae	**DQ986252**
P. argillospora Rick ex. Sing.	R. Singer B6165/F, paratype	Colombia	Psathyrella/ Subatratae	Psathyra/ Spintrigerae	**DQ986241**
P. atomata (Fr.) Quél.	J.S. Hopple 139/ DUKE	N. America	Psathyrella/ Psathyrella	Psathyrella/ Atomatae	**DQ986230**
P. brooksii A.H. Sm.	M. Padamsee 98/MIN	Washington, USA	Pannucia/ Pannucia	Psathyrella/ Atomatae	**DQ986269**
P. camptopoda (Peck) A.H. Sm.	DAOM214256	N. America	Homophron/ Homophron	Psathyra/ Hydrophilae	**AF261489**
P. candolleana[1] (Fr.) Maire	P.B. Matheny 978/ WTU	Washington, USA	Candolleana/ Candolleana	Psathyra/ Spintrigerae	**DQ986250**
P. candolleana[2] (Fr.) Maire	M. Padamsee 001/ MIN	Minnesota, USA	Candolleana/ Candolleana	Psathyra/ Spintrigerae	**DQ986225**
P. candolleana[3] (Fr.) Maire	JCS0804A	—	Candolleana/ Candolleana	Psathyra/ Spintrigerae	**DQ110874**
P. candolleana[4] (Fr.) Maire	GLM 46005	Europe	Candolleana/ Candolleana	Psathyra/ Spintrigerae	**AY207279**
P. candolleana[5] (Fr.) Maire	J.S. Hopple 181/ DUKE	England	Candolleana/ Candolleana	Psathyra/ Spintrigerae	**AF041531**
P. carbonicola A.H. Sm.	P.B. Matheny 4952/ WTU	Washington, USA	Pannucia/ Pannucia	Psathyra/ Pennatae	**DQ986253**
P. conissans (Peck) A.H. Sm.	D.J. McLaughlin 916/ MIN	Minnesota, USA	Homophron/ Homophron	Psathyra/ Spadiceae	**DQ986266**
P. conopilus[1] (Fr.) A. Pearson & Dennis	TUB 011587	Europe	Psathyrella/ Subatratae	Psathyrella/ Subatratae	**DQ071706**
P. conopilus[2] (Fr.) A. Pearson & Dennis	T. Tang s.n./ MIN	California, USA	Psathyrella/ Subatratae	Psathyrella/ Subatratae	**DQ986249**
P. coprophila Watling	J.S. Hopple 171/ DUKE	Scotland	Psathyrella/ Atomatae	Psathyrella/ Atomatae	**DQ986232**
P. corrugis (Pers.) Konrad & Maubl.	GLM 46007	Europe	Psathyrella/ Psathyrella	Psathyrella/ Psathyrella	**AY207281**
P. cystidiosa (Peck) A.H. Sm.	D.J. McLaughlin 1157/ MIN	Minnesota, USA	Homophron/ Cystidiosae	Psathyra/ Spadiceae	**DQ986226**
P. delineata[1] (Peck) A.H. Sm.	J.S. Hopple 156/ DUKE	N. Carolina, USA	Pannucia/ Appendiculatae	Psathyra/ Hydrophilae	**AF041532**
P. delineata[2] (Peck) A.H. Sm.	M. Padamsee 142/ MIN	Tennessee, USA	Pannucia/ Appendiculatae	Psathyra/ Hydrophilae	**DQ986262**
P. delineata[3] (Peck) A.H. Sm.	M. Padamsee 144/ MIN	Tennessee, USA	Pannucia/ Appendiculatae	Psathyra/ Hydrophilae	**DQ986263**
P. echiniceps (G.F. Atk.) A.H. Sm.	D.J. McLaughlin 1153/ MIN	Minnesota, USA	Lacrymaria/-	Psathyra/ Pseudostropharia	**DQ986260**
P. ellenae var. *yubaensis* Thiers & A.H. Sm.	P.B. Matheny 501/WTU	California, USA	Pseudostropharia/ Pseudostropharia	Psathyra/Pennatae	**DQ986255**
P. aff. *elwhaensis* A.H. Sm.	WS292/WTU	Washington, USA	Candolleana/ Fragillissimae	Psathyra/ Spintrigerae	**DQ986259**
P. epimyces (Peck) A.H. Sm.	B.C. Dentinger s.n./ MIN	Minnesota, USA	Mycophila/-	Psathyra/ Pseudostropharia	**DQ986234**

(*continued on next page*)

Table 1 (*continued*)

Species[a]	Collector No./Herb.[b]/ GenBank Voucher ID	Collection location	Subgenera/ sections (Smith)	Subgenera/sections (Kits van Waveren)	GenBank ID
P. floccosa (Earle) A.H. Sm.	Singh 11/F	India	Pseudostropharia/ Spintrigerae	Psathyra/ Pseudostropharia	**DQ986235**
P. fulvescens (Romagn.) M.M. Moser ex A.H. Sm.	Rolle s.n./F	Switzerland	Psathyrella/ Obtusatae	Psathyra/ Pennatae	**DQ986245**
P. gracilis[1] (Fr.) Quél.	J.S. Hopple 130/ DUKE	N. America	Psathyrella/ Psathyrella	Psathyrella/ Psathyrella	**AF041533**
P. gracilis[2] (Fr.) Quél.	P.B. Matheny 1391/ WTU	Washington, USA	Psathyrella/ Psathyrella	Psathyrella/ Psathyrella	**DQ986251**
P. gracilis[3] (Fr.) Quél.	S. Lundell 2978/ MICH	Sweden	Psathyrella/ Psathyrella	Psathyrella/ Psathyrella	**EF532404**
P. gracilis forma *corrugis* (Pers.) Kits van Wav.	M. Vašutová 05/147/ MIN	Czech Republic	Psathyrella/ Psathyrella	Psathyrella/ Psathyrella	**DQ986243**
P. hirta Peck	GLM 46008	Europe	Psathyrella/ Atomatae	Psathyrella/ Atomatae	**AY207282**
P. huronensis A.H. Sm.	M. Padamsee 102/ MIN	Minnesota, USA	Candolleana/ Fragillissimae	Psathyra/ Spintrigerae	**DQ986270**
P. hymenocephala (Peck) A.H. Sm.	M.G. Weaver 2600/ MIN	Minnesota, USA	Candolleana/ Candolleana	Psathyra/ Spintrigerae	**DQ986248**
P. impexa (Romagn.) Bon	GLM 46009	Europe	Pannucia/ Pannucia	Psathyra/ Pennatae	**AY207283**
P. incerta (Peck) A.H. Sm.	M.G. Weaver 2127/ MIN	Minnesota, USA	Candolleana/ Candolleana	Psathyra/ Spintrigerae	**DQ986246**
P. iterata A.H. Sm.	M. Padamsee 99/ MIN	Washington, USA	Pannucia/ Appendiculatae	Psathyra/ Pennatae	**DQ986271**
P. kauffmanii A.H. Sm.	J.S. Hopple 99/ DUKE	N. America	Pseudostropharia/ Pseudostropharia	Psathyra/ Spadiceogriseae	**DQ986231**
P. larga (Kauffman) A.H. Sm.	D.J. McLaughlin 1170/ MIN	Minnesota, USA	Pannucia/ Appendiculatae	Psathyra/ Spadiceogriseae	**DQ986265**
P. leucotephra (Berk. & Broome) P.D. Orton	R. Singer M8920/ F	Mexico	Pseudostropharia/ Spintrigerae	Psathyra/ Spintrigerae	**DQ986240**
P. longicauda P. Karst.	GLM 46010	Europe	Psathyrella/ Psathyrella	Psathyrella/ Psathyrella	**AY207292**
P. madeodisca (Peck) A.H. Sm.	M. Padamsee NAMA84/ MIN	Minnesota, USA	Pannucia/ Appendiculatae	Psathyra/ Pennatae	**DQ986273**
P. marcescibilis (Britzelm.) Singer	GLM 46011	Europe	Candolleana/ Fragillissimae	Psathyra/ Spintrigerae	**AY207285**
P. melleipallida A.H. Sm.	D.J. McLaughlin 1138/ MIN	Minnesota, USA	Psathyrella/ Fatuae	Psathyra/ Pennatae	**DQ986272**
P. microrrhiza[1] (Lasch) Konrad & Maubl.	GLM 46012	Europe	Pannucia/ Pannucia	Psathyrella/ Psathyrella	**AY207286**
P. microrrhiza[2] (Lasch) Konrad & Maubl.	M. Vašutová 05/191/ MIN	Czech Republic	Pannucia/ Pannucia	Psathyrella/ Psathyrella	**DQ986244**
P. murcida (Fr.) Kits van Wav.	GLM 46013	Europe	Psathyrella/ Psathyrella	Psathyra/ Pennatae	**AY207284**
P. nolitangere (Fr.) A. Pearson & Dennis	M. Padamsee 127/ MIN	Minnesota, USA	Pannucia/ Pannucia	Psathyra/ Spadiceogriseae	**DQ986264**
''*P. oahuensis*'' nom. invalid.	R. Singer SH4/ F	Hawaii, USA	Psathyrella/ Subatratae	Psathyra/ Spintrigerae	**DQ986242**
P. ogemawensis A.H. Sm.	D.J. McLaughlin 1201/ MIN	Minnesota, USA	Pannucia/ Appendiculatae	Psathyra/ Hydrophilae	**DQ986274**
P. aff. olympiana A.H. Sm.	J-M. Moncalvo CR119/ DUKE	Costa Rica	Homophron/ Cystidiosae	Psathyra/ Spadiceae	**AF261488**
P. olympiana A.H. Sm.	J.F. Ammirati s.n./ WTU	Washington, USA	Homophron/ Cystidiosae	Psathyra/ Spadiceae	**DQ986254**
P. panaeoloides (Maire) M.M. Moser	GLM 46014	Europe	Psathyrella/ Fatuae	Psathyra/ Spadiceogriseae	**AY207287**
P. piluliformis[1] (Bull.) P.D. Orton	P.B. Matheny 390/ WTU	Washington, USA	Pannucia/ Appendiculatae	Psathyra/ Hydrophilae	**DQ986257**
P. piluliformis[2] (Bull.) P.D. Orton	GLM 46015	Europe	Pannucia/ Appendiculatae	Psathyra/ Hydrophilae	**AY207288**
P. prona (Fr.) Gillet	GLM 46016	Europe	Psathyrella/ Atomatae	Psathyrella/ Atomatae	**AY207289**

