



UNIVERSIDAD DE GUADALAJARA

Centro Universitario de Ciencias Biológicas y Agropecuarias

**Taxonomía y análisis filogenético del
género *Psilocybe sensu lato* (Fungi,
Agaricales)**

**Tesis
que para obtener el grado de**

**Doctora en Ciencias en Biosistemática,
Ecología y Manejo de Recursos Naturales y
Agrícolas**

Presenta

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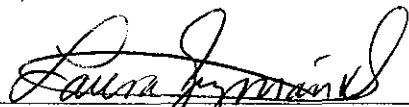
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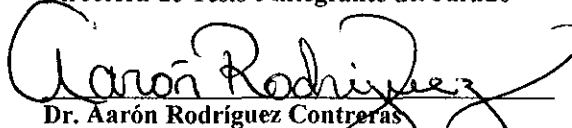
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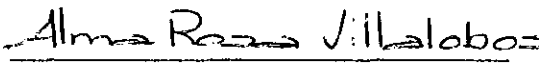
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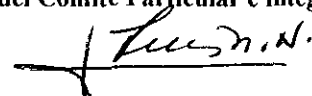


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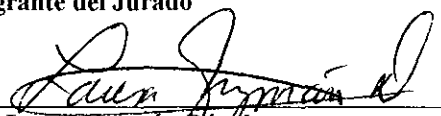
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Resumen

El género *Psilocybe sensu lato* es mundialmente conocido porque a él pertenecen los hongos alucinógenos que contienen psilocibina, redescubiertos en la sierra mazateca en el estado de Oaxaca en México. Ahora se sabe que estos hongos están presentes en las ceremonias tradicionales de varios grupos étnicos en México, además de los mazatecos. *Psilocybe* presenta una amplia distribución en el mundo, con la mayor diversidad concentrada en los trópicos. Por mucho tiempo se creyó que el género incluía además a especies no alucinógenas; pero desde hace una década, con base en estudios filogenéticos, se sabe que son independientes, *Deconica* y *Psilocybe sensu stricto*. Las clasificaciones infragenéricas de ambos se basan en morfología y difieren de acuerdo al autor. Este trabajo representa hipótesis sobre las relaciones de parentesco en estos géneros con base en secuencias de ADN. Se presentan aquí los resultados del estudio de ejemplares tipo en *Psilocybe* s.l., con el fin de verificar la determinación de las especies. En los análisis filogenéticos se usaron secuencias de tres regiones de ADN (28S, 5.8S y *rpb1*) de *Deconica* y *Psilocybe*. Se confirma que son géneros independientes, sin una relación de grupos hermanos y se recupera su posición filogenética en relación con otros Agaricales. *Deconica* pertenece a la familia Strophariaceae y *Psilocybe* a Hymenogastraceae. Se mapearon tres caracteres de importancia taxonómica en la topología obtenida. De los tres, sólo los crisocistidios representan una sinapomorfia en *Deconica*. Con el propósito de profundizar en las relaciones infragenéricas se llevaron a cabo análisis de *Deconica* y *Psilocybe* por separado, usando sólo la región ITS del ADN ribosomal (ITS1, 5.8S y ITS2) e incrementando el número de taxa en ambos géneros. De los clados obtenidos, algunos corresponden con las diagnósis de secciones propuestas por autores previos, tal es el caso de *Cordisporae*, *Cubensae* y *Zapotecorum* en *Psilocybe*. Mientras que en *Deconica* sólo un clado corresponde con la sección *Chrysocystidiatae*. Por último, se confirma la presencia de crisocistidios únicamente en el género *Deconica*, ya que las células que algunos autores llamaron crisocistidios en *Psilocybe* corresponden a otro tipo de cistidios.

