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ARTICLE

Phylogenetic inference and trait evolution of the psychedelic mushroom genus *Psilocybe* sensu lato (Agaricales)

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Abstract: The genus *Psilocybe* contains iconic species of fungi renowned for their hallucinogenic properties. Recently, *Psilocybe* also included non-hallucinogenic species that have since been shifted to the genus *Deconica*. Here, we reconstruct a multigene phylogeny for *Psilocybe*, *Deconica*, and other exemplars of the families Hymenogastraceae and Strophariaceae sensu stricto (s. str.), using three nuclear markers (nLSU-rRNA, 5.8S rRNA, and *rpb1*). Our results confirm the monophyly of *Deconica* within Strophariaceae s. str., as well as numerous robust infrageneric relationships. *Psilocybe* is also recovered as a monophyletic group in the Hymenogastraceae, in which two principal lineages are recognized, including several nested subgroups. Most sections of *Psilocybe* following classifications based on morphological features are not supported in these analyses. Ancestral character state reconstruction analyses suggest that basidiospore shape in frontal view and spore wall thickness, commonly used to characterize sections in *Deconica* and *Psilocybe*, are homoplastic. Chrysocystidia, sterile cells located in the hymenium, evolved on at least two occasions in the Strophariaceae s. str., including in a novel lineage of *Deconica*.

Key words: Basidiomycota, chrysocystidia, *Deconica*, molecular systematics, psilocybin, psychedelic mushrooms.

Résumé : Le genre *Psilocybe* comporte des espèces icônes de champignons réputées pour leurs propriétés hallucinogènes. Récemment, on a attribué au genre *Psilocybe* des espèces non hallucinogènes transférées depuis au genre *Deonica*. Les auteurs ont construit une phylogénie multigénique pour les *Psilocybe*, *Deconica* ainsi que d'autres entités des familles Hymenogastraceae et Strophariaceae sensu stricto (s. str.), en utilisant trois marqueurs nucléiques (nLSU-rARN, 5.8S rARN, et rpb1). Les résultats confirment la monophylie des *Deconica* au sein des Strophariaceae s. str., ainsi que de nombreuses relations infra génériques robustes. Le genre *Psilocybe* recouvre aussi sont statut monophylétique parmi les Hymenogastraceae, où on reconnait deux lignées principales, incluant plusieurs sous-groupes nichés. Dans cette analyse, la plupart des sections du genre *Psilocybe* basées sur les caractères morphologiques ne trouvent aucun support. Les analyses de reconstruction de l'état des caractères ancestraux suggèrent que la forme des basidiospores en vue frontale ainsi que l'épaisseur de la paroi sporale, généralement utilisées pour caractériser les sections au sein des *Deconica* et *Psilcybe*, sont homoplastiques. Les chrysocystides, cellules stériles localisées dans l'hyménium, ont évolué au moins en deux occasions chez les Strophariaceae s. str., incluant une nouvelle lignée parmi les *Deconica*. [Traduit par la Rédaction]

Mots-clés : Basidiomycota, chrysocystidia, *Deconica*, systématique moléculaire, psilocybine, champignons psychédéliques.

Introduction

The genus *Psilocybe* (Fr.) P. Kumm. is an important and iconic group of mushroom-forming fungi famous for its neurotropic use, especially in sacred religious ceremonies. *Psilocybe* sensu lato (s.l.) is widely distributed around the world (Guzmán et al. 1998; Guzmán 2005) and numbers between 277 and 300 species (Guzmán 2005; Kirk et al. 2008), growing on stems, leaves, seeds, earth, dung, sawdust, straw, dead wood, or among mosses. Species of *Psilocybe* have been embraced by some cultures that consider them to be divine mushrooms (Wasson 1957). Their traditional use was rediscovered in Mexico in the 1950s among the Mazatecs of Oaxaca (Heim 1956*a*; Wasson and Wasson 1957). Furthermore, other Mexican indigenous people — e.g., Chatinos, Chinantecs, Mixes, Nahuas, and Zapotecs — used them for ceremonial purposes (Heim 1956*b*, 1957*a*, 1957*b*; Heim and Cailleux 1958; Guzmán 1960; Rubel and Gettelfinger-Krejci 1976). Hallucinogenic, psychoactive, or "magic mushrooms" have since generated considerable interest and have a wide recreational use (Stamets 1996; Guzmán 2003). Several works on the ethnomycology, taxonomy, and chemistry of these mushrooms have been published (e.g., Singer 1958; Singer and Smith 1958*a*, 1958*b*; Guzmán 1959, 1978*a*, 1978*b*, 1978*c*; Heim 1959; Heim and Wasson 1958; Heim et al. 1967; Hoffman 1978; Guzmán et al. 1979).

Species of *Psilocybe* and *Deconica* (W.G. Sm.) P. Karst. were once considered members of a single genus, *Psilocybe* s.l. (Singer 1951, 1986; Guzmán 1983, 1995). Recently, phylogenetic analyses by Moncalvo et al. (2002) and Matheny et al. (2006), based on molecular data, have demonstrated that *Psilocybe* is a polyphyletic group composed of two separate genera. However, as the aim of these works was to establish the relationship in Agaricales, they did not attempt to solve all questions about *Psilocybe* s.l. Moncalvo et al. (2002) recovered two poorly supported separate groups (clades "/psychedelia" and "/psilocybe"), but their relationships with the other members of Strophariaceae were not resolved. Matheny et al. (2006) recovered two well-supported clades in *Psilocybe* s.l., and their relationships with other Agaricales was supported, but *Psilocybe* s.l. was poorly represented, as expected in a broad-scope work. Since the acceptance of the nomenclatural proposal presented by Redhead et al. (2007), the name *Psilocybe* is now applied to the clade of psychoactive species (*Psilocybe* sensu stricto (s. str.), Fig. 1),

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Fig. 1. Macro- and micro-morphologic features of *Psilocybe*: (*a*–*f*) Basidiomata: (*a*) *P. cubensis* (photo: F. Landeros); (*b*) *P. zapotecorum* (photo: E. Fanti); (*c*–*d*) *P. fagicola* (photos: M.A. Gómez); (*e*) *Psilocybe* sp. (photo: M. No-Line); (*f*) *P. subaeruginosa* (photo: P.B. Matheny); (*g*) basidiospores without angles, *P. hispanica*; (*h*) hexagonal basidiospores, *P. mexicana*; (*i*) hexagonal basidiospore, *P. cubensis*; (*j*) subrhomboid basidiospores, *P. neoxalapensis*; (*k*) pleurocystidium (deuterocystidium) in Congo red originated from hymenophoral trama, *P. zapotecorum*; (*l*) pleurocystidium (deuterocystidium) in KOH, originated from hymenophoral trama, *P. zapotecorum*; (*m*) pleurocystidium in KOH, *P. zapotecorum*; (*n*) cheilocystidia, *P. hispanica*; (*o*) radial pileus trama, *P. yungensis*; (*p*) setoid hyphae, *P. yungensis*. Scale bars: (*a*) 20 mm; (*b*) 15 mm; (*c*) 3 mm; (*d*) 10 mm; (*e*) 25 mm; (*f*) 30 mm; (*g*–*p*) 10 μm.

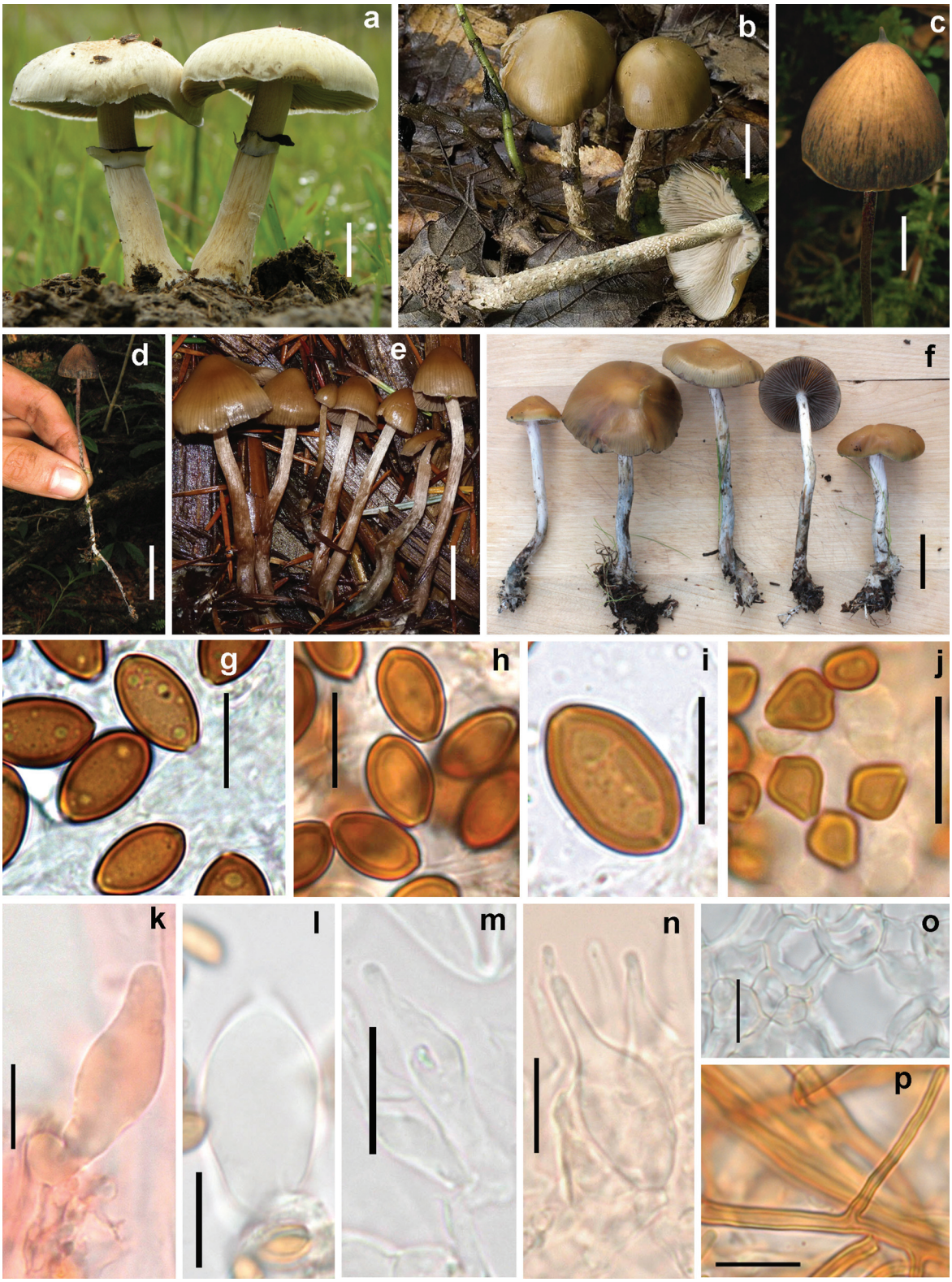


Fig. 2. Macro- and micro-morphologic features of *Deconica*: (*a*–*d*) basidiomata: (*a*) *D. coprophila* (photo: L. Guzmán-Dávalos); (*b*) *D.* aff. *montana* (photo: V. Ramírez-Cruz); (*c*) *Deconica* sp. (photo: V. Ramírez-Cruz); (*d*) *Deconica* sp. (photo: C. Braaten); (*e*) basidiospore without angles, *Deconica* sp. (CCB 45); (*f*) rhomboid and subrhomboid basidiospores, *D. umbrina*; (*g*) subrhomboid basidiospores, *Deconica* sp. (PBM 2790); (*h*) subrhomboid basidiospores; *Deconica* sp.; (*i*) hexagonal basidiospore; *D. coprophila*; (*j*–*l*) chrysocystidia in KOH: (*j*) *D. subbrunneocystidiata*; (*k*) *Deconica* sp. (CCB 45), (*l*) *D. aureicystidiata*; (*m*–*o*) chryoscystidia in Patent blue V: (*m*) *D. thailandensis;* (*n*) *Deconica* sp. (CCB 45); (*o*) *D. aureicystidiata*; (*p*) cheilocystidia (leptocystidia) in KOH, *Deconica* sp. (TFB 6422); (*q*) cheilocystidia in Congo red, *D. montana*; (*r*) cheilocystidia in KOH, *Deconica* sp. Scale bars: (*a*) 10 mm; (*b*–*c*) 6 mm; (*d*) 7 mm; (*e*–*r*) 10 μm.