Table 1 (*continued*)

Species[a]	Collector No./Herb.[b]/ GenBank Voucher ID	Collection location	Subgenera/ sections (Smith)	Subgenera/sections (Kits van Waveren)	GenBank ID
P. ramicola A.H. Sm.	P.B. Matheny 871/ WTU	Washington, USA	Psathyrella/ Umbonatae	Psathyrella/ Atomatae	**DQ986258**
P. rhizophorae Singer	J. Kohlmeyer 2564/ F, holotype	Hawaii, USA	Psathyrella/ Fatuae	Psathyrella/ Atomatae	**DQ986239**
P. rhodospora[1] M.G. Weaver & A.H. Sm.	D.J. McLaughlin 1226/ MIN	Minnesota, USA	Homophron/ Homophron	Psathyra/ Spadiceogriseae	**DQ986227**
P. rhodospora[2] M.G. Weaver & A.H. Sm.	M. Padamsee 133/ MIN	Minnesota, USA	Homophron/ Homophron	Psathyra/ Spadiceogriseae	**AY645058**
P. rugocephala (G.F. Atk.) A.H. Sm.	R. Vilgalys 778/ DUKE	Papua New Guinea	Lacrymaria/-	Psathyra/ Spadiceogriseae	**DQ986233**
P. spadicea[1] (Schaeff.) Singer	GLM 46017	Europe	Homophron/ Homophron	Psathyra/ Spadiceae	**AY207291**
P. spadicea[2] (Schaeff.) Singer	P.B. Matheny 2710	Colorado	Homophron/ Homophron	Psathyra/ Spadiceae	**DQ470822**
P. spadiceogrisea[1] (Schaeff.) Maire	GLM 46018	Europe	Psathyrella/ Fatuae	Psathyra/ Spadiceogriseae	**AY207290**
P. spadiceogrisea[2] (Schaeff.) Maire	P.B. Matheny 2292/ WTU	Washington, USA	Psathyrella/ Fatuae	Psathyra/ Spadiceogriseae	**DQ986256**
P. squamifera P. Karst.	R. Singer C5321/ F	Italy	Pannucia/ Pannucia	Psathyrella/ Psathyrella	**DQ986237**
P. subamara A.H. Sm.	M. Padamsee NAMA85/ MIN	Minnesota, USA	Psathyroides/-	Psathyra/ Spadiceogriseae	**DQ986261**
P. subatrata (Batsch) Gillet	R. Singer C5319/F	Italy	Psathyrella/ Subatratae	Psathyrella/ Subatratae	**DQ986236**
P. cf. *subhyalinispora* A.H. Sm.	D.J. McLaughlin 1158/ MIN	Minnesota, USA	Psathyrella/ Subatratae	Psathyra	**DQ986268**
P. sublateritia A.H. Sm.	D.J. McLaughlin 1156/ MIN	Minnesota, USA	Homophron/ Homophron	Psathyra/ Spadiceae	**DQ986228**
P. subtruncatispora A.H. Sm.	A. Methven NAMA207/ MIN	Minnesota, USA	Psathyroides/-	Psathyra/ Spadiceogriseae	**DQ986267**
P. tephrophylla (Romagn.) M.M. Moser	GLM 46019	Europe	Psathyrella/ Umbonatae	Psathyra/ Spadiceogriseae	**AY207293**
P. typhae (Kalchbr.) A. Pearson & Dennis	J.S. Hopple 1789/ DUKE	Montana, USA	Pannucia/ Pannucia	Psathyra/ Spintrigerae	**DQ986229**
P. aff. *vanhermanii* A.H. Sm.	J-M. Moncalvo CR31/ DUKE	Costa Rica	Pseudostropharia/ Spintrigerae	Psathyra/ Pseudostropharia	**AF261487**
P. vernalis (J.E. Lange) M.M. Moser	R. Singer C5257/F	Switzerland	Psathyrella/ Obtusatae	Psathyra/ Spadiceogriseae	**DQ986238**

[a] If a species is represented by more than one collection, it is distinguished by consecutive numbers (also see Fig. 1).

[b] Herbarium abbreviations. Holmgren and Holmgren (1998) (continuously updated). Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. http://sweetgum.nybg.org/ih/.

analyses were acquired from GenBank and were included in the dataset (Supplementary Table 1). Sequences of *Cystoagaricus strobilomyces*, *Crucibulum laeve*, *Podaxis pistillaris*, *Montagnea arenaria*, *Cyathus stercorarius*, *Mythicomyces corneipes*, *Parasola plicatilis*, *Parasola leiocephala*, *Coprinellus impatiens*, and *Stagnicola perplexa* were also obtained from GenBank (Supplementary Table 1) and included in our dataset. The generic type *Coprinellus deliquescens* (synonym ''*Coprinus*'' *silvaticus*) was obtained as a dried specimen from R. Watling (RW27209) and was used to generate a LSU sequence. An additional 21 sequences of *Psathyrella* were obtained from GenBank; all others were original sequences generated for this study (Table 1).