Capítulo I. Introducción

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El presente trabajo es un estudio taxonómico y filogenético de *Psilocybe sensu lato* (s.l.). Se estiman aquí las relaciones filogenéticas de los géneros *Deconica* (W.G. Sm.) P. Karst. y *Psilocybe* (Fr.) P. Kumm. con secuencias de ADN y se evalúan las propuestas de clasificación infragenérica para cada género. Este trabajo consta de ocho capítulos: el primero incluye generalidades sobre *Psilocybe* s.l. El capítulo II se refiere a la revisión de material tipo de 42 especies de *Psilocybe* s.l., en donde se aportan datos nuevos que amplían los protólogos de las especies estudiadas. Se proponen tres nombres en sinonimia y tres combinaciones en *Deconica*. En el capítulo III se analiza la posición filogenética de *Deconica* y *Psilocybe sensu stricto* (s.s.) en relación con otros Agaricales. Para ello se construyó una matriz con secuencias de tres regiones de ADN (28S, 5.8S y *rpb1*). Además se analizó la evolución de tres caracteres morfológicos.

El capítulo IV muestra las relaciones filogenéticas en *Psilocybe* s.s. con base en secuencias de la región ITS del ADN ribosomal. Resultaron nueve grupos infragenéricos, de ellos cinco coinciden a *grosso modo* con las propuestas de clasificación infragenérica de algunos autores. El capítulo V es un análisis filogenético del género *Deconica*; se encontró que hay seis clados bien soportados, pero no tienen coincidencia con las clasificaciones tradicionales, excepto uno que incluye especies con crisocistidios.

En los capítulos VI y VII se presentan algunas combinaciones en *Deconica* de hongos que se describieron como *Psilocybe*. Finalmente, en el capítulo VIII se discute la importancia de la determinación correcta de los especímenes que se usan en un estudio filogenético con secuencias de ADN, así como la relevancia de estudiar ejemplares tipo para entender el concepto de la especie de acuerdo a los autores que la describieron. Se mencionan las conclusiones y los alcances de este trabajo y lo que queda pendiente para llegar a una mejor comprensión de las relaciones de parentesco en este grupo de hongos.

Psilocybe sensu lato

El género *Psilocybe* es conocido ampliamente en el mundo porque a él pertenecen la mayoría de los hongos alucinógenos, los cuales fueron redescubiertos en Oaxaca. Se sabe que los indígenas mazatecos, chatinos, chinantecos, mixes y zapotecos en Oaxaca los usaban y usan con fines ceremoniales (Heim 1956a, 1956b; Wasson y Wasson 1957; Heim y Cailleux 1958; Rubel y Gettelfinger-Krejci 1976). También los usaron los nahuas del Estado de México y Puebla (Guzmán 1958, 1960, 1983, 2005, 2012). La palabra *Psilocybe* viene del griego *psilós* que significa liso o desnudo y *kýbe* que significa píleo, por carecer de ornamentación en el píleo (Ulloa y Herrera 1994).

Psilocybe fue descrito por Fries (1821) como un grupo de la serie *Pratella* Fr. dentro del género *Agaricus* L., y más tarde Kummer (1871) lo elevó a género. Por otro lado, *Deconica* fue un subgénero dentro de *Agaricus* (Smith 1870), que después fue erigido a género por Karsten (1879). A mediados del siglo XX algunos autores consideraron a *Deconica* como un género independiente de *Psilocybe* (Singer y Smith 1946; Singer 1951); sin embargo, en ese mismo siglo se empezó a tratar como sinónimo de *Psilocybe* (Singer 1962, 1975, 1986; Pegler 1977; Guzmán 1983; Watling y Gregory 1987). Estudios filogenéticos con secuencias de ADN

sugieren que *Psilocybe* s.l. no constituye un grupo monofilético (Moncalvo et al. 2002; Matheny et al. 2006), sino dos clados independientes: *Deconica* y *Psilocybe* s.s., y que la sinapomorfia de este último es la presencia de compuestos alucinógenos.