Table 1. Infrageneric classification of *Psilocybe* sensu lato (s.l.).

Guzmán (1983, 1995, 2004) and Guzmán et al. (2007*a*)	Singer (1986)	Noordeloos (2011)
Psilocybe s.l.	*Psilocybe* s.l.	*Deconica*
Sect. *Atrobrunneae*	Sect. *Atrobrunneae*	Sect. *Deconica**
Sect. *Aztecorum*	**Sect. *Caerulescentes****	Subsect. *Deconica**
Sect. *Bisporae*	**Stirp *Caerulescens****	Subsect. *Inquilinae**
Sect. *Brunneocystidiatae**	**Stirp *Caerulipes***	Sect. *Melanotus**
Sect. *Blattariopsidae*	**Stirp *Cubensis****	Sect. *Merdariae**
Sect. *Coprophilae**	**Stirp *Cyanescens****	
Sect. *Cordisporae**	**Stirp *Mexicanae****	*Psilocybe* sensu stricto
Sect. *Cubensae**	**Stirp *Silvatica***	**Sect. *Atrobrunneae***
Sect. *Merdariae*	**Stirp *Yungensis****	**Sect. *Caerulescentes****
Sect. *Mexicanae**	Sect. *Chrysocystidiatae*	**Stirp *Caerulescentes****
Sect. *Neocaledonicae**	Sect. *Merdariae**	**Stirp *Cyanescens****
Sect. *Pratensae*	Sect. *Psilocybe**	**Stirp *Serbica****
Sect. *Psilocybe**	Sect. *Septembres*	**Sect. *Psilocybe****
Sect. *Semilanceatae**	**Sect. *Tenaces***	**Stirp *Psilocybe****
Sect. *Singerianae*		**Stirp *Puberula***
Sect. *Squamosae*		
Sect. *Stuntzae**		
Sect. *Subaeruginosae**		
Sect. *Zapotecorum**		

Note: Sections represented in this work are indicated with *, and hallucinogenic sections in bold.

whereas the name *Deconica* is applied to those former species of *Psilocybe* and *Melanotus* Pat. that lack hallucinogenic compounds (Fig. 2). This scheme has been approved by Barrie (2011), McNeill et al. (2011), and Norvell (2011).

Species of *Psilocybe* s. str. contain psilocybin, psilocin, and baeocystin (Beug and Bigwood 1981; Koike et al. 1981; Ott 1993; Gartz 1994), whereas *Deconica* has none of these compounds (Marcano et al. 1994). However, hallucinogenic compounds are also present in other genera of Agaricales, such as *Conocybe* Fayod, *Copelandia* Bres., *Gymnopilus* P. Karst., *Inocybe* (Fr.) Fr. s. str., *Panaeolina* Maire, *Panaeolus* (Fr.) Quél., and *Pluteus* Fr. (Stamets 1996; Wurst et al. 2002). Psilocin and psilocybin are controlled substances under Schedule 1 of the United Nations Convention on Psychotropic Substances of 1971. In the United States, possession of psilocybin-containing mushrooms is illegal, and in Mexico, psilocin and psilocybin are forbidden under the "Ley General de Salud" (General Health Law) of 1984.

Three different classifications have been proposed for *Psilocybe* by Guzmán (1983), Singer (1986), and Noordeloos (2011) (Table 1). Guzmán (1983), in his worldwide monograph, published an infrageneric classification based on the bluing reaction of basidiomata; pileus shape; presence and type of annulus; growth substrate; form, color, and wall thickness of basidiospores; and content color of pleurocystidia and cheilocystidia. Thus far, Guzmán (1983, 1995, 2004) and Guzmán et al. (2007*a*) have recognized 19 sections in *Psilocybe* s.l. Singer (1986) pointed out the presence of chrysocystidia, differentiated sterile cells located in the hymenium that possess a golden inclusion in alkali solutions, as an important character for *Psilocybe* classification, in addition to many of the abovementioned characteristics. However, Singer did not consider spore shape and content color of cystidia to be meaningful. Singer (1986) recognized only seven sections, one of them (sect. *Caerulescentes*) with seven stirps. Recently, Noordeloos (2011) divided *Deconica* and *Psilocybe* into three sections, each based on the same features considered by previous authors except for the presence of chrysocystidia. Although the characters on which classifications were based are the same, the importance that individual characters receive and the interpretation of each author is different. Traditionally, morphological basidiospore features (i.e., shape and wall thickness) have been widely used for infrageneric circumscription in *Psilocybe* s.l. The basidiospores have two views: frontal and lateral. In frontal view, the shape varies from hexagonal (Figs. 1*h*–1*i*, 2*i*, 3*a*–3*b*), rhomboid to subrhomboid (Figs. 1*j*, 2*f*–2*h*), or without angles (Figs. 1*g*, 2*e*). In lateral view, the angles are not apparent. Some species of *Deconica* and *Psilocybe* feature unusually shaped basidiospores, which are narrower in profile than in frontal view. Such an unusual spore shape was referred to by Singer (1986: 73) as "lentiform." However, lentiform-shaped spores also occur in unrelated species of *Conocybe* and coprinoid genera in the Psathyrellaceae.

Despite the attention that psychedelic mushrooms receive in popular culture, little is known about their evolutionary relationships, other than preliminary single gene phylogenetic studies. Furthermore, classifications within the group are based on morphological features only. Here, we produce a multigene phylogeny of *Psilocybe* s.l. Our objectives are to (*i*) provide an overview of the family-level classification of *Psilocybe* and *Deconica*, (*ii*) resolve infrageneric phylogenetic relationships within *Psilocybe* and *Deconica* and evaluate previous morphological-based classifications; (*iii*) analyze the evolution of their unusual basidiospore shape and spore wall thickness in both clades; and (*iv*) evaluate the evolution of chrysocystidia in Strophariaceae s. str.

Materials and methods

Taxon sampling

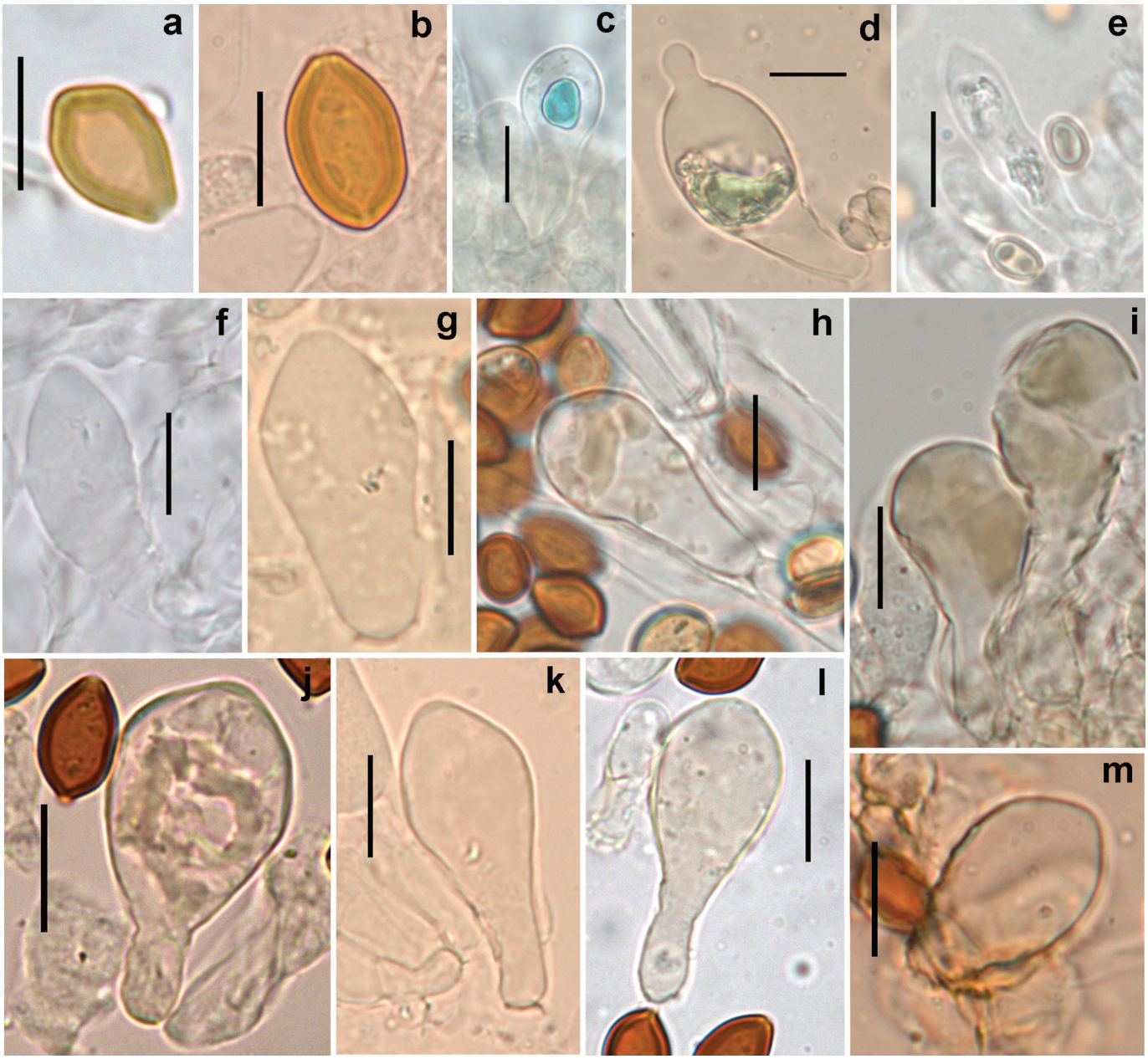
We sampled 14 specimens of *Deconica*, 28 of *Psilocybe*, and 24 outgroup taxa for our molecular analyses (Table 2). Ingroup sequences were obtained from herbarium specimens, including four type specimens of *Psilocybe* s. str. Taxon sampling included species of most sections within *Psilocybe* (Table 1). Of the 19 taxonomic sections proposed by Guzmán (1983, 1995) and Guzmán et al. (2007*a*) for *Psilocybe* s.l., 11 were sampled here. Following the classification of Singer (1986) and Noordeloos (2011), three and five sections were sampled, respectively. Although taxon sampling included only 14.5% of the world total estimate of *Psilocybe* s.l., we sampled species exhibiting all morphological variation in both genera. About 25 species are known only from type collections, so these have not been included, other than five recently collected type specimens from which DNA was successfully sequenced.

DNA extraction, amplification, and sequencing

DNA was extracted from small pieces (ca. 4 mg) of the pileus (including cutis, context, and lamellae), using one of the following procedures: Doyle and Doyle (1987), Aljanabi and Martinez (1997), or the EZNA Fungal DNA Kit (Omega Bio-Tek Inc., Norcross, Ga., USA). The DNA extracts were undiluted or diluted (1:10, 1:100) for polymerase chain reactions (PCR).