2.2. DNA extraction, PCR amplification, and sequencing

DNA for sequencing was extracted from dried fruit bodies using one of three methods: (1) QIAGEN DNeasy Tissue kit (QIAGEN Inc., Valencia, CA); (2) REDExtract-N-Amp Tissue PCR Kit (Sigma–Aldrich Ltd., St. Louis, MO); (3) a modified cetyltrimethylammonium bromide (CTAB) protocol (Anikster et al., 2004). In the modified CTAB method, 2–3 gills per sample were frozen in liquid nitrogen and crushed using a micropestle in 1.5 ml tubes. CTAB extraction buffer (Anikster et al., 2004) was added to the samples followed by sodium dodecyl sulfate and incubated overnight at 65 °C. After incubation, the samples were extracted with an equal volume of phenol/chloroform/iso-amyl alcohol (25:24:1) and centrifuged at 11,500 rpm for 15 min. The supernatant was transferred to another tube and RNA was removed by digestion with 10 μl of 10 μg/ml RNase A (Sigma–Aldrich Ltd.) at 37 °C for 2 h. DNA was precipitated with cold isopropanol (0.6× volume) in the freezer at −20 °C overnight. DNA pellets were washed with 500 μl of cold 70% ethanol and resuspended in 50 μl of TE buffer.

For PCR amplification several dilutions were tested: 1:10, 1:100, 1:1000. Each PCR reaction had a total volume of 18.8 μl and contained 4 μl of the diluted DNA, 0.4 μl each of LSU primers, 10 μl of either REDExtract-N-Amp PCR Reaction Mix or JumpStart Taq ReadyMix (Sigma–Aldrich Ltd.) and 4 μl of water or 5 M Betaine (Sigma–Aldrich Ltd.). Primers for PCR amplification of the LSU region were: ITS-1F and Tw14 (http://plant-bio.berkeley.edu~/bruns/primers.html); 5.8 SR, LROR, LR7, LR16, LR3R, LR22R, and LR5 (http://www.biol-ogy.duke.edu/fungi/mycolab/primers.htm); and a newly designed primer LROR-A (CAAGGATTCCCCTAC-TAACTGC, Dentinger, B.T.M.). PCR amplification was performed using a MJ Research PTC 100 thermocycler (MJ Research Inc., Incline Village, Nevada). PCR was performed using either the protocol outlined in Gardes and Bruns (1993) or a touchdown program with the following parameters: 1 min at 95 °C; 1 min at 95 °C; 1 min at 60 °C (add −1 °C per cycle); 1 min at 72 °C; cycle 5 times; 1 min at 95 °C; 1 min at 55 °C; 1 min at 72 °C; cycle 25 times; 7 min at 72 °C.

PCR products were purified using the QIAGEN QIA-quick PCR Purification kit (QIAGEN Inc.). Primers for sequencing were Tw14, CTB6, LROR, LR5, LR22R, LR16, and LROR-A. Sequencing was done through the BioMedical Genomics Center, University of Minnesota; samples were run on an Applied Biosystems model 3730 automated capillary DNA sequencer using ABI BigDye Terminator Chemistry (Applied Biosystems, Foster City, CA). Sequences were edited with Sequencher v 3.0 or 4.6 software (GeneCodes Corp., Ann Arbor, MI).

2.3. Phylogenetic analyses

Initially sequences of *Psathyrella* were aligned manually in the editor window of PAUP* 4.0b10 (Swofford, 2002) with a LSU dataset containing 945 taxa with an extensive sampling from the euagarics (Dentinger and McLaughlin, 2006; Moncalvo et al., 2002). A sequence of *Cystoagaricus strobilomyces*, recently assigned to Psathyrellaceae (Vel-linga, 2004), was also included in this dataset. This dataset was analyzed using a heuristic search and parsimony criterion (search methods outlined below) to identify possible outgroups and to evaluate the effect of expanded taxon sampling on the phylogeny of *Psathyrella*. *Mythicomyces corneipes* and *Stagnicola perplexa* appeared to be sister to the Psathyrellaceae in an 877 taxa euagarics dataset (Mon-calvo et al., 2002, see their Fig. 1, p. 367) and were included in this dataset; *M. corneipes* was also included in Psathy-rellaceae (Matheny et al., 2006, see their Fig. 1, p. 987).

Sequences from Psathyrellaceae and allied taxa (Table 1 and Supplementary Table 1) were aligned manually in PAUP* using an alignment of only coprinoid taxa (Hopple and Vilgalys, 1999) available on TreeBASE (study acces-sion number S605; www.treebase.org/treebase/) as a start-ing point. Equally weighted parsimony analysis was performed with PAUP* with only parsimony-informative

characters included and gaps treated as missing data. Ambiguously aligned nucleotides (positions 567–580) and positions of missing sequence information resulting from the primers utilized (1–66 and 854–1380) were excluded from analyses. Three sequences were much shorter than the rest of the matrix (*Psathyrella hymenocephala*, *P. psam-mophila*, *P. michiganensis*), and were excluded. A heuristic search (1000 replicates) was performed with maxtrees set to auto-increase, random taxon addition sequences, with branch swapping set to tree bisection-reconnection (TBR), and saving not more than 100 trees with score greater than or equal to one per replicate. An appropriate outgroup for this dataset was the family Agaricaceae as suggested by a phylogeny inferred from six gene regions (Matheny et al., 2006). Four taxa from the Agaricaceae (*Podaxis pistillaris*, *Montagnea arenaria*, *Coprinus comatus*, and *C. sterquilinus*) were used for rooting purposes. Non-parametric bootstrap (NPB; significant at 70%; Felsenstein, 1985) analysis was conducted in PAUP* using 1000 pseu-doreplicates with 10 random taxon sequence additions per replicate, with branch swapping set to TBR, and no more than 500 trees per replicate were saved. Ambiguously aligned regions were coded using INAASE (Lutzoni et al., 2000), appended to the end of the data matrix, and incor-porated in the bootstrap analyses. Several taxa have over 30 gaps in a row between positions 551–585 (except for a TTG insert within this region, 572–574); the presence of this region of gaps was coded as present (=1) in these taxa and absent (=0) in all others and also appended to the end of the data matrix for bootstrap analyses. All coded char-acters were equally weighted. The coded characters were also utilized in a heuristic search as mentioned above; the most parsimonious trees (MPT) from this search were com-pared with the MPT from the search that only used DNA sequence data with a Wilcoxon Signed Ranks test (Larson, 1998; Templeton, 1983) as implemented in PAUP*.

The Akaike Information Criterion (AIC) was used to select the best-fit model for Bayesian analyses in Modeltest version 3.7 (Posada and Crandall, 1998). Maximum likeli-hood (ML) estimates were conducted with a model that had six categories of base-substitution, a gamma-distrib-uted rate parameter, and a proportion of invariant sites (GTR+Γ+I) in Mr.Bayes v3.1 (Hulsenbeck and Ronquist, 2001). Two independent runs each with four Metropolis-coupled Markov Chains with Monte Carlo simulation (MCMCMC) were executed in Mr.Bayes for two million generations; trees were sampled every 100th generation. Ends of the sequences (1–66 and 854–1380) and ambigu-ously aligned regions (567–580) were excluded. After the likelihoods of the trees in each chain converged, the "burn-in" was discarded. Branch lengths of the trees were saved and a Bayesian consensus phylogram based on mean branch lengths was calculated from the remaining trees. Bayesian MCMCMC-estimated posterior probabilities (BPP; significant at 95%) were made by constructing a majority-rule consensus tree in PAUP* based on the trees that remained after the burn from both the Bayesian runs

and were used to evaluate the level of support in the ML estimation.