El conocimiento de que *Psilocybe* s.l. es polifilético, llevó a que se hicieran las propuestas nomenclaturales pertinentes, ya que la especie tipo del género correspondía a un hongo no alucinógeno, *P. montana* (Pers.) P. Kumm. Dada la importancia cultural y los términos derivados del nombre del género, se conservó el término *Psilocybe* para especies con propiedades alucinógenas y el de *Deconica* se mantuvo para aquéllas especies sin compuestos psicoactivos (Redhead et al. 2007). Estas propuestas fueron aprobadas en el XVII Congreso Internacional de Botánica y reportadas por Barrie (2011), McNeill et al. (2011) y Norvell (2011).

Las sustancias que producen los efectos alucinógenos en *Psilocybe* s.s. son psilocibina, psilocina y baeocystina (Beug y Bigwood 1981; Koike et al. 1981; Ott 1993; Gartz 1994). La psilocibina y psilocina no son exclusivas de *Psilocybe* s.s., ya que también se encuentran en otros linajes dentro de los Agaricales (Kosentka et al. 2013). De acuerdo con Guzmán et al. (1998), la psilocibina está presente en los géneros *Conocybe* Fayod, *Copelandia* Bres., *Panaeolina* Maire y *Panaeolus* (Fr.) Quél. (todos los anteriores en Bolbitiaceae), *Inocybe* (Fr.) Fr. s.s. (Inocybaceae), *Gymnopilus* P. Karst. e *Hypholoma* (Fr.) P. Kumm. (Strophariaceae) y en *Pluteus* Fr. (Pluteaceae).

Psilocybe s.l. se distribuye en todo el mundo y se estiman entre 277 y 300 especies (Guzmán 2005; Kirk 2008, respectivamente). Sin embargo, su uso tradicional sólo está registrado en México y probablemente en África y España (Guzmán 2012), aunque su uso recreativo (Ott 1975) se encuentra mucho más extendido. Los compuestos alucinógenos también tienen uso terapéutico; Montagne (2007) utilizó la psilocibina como tratamiento para la depresión, Moreno y Delgado (2007) para el desorden obsesivo-compulsivo, y Sewell y Halpern (2007) lo emplearon para cefaleas muy fuertes causadas por el nervio trigémino, por citar algunos ejemplos.

De acuerdo con Guzmán (1983, 1995), *Psilocybe* s.l. puede reconocerse por una combinación de características. El píleo es hemisférico, cónico, convexo o campanulado, a veces umbonado o papilado, en ocasiones plano y deprimido en adultos, frecuentemente higrófono, glabro o si presenta escamas, éstas son restos del velo. La superficie del píleo es viscosa, subviscosa, lubricosa o pocas veces seca, y es de color café amarillento o café oscuro, con el borde más claro o más oscuro. El margen del píleo es liso o ligeramente estriado. Las láminas son adnexadas, adnadas o sinuadas, de color café, gris violeta, amarillo violáceo, café violáceo, café chocolate o en ocasiones café púrpura. El estípite es central, cilíndrico, uniforme o engrosado en la base, no viscoso, hueco a relleno. La superficie del estípite es lisa o flocosa-fibrilosa, blanca, blanquecina, amarilla o de color café, a veces concolora con el píleo. En ocasiones los basidiomas presentan pseudorriza o cordones miceliares. El velo parcial esta presente como cortina aracnoide o puede ser membranoso. El contexto es carnoso en el píleo y en el estípite puede ser fibriloso, carnoso o cartilaginoso, y es blanco, blanquecino o de color café. Todo el basidioma se puede manchar de azul cuando se toca. La esporada es de color café violeta oscuro, gris violeta oscuro, morado oscuro, café púrpura o lila oscuro. Las especies alucinógenas tienen olor y sabor farináceo; por el contrario las no alucinógenas no tienen un olor ni sabor particular.