PCR was performed to amplify the internal transcribed spacer 1 (ITS1), the 5.8S rRNA gene, the internal transcribed spacer 2 (ITS2), and a partial sequence (~600 bp) of the large subunit (LSU) of the rRNA gene, including the D1–D2 domains (Lapeyre et al. 1993). The primer pairs ITS1F/ITS4S, ITS1F/ITS4, ITS1/ITS4, and ITS5/ITS4 were used to amplify the entire ITS. ITS1F/ITS2, ITS1/ITS2, and ITS5/ITS5.8S were used to amplify the ITS1, and ITS3/ITS4, ITS5.8SR/ITS4, ITS3/ITS4S, and 5.8SR/ITS4S to amplify the ITS2 (Vilgalys and Hester 1990; White et al. 1990; Gardes and Bruns 1993). The primer combination 5.8SR/LR3 and LR0R/LR3 (Vilgalys and Hester 1990; Moncalvo et al. 2000) was used to amplify the partial sequence of LSU. Furthermore, two primers (LPs1 5-ATGCAGCTCAAAATGGGTGGTAAA-3 and LPs1R 5-CTTTCATTACGCGCTCGGGTTTTC-3) specific to *Psilocybe* were designed, using the software Lasergene Primer Select vs. 7.1.0 (DNASTAR, Inc.). LR0R/LR21 and LPs1/LPs1R were used to amplify the partial LSU in two fragments of 300 bp each. Conserved domains A to C of *rpb1* were amplified with the primer pair gRPB1-A/fRPB1-C (Matheny et al. 2002). Additionally, three primers (Ps-int2F 5-GGCWGAACGAGSAGTGCG-3, Ps-Ex2R 5-GCGTAYTCTTCCGAGAGACC-3, and Ps-Ex3R 5-GCATRACAGTAAGAATCATCC-3) were designed to amplify *rpb1* in *Deconica* and *Psilocybe*. When it was not

Fig. 3. Micromorphologic features of Strophariaceae: (*a*–*b*) Hexagonal basidiospores with three layers under light microscope; (*a*) *Psilocybe caerulescens*; (*b*) *P. cubensis*; (*c*–*d*) chrysocystidia in Patent blue V; (*c*) *Hypholoma fasciculare*; (*d*) *Hypholoma* sp.; (*e*) chrysocystidium in KOH, *Pholiota* aff. *gummosa*; (*f*) pleurocystidium in KOH, *Psilocybe magnispora*; (*g*) pleurocystidium in Patent blue V, *P. magnispora*; (*h*) pleurocystidium in KOH, *P. thaiaerugineomaculans*; (*i*) pleurocystidium in Patent blue V, *P. thaiaerugineomaculans*; (*j*) pleurocystidium in KOH, *P. ovoideocystidiata*; (*k*) pleurocystidium in Patent blue V, *P. ovoideocystidiata*; (*l*) pleurocystidium in KOH, P. *thaiduplicatocystidiata*; (*m*) pleurocystidium in Patent blue V, *P. thaiduplicatocystidiata*. Scale bars: (*a*–*m*) 10 μm.



possible to amplify across domains A to C, the primer pairs Ps-int2F/fRPB1-C, Ps-int2F/Ps-Ex3R, Psint2F/Ps-Ex2R, int2F/Ps-Ex2R, and int2F/Ps-Ex3R were used to amplify shorter fragments.

DNA amplification was done with two protocols. In the first one, each 20 μL of PCR reaction contained 11.85 μL water, 2 μL 10× Taq reaction buffer, 1 μL of 50 mmol·L^{-1} $MgCl_2$,1 μL of 5 mmol·L^{-1} dNTP, 2 μL BSA, 0.5 μL of each 10 μmol·L^{-1} primer, 0.15 μL of Taq polymerase (5 U·μL^{-1}), and 1 μL of DNA template to amplify ITS and LSU. Similarly, on the second protocol, each 24 μL of PCR reaction contained 14.875 μL water, 5 μL 5× Taq reaction buffer, 0.5 μL of 5 mmol·L^{-1} dNTP, 1.25 μL of each 10 μmol·L^{-1} primer, 0.125 μL of Taq polymerase (5 U·μL^{-1}), and 2 μL of DNA template to amplify *rpb1*. PCR reactions were performed in Swift MaxPro (ESCO, Portland, Ore., USA) and Techne TC-312 thermocyclers (Bibby Scientific Limited, Staffordshire, UK). The ITS region was amplified with the program described by Guzmán-Dávalos et al. (2003). To amplify LSU, the DNA was denatured at 95 °C for 3 min, followed by 34 cycles of denaturing at 95 °C for 1 min, annealing at 56 °C for 45 s, extension at 72 °C for 2 min, and a final extension step of 72 °C for 10 min, and finally refrigerated at 4 °C. The *rbp1* region was amplified according to the protocols of Matheny et al. (2002) and Matheny (2005). Amplification products were visualized by electrophoresis in 1.5%–2% TBE agarose gels (UltraPure grade, Invitrogen, Carlsbad, Calif., USA) using a 100 bp DNA size marker, and then stained in an ethidium bromide solution (20 μL·500 mL^{-1}, from a 10 mg·mL^{-1} stock solution). PCR products

Table 2. Specimens of *Deconica*, *Psilocybe*, and outgroups used in this study.

Specimen-DNA number	Species name	Section 1. Guzmán (1983, 1995, 2004) and Guzmán et al. (2007*a*), 2. Singer (1986), 3. Noordeloos (2011)	Herbarium and specimen voucher	Collector, number	Date of collection	Country	Accession Numbers ITS	LSU	*rbp1*
	INGROUP								
	Hymenogastraceae								
Ps-329	*Psilocybe caerulescens* Murrill	1. *Cordisporae* 2. *Caerulescentes*, stirp *Caerulescens*	IBUG	I.J. Franco-Galván 1	31 August 2005	Mexico	**KC669281**	**KC669317**	**KC669342**
UT 1609	*P. caerulipes* (Peck) Sacc.	1. *Semilanceatae* 2. *Caerulescentes*, stirp *Caerulipes*	TENN-064502	SAT09–216-06	8 August 2009	USA	**KC669282**	—	**KC669343**
Ps-59	*P. cubensis* Earle (Singer)	1. *Cubensae* 2. *Caerulescentes*, stirp *Cubensis* 3. *Caerulescentes*, stirp *Caerulescentes*	XAL	V. Ramírez-Cruz 87	1 July 2004	Mexico	**KC669283**	**KC669318**	**KC669344**
UT 1524	*P. cubensis*	1. *Cubensae* 2. *Caerulescentes*, stirp *Cubensis* 3. *Caerulescentes*, stirp *Caerulescentes*	TENN-051528	RHP 5203	7 May 1992	Costa Rica	**KC669284**	—	**KC669345**
Ps-66	*P. cyanescens* Wakef.	1. *Semilanceatae* 2. *Caerulescentes*, Stirp *Cyanescens* 3. *Psilocybe*, stirp *Psilocybe*	XAL	J. Workman & P. Werner s.n.	No date	USA	**KC669285**	**KC669319**	**KC669346**
Ps-113	*P. cyanescens*	1. *Semilanceatae* 2. *Caerulescentes*, stirp *Cyanescens* 3. *Psilocybe*, stirp *Psilocybe*	F-1021111	Ower 2157	No date	USA	**KC669286**	**KC669320**	**KC669347**
Ps-466	*P. cyanescens*	1. *Semilanceatae* 2. *Caerulescentes*, stirp *Cyanescens* 3. *Psilocybe*, stirp *Psilocybe*	IBUG	S. Chornick s.n.	November 2011	USA	**KC669287**	**KC669321**	**KC669348**
Ps-364	*P. fagicola* R. Heim & Cailleux	1. *Cordisporae*	IBUG	M.A. Gómez 22731	July 2010	Mexico	**KC669288**	**KC669322**	**KC669349**
Ps-92	*P. hispanica* Guzmán	1. *Semilanceatae* 3. *Psilocybe*, stirp *Psilocybe*	XAL	R. Fernández-Sasia s.n.	15 January 2005	Spain	**KC669289**	**KC699323**	**KC669350**
Ps-333	*P. mescaleroensis* Guzmán, Walstad, E. Gándara & Ram.-Guill.	1. *Stuntzae*	XAL	L. Field Walstad s.n., **Holotype**	August 2005	USA	**KC669290**	**KC669324**	**KC669351**
Ps-308	*P. mexicana* R. Heim	1. *Mexicanae* 2. *Caerulescentes*, stirp *Mexicanae*	IBUG	M.R. Sánchez-Jácome 1038	30 June 2002	Mexico	**KC669291**	**KC669325**	**KC669352**

Table 2 (*continued*).

Specimen-DNA number	Species name	Section 1. Guzmán (1983, 1995, 2004) and Guzmán et al. (2007*a*), 2. Singer (1986), 3. Noordeloos (2011)	Herbarium and specimen voucher	Collector, number	Date of collection	Country	Accession Numbers		
							ITS	LSU	*rbp1*
Ps-324	*P. neoxalapensis* Guzmán, Ram.-Guill. & Halling	1. *Cordisporae*	XAL	M.A. Gómez 1883	5 September 2007	Mexico	—	**KC669326**	**KC669353**
Ps-467	*P. pelliculosa* (A.H. Sm.) Singer & A.H. Sm.	1. *Semilanceatae*	IBUG	S. Chornick s.n.	December 2011	USA	**KC669292**	—	**KC669354**
Ps-470	*P. samuiensis* Guzmán, Bandala & J.W. Allen	1. *Mexicanae*	XAL	Sihanonth & J. Allen	18 June 2004	Thailand	**KC669293**	—	**KC669355**
Ps-67	*P. serbica* M.M. Moser & E. Horak	1. *Semilanceatae* 2. *Caerulescentes*, stirp *Cyanescens* 3. *Caerulescentes*, stirp *Serbica*	WU-4448	I. Krisai s.n.	5 November 1987	Austria	**KC669294**	**KC669327**	**KC669356**
Ps-468	*P. stuntzii* Guzmán & J. Ott	1. *Stuntzae* 3. *Caerulescentes*, stirp *Cyanescens*	IBUG	S. Chornick s.n.	October 2011	USA	**KC669295**	—	**KC669357**
Ps-459	*P. subaeruginosa* Cleland	1. *Subaeruginosae*	PDD	L. Taylor	16 May 2008	New Zealand	**KC669296**	—	**KC669358**
UT 1608	*P. subaeruginosa*	1. *Subaeruginosae*	TENN-065481	PBM 3218	9 June 2009	Australia	**KC669278**	—	**KC669359**
Ps-211	*P. subcubensis* Guzmán	1. *Cubensae* 2. *Caerulescentes*, stirp *Cubensis*	XAL	G. Guzmán 35102	8 October 2001	Nepal	**KC669297**	**KC669328**	**KC669360**
Ps-434	*P. thaiaerugineomaculans* Guzmán, Karunarathna & Ram.-Guill.	1. *Stuntzae*	XAL	S.C. Karunarathna NTS-121, **Holotype**	27 July 2010	Thailand	**KC669298**	—	**KC669361**
Ps-433	*P. thaiduplicatocystidiata* Guzmán, Karunarathna & Ram.-Guill.	1. *Cordisporae*	XAL	S.C. Karunarathna NTS-120, **Isotype**	27 July 2010	Thailand	**KC669299**	**KC669329**	**KC669362**
Ps-440	*P. thaizapoteca* Guzmán, Karunarathna & Ram.-Guill	1. *Zapotecorum*	XAL	G. Guzmán 38342, **Holotype**	12 July 2010	Thailand	**KC669300**	—	**KC669363**
Ps-455	*P. yungensis* Singer	1. *Cordisporae*	XAL	A. Cortés-Pérez 549	30 October 2010	Mexico	**KC669301**	**KC669330**	**KC669364**
Ps-243	*P. zapotecoantillarum* Guzmán, T.J. Baroni & Lodge	1. *Zapotecorum*	XAL	S. Cantrell & Salgado s.n., **Isotype**	23 May 2000	Puerto Rico	**KC669302**	**KC669331**	**KC669365**
Ps-317	*P. zapotecorum* R. Heim	1. *Zapotecorum* 2. *Caerulescentes*, stirp *Caerulescens*	IBUG	V. Ramírez-Cruz 1094	30 July 2009	Mexico	**KC669303**	**KC669332**	**KC669366**
Ps-315	*Psilocybe* sp.	1. *Cordisporae*	IBUG	V. Ramírez-Cruz 551	25 August 2006	Mexico	**KC669304**	**KC669333**	**KC669367**
Ps-369	*Psilocybe* sp.	1. *Cordisporae*	IBUG	V. Ramírez-Cruz 1328	12 August 2010	Mexico	**KC669305**	**KC669334**	**KC669368**

Table 2 (*continued*).