For ML analyses the best fitting model of DNA evolution was chosen using the AIC as implemented in Modeltest. ML searches were performed using a GTR+Γ+I model in GARLI v0.951 (Zwickl, 2006). The run was repeated three times from random starting trees using the auto-terminate setting and default parameters. Branch lengths for Fig. 1 are ML estimates and are scaled in terms of expected numbers of nucleotide substitutions per site.
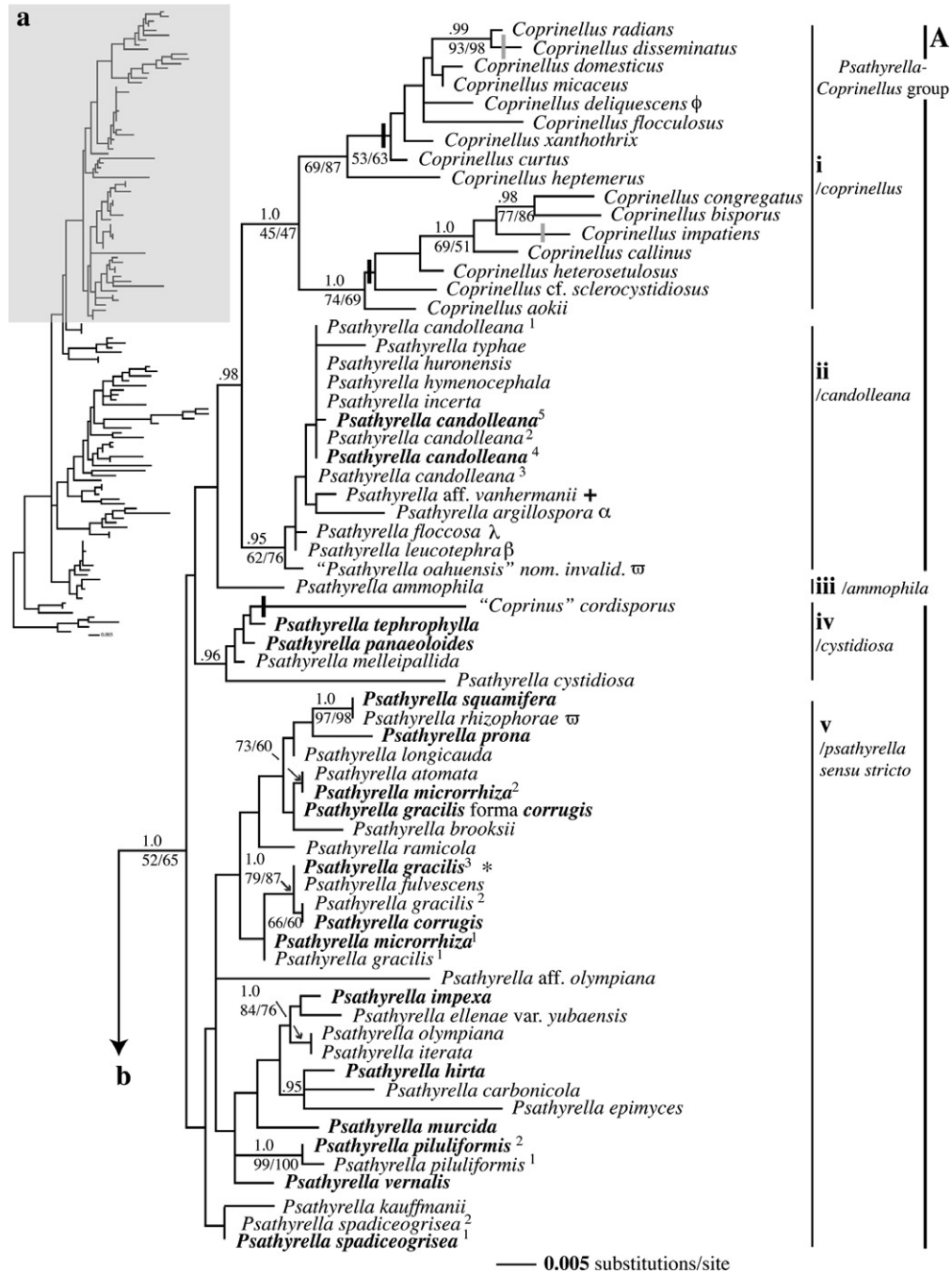

Fig. 1. The maximum likelihood tree (GTR+Γ+I model of evolution, $-\ln L = 6270.15$). Numbers above branches represent the Bayesian posterior probabilities, numbers below branches are maximum likelihood bootstrap values (100 replicates, represented as percentages) followed by nonparameteric bootstrap values (1000 pseudoreplicates with 10 random taxon sequence additions per replicate, represented as percentages). Branches indicated by letters (A–H) and Roman numerals (i–v) identify clades discussed in detail in the text. Species names in bold are European collections; other names without symbols are North American collections. Symbols: α, Colombia; λ, India; β, Mexico; ϖ, Hawaii, +, Costa Rica; ζ, Japan; * type species of *Psathyrella*; φ, type species of *Coprinellus*; δ, type species of *Coprinopsis*; φ, type species of *Parasola*; superscript numbers/letters correspond to multiple collections (Table 1). Black bars on the branches reflect the maximum number of gains of deliquescence as reconstructed in MacClade; gray bars on the branches reflect subsequent losses of deliquescence (Table 3 and Supplementary Table 2).
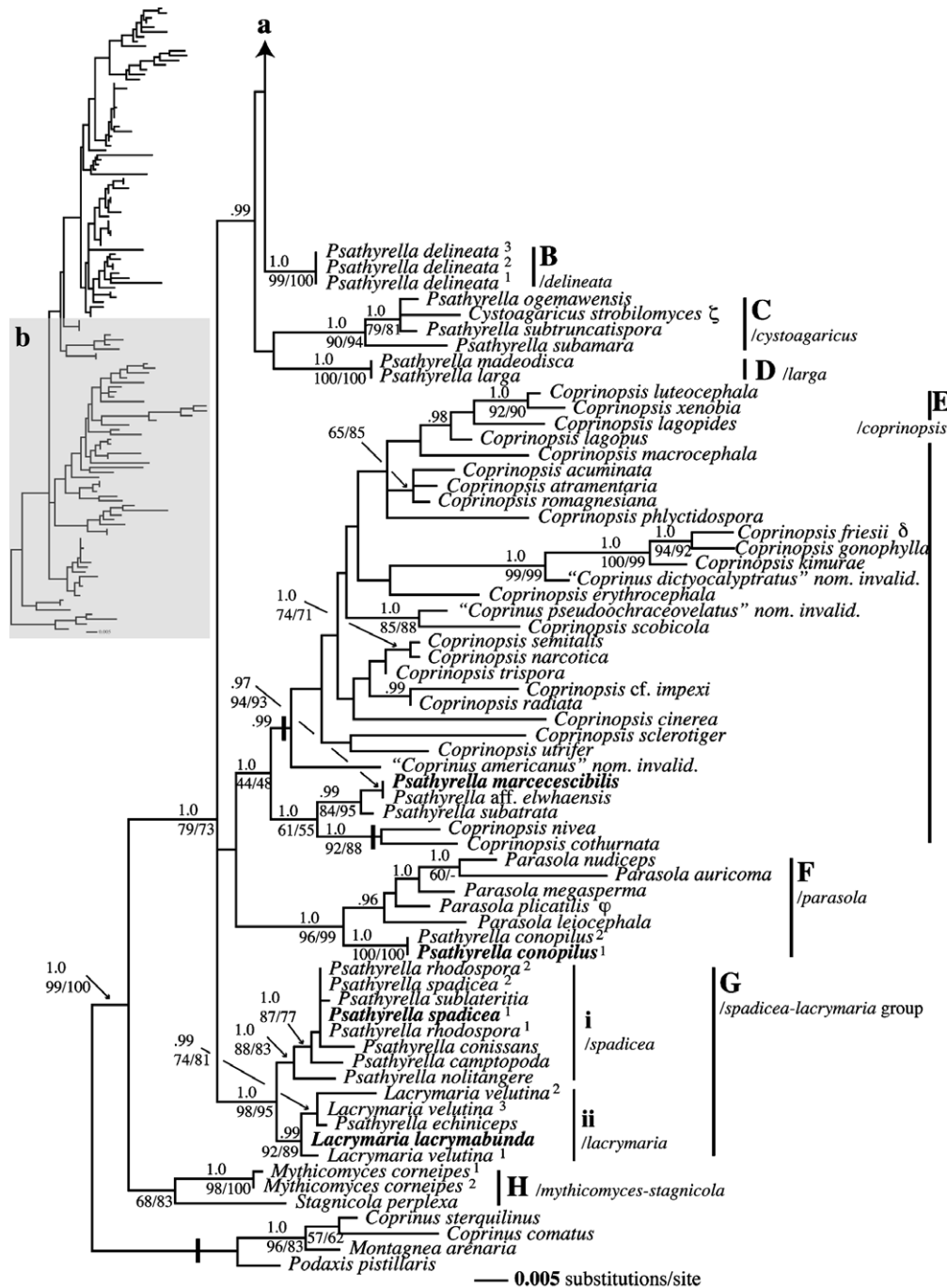
Fig. 1 (*continued*)