Las basidiosporas son pequeñas (4–5 μm de longitud), grandes (12–14 μm) o muy grandes (16–22 μm). La variación en forma se observa en vista frontal, y pueden ser elipsoides a subelipsoides, romboides, subromboides o subhexagonales. Su pared es delgada o gruesa, lisa, con poro germinal ancho que provoca que el ápice se vea truncado, de color café oscuro o café amarillento en KOH. Los basidios son ventricosos o subcilíndricos, usualmente con una constricción media o superior, generalmente tetraspóricos, hialinos, en ocasiones amarillentos o de color café pálido. Los pleurocistidios son hialinos o con contenido de color café o café amarillento, aunque en algunas especies están ausentes. El borde de la lámina es estéril. Los queilocistidios siempre están presentes, generalmente son hialinos, y pocas veces tienen contenido de color café amarillento. El subhimenio es hialino a color café amarillento, con o sin incrustaciones de color café amarillento en la pared de las hifas. La trama himenófora es regular o subregular. El pileipellis es un cutis o ixocutis, con hifas hialinas, y con pared delgada.

Tradicionalmente, *Psilocybe* y *Deconica* se clasificaron en la familia Strophariaceae (Singer y Smith 1946). El grupo contiene a los géneros: *Deconica*, *Flammula* (Fr.) P. Kumm., *Kuehneromyces* Singer & A.H. Sm., *Melanotus* Pat., *Hypholoma* (Fr.) P. Kumm. (= *Naematoloma* P. Karst.), *Pholiota* (Fr.) P. Kumm., *Pleuroflammula* Singer, *Psilocybe* y *Stropharia* (Fr.) Quél. Singer (1986) reconoció dos subfamilias: Stropharioideae y Pholiotoideae, separadas por el color de la esporada. En Stropharioideae las basidiosporas son de color café amarillento y la esporada es lila, sepia, marrón oscuro o marrón violáceo, e incluye a los géneros *Hypholoma*, *Melanotus*, *Psilocybe* s.l. y *Stropharia*. En Pholiotoideae las basidiosporas tienen tonos amarillo-anaranjados o color café amarillento y la esporada tiene tonos rojizos a color café-rojizo. Los géneros *Kuehneromyces*, *Pachylepyrium* Singer, *Phaeomarasmius* Scherff., *Pholiota* y *Pleuroflammula* están incluidos aquí.

Existen otros puntos de vista en relación a Strophariaceae. Kühner (1980, 1984) consideró a la familia en un sentido más amplio e incluyó a *Gymnopilus* y *Galerina* Earle dentro de ella, géneros que para Singer (1986) pertenecen a la familia Cortinariaceae. Por su parte, Noordeloos (2011) incluyó a los géneros: *Deconica*, *Flammula*, *Kuehneromyces*, *Leratiomyces* Bresinsky & Manfr. Binder ex Bridge, *Hemipholiota* (Singer) Kühner ex Bon, *Hemistropharia* Jacobsson & E. Larss., *Hypholoma*, *Meotatomyces* Vizzini, *Phaeonematoloma* (Singer) Bon, *Pholiota*, *Psilocybe* y *Stropharia* en Strophariaceae s.l.

La filogenia de Strophariaceae ha sido analizada con base en secuencias de ADN. La hipótesis más robusta es la de Matheny et al. (2006), quienes recuperaron a algunos miembros de Strophariaceae en el sentido de Singer (1986). Este grupo fue nombrado como Strophariaceae s.s., con los géneros *Agrocybe* s.s., *Deconica* (representada por especies no alucinógenas de *Psilocybe* s.l.), *Hypholoma*, *Kuehneromyces*, *Nivatogastrium* Singer & A.H. Sm., *Pholiota* y *Stropharia* (Moncalvo et al. 2002, Matheny 2006). Por otro lado, Gulden et al. (2005) presentaron la hipótesis de Strophariaceae en el sentido de Kühner (1980); sin embargo, resultó débilmente soportada.