Specimen-DNA number	Species name	Section 1. Guzmán (1983, 1995, 2004) and Guzmán et al. (2007*a*), 2. Singer (1986), 3. Noordeloos (2011)	Herbarium and specimen voucher	Collector, number	Date of collection	Country	Accession Numbers ITS	LSU	*rbp1*
Ps-464	*Psilocybe* sp.	1. *Semilanceatae*	IBUG	M. No-Line	19 December 2011	USA	**KC669306**	**KC669335**	**KC669369**
	***Strophariaceae* s. str.**								
Ps-15	*Deconica argentina* Speg.	1. *Coprophilae* 2. *Merdariae*	XAL	M. Contu s.n.	7 October 2002	Italy	**KC669307**	**KC999956**	**KC669370**
Ps-50	*D. coprophila* (Bull.) P. Karst.	1. *Coprophilae* 2. and 3. *Merdariae*	XAL	V. Ramírez-Cruz 114	1 July 2004	Mexico	**KC669308**	**KC669336**	**KC669371**
Ps-463	*D. horizontalis* (Bull.) Noordel.	3. *Melanotus*	ICN-154677	P.S. Silva 253/10	14 May 2010	Brazil	**KC669309**	**KC669337**	**KC669372**
Ps-443	*D.* aff. *Horizontalis*	3. *Melanotus*	IBUG	V. Ramírez-Cruz 1520	23 July 2011	Costa Rica	**KC669310**	**KC669338**	**KC669373**
	D. inquilina (Fr.) Romagn.	1. *Psilocybe* 3. *Deconica*, subsect. *Inquilinae*	GLM-51242				—	DQ071689	DQ067969
Ps-370	*D. montana* (Pers.) P.D. Orton	1. *Psilocybe* 3. *Deconica*, subsect. *Deconica*	IBUG	V. Ramírez-Cruz 1323	12 August 2012	Mexico	**KC669311**	—	**KC669374**
Ps-96	*Deconica* aff. *montana*	1. *Psilocybe* 3. *Deconica*, subsect. *Deconica*	XAL	J. Trappe 10065	12 July 1986	USA	—	—	**KC669375**
Ps-456	*D. neorhombispora* nom. prov. = *Psilocybe subbrunneocystidiata* P.S. Silva & Guzmán	1. *Neocaledonicae* 1. *Brunneocystidiatae*	XAL	A. Cortés-Pérez 739	12 October 2011	Mexico	—	**KC669339**	**KC669376**
Ps-239	*D. xeroderma* (Huijsman) Noordel.	1. *Psilocybe* 3. *Deconica*, subsect. *Deconica*	WU	Oswald s.n.	24 August 2004	Austria	**KC669312**	**KC669340**	**KC669377**
Ps-367	*Deconica* sp.	1. *Psilocybe*	IBUG	V. Ramírez-Cruz 1269	17 July 2010	Mexico	—	**KC669341**	**KC669378**
UT 1574	*Deconica* sp.		TENN-062238	TFB 12591	11 August 2005	USA	**KC669313**	—	**KC669379**
UT 1606	*Deconica* sp.		TENN-067047	PBM 3781	3 March 2012	Australia	**KC669314**	—	**KC669380**
UT 1613	*Deconica* sp.		TENN-067013	CCB 45	19 July 2012	USA	**KC669315**	—	**KC669381**
UT 1581	*Deconica* sp.	1. *Psilocybe*	TENN-062588	PBM 2790	23 July 2006	USA	**KC669316**	—	**KC669382**
	OUTGROUP								
	Crepidotaceae								
	Crepidotus applanatus (Pers.) P. Kumm.		WTU	P.B. Matheny 717			DQ202273	AY380406	AY333303
	Crepidotus mollis (Schaeff.) Staude		TUB-011566				—	DQ071698	DQ067977
	Simocybe serrulata (Murrill) Singer		CUW	PBM 2536		USA	DQ494696	AY745706	DQ447940
	Simocybe sp.		TENN-062784	PBM 3031	31 August 2008	USA	—	—	KC669280
	Gymnopileae								
	Gymnopilus penetrans (Fr.) Murrill		GLM-45929			Germany	—	AY207208	DQ068014
	Gymopilus sapineus (Fr.) Murrill		WTU	PBM 1541			—	AY380362	AY351789

Table 2 (*concluded*).

Specimen-DNA number	Species name	Section 1. Guzmán (1983, 1995, 2004) and Guzmán et al. (2007*a*), 2. Singer (1986), 3. Noordeloos (2011)	Herbarium and specimen voucher	Collector, number	Date of collection	Country	Accession Numbers		
							ITS	LSU	*rbp1*
	Hymenogastraceae								
	Alnicola eschariodes (Fr.) Romagn.		WTU	PBM 1719		USA	—	—	AH013186
	Alnicola solecina var. *umbrina* Singer		TU-110280	L. Tedersoo	16 September 2010	Estonia	—	JN938854	JQ014106
	Flammula alnicola (Fr.) P. Kumm.		CUW	P.B. Matheny 2608			DQ486703	DQ457666	DQ447900
	Galerina badipes (Pers.) Kühner		GLM-45922			Germany	—	AY207201	DQ067975
	Galerina marginata (Batsch) Kühner		CUW	PBM 2518		USA	DQ192182	DQ457669	DQ447901
	Hebeloma mesophaeum (Pers.) Quél.		TUB-011577				—	DQ071690	DQ067971
	Hebeloma olympianum A.H. Sm., V.S. Evenson & Mitchel		UTC	BK 21-Nov-98–20			—	AY038310	AF389532
	Strophariaceae s. str.								
	Hypholoma fasciculare (Huds.) P. Kumm.		WTU	PBM 1844			—	AY380409	AY351829
	Hypholoma subviride (Berk. & M.A. Curtis) Krieglst.		TENN-062712	P.B. Matheny 2954	24 July 2008	USA	—	—	KC669279
	Kuehneromyces rostratus Singer & A.H. Smith		CUW	P.B. Matheny 2703		USA	—	DQ457684	DQ447918
	Pholiota flammans (Bastch) P. Kumm.		TUB-011573				—	DQ071688	DQ067973
	Pholiota squarrosa (Oeder) P. Kumm.		CUW	PBM 2735			DQ494683	DQ470818	DQ447931
	Stropharia coronilla (Bull.) Quél.		GLM-46074				—	DQ071687	DQ067966
	Stropharia aeruginosa (Curtis) Quél.		TUB-012151				—	DQ071686	DQ067967
	Tubarieae								
	Flammulaster muricatus (Fr.) Watling		TUB-012150				—	DQ071740	DQ068012
	Phaeomarasmius curcuma (Berk. & M.A. Curtis) Singer		WTU	JFA 11323			—	AY038329	AF389551
	Tubaria hiemalis Romagn. ex Bon		GLM-46038			Germany	—	AY207311	DQ067966
	Tubaria serrulata (Cleland) Bougher & Matheny					Australia	DQ182507	DQ156128	DQ447930

Note: Sequences produced for this work are in bold.

were cleaned using Illustra GFX columns (GE Healthcare, Little Chalfont, UK) or PCR Purification Kit (Qiagen, Venlo, the Netherlands) following the manufacturers' protocols, and in some cases with the enzymatic method USB Exo-sap-IT (Affymetrix, Santa Clara, Calif., USA).

Sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA) in 20 μL or 10 μL final volumes following the manufacturer's protocol and using the same primers as in the DNA amplification for ITS, LSU, and *rpb1* regions. The *rpb1* region was sequenced with two additional primers, int2.1F and int2.1R (Frøslev et al. 2005). Sequencing reactions were purified with AutoSeq G-50 column (GE Healthcare, Little Chalfont, UK), and 18 μL of formamide was added. Sequences were visualized by capillary electrophoresis on an ABI-Prism 310 Genetic Analyzer (Applied Biosystems) at the Institute of Botany, University of Guadalajara, or in an ABI 3730 (Applied Biosystems) 48 Capillary Electrophoresis Genetic Analyzer at the Molecular Biology Resource Facility at the University of Tennessee.

Alignments and phylogenetic analyses

Sequence annotations were made with Chromas Pro 1.41 (McCarthy 1996–1998) and Sequencher v.4.9 (Gene Codes Inc., Ann Arbor, Mich., USA). Sequence assembly and alignment were carried out with MacClade 4.08 (Maddison and Maddison 2000). Aligned sequences have been deposited at TreeBASE (http://purl.org/phylo/treebase/study/TB2:S14204). Alignments of each gene were subjected to maximum likelihood (ML) searches using RAxML 7.0.3 (Stamatakis 2006) to test for strongly supported gene conflict. ML trees with bootstrap labels from each gene tree were compared and inspected for gene conflict between nodes, where conflict is gauged by >70% bootstrap support for contradictory nodes.

Trees were obtained using Bayesian and ML criteria. The Bayesian analysis was executed using MrBayes 3.1 (Ronquist and Huelsenbeck 2003). For the Bayesian analyses, the most likely model of evolution was determined using Modeltest 3.7 with the Akaike criterion (Posada and Crandall 1998). The Bayesian analysis was run for 10 million generations, with trees sampled every 1000 generations. The standard deviation of the split frequencies was examined to confirm that independent runs had converged to similar tree scores. The first 2501 trees were burned, and posterior probabilities (PP) were calculated from a consensus of the remaining 7500 trees from two runs. This analysis was repeated twice. In ML analyses, 1000 rapid bootstrap inferences were performed with all free model parameters estimated by RAxML using a GTRGAMMAI model and empirical base frequencies. The trees were visualized in FigTree v1.3.1 (Rambaut 2010).

Outgroup choices were based on the work of Matheny et al. (2006). Hymenogastraceae is represented by *Alnicola* Kühner, *Galerina* Earle, *Flammula* (Fr.) P. Kumm., and *Hebeloma* P. Kumm. Strophariaceae s. str. is represented by *Hypholoma* (Fr.) P. Kumm., *Kuehneromyces* Singer & A.H. Sm., *Pholiota* (Fr.) P. Kumm., and *Stropharia* (Fr.) Quél. The remaining outgroups include genera of Crepidotaceae, Gymnopileae, and Tubariaceae. All outgroup sequences were obtained from GenBank (Table 2).

Light microscopy studies

Microscopic characteristics were observed from dried material mounted in 3% potassium hydroxide (KOH) or in Congo red. Patent blue V 0.1% (Jahnke 1984) was used to detect the presence of chrysocystidia. Measurements and drawings were made using a 100× oil-immersion objective on a Zeiss K7 or a Zeiss Axioskop 40 microscope. Basidiospore walls were measured through the Axio Vision 4 software in the Zeiss Axioskop 40 microscope, with 1250× magnifications. Structures were photographed through Axio Vision 4 software on the Zeiss Axioskop 40.

Character evolution

We studied the evolution of the basidiospore shape in frontal view, basidiospore wall thickness, and presence and (or) absence of chrysocystidia using a Bayesian approach. Evolutionary models that best fit the data and ancestral state reconstructions (ASR) were obtained using BayesTraits 1.0 (Pagel and Meade 2007) with the Reversible-Jump MCMC algorithm (RJ-MCMC) (Pagel and Meade 2006). For both analyses (estimation of models and ASR), 1000 trees from the posterior distribution were analyzed. Trees were re-rooted and dichotomized in Mesquite 2.5 (Maddison and Maddison 2011).