GARLI was also used to generate 100 ML nonparametric bootstrap replicates (MLBP; significant at 70%) with the generation threshold (without an improvement in topology) halved to 5000 as suggested by the program; the replicates were used to calculate a majority rule consensus tree in PAUP* to assess clade support.

2.4. Evaluation of taxonomic treatments and morphology

A series of Shimodaira-Hasegawa (SH) tests of constrained topologies was performed to evaluate various hypotheses (Table 2) (Shimodaira and Hasegawa, 1999). Constraint trees were created in MacClade v4.03 (Maddison and Maddison, 2001); an unresolved tree was constructed and all taxa for the genus or group under consideration were joined into a single branch with the internal topology unresolved. Two ML searches were conducted in GARLI as described above for each of the constraint trees to test the reproducibility of the results. A comparison of the likelihood values between the constrained and unconstrained ML trees that resulted was performed with the use of the SH test in PAUP*

Table 2
Shimodaira-Hasegawa tests (one-tailed RELL bootstrap with 1000 replicates) for constrained vs. unconstrained tree topologies (resulting from maximum likelihood search, GTR+Γ+I model of evolution) indicating significant values for constraints ($^*p < 0.05$)

Constraints to monophyly	$-\ln L$	Diff.	p
None	7247.92	0	Best
Psathyrella including subgenus *Lacrymaria*	7436.19	188.27	0.000[*]
Psathyrella without *Lacrymaria*	7512.20	264.27	0.000[*]
Psathyrella with Smith's taxonomic arrangement	10581.05	3333.13	0.000[*]
Psathyrella with Kits van Waveren's taxonomic arrangement	10303.82	3055.9	0.000[*]
Single gain of deliquescence	7644.35	396.43	0.000[*]
Coprinellus with *C. cordisporus*	7267.33	19.4	0.412
Coprinopsis	7280.73	32.81	0.133
Psathyrella Clade A, ii–v	7975.93	728.0	0.000[*]

with "RELL" approximation and 1000 bootstrap replicates.

The phylogenetic utility of traditional morphological characters used to separate certain genera, subgenera, and sections was evaluated in MacClade. The following features were coded as binary characters: deliquescence, crystals/incrustations on cystidia (sterile cells), brachycystidia (short cystidia), pleurocystidia (cystidia on face of gill), thick cystidial wall, spore ornamentation, persistence of spore pigmentation in sulfuric acid, germ pore (on apex of spore), caulocystidia (cystidia on stipe), pileocystidia (cystidia on cap), perisporium (membrane enveloping spores), and spore color (Supplementary Table 2). The one multistate character, spore shape, had six states (Supplementary Table 2) and it was treated as nonadditive (= unordered). Character states were obtained from published sources (Hopple, 1994; Kits van Waveren, 1985; Moser, 1983; Orton and Watling, 1979; Singer, 1947; Smith, 1972; Uljé, 2005) and our own observations (tissue mounted in 5% aqueous potassium hydroxide solution and viewed with a Zeiss Axioskop with a Plan-Neofluor 100X/ 1.3 N.A. lens and an Achromatic/Aplanetic 1.4 N.A. condensor at 1250X).

The ML tree and all of the MPTs were each imported into MacClade. Polytomies were assumed to be of uncertain resolution or "soft." The characters were plotted on the topologies using "Trace" and "choose character" to evaluate character evolution with "show all parsimonious states at each node" selected. The "minimum and maximum number" of changes per event was charted with the "resolve polytomies" option selected for each traced character. The retention index (RI) per character was calculated only on the ML tree.

3. Results

3.1. DNA sequencing and phylogenetic analyses

Approximately the first 900 base pairs of the 5′ end of the nuclear LSU region were sequenced for 54 collections and represented 50 species to which were added 7 species from GenBank. These were aligned with 1026 previously published sequences (Dentinger and McLaughlin, 2006; Moncalvo et al., 2002). The data matrix was composed of 1080 taxa and 1585 characters; only parsimony informative characters (457) were included for the analyses. Parsimony analyses revealed that three collections identified as *Psathyrella* cf. *subhyalinispora*, *P. rugocephala*, and *P. coprophila* fell outside Psathyrellaceae and grouped together with *Simocbye*, *Hebeloma*, and *Conocybe*, respectively. They were excluded from further analyses (data not shown).

The *Psathyrella* dataset was combined with 84 previously published LSU sequences available from GenBank (Supplementary Table 1). The data matrix was initially composed of 135 taxa and 1380 characters. The final matrix was composed of 132 taxa and 169 parsimony-informative characters. The heuristic search resulted in 10,500 MPT (L = 859, CI = 0.25, RI = 0.75, 114 islands). The Bayesian analyses of the data set were examined and the burn-in assessed at 7850 trees for each run using the standard deviation of split frequencies ($p < 0.05$) and the potential scale reduction factors (near 1). The BPP were calculated using the remaining 24,302 trees. The topology of the parsimony strict consensus tree (data not shown) differed from the Bayesian consensus and the ML phylogram (Fig. 1) by the lower number of resolved relationships; the backbone of the cladogram is a polytomy in the parsimony strict consensus. There was no significant difference between the Bayesian consensus tree and the ML tree ($p > 0.05$).