Las características morfológicas útiles para delimitar géneros dentro de Strophariaceae son: presencia de velo parcial, color de las láminas, características de las basidiosporas (forma,

tamaño, presencia o ausencia de poro germinal), presencia de crisocistidios, arreglo del subpellis (como hipodermio), entre otros (Singer 1986, Watling y Gregory 1987, Noordeloos 2011). Recientemente se propuso la presencia de acantocitos como característica clave para separar al género *Stropharia* del resto de los miembros de Strophariaceae (Cortez 2008a, 2008b; Cortez y Silveira 2008).

Clasificación infragenérica

En la Figura 1 se presentan diferentes propuestas de clasificación infragenérica. Guzmán (1983) dividió a *Psilocybe* s.l. en 18 secciones. Para su clasificación, el autor utilizó la forma y grosor de la pared de las basidiosporas, color del contenido de los cistidios, presencia de anillo y si las fructificaciones se manchan de azul al maltratarse. Las secciones reconocidas por Guzmán (1983) son *Atrobrunneae*, *Aztecorum*, *Blattariopsidae*, *Brunneocystidiatae*, *Coprophilae*, *Cordisporae*, *Cubensae*, *Cyanescens*, *Merdariae*, *Mexicanae*, *Pratensae*, *Psilocybe*, *Stuntzae*, *Singerianae*, *Squamosae*, *Subaeruginosae*, *Semilanceatae* y *Zapotecorum*. Posteriormente, Guzmán (1995) disminuyó a 17 secciones, al combinar *Semilanceatae* y *Cyanescens* en una. Más tarde, propuso dos secciones nuevas, *Neocaledonicae* (Guzmán 2004) y *Bisporae* (Guzmán et al. 2007).