Estimation of evolutionary models and ASR were made using the gamma hyperprior probabilistic model (Pagel and Meade 2006). The amount of change in rate coefficients among generations in the MCMC (ratedev parameter) was set to achieve acceptance rates in the range of 20%–40%. Parameters to estimate models were left at their default values (the Markov chain ran for more than five million generations, with a sampling frequency of every 100th generation and burn-in value of 50 000 generations). The best-fit model appears most frequently in the posterior sample.

In the ASR analysis, the Markov chain ran for 10 million generations, with a sampling frequency of every 1000 generations and a burn-in value of 10 000. We used the "addmrca" command to reconstruct the ancestral state at each node. With the "fossil" command, we tested whether there was support for one state over the others. These analyses were repeated three times. To test hypotheses, Bayes factors (BF) were used. According to Kass and Rafteri (1995), BF values between 2 and 5 are interpreted as positive evidence, BF up to 10 as strong evidence, and BF greater than 10 as very strong evidence in favor of the hypothesis having better log-likelihoods.

The three microscopic characters were scored using the following criteria: *basidiospore shape in frontal view*: (0) without angles (Figs. 1*g*, 2*e*), or (1) angled (rhomboid to subrhomboid or hexagonal) (Figs. 1*h*–1*j*, 2*f*–2*i*). Initially, three character states were coded: (0) without angles, (1) rhomboid to subrhomboid, and (2) hexagonal. This option was problematic because it lacked discrete states at times, or the interpretation of the shape was subjective. *Basidiospore wall*: (0) thin-walled (0.3–0.5 μm), (1) slightly thick-walled (0.51–0.84 μm) (Fig. 1*j*), and (2) thick-walled (0.85–1.89 μm) (Figs. 1*i*, 2*i*, 3*a*–3*b*). In the Agaricales, Garnica et al. (2007) split the basidiospore wall thickness into two classes corresponding to whether the thickness of the spore wall exceeded 200 nm (0.2 μm). However, in *Deconica* and *Psilocybe* the basidiospore wall varies from 0.3 to 1.89 μm thick; therefore, in the sense of Garnica et al. (2007), all the spores would be thick-walled. The ranges of wall thickness used here were established based on the division of data in quartiles of 4900 basidiospore wall measurements. The first state (0) includes values within the first quartile. Values within the second and third quartiles were assigned to state (1). Lastly, state (2) corresponds to values greater than the third quartile. *Chrysocystidia*: (0) absent, (1) present. This structure has been observed in some lineages of the Strophariaceae, including some taxa of *Psilocybe* s.l. (Singer 1986, in sect. *Chrysocystidiata*; Guzmán 2004, in sect. *Neocaledonicae*).

Results

DNA sequence data

This study generated 104 new sequences (37 ITS, 26 LSU, 41 *rpb1*). Fifteen ingroup taxa lacked the LSU sequences and five others lacked the ITS region. The LSU, 5.8S, and *rpb1* matrix contained 66 terminals and 1747 aligned characters, distributed as follows: *rpb1* exons (1–117, 438–1043), *rpb1* conserved region of intron 2 (118–437), LSU (1044–1588), and 5.8S (1589–1747). The *rpb1* introns 1 and 3, and ITS1 and ITS2 were removed due to alignment ambiguities.

Phylogenetic analyses

The best-fit model of molecular evolution was the GTR + I + G for LSU and *rpb1* partitions. The JK model was best fit to the 5.8S partition (Posada and Crandall 1998). The tree topology recovered by ML and Bayesian inference was the same. Gene sequences were concatenated, as strongly supported topological conflicts were not observed when the loci were analyzed separately. Phylogenetic relationships inferred from the matrix dataset are shown in Fig. 4. *Deconica* and *Psilocybe* are monophyletic. Within *Deconica*, four main clades can be observed (Fig. 4, clades E–G). *Psilocybe* contains two main clades, each one subdivided into two (Fig. 4, clades A–D). Below, we report only the lineages receiving significant support.

Monophyletic groups within *Psilocybe* s.str.

Two clades, I and II, are recovered in *Psilocybe*. Clade I also includes groups A and B, whereas clade II includes C and D. Clade A comprises tropical species and splits into three groups: "cordisporae," "mexicanae," and "zapotecorum."

Subclade "cordisporae" is composed of at least four neotropical species: *P. fagicola* (Figs. 1*b*, 1*f*), *P. neoxalapensis, P. yungensis*, and *Psilocybe* sp., all belonging to sect. *Cordisporae* (Guzmán 1983, 1995). However, not all the studied species from sect. *Cordisporae* are in this clade. In our "cordisporae" clade, the basidiomata are small, with conical and papillate pilei. The micromorphological features of this group include slightly thick-walled, small, rhomboid to subrhomboid basidiospores 4–6 μm long; small lageniform pleurocystidia up to 24 μm long; lageniform, cylindrical, utriform and occasionally branched cheilocystidia; thick-walled (up to 1.6 um) pigmented hyphae from the pileus trama (Fig. 1*o*) and hymenophoral trama; and basal mycelium of the stipe composed by setoid hyphae (Fig. 1*p*). Species of this clade grow in the subtropics in soil or muddy soil, or sometimes on rotting wood.

Subclade "mexicanae" is composed by *P. caerulescens*, *P. mexicana*, and *P. samuiensis*. The former is part of sect. *Cordisporae* (Guzmán 1983, 1995), whereas the last two species are in sect. *Mexicanae* (Guzmán 1983, 1995). This group has slightly thick or thick-walled and angular (rhomboid and hexagonal) basidiospores, 6–11 μm long. *Psilocybe caerulescens* and *P. mexicana* grow in tropical and subtropical meadows and forests in the Americas, but *P. samuiensis* occurs in tropical habitats in Thailand (Guzmán et al. 1993). Clade "mexicanae" shows a sister group relationship with clade "cordisporae" but with low bootstrap support.

Subclade "zapotecorum" includes *P. thaizapoteca*, *P. zapotecoantillarum*, and *P. zapotecorum* (Fig. 1*b*), all grouped in sect. *Zapotecorum* by Guzmán (1983, 1995, 2012). The macromorphological features are very variable in this group; e.g., robust to delicate basidiomata. The basidiospores are 6–9 μm long, without angles, and thin-walled. The species in this clade fruit on muddy soils in tropical and subtropical forests. They have been collected from Mexico to Argentina, except for *P. thaizapoteca*, which was recently described from Thailand (Guzmán et al. 2012).

Clade B groups *P. caerulipes*, *P. hispanica*, *P. pelliculosa*, *P. stuntzii*, and *Psilocybe* sp. (Fig. 1*e*). Sections *Stuntzae* and *Semilanceatae* in the sense of Guzmán (1983, 1995), and sections *Caerulescentes* and *Psilocybe* in the sense of Noordeloos (2011) are represented here. Morphologically, they are characterized by slightly thick to thick-walled basidiospores, and lageniform or sometimes branched pleurocystidia and cheilocystidia with long necks. These species are distributed in temperate zones in Europe and North America.

Clade II of *Psilocybe* contains a mixture of tropical and temperate species. All share slightly thick to thick-walled, with or without angles, large basidiospores 10–14 μm long. Subgroups C and D are recovered within this clade. Clade C comprises the temperate taxa *P. serbica* and *P. mescaleorensis*, which are closely related to *P. cyanescens* and *P. subaeruginosa* (Fig. 1*f*) (*cyanescens* complex). Species of clade C produce medium-sized basidiomata, basidiospores 10–13 μm long and without angles, and have pleurocystidia and cheilocystidia.

Clade D includes *P. cubensis* (Fig. 1*a*), *P. subcubensis*, *P. thaiaeruginеomaculans*, and *P. thaiduplicatocystidiata*. This clade is composed of a mixture of species from different sections (sect. *Cubensae*, sect. *Stuntzae*, and sect. *Cordisporae*, respectively). In general, the species have robust basidiomata, hexagonal basidiospores, and ovoid to clavate or sometimes broadly fusiform cystidia. *Psilocybe cubensis* and *P. subcubensis* are the most widely distributed species in the world, occurring in tropical and subtropical habitats, whereas *P. thaiaerugineomaculans* and *P. thaiduplicatocystidiata* are known to date from Thailand in tropical habitats.

Infrageneric relationships in *Deconica*

Two clades, I and II, within *Deconica* are recovered. Clade I includes groups E, F, and G, whereas clade II harbors two coprophilous species (Fig. 4).

Clade E, or "chrysocystidiatae," contains *D. neorhombispora* = *Psilocybe subbrunneocystidiata*, which was described as bluing in sect. *Brunneocystidiatae* (Silva et al. 2007), and three undetermined species, each annotated as *Deconica* sp. (Fig. 2*d*). Species in this clade produce small basidiomata with convex and often umbonate pilei, small basidiospores up to 7 μm long, with or without angles, and chrysocystidia (Figs. 2*j*–2*o*). Species in this group fruit on rotten wood or soil in the tropics.

Clade F includes *D. horizontalis* and *D.* aff. *horizontalis*, both in sect. *Melanotus* in the sense of Noordeloos (2011). Both species produce basidiomata with a reduced stipe, or no stipe at all, and the basidiospores are small and thin-walled. Clade G contains *D. montana* (Fig. 2*b*), *D. inquilina*, and two undetermined species (Figs. 2*c*, 2*g*–2*h*). The members of this clade fruit on sticks, leaves, or mosses and are known from temperate areas. They are characterized by small basidiomata, thin- to slightly thick-walled basidiospores up to 10 μm long with or without angles, and absence of pleurocystidia but presence of lageniform cheilocystidia. Clades E, F, and G form a moderately well-supported group with a sister relationship to *D. xeroderma*.

Clade II is composed of two species, *D. argentina* and *D. coprophila* (Fig. 2*a*), both in sect. *Coprophilae* sensu Guzmán (1983, 1995) or sect. *Merdariae* sensu Singer (1986). They are characterized by hexagonal, thick-walled (more than 1 μm thick) basidiospores that are 12–14 μm long. This group represents a natural assembly of coprophilous species that grow in disturbed tropical and subtropical habitats. This clade is the sister group of all other species of *Deconica*.

Basidiospore and cystidia morphology

Basidiospores in *Deconica* and *Psilocybe* have a complex wall. According to Ruch and Motta (1987), it is composed of three layers. However, in most of our light microscope observations, only two layers were seen, and only in a few cases was it possible to detect three layers (Figs. 3*a*–3*b*). In *Deconica*, two types of cystidia can be observed according to their contents: leptocystidia (euplasmatic cystidia) (Figs. 2*p*–2*r*) and chrysocystidia (deuteroplasmatic cystidia) (Figs. 2*j*–2*o*). Chrysocystidia are entirely absent from species of *Psilocybe* s. str. Species of *Psilocybe* do possess leptocystidia (Figs. 1*m*–1*n*) and a second type of deuteroplasmatic cystidia that do not stain with Patent blue V (Figs. 1*k*–1*l*, 3*f*–3*m*) (see below).

Models of character evolution

Character evolution models are presented in Tables 3 and 4. The best-fit model for the basidiospore shape in frontal view shows equal evolutionary rates between states $q_{01} = q_{10}$ (Table 3). In other words, transitions in spore shape from "without angles" to "with angles" are permitted to change along the phylogeny symmetrically. This model is strongly supported (BF = 6.7924) over the second most frequently sampled model ($q_{10} = 0$). In relation to the basidiospore wall, 703 models were sampled during the RJ-MCMC

Fig. 4. Maximum likelihood tree with average branch lengths from the combined analyses of 5.8S, partial nLSU rDNA, and *rpb1* dataset of *Psilocybe* and *Deconica*. Maximum likelihood bootstrap support and posterior probability obtained from the Bayesian inference are indicated over the branches. Branch lengths are scaled to the expected number of nucleotide substitutions per site. Species of *Deconica* are in orange, *Psilocybe* in green, and outgroups in black. Taxonomic types are indicated by a "T" at the end of the species name.