Psathyrella is polyphyletic and distributed throughout the ingroup within 11 clades (A, ii–v; B–F; G, i–ii) in the maximum likelihood tree (Fig. 1); there are a total of 13 clades within Psathyrellaceae. There are 51 well-resolved clades with significant MLBP, NPB, and BPP. Most of the *Psathyrella* species (49 species, Fig. 1, Clades A, ii–v), including the conserved generic type, *P. gracilis* (Fig. 2G), are placed in Clade A (/*Psathyrella–Coprinellus* group; BPP 1.0, MLBP 52%, NPB 65%) with *Coprinellus* (Clade A, i, /*coprinellus*, BPP 1.0, MLBP 45%, NPB 47%). Within Clade A, the well-supported clade (Clade A, ii, /*candolleana*; BPP.95, MLBP 62%, NPB 76%) containing *P. candolleana* (Fig. 2A) appears to have a sister relationship (BPP 0.98) to the clade containing two *Coprinellus* clades (Fig. 1, Clade A, i, /*coprinellus*). Various representatives of the type species of the genus, *Psathyrella gracilis* (including *P. corrugis* and *P. gracilis* var. *corrugis*) are distributed within Clade A, v (/*psathyrella sensu stricto*), which we therefore regard as *Psathyrella sensu stricto*. Species of *Psathyrella* separate into four clades (one with only one species) within Clade A, which is expected to have a significant impact on the nomenclature of *Psathyrella*. Clade B (/*delineata*) is solely comprised of three representatives of *P. delineata* (BPP 1.0, MLBP 99%, NPB 100%). Clade C (/*cystoagaricus*; BPP 1.0, MLBP 90%, NPB 94%), composed of *P. ogemawensis*, *P. subtrun-*

Fig. 2. *Psathyrella* species from the various clades. Species represented are: (A) *Psathyrella candolleana*, M. Padamsee 166, basidiospores (B) *Psathyrella echiniceps*, D.J. McLaughlin 1153, basidiospores, (C) *Psathyrella spadiceogrisea*, (D) *Psathyrella larga*, M. Padamsee 209, (E) *Psathyrella piluliformis*, (F) *Psathyrella nolitangere*, M. Padamsee 127, (G) *Psathyrella gracilis* forma *corrugis*, M. Vašutová 05/147. (A), (B), (D), (F) are copyright by the Bell Museum of Natural History, University of Minnesota and are used with permission. (C and E) are courtesy of P.B. Matheny. (G) is courtesy of M. Vašutová. Scale bar = 1 cm. Scale bar within basidiospore insets = 5 μm.

catispora, *P. subamara*, and *Cystoagaricus strobilomyces*, is a well-resolved clade and appears to be sister (no support) to the small Clade D (*/larga*; BPP 1.0, MLBP 100%, NPB 100%), which contains *P. larga* (Fig. 2D) and *P. madeodisca*. Clade E (*/coprinopsis*), composed of the genus *Coprinopsis* and three species of *Psathyrella*, is moderately supported (BPP 1.0, MLBP 44%, NPB 48%). Clade F

(*/parasola*) is strongly supported (BPP 1.0, MLBP 96%, NPB 99%) with the genus *Parasola* in a sister relationship to two representatives of *Psathyrella conopilus*. Clade G (*/spadicea–lacrymaria*) is strongly supported (BPP 1.0, MLBP 98%, NPB 95%) and consists of two clades: one (Clade G, ii, */lacrymaria*) with *Lacrymaria* and one *Psathyrella* taxon (BPP 0.99, MLBP 92%, NPB 89%) and the

other (Clade G, i, /*spadicea*) with six *Psathyrella* species (BPP 1.0, MLBP 92%, NPB 83%). The relationship between *Mythicomyces* and *Stagnicola* and the rest of Psathyrellaceae is strongly supported (Fig. 1, Clade H, /*mythicomyces–stagnicola*; BPP 1.0, MLBP 99%, NPB 100%).

3.2. Evaluation of taxonomic treatments and morphology

The SH test was used (Table 2) to compare constrained maximum likelihood trees that force monophyly of *Psathyrella* with and without *Lacrymaria*. For both hypotheses, the constrained trees were found to be significantly worse than the unconstrained tree ($p < 0.000$) and the monophyly of *Psathyrella* was rejected. Both of the trees constrained using Smith's (1972) and Kits van Waveren's (1985) taxonomic systems were found to be significantly worse ($p < 0.000$) than the unconstrained tree and were rejected. The tree constrained with a single event of a gain of deliquescence was also significantly worse ($p < 0.000$) than the unconstrained tree. Constraining all *Psathyrella* species within Clade A (ii–v) as well as all the *Psathyrella* (A; iv, v) except the *P. candolleana*/*P. ammophila* clades (A; ii, iii) to be monophyletic resulted in significantly worse ML trees ($p < 0.000$). Constraining the monophyly of *Coprinellus* and "*Coprinus*" *cordisporus* did not result in a significantly worse ML tree ($p = 0.412$). Constraining *Coprinopsis* to be monophyletic with the exclusion of *P. marcescibilis*, *P.* aff. *elwhaensis*, and *P. subatrata* did not result in a significantly worse ML tree ($p = 0.133$) and thus the monophyly of *Coprinopsis* cannot be rejected.

Mapping characters on the cladogram illustrates homoplasy of traditionally used taxonomic features across Psathyrellaceae, although the relatively high RIs suggest that these features are useful at the species level (Table 3). Plotting the steps per character, without resolving polytomies in the ML tree, resulted in the same maximum and minimum number as plotting the changes per character with the polytomies randomly resolved. Deliquescence has been

Table 3
Morphological character changes optimized on ML and MP trees using parsimony

Character (No. of states)	ML gain/ MP gain	ML loss/ ML loss	RI on ML only
1. Deliquescence (2)	3–6/3–5	2–5/2–5	0.83
2. Presence of Brachycystidia (2)	13–15/7–21	4–6/1–15	0.66
3. Pleurocystidia present (2)	5–6/3–9	10–11/9–15	0.61
4. Thick-walled cystidia (2)	5/5	0/0	0.64
5. Crystals on cystidia (2)	4–6/5–6	0–2/0–1	0.50
6. Spore shape (6)	27–53/22–54	0–5/0–7	0.25
7. Spore color (2)	2/1–2	0/0–1	1.00
8. Spore decoration (2)	3/3	0/0	0.71
9. Spore pigment in sulfuric acid persistent (2)	0/0	0/2	0.67
10. Germ pore present (2)	0/0–4	7/3–7	0.14
11. Caulocystidia present (2)	1/1–7	14/9–15	0.71
12. Pileocystidia present (2)	7–11/7–13	1–5/0–5	0.39
13. Perispore present (2)	3/2–4	0/0–2	0.50

gained three to six and lost two to five times (RI = 0.83). The presence of brachycystidia (character 2), pleurocystidia (character 3), and thick-walled cystidia (character 4) has evolved independently multiple times as have cystidia with ornamentations or crystals (character 5, RI = 0.5). All taxa (except ones for which the character state was unknown) within Psathyrellaceae have pigmented spores but appear to lose this pigment in sulfuric acid except for *Coprinopsis lagopus* (Table 3). Spore shape overall is highly homoplastic (RI = 0.25) but can be informative at the level of a species group.

4. Discussion

4.1. Psathyrella and intrafamilial taxonomic concepts

Although previous studies have suggested that *Psathyrella* is not monophyletic (Hopple and Vilgalys, 1999; Walther et al., 2005), this study demonstrates conclusively that *Psathyrella*, as currently defined (Kits van Waveren, 1985; Singer, 1986; Smith, 1972), is polyphyletic. Psathyrellaceae is monophyletic and composed of at least 13 clades (Fig. 1, Clade A, i–v; B–F; G, i–ii; H). Previously inferred relationships between genera in Psathyrellaceae (Hopple and Vilgalys, 1999; Redhead et al., 2001) will have to be reevaluated with this increased taxon sampling of *Psathyrella*. This study also demonstrates that generic limits in Psathyrellaceae will need to be modified to reflect these results. Here we comment on how generic limits need to be reinterpreted and when possible indicate potential morphological synapomorphies.