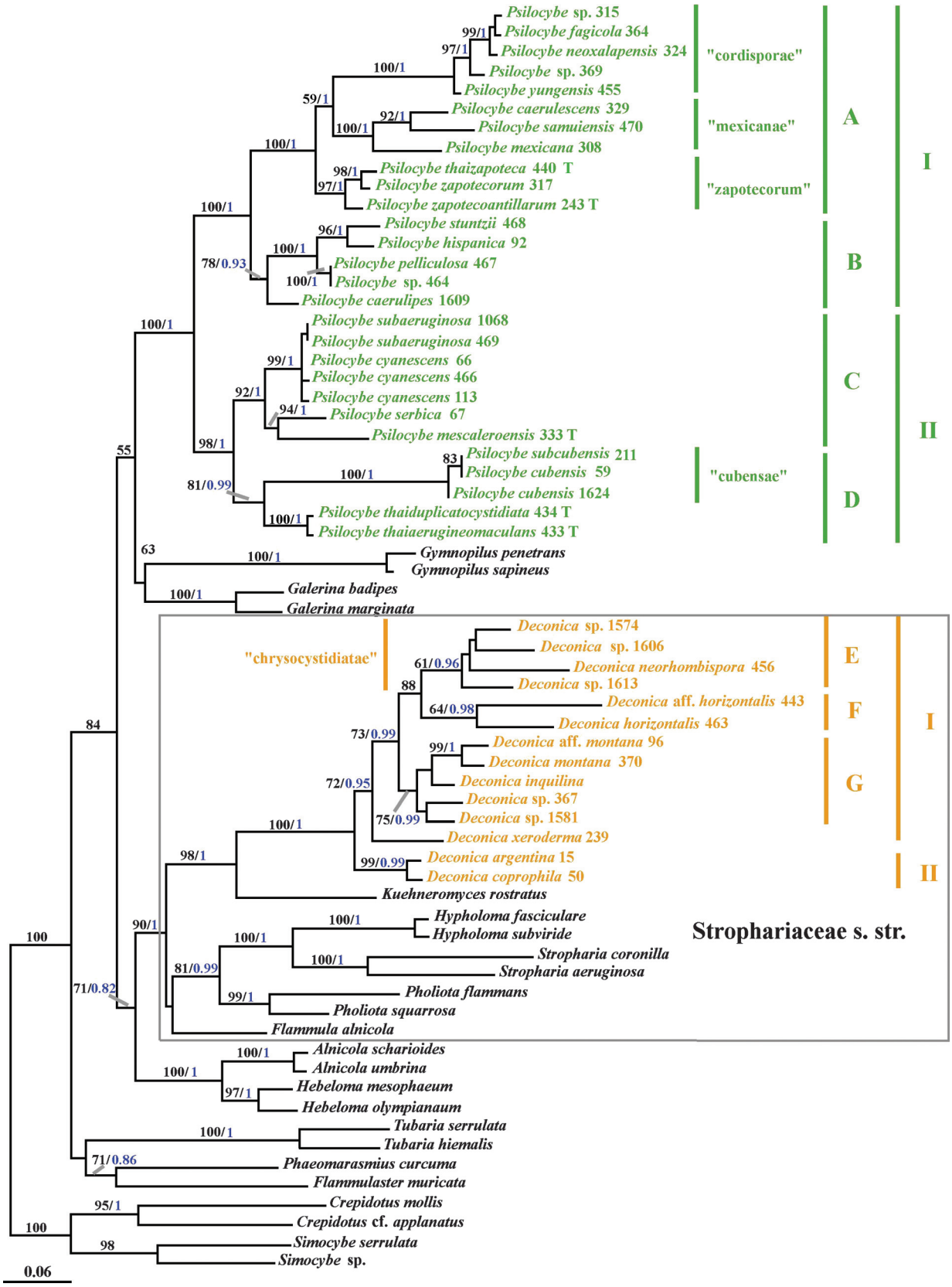


Table 3. Frequencies and likelihoods of models sampled during RJ-MCMC analyses.

	Two-parameter model ($q_{01} > q_{10}$)		Two-parameter model ($q_{01} < q_{10}$)		One-parameter model ($q_{01} = q_{10}$)		Restricted model ($q_{01} = 0$)		Restricted model ($q_{10} = 0$)	
Character	Frequency	–log L	Frequency	–log L	Frequency	–log L	Frequency	–log L	Frequency	–log L
Chrysocystidia	67	11.1098	75	11.3812	19 541	11.1316	**30 284**	**10.7497**	34	22.3421
Basidiospore shape in frontal view	437	34.1660	483	34.1047	**48 349**	**35.0359**	38	44.3821	699	38.4321

Note: The frequency and likelihood of the best-fit model are in bold. RJ-MCMC, Reversible-Jump Markov chain Monte Carlo algorithm; –log L, negative log-likelihood.

Table 4. The 15 most prevalent models of character state transition for basidiospore wall thickness sampled during RJ-MCMC.

q_{01}	q_{02}	q_{10}	q_{12}	q_{20}	q_{21}	Model frequency
1.5939	1.5939	0	1.5939	0	1.5939	4113
1.6110	1.6110	0	0	0	1.6110	3764
1.6357	1.6357	1.6357	1.6357	0	1.6356	3393
1.6110	1.6110	0	0	0	1.6110	3341
1.6618	1.6618	0	0	1.6618	1.6618	3031
1.7387	1.7387	1.7387	1.7387	1.7387	1.7387	2798
1.7614	1.7614	1.7614	0	1.7614	1.7614	2690
1.8027	1.8027	1.8026	0	0	1.8027	2017
4.4997	0	4.4997	4.4998	0	4.4997	713
0	4.6343	0	0	4.6342	4.6342	628
4.3468	0	4.3468	4.3468	0	0	574
5.1042	0	5.1042	5.1042	5.1042	5.1042	534
0	5.1846	5.1846	5.1846	5.1846	5.1846	354
3.5439	0	0	3.5439	0	3.5439	352
1.4118	1.4118	0	5.8612	0	5.8612	278

Note: Rate parameters and the number of times each model was sampled are shown. 0, thin-walled; 1, slightly thick-walled; 2, thick-walled; RJ-MCMC, Reversible-Jump MCMC algorithm.

runs. Table 4 shows the 15 more frequently sampled models. The most frequently sampled model (4113 times) disallows transitions from "slightly thick-walled" to "thin-walled" ($q_{10} = 0$) and "thick-walled" to "thin-walled" ($q_{20} = 0$). It also assumes that a reversal is not possible once the thickness of the wall is gained. The second most frequent model (3764) assumes that the change from "slightly thick-walled" to "thick-walled" is not possible ($q_{12} = 0$).

The best-fit model for the evolution of chrysocystidia rejects the capability of their loss ($q_{10} = 0$). However, the second most frequently sampled model permits equal rates of change between gains and losses ($q_{01} = q_{10}$) (Table 3). Based on the Bayes factor (BF = 0.7638), the second model cannot be rejected in favor of the first one.

Ancestral state reconstruction analyses

Character evolution at nodes of interest is depicted in Fig. 5 (see also Tables 5 and 6). Evolution of basidiospore shape in frontal view reveals that a non-angular shape is ancestral in *Psilocybe* (node 7). Three transitions to angular spores in frontal view occurred: in clade D (node 8), in the clade uniting "cordisporae" and "mexicanae" (node 13), and in *P. stuntzii* within clade B. However, in *Deconica* the angular basidiospore in frontal view is indicated as ancestral (node 2), but the strength for this hypothesis is weak (Table 5). While most species of *Deconica* feature angular spores in frontal view, non-angular spores are found in three different lineages.

The most recent common ancestor of *Psilocybe* had thin-walled basidiospores (node 7, Table 6). Clades C and D (nodes 8 and 9) have thick-walled basidiospores, and clades A and B (nodes 10 and 11) thin-walled spores. The "cordisporae" clade is characterized by slightly thick-walled spores (node 15). Similarly, the most recent common ancestor of *Deconica* has thin-walled basidiospores (node 2). Thick-walled basidiospores evolved at least once in a coprophilous group of *Deconica* species and independently on at least three occasions in *Psilocybe*.

The most recent common ancestor of Strophariaceae s. str. (node 1) lacked chrysocystidia, a character that unites an assemblage of species in *Hypholoma*, *Stropharia*, and *Pholiota* s. str. (Fig. 5). The common ancestor of *Deconica* (node 2) also lacked chrysocystidia. However, they were gained in a poorly known group of *Deconica* species related to *D. neorhombispora* (clade E, Fig. 5).

Discussion

Family-level classification of *Psilocybe* and *Deconica*

The family placement of *Psilocybe* and *Deconica* has been the subject of much debate. Guzmán (1983) and Singer (1986) considered *Psilocybe* s.l. in the Strophariaceae. Singer (1986) recognized two subfamilies in Strophariaceae: Strophrioideae and Pholiotoideae, based on the basidiospore morphology and spore print color. In Strophrioideae, the basidiospores are yellowish brown, and the spore print is deep lilac, sepia, dark brown, or purplish brown. This subfamily comprises the genera *Hypholoma*, *Melanotus*, *Psilocybe* s.l., and *Stropharia*. In the Pholiotoideae, the basidiospores are orange yellowish or brown yellowish, and the spore print has reddish, rusty-brown, or red-brown tones. The genera *Kuehneromyces*, *Pachylepyrium* Singer, *Phaeomarasmius*, *Pholiota*, and *Pleuroflammula* Singer belong to Pholiotoideae. Traditional characters that distinguish genera in the Strophariaceae sensu Singer (1986) are inadequate for some groups. In this family, emphasis has been placed on the presence of chrysocystidia, a subcellular hypodermium, and the presence of acanthocytes in rhizomorphs and basal mycelium of the stipe. This last character is only useful for distinguishing the genus *Stropharia* (Cortez 2008*a*, 2008*b*; Cortez and da Silveira 2008) because these structures are not present in any other genera in the family. Some residual species of *Stropharia* lack acanthocytes and have been moved to *Leratiomyces* Bresinsky & Manfr. Binder ex Bridge, Spooner, Beever & D.C. Park (Redhead and McNeill 2008) and *Protostropharia* Redhead, Moncalvo & Vilgalys (Redhead 2013). Guzmán (1983) and Singer (1986) considered the subcellular hypodermium to be a key character for distinguishing *Hypholoma*; however, not all the members of this genus have this feature. It has been suggested that chrysocystidia were exclusive to *Pholiota*, *Stropharia*, and *Hypholoma* (but see below).

Moncalvo et al. (2002), using partial sequences from a single gene region, recovered *Psilocybe* s. str. and *Deconica* in the separate clades "/psychedelia" and "/psilocybe," respectively. Additionally, this analysis recovered the "/strophrioid" group, composed of *Hypholoma*, *Leratiomyces*, *Pachylepyrium*, *Phaeonematoloma* (Singer) Bon, *Pholiota*, *Stropharia*, and *Weraroa* Singer. These results were incongruent with the Strophariaceae in the sense of Singer (1986). *Psilocybe* s. str. was related to the "/strophrioid" clade, but without support. Similar results were generated by Bridge et al. (2008).

Other ideas regarding the circumscription of Strophariaceae have been proposed. Gulden et al. (2005) suggested the family Strophariaceae should be considered in the broad sense of Kühner (1980), including the following genera: *Agrocybe* Fayod, *Flammula*, *Flammulaster*, *Galerina*, *Gymnopilus*, *Hebeloma*, *Hemipholiota* (Singer) Kühner ex Bon, *Hypholoma*, *Kuehneromyces*, *Melanotus*, *Naucoria* (Fr.) P. Kumm., *Pachylepyrium*, *Phaeocollybia* R. Heim, *Phaeogalera* Kühner,

Fig. 5. Characters traced in a phylogram obtained with the Bayesian inference with 5.8S, partial nLSU rDNA, and *rpb1* dataset of *Psilocybe* and *Deconica*. Numbers indicate the nodes.

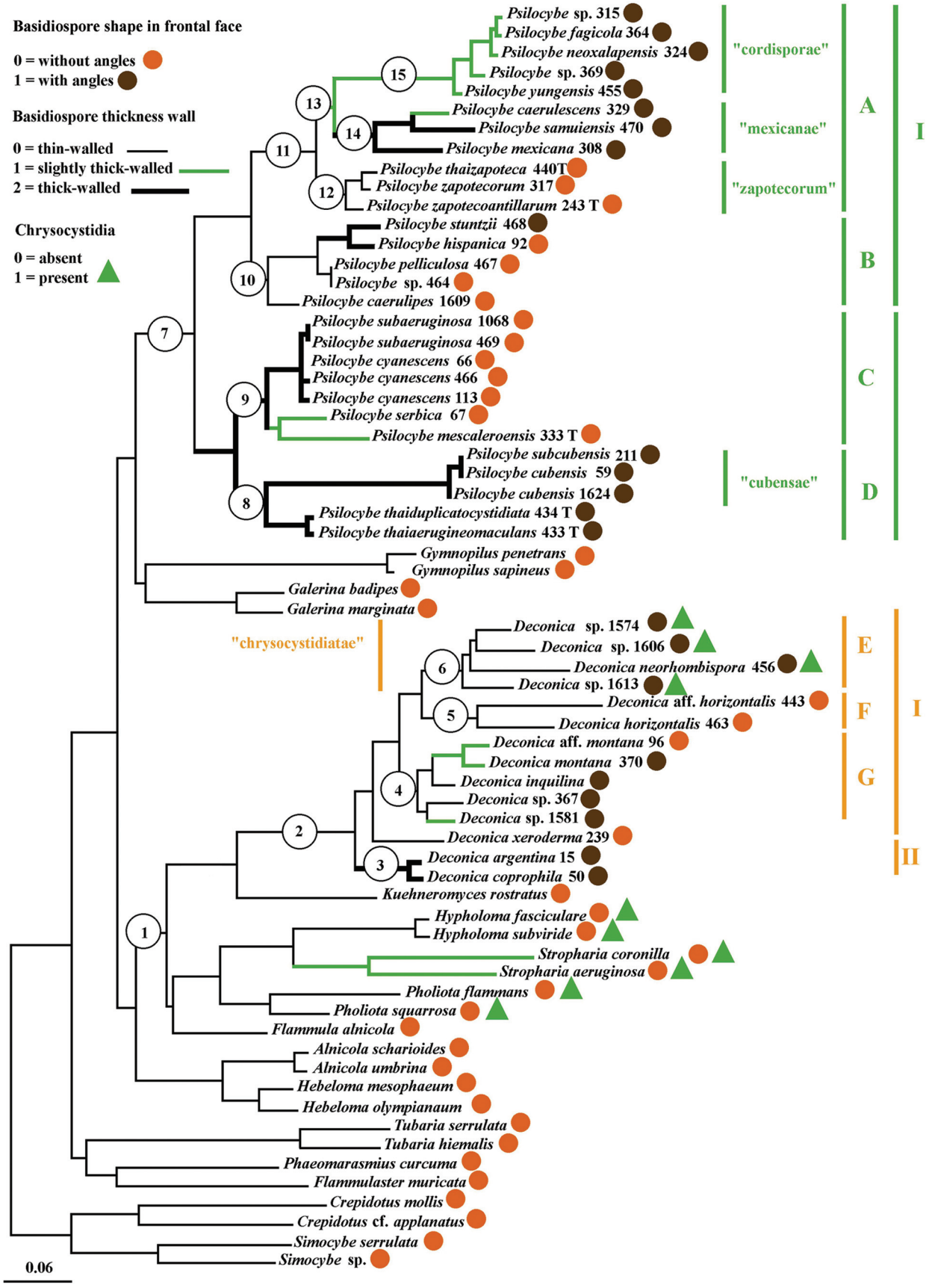


Table 5. Probabilities of ancestral state reconstructions for presence or absence of chrysocystidia and basidiospore shape (without angles or with angles).

Character	Probability of reconstructed ancestral states		Harmonic mean of likelihood when fixed at a state (–log L)		Bayes factor
Chrysocystidia	0	1	0	1	
Node 1	0.9613	0.0386	12.7884	20.0468	**14.516*****
Node 2	0.9997	0.0002	13.0533	23.1959	**20.285*****
Node 6	0.0029	0.9970	16.6718	12.1696	**9.004****
Basidiospore shape	0	1	0	1	
Node 1	0.9133	0.0866	35.5131	40.5988	**10.171*****
Node 2	0.0849	0.9151	39.0313	38.5259	**1.011**
Node 3	0.0045	0.9955	42.4146	37.6366	**9.556****
Node 4	0.0075	0.9926	42.1596	37.3903	**9.539****
Node 5	0.5737	0.4263	38.5235	37.7175	**1.612**
Node 6	0.2057	0.7942	39.5820	37.6700	**3.8240***
Node 7	0.8170	0.1829	37.2908	40.3837	**6.186****
Node 8	0.0623	0.9377	38.4279	37.9411	**0.9736**
Node 9	0.9818	0.0181	37.7958	41.2563	**6.921****
Node 10	0.8994	0.1004	37.2804	40.2990	**6.037****
Node 11	0.5386	0.4613	37.3487	39.3038	**3.910***
Node 12	0.9963	0.0037	37.4092	42.5221	**10.226****
Node 13	0.0571	0.9428	38.2675	37.7688	**0.998**
Node 14	0.0364	0.9635	39.6392	37.5509	**4.177***
Node 15	0.0029	0.9971	42.8949	37.5020	**10.786*****

Note: Bayes factors are in bold. *, positive evidence; **, strong evidence; ***, very strong evidence; –log L, negative log-likelihood.

Table 6. Ancestral state reconstructions of character basidiospore wall thickness.

Nodes	Probability of reconstructed ancestral states			Harmonic mean of likelihoods when fixed at a state (–log L)		
	0	1	2	0	1	2
Node 2	0.7177	0.0559	0.2264	**44.4403**$^{**/+}$	47.7693	46.7302
Node 3	0.0006	0.0020	0.9973	49.2396	49.7237	**44.4382**$^{**/++}$
Node 4	0.6774	0.2971	0.0255	**44.5045**$^{**/++}$	47.5280	48.1028
Node 5	0.9584	0.0218	0.0197	**44.1009**$^{***/+++}$	49.2103	49.7673
Node 6	0.9914	0.0050	0.0035	**44.0846**$^{***/++}$	50.2842	51.2942
Node 7	0.5722	0.0859	0.3419	**44.2445**$^{**/+}$	47.8057	46.8224
Node 8	0.0158	0.0287	0.9555	48.3355	47.2632	**44.1366**$^{**/++}$
Node 9	0.0236	0.4312	0.5452	48.92244	46.1961	**44.4534**$^{**/+}$
Node 10	0.9519	0.0152	0.0328	**44.14695**$^{**/++}$	49.1815	48.0900
Node 11	0.7187	0.1239	0.1574	**44.08254**$^{**/++}$	47.6208	47.5632
Node 12	0.9980	0.0012	0.0008	**44.2165**$^{***/+++}$	51.5959	51.0392
Node 14	0.0159	0.1477	0.8364	46.6692	46.7046	**44.2949**$^{*/+}$
Node 15	0.0001	0.9977	0.0017	51.5037	**44.7996**$^{**/++}$	51.8551

Note: Asterisks and plus signs denote positive (* or +), strong (** or ++), or very strong (*** or +++) evidence (in bold) against the other states (*, first state; +, second state). –log L, negative log-likelihood.

Phaeomarasmius, *Pholiota*, *Panaeolus*, and *Tubaria*. However, this hypothesis received weak support.

Matheny et al. (2006), using a supermatrix of six gene regions, recovered members of Strophariaceae sensu Singer (1986) in two clades. The first one included *Agrocybe* s. str., *Deconica* (represented by *Psilocybe montana* and *P.* "*silvatica*," the latter a misapplied name because the name is for a bluing mushroom, but the sequenced specimen corresponds to *Deconica*), *Hypholoma* including *H. udum*, now *Bogbodia* Redhead (Redhead 2013), *Kuehneromyces*, *Nivatogastrium* Singer & A.H. Sm., *Pholiota*, and *Stropharia*. Matheny et al. (2006) circumscribed this clade as Strophariaceae s. str. The second clade, named Hymenogastraceae, included *Alnicola*, *Galerina*, *Hebeloma*, *Phaeocollybia*, and *Psilocybe* s. str. (represented by *P. cyanescens* and *P. stuntzii*). Strophariaceae s. str. and Hymenogastraceae showed a sister group relationship. *Galerina* and *Phaeocollybia* were the sister group of *Psilocybe* s. str. with significant support from Bayesian posterior probabilities (Matheny et al. 2006). Recently, Noordeloos (2011) considered *Deconica*, *Flammula*, *Kuehneromyces*, *Leratiomyces*, *Hemipholiota*, *Hemistropharia* Jacobsson & E. Larss., *Hypholoma*, *Meottomyces* Vizzini, *Phaeonematoloma*, *Pholiota*, *Psilocybe*, and *Stropharia* in Strophariaceae, but excluding *Galerina*, *Gymnopilus*, and *Phaeocollybia*.

Here, Strophariaceae s. str. was strongly supported and includes *Deconica*, *Flammula*, *Hypholoma*, *Kuehneromyces*, *Pholiota*, and *Stropharia* (and likely *Bogbodia*). Sequences of *rpb1* for *Agrocybe* s. str. were lacking and thus do not appear in our trees. The position of *Flammula* within Strophariaceae and the sister position of *Alnicola* and *Hebeloma* with respect to Strophariaceae s. str. contradict results shown in Matheny et al. (2006). This is most likely due to differences in gene sampling, as our study included only three gene regions. Nevertheless, within Strophariaceae, *Kuehneromyces* was recovered as the sister group of *Deconica* with strong measures of statistical support. Our results also suggest that a clade of *Galerina* and *Gymnopilus* may be the sister group to *Psilocybe* s. str., but support for this hypothesis is weak.

Psilocybe s.l. represents a polyphyletic assembly

Our results are congruent with those of Moncalvo et al. (2002), Walther et al. (2005), and Matheny et al. (2006) in that *Psilocybe* s.l. is a polyphyletic group. We also confirmed the monophyly of *Deconica* and *Psilocybe* s. str. Moncalvo et al. (2002) recovered the clade "/psychedelia" but with poor support composed of *P. cubensis*, *P. cyanescens*, *P. fimetaria* (P.D. Orton) Watling, *P. liniformans* Guzmán & Bas, *P. semilanceata* (Fr.) P. Kumm., *P. stuntzii*, and *P. subaeruginosa*. Sister to "/psychedelia" was the clade "/strophariod." Moncalvo et al. (2002) also recovered what is now known as *Deconica*, in their work labeled "/psilocybe," which contains the non-hallucinogenic fungi of *Psilocybe* s.l. and *Melanotus*. Walther et al. (2005) obtained three supported clades within *Psilocybe* s.l. from neighbor-joining analyses. Their clade 1 included *D. coprophila* (Fig. 2*a*), *D. inquilina*, and *Deconica* sp., and a second grouping comprised *P. semilanceata* and *P. stuntzii* (both with hallucinogenic properties) together with *Tubaria hiemalis* Romagn. ex Bon. A third taxon labeled *Psilocybe* sp. was found independently of the other two clades; the name *Psilocybe* in this instance may be misapplied. Similarly, Matheny et al. (2006) recovered non-hallucinogenic *Psilocybe* (Strophariaceae s. str.) apart from hallucinogenic species of *Psilocybe* (Hymenogastraceae). Our results and previous papers have shown that presence of psilocybin is a synapomorphy of the genus *Psilocybe*. Psilocybin, however, has multiple origins, as it is present in several distantly related lineages of Agaricales (Stamets 1996; Guzmán et al. 1998; Kosentka et al. 2013).