In Clade F (/*parasola*), five representatives of *Parasola* (including the type species *P. plicatilis*) group with *Psathyrella conopilus*. Walther et al. (2005) suggest a possible morphological shared feature for this clade: the presence of setae (thick-walled bristles) in the pileipellis (cap tissue) as seen in both *Parasola auricoma* and *Psathyrella conopilus*. Also, *P. conopilus* has a strongly striate cap, which is reminiscent of the plicate cap of members of *Parasola*. Although strong support overall exists for Clade F, there is only significant BPP (.96) support for the clade containing *Parasola*; MLBP is 45% and in the MP strict consensus tree *Parasola auricoma* falls between the *Psathyrella conopilus* clade and the *Parasola* clade, which now has 57% NPB support. *Psathyrella conopilus* could be assigned to *Parasola*.

We also evaluated whether *Coprinellus* required redefinition. Redhead et al. (2001) proposed the recognition of *Coprinellus* and transferred 38 species into this genus (Fig. 1, Clade A, i, /*coprinellus*), but without obtaining a DNA sequence of the generic type *C. deliquescens* (= "*Coprinus*" *silvaticus*). This is the first molecular study of Psathyrellaceae that includes the type of *Coprinellus*. Most of *Psathyrella*, including its type *P. gracilis*, are included in Clade A (/*Psathyrella-Coprinellus* group; BPP 1.0, MLBP 52%, NPB 65%) along with the two *Coprinellus* clades. However, within this clade there are only eight rela-

tionships supported with significant BPP and greater than 60% ML and MP BS support (Fig. 1). Redhead et al. (2001) state that a clear separation of a group of coprinoid taxa from psathyrelloid species was difficult (in reference to Clade II, node 18, Hopple and Vilgalys, 1999). This roughly corresponds with our study's Clade A (Fig. 1).

To assess generic limits of *Psathyrella sensu stricto*, a series of constrained ML analyses were performed (Table 2). All of the *Psathyrella* species within Clade A (Fig. 1, Clades A, ii–v) were constrained to be monophyletic; this hypothesis was significantly worse (*p* < 0.000) than the unconstrained analysis. Clade A, iv (Fig. 1, /*cystidiosa*), consisting of *P. tephrophylla* to *P. cystidiosa*, was also constrained to be monophyletic with Clade A, v (Fig. 1, /*psathyrella sensu stricto*) with and without the inclusion of "*Coprinus*" *cordisporus*. Both of these hypotheses were also rejected. The results of the constraint analyses demonstrate that Clade A, v (Fig. 1) best represents the generic limits of *Psathyrella sensu stricto.*

There is a possible sister relationship between *Coprinellus* and *Psathyrella* species in Clade A, ii (Fig. 1, /*candolleana*) containing the well-known and easily recognized *P. candolleana* (BPP only). In the parsimony strict consensus (data not shown), *Coprinellus* is not resolved as monophyletic but is recovered as two clades corresponding to the two major clades within Clade A, i (Fig. 1). *Coprinellus* was thus constrained to be monophyletic and loaded as a filter in PAUP* to find the trees that support this constraint. *Coprinellus* is recovered as monophyletic in approximately 32% of the MPT; interestingly all resulting MP trees show a sister relationship between Clade A, i and ii (Fig. 1). Clade A, ii appears to represent an independent psathyrelloid lineage. *Psathyrella ammophila* (Clade A, iii, /*ammophila*) occurs in sand dunes; it is basal to Clades A, i–ii and could also represent a distinct psathyrelloid lineage. In the Walther et al. study (2005), *Coprinellus* appears to be polyphyletic but with poor support values (see their Fig. 5, p. 532), with *Coprinellus impatiens* sister to a large *Psathyrella* clade. This result is likely an artifact of their taxon sampling because in our study the monophyly of *Coprinellus* (including *C. impatiens*) cannot be rejected.

"*Coprinus*" *cordisporus* occupies a long branch (Fig. 1, Clade A, iv) suggesting that its relationship to Clade A, iv may be an artifact (Felsenstein, 1978). Recent work (Keirle et al., 2004) using nuclear ribosomal internal transcribed spacer region (ITS) sequence data shows that *C. cordisporus* (18 isolates) forms a clade sister to *Coprinellus* with 82% NPB (see their Fig. 16 C, p. 89). Also *P. candolleana* in this dataset (Keirle et al., 2004) is sister to the *Coprinellus* and the *C. cordisporus* clades. Constraining *Coprinellus* and *C. cordisporus* to be monophyletic in our dataset resulted in a ML tree that was not significantly worse (*p* = 0.412) than the unconstrained ML tree; this agrees with the results of Keirle et al. (2004). Interestingly, the four *Psathyrella* species in Clade A, iv (/*cystidiosa*) are sister to the *Coprinellus* plus *C. cordisporus* clade in the constrained analysis. Keirle et al. (2004) and Redhead et al. (2001) suggest that on the basis of the molecular and morphological data "*Coprinus*" *cordisporus* may represent a distinct coprinoid lineage; it also represents an additional gain of deliquescence.

Coprinopsis is paraphyletic with the addition of three *Psathyrella* species to the clade containing all *Coprinopsis* species (Fig. 1, Clade E, /*coprinopsis*; BPP 1.0, MLBP 44%, NPB 48%). However, constraining *Coprinopsis* to be monophyletic does not result in a significantly worse (*p* = 0.133) ML tree than the unconstrained analyses. Additional data are required to determine whether or not *Coprinopsis* should be expanded to include these species of *Psathyrella*. Clades B–D (Fig. 1) all represent additional psathyrelloid lineages. *Mythicomyces corneipes* (Fig. 1, Clade H, /*mythicomyces–stagnicola*) is included in Psathyrellaceae in Matheny et al. (2006) or with *Stagnicola perplexa* represents the sister group to the Psathyrellaceae.

The landmark study of Hopple and Vilgalys (1999) and the challenging, monumental taxonomic decisions that followed (Redhead et al., 2001) paved the way for this study of Psathyrellaceae. However, with the under-sampling of *Psathyrella* species, the story was not complete. Redhead et al. (2001) realized that the under-sampling of *Psathyrella* and psathyrelloid taxa caused the generic limits within Psathyrellaceae to be unresolved and as such were opposed to merging *Psathyrella* with coprinoid species (in their case all the rest of Psathyrellaceae, including *Lacrymaria*). The additional sampling in our study distilled the issue faced by Redhead et al. (2001) down to the generic limits of *Coprinellus* and *Psathyrella* because the generic name *Psathyrella* (Quélet, 1872) has priority over the generic name *Coprinellus* (Karsten, 1879).

Our analyses have now established the generic limits of *Psathyrella sensu stricto* but before any taxonomic decisions are undertaken the matter of *Psathyrlla gracilis*, the generic type should be resolved; there is evidence that *P. gracilis* may be a species complex (Fig. 1). An isolate of *P. gracilis* from Sweden (type locality) was included in the analyses and forms a well-supported clade with another *P. gracilis* isolate from N. America, *P. corrugis* (synonymized with *P. gracilis* by Smith, 1972 and Kits van Waveren, 1985) from Europe (possibly Germany), and *P. fulvescens*. A lectotype of *P. gracilis* should be designated preferably from the type locality so that taxonomic action can ensue.

A clearer picture is emerging with the inclusion of additional species of *Psathyrella*. Generic concepts will continue to evolve as more *Psathyrella* diversity is included in future analyses. One such concept that may change is whether *Psathyrella conopilus* should be included in *Parasola*. In an "artificial key to phylogenetically separated *Coprinus*-like genera", Redhead et al. (2001) mention that species within *Parasola* always have pleurocystidia; *P. conopilus*, lacks them (Kits van Waveren, 1985). However, *Parasola megasperma* may (Uljé, 2005) or may not (Orton and Watling, 1979) have pleurocystidia. Uljé (2005) also reports that *Parasola miser* (as *Coprinus miser*) lacks pleur-

ocystidia but this species placement in *Parasola* needs to be confirmed. *P. conopilus* also has pileocystidia (thin-walled cystidia in the cap), a character that is not present in any species of *Parasola*. On the basis of these morphological differences, *P. conopilus* could represent a unique genus.