This work confirmed the phylogenetic placement of two species of *Melanotus* in *Deconica*. Guzmán (1983) and Singer (1986) considered *Melanotus* to be a non-stipitate genus in Strophariaceae. Noordeloos (2011), likely following Moncalvo et al. (2002), transferred species of *Melanotus* to *Deconica*. Our analyses reaffirm that *Melanotus* is nested within *Deconica* (Figs. 4–5, as *D. horizontalis* and *D.* aff. *horizontalis*). However, the type of the genus, *M. bambusinis* (Pat.) Pat., has yet to be sequenced.

Nested groupings within *Psilocybe* s. str.

Our results show four groups within *Psilocybe* (Fig. 4, A–D), but none match with previous proposed sections. We observe some branches congruent with traditional taxonomic groups: sect. *Cubensae* of Guzmán (1983), or stirp *Cubensis* of Singer (1951, 1986), match our "cubensae" clade, sect. *Cordisporae* (in part), and sect. *Zapotecorum* (Guzmán 1983) corresponds to "cordisporae" and "zapotecorum" clades.

Sect. *Cordisporae*, in the sense of Guzmán (1983), is the most diverse section in the genus. *Psilocybe thaiduplicatocystidiata* and *P. caerulescens* were originally classified in this section. However, *P. thaiduplicatocystidiata* is distantly related to the "cordisporae" clade. Instead, it groups in clade D, together with *P. thaiaerugineomaculans*, *P. cubensis*, and *P. subcubensis*. Likewise, *P. caerulescens* groups with *P. mexicana* and *P. samuiensis*, representatives of sect. *Mexicanae* sensu Guzmán (1983). Macromorphological features of *P. caerulescens* are different from the abovementioned species although the micromorphology is similar.

Sect. *Semilanceatae* in the sense of Guzmán (1983) is not monophyletic. *Psilocybe caerulipes*, *P. hispanica*, and *P. pelliculosa* form clade B, but others, *P. cyanescens* and *P. serbica*, were recovered in clade C. On the other hand, clade C is an unanticipated mixture of species traditionally placed in sections *Semilanceatae* (Guzmán 1995) and *Stuntzae*. *Psilocybe cyanescens* and *P. serbica* have been reported from temperate zones in Europe and North America, while *P. subaeruginosa* was described from Australia, and *P. mescaleroensis* (Sect. *Stuntzae*, Guzmán et al. 2007*b*) has been found in the United States. We suspect that *P. cyanescens* and *P. subaeruginosa* (sect. *Subaeruginascens*, Fig. 1*f*) represent the same species, based on their high genetic similarity (Fig. 4) and shared micromorphological features. Clade D contains two species from Thailand as well as clade "cubensae." It seems to be a natural group, based on the angular basidiospores in frontal view (Fig. 5, node 8) and the shape of the pleurocystidia and cheilocystidia (Figs. 3*f*–3*m*).

Members of sect. *Stuntzae* (Guzmán 1983; Guzmán et al. 2007*b*, 2012; Horak et al. 2009) clustered in different clades. *Psilocybe stuntzii* grouped in clade B and *P. mescaleroensis* in clade C. *Psilocybe subbrunneocystidiata,* traditionally classified in sect. *Brunneocystidiatae*, is a member of *Deconica* (Fig. 4), an understandable outcome in that this species is a non-bluing mushroom, based on observations from fresh specimens.

Lastly, sect. *Neocaledonicae* (Guzmán 2004) contains a mixture of species belonging to *Deconica* and *Psilocybe* s. str. The section was described with species that have hallucinogenic properties, rhomboid basidiospores, and chrysocystidia. *Psilocybe thailandensis* E. Horak, Guzmán & Desjardin and *P. umbrina* E. Horak, Guzmán & Desjardin were considered as bluing and placed in sect. *Neocaledonicae* (Horak et al. 2009; Guzmán et al. 2012) but were recently transferred to *Deconica* (Ramírez-Cruz et al. 2012).

Nested groupings within *Deconica*

Our analysis resolved several phylogenetic relationships within *Deconica* (Fig. 4). Sect. *Psilocybe* (excluding *D. xeroderma*) from Guzmán (1983), or sect. *Deconica* from Noordeloos (2011), was recovered as a monophyletic group (clade G). *Deconica* sect. *Melanotus* (Noordeloos 2011) was recovered in clade F. In addition, we recovered a natural group with species producing chrysocystidia as clade E, which corresponds with sect. *Chrysocystidiatae* in the sense of Singer (1986).

Evolution of morphological characters

Basidiospore shape

Basidiospore shape and basidiospore wall thickness are commonly used in the taxonomy of *Psilocybe* and *Deconica*. However, they have not been discussed within a phylogenetic framework. According to Kauserud et al. (2008), the shape of a spore is important because it will influence aerodynamic properties. For example, spherical spores travel faster, and narrow spores (e.g., ellipsoid) float better through the air. Nevertheless, there is no information about the advantages of angled spores. Here, basidiospore evolution was investigated according to shape in frontal view and wall thickness. The spore angles have evolved independently on multiple occasions during the history of these genera. Some works supported the idea that having angles is a derived character. For instance, in the "crown" group of the genus *Parasola* Redhead, Vilgalys & Hopple (Psathyrellaceae), all members possess rounded triangle or "heart" shaped spores, but the rest of the group has ellipsoid spores (Nagy et al. 2009). A similar scenario does not occur in *Deconica* and *Psilocybe*. According to the best-fit evolutionary model, rates of change have equal probabilities ($q_{01} = q_{10}$) to transform from one state to another, such that in *Deconica* and *Psilocybe* the basidiospores with angles are phylogenetically dispersed.

Basidiospore wall

Garnica et al. (2007) pointed out that the thickness of the basidiospore wall represents an evolutionary advantage to some fungi. Thick-walled spores may be more resistant to dehydration and UV radiation than thin-walled spores. Here we observe four ensembles of species that have exclusively thick-walled basidiospores. Two of them include coprophilous species, one with *Deconica* species and the clade "cubensae" of *Psilocybe*. This result supports the hypothesis that some of these spores are adapted to survive the digestive tract of herbivores. Although this character has biological importance, the thickness of the basidiospore wall is evolutionarily labile in both genera, and sometimes the two states (slightly thick and thin-walled) are observed among species in the same clade (Fig. 5, node 4).

Chrysocystidia

It has been thought that "true" chrysocystidia were exclusive to *Pholiota*, *Stropharia*, and *Hypholoma*. Clémençon (2012) defines chrysocystidia as deuteroplasmatic cystidia, "vesicular or fusiform, often mucronate, …. whose deuteroplasm contains one or a few highly refractive masses …. becoming yellow in age and in alkali solutions." This kind of cystidium is present in *Hypholoma* (Figs. 3*c*–3*d*). However, we have observed that refractive masses are not always present, nor do they always become yellow in KOH. Thus, we identified chrysocystidia by staining their content with Patent blue V following Jahnke (1984). As a result, we observed two types of chrysocystidia in *Deconica*. One type is hyaline with a refringent content, sometimes with granulose or crystal-like content (Figs. 2*j*–2*k*, 2*n*) as in *D. neorhombispora* and *Deconica* spp. (1606, 1613). This type of cystidium is very similar to that in *Pholiota* aff. *gummosa*, which are considered chrysocystidia by Holec (2001) (Fig. 3*e*). In the second type, the cystidia have homogeneous hyaline and never yellowish content and were observed in *D. aequatoriae* (Singer) Ram.-Cruz & Guzmán, *D. aureicystidiata* nom. prov., *D. thailandensis* (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán (Figs. 2*l*–2*m*, 2*o*), *D. umbrina* (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán, and *Deconica* sp. (1574) (recovered in clade E). Chrysocystidium type 2 does not fit the definition of chrysocystidia of Largent et al. (1977), Holec (2001), and Clémençon (2012) because they lack highly refractive masses, but they do stain with Patent blue V (Jahnke 1984).

Recently, Guzmán (2012) used the term "pseudocystidia" to name cells differing from the typical pleurocystidia (Figs. 1*m*–1*n*) and chrysocystidia. According to him, pseudocystidia are fusiform, ventricose, cylindrical, broadly lageniform, or frequently irregularly branched. In addition, they are hyaline, grayish, yellowish brown, or orange brown in KOH (Fig. 1*k*–1*l*), and larger than typical pleurocystidia. Initially, we misinterpreted the pseudocystidia of *P. zapotecorum* as chrysocystidia, until we observed their negative reaction to Patent blue V. Currently, we conclude that they are deuteroplasmatic cystidia, but with a content different to that present in true chrysocystidia.

In *Psilocybe* s.l., interpretation of chrysocystidia has been controversial. Singer (1986) and Horak and Desjardin (2006) indicated their presence, but Guzmán (1983, 1995) interpreted their absence. Later, Guzmán (2004) proposed the bluing sect. *Neocaledonicae* to include species with chrysocystidia. This section does not correspond to sect. *Chrysocystidiata* Singer, which Guzmán (1980) considered as belonging to the genus *Hypholoma*. According to Singer (1986), sect. *Chrysocystidiatae* "differs from sect. *Psilocybe* only in the presence of chrysocystidia on the sides of the lamellae." Although they do not show an amorphous yellowish content in KOH, their shape is similar to that of chrysocystidia. Based on *P. magnispora* E. Horak, Guzmán & Desjardin (sect. *Neocaledonicae*), it was suspected that chrysocystidia were present in *Psilocybe* s. str. (Horak et al. 2009; Guzmán et al. 2012). Using Patent blue V, we tested this species together with *P. ovoideocystidiata* Guzmán & Gaines (sect. *Stuntzae*), *P. thaiaerugineomaculans* (sect. *Stuntzae*), and *P. thaiduplicatocystidiata* (sect. *Cordisporae*); contrary to *Hypholoma* (Figs. 3*c*–3*d*) and *Deconica* (Figs. 2*m*–2*o*), none of them showed any reaction (Figs. 3*g*, 3*i*, 3*k*, 3*m*). Therefore, our observations confirmed that chrysocystidia are present in *Deconica* (Fig. 5, clade E), at times lacking refractive masses, and absent from *Psilocybe*. Chrysocystidia are key structures in Strophariaceae s. str., although they are not present in all lineages of the family. Indeed, the absence of chrysocystidia is a symplesiomorphic state and evolved twice within the family in our analysis.

Molecular phylogenetic analyses confirm the separation of *Deconica* and *Psilocybe* s. str., which has been known for some time (Moncalvo et al. 2002). These genera have been separated based on the presence of hallucinogenic compounds in *Psilocybe*. Thus, as a general rule, basidiomata in *Deconica* never turn blue when bruising, as it is assumed that the blue reaction in *Psilocybe* is due to the oxidation of psilocin (Blaschko and Levine 1960). However, because some species of *Deconica* turn blackish, care must be paid not to confuse this stain with a bluish one. Besides the bluing reaction, no single macro or micromorphological character can be used to separate these two genera.

Finally, *Deconica* and *Psilocybe* do not have a sister group relationship, so their morphological similarities represent homoplasies. None of the traditional infrageneric classifications were recovered here. Our results show that the characters used by Guzmán (1983, 1995), Singer (1986), and Noordeloos (2011) are highly homoplastic. Some nested clades match few of the traditional sections. Therefore, the relationships obtained here cannot be transferred to a formal classification yet, because a broader sample, especially in *Deconica*, would be necessary to attempt it.

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