4.2. Systematic treatments

The constrained trees based on the classification systems of Smith (1972) and Kits van Waveren (1985) were both significantly worse than the unconstrained tree. The tree based on Kits van Waveren's treatment had a higher likelihood score than did the one based on Smith's, possibly because it has fewer taxonomic divisions and therefore fewer constraints on the topology. Neither system is adequate to address the phylogenetic relationships and to form the basis for a new systematic arrangement for *Psathyrella*.

Lacrymaria, distinguished from *Psathyrella* primarily by its ornamented spores, has historically been considered part of *Psathyrella* and treated as a subgenus by Smith (1972) and Singer (1986). However, *Lacrymaria* also has been recognized as a distinct genus (Kits van Waveren, 1985; Watling, 1979). The *Lacrymaria* clade (Fig. 1, Clade G, ii, /lacrymaria) contains *Psathyrella echiniceps* (Fig. 2B), which has smooth to obscurely warty spores. The ornamented spores may be characteristic of this group. There are also other species with ornamented spores that have not been part of this analysis, such as *P. nigeriensis* (Pegler and Young, 1992). The parsimony, likelihood, and Bayesian analyses strongly support the sister relationship of smooth-spored species of the *P. spadicea* group (Fig. 1, Clade G, i, /spadicea) with the rough-spored species of *Lacrymaria* (Clade G, ii, /lacrymaria). *Psathyrella echiniceps* should be transferred to *Lacrymaria*.

With more than 10% of the species sampled and consulting multiple analytical approaches, the organization of *Psathyrella sensu stricto* is taking shape. The 11 psathyrelloid lineages (Clades A, ii–v; B–F; G, i–ii) impact the taxonomic circumscription of *Psathyrella*, and could well change the generic limits of *Parasola* and *Coprinopsis*. At least five clades can be raised to generic status. Relationships within genera in Psathyrellaceae are elucidated in the context of a greatly expanded sampling of *Psathyrella*. However, LSU sequence data alone do not provide sufficient phylogenetic support for the backbone structures within Clades A and E. Adding other genes such as *rpb1* (the gene that encodes the largest subunit of RNA polymerase II, Matheny et al., 2002) may improve support values but can only effectively delimit generic limits if there is adequate taxon sampling as well. This study provides a targeted sampling blueprint for future multigene analyses and for a comprehensive taxonomic revision of Psathyrellaceae.

Confusion over species limits still needs to be addressed. Smith (1972) described over 400 species, many of them new; Kits van Waveren (1985) synonymized many of these species. This problem is compounded by the fact that many species in Smith's monograph (1972) were based on descriptions of European types. Smith (1972) did not have access to molecular tools and was of the opinion that many species did not co-occur in Europe and North America. Certain sequences included in our analyses are from specimens from both regions, and in the case of *Psathyrella conopilus* and *P. spadiceogrisea* (Fig. 2C), the sequences cluster together (Fig. 1) indicating a broad geographic range for these species. Keying *Psathyrella cystidiosa*, a Minnesota endemic, through Kits van Waveren (1985) leads to identifying the collection as the widely distributed *P. olympiana*; however, the two species do not group together in the phylogeny (Fig. 1). Neither did *Psathyrella ogemawensis* and *P. piluliformis* (Fig. 2E), which were treated as synonymous by Kits van Waveren (1985). ITS sequence data may aid in delimiting species found in Europe and North America (Padamsee, unpublished).

4.3. Evolution of morphology and a reevaluation of its systematic utility

In the taxonomic treatments of Psathyrellaceae and the genera within, 13 characters (Table 3 and Supplementary Table 2) have been prominently used to define genera and their relationships among one another. Our results imply that these morphologies are not conserved at the level of family or tribe, but rather at much finer taxonomic levels, such as species groups and genera. Others have also expressed reservations about the conservation of these morphologies. Redhead et al. (2001) states that autodigestion of gills has obviously arisen several times in the *Psathyrella* clade and possibly also been lost once. Within our study, deliquescence (autodigestion) has evolved independently within Psathyrellaceae (and Agaricaceae) for a maximum of six times and been lost a maximum of five times (Table 3). However, the character has a high retention index (0.83) implying low scatter (Mickevich and Lipscomb, 1991) and phylogenetic usefulness at more recent divergences (e.g., among closely related species). Like deliquescence, several fungal morphologies traditionally used to distinguish genera exhibit homoplasy within Psathyrellaceae yet have high retention indices (Table 3, >0.50 for binary). These characters are potentially useful to define species and species groups in future systematic treatments.

One exception to the family-level homoplasy is character 9 (observable loss of spore pigment in sulfuric acid). With an RI of 0.67, this feature may be diagnostic for Psathyrellaceae but information is needed about *Mythicomyces* and *Stagnicola* spores. Previous studies (Kretzer et al., 1996; Singer, 1986) have mentioned the homoplasious nature of spore color in dark-spored genera and thus it is surprising that there is high support for the close relationship (BPP 0.98, MLBP 88%, NPB 89%, RI = 1.0) between several species with red spores (Fig. 1, Clade G, i, /spadicea). Further, not all red-spored species of *Psathyrella* were sampled in our analyses. Data from these additional species will be needed to resolve this subclade.

Spore shape (character 6, Table 3) is a homoplastic character (RI = 0.25), although individual states may be conserved and informative. For example, the state "subglobose to globose spores" is informative (within Clade E/*coprinopsis*; *Coprinopsis friesii–C. dictyocalyptratus*) even though across the phylogeny the shape of spores is homoplasious. The majority of Psathyrellaceae and the outgroup have elliptic spores, which may imply that this shape is the pleisiomorphic state (Supplementary Table 2). "Hexagonal-" and "sub-triangular-" shaped spores are autapomorphic (Supplementary Table 2) and as such provide little phylogenetic information. Modifications of spore shape (e.g., globose) may serve as a diagnostic feature but should be viewed with caution. For example, *Psathyrella ogemawensis*, *P. subtruncatispora*, *P. subamara*, and *Cystoagaricus strobilomyces* (Clade C, /*cystoagaricus*) all have ovate to wedge-shaped spores but this is also a highly homoplastic character state (Supplementary Table 2).

4.4. Conclusions

Psathyrella is the archetypal little brown mushroom genus with few easily discernable characters to delimit the genus or the species. Molecular sequence data should be combined with morphology to contextualize the genera and sections and to understand morphological evolution within the group. Smith (N.S. Weber, personal communication) commented that his work with *Psathyrella* was just a step on the ladder toward a better understanding of this genus; this research adds another step towards this goal. LSU DNA sequence data can be considered the "industry standard" for fungal systematics. Unfortunately, this gene alone is not sufficient to resolve relationships at all taxonomic levels (Moncalvo et al., 2002). However, increasing the number of genes used without a corresponding increase in sampling can also hamper the ability to infer relationships (Hibbett, 2006). Achieving a definitive understanding of relationships in Psathyrellaceae and other mushroom families will require assembling intensively sampled, multigene datasets. With just 10% of *Psathyrella* sampled, there is evidence for raising five psathyrelloid lineages to generic status and for 13 clades within Psathyrellaceae. This study increases the feasibility of defining new genera based on diagnostic, morphological synapomorphies and interpreting the evolution of morphological characters. Once a polyphyletic assemblage of species, *Psathyrella* can be redefined as a monophyletic genus.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2007.11.004.

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