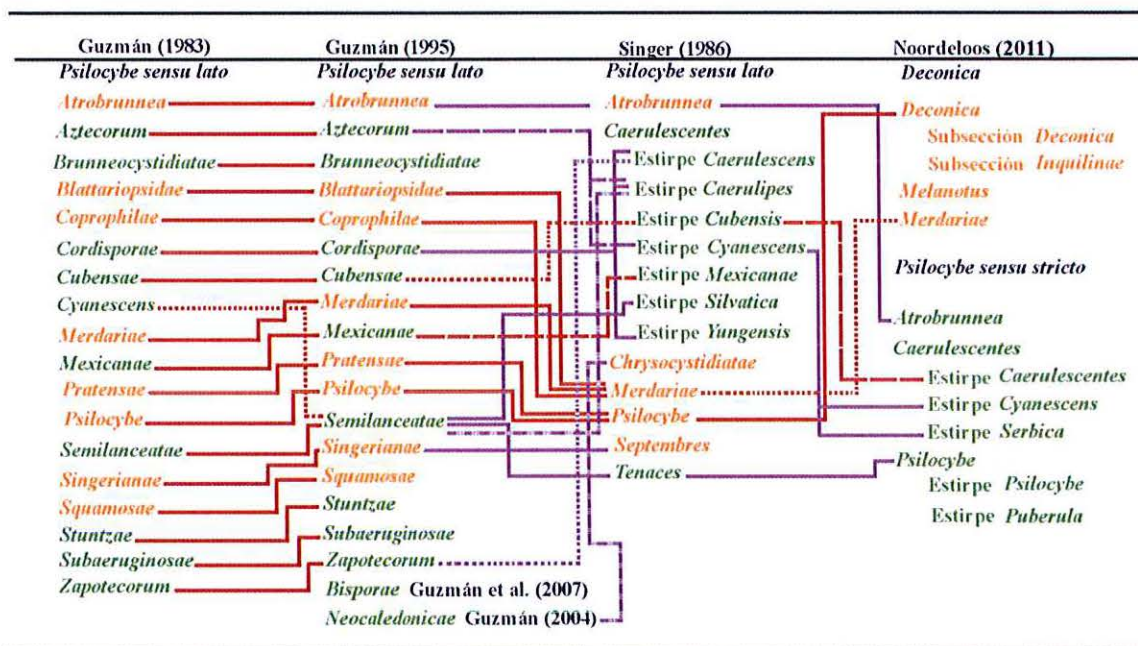


Fig. 1. Clasificación infragenérica de *Psilocybe* s.l. Todas son secciones excepto cuando se indica otro nivel infragenérico. En verde *Psilocybe* s.s. y en anaranjado *Deconica*. Las líneas rojas señalan la correspondencia, las líneas en morado indican que el taxón concuerda solo parcialmente. Se usaron en ocasiones líneas punteadas para una mejor visualización.

Hasta la fecha, las propuestas de Guzmán (1983, 1995, 2004) y Guzmán et al. (2007) incluyen a 19 secciones, de las cuales 11 contienen especies alucinógenas. Actualmente, Guzmán (com. pers.) está revisando varias especies de *Psilocybe* s.s. para presentar una nueva edición de su trabajo de 1983, del cual ya tiene terminada la sección *Zapotecorum*.

Por otro lado, Singer (1986) clasificó *Psilocybe* s.l. en siete secciones, las cuales son *Atrobrunneae*, *Caerulescentes*, *Chrysocystidiatae*, *Merdariae*, *Psilocybe*, *Septembres* y *Tenaces*. De éstas *Caerulescentes* y *Tenaces* agrupan a las especies alucinógenas. Noordeloos (2011) reconoció a *Deconica* y *Psilocybe* s.s. En el primero incluyó a las secciones *Deconica*, *Melanotus* y *Merdariae*; mientras que en el segundo a *Atrobrunneae*, *Caerulescentes* y *Psilocybe*, esta última no en el sentido de Guzmán (1983, 1995) ni de Singer (1986), que corresponde ahora a *Deconica*. Aunque las clasificaciones mencionadas difieren en el número de secciones o los taxones incluidos en cada una de ellas, se basan prácticamente en los mismos caracteres morfológicos. Noordeloos (2011) separó a *Deconica* y *Psilocybe* a partir de los resultados de los análisis filogenéticos de secuencias de ADN de Moncalvo et al. (2002) y Matheny et al. (2006), pero su clasificación infragenérica es con base en caracteres morfológicos.

Estudios filogenéticos

Fue ya hace más de una década que Moncalvo et al. (2002), con secuencias de LSU del ADN ribosomal, encontraron que *Psilocybe* s.l. se recuperó en dos clados independientes. Los hongos alucinógenos (*Psilocybe* s.s.) resultaron en el clado /psychodelia y con una relación de grupo hermano con el clado /stropharioid. A su vez, /stropharioid incluyó a los géneros *Hypholoma*, *Leratiomyces*, *Pachylepyrium*, *Phaeonematoloma*, *Pholiota*, *Stropharia* y *Weraroa* Singer. Por otro lado, *Deconica* se recuperó en un grupo independiente, junto con *Melanotus*. De forma similar, Matheny et al. (2006), con una matriz de secuencias de seis genes, recuperaron a *Psilocybe* s.l. en dos clados independientes bien soportados. *Deconica* se agrupó en el clado Strophariaceae s.s. Las especies alucinógenas de *Psilocybe* (representadas por *P. cyanescens* y *P. stuntzii*), quedaron en el clado Hymenogastraceae junto con los géneros *Anamika* K.A. Thomas, Peintner, M.M. Moser & Manim., *Alnicola* Kühner, *Galerina*, *Hebeloma* P. Kumm. y *Phaeocollybia* R. Heim. Hymenogastraceae y Strophariaceae mostraron una relación de grupos hermanos.

En relación a trabajos específicos, Boekhout et al. (2002) presentaron una revisión de la sección *Psilocybe* (ahora en *Deconica*) con datos morfológicos, de apareamientos de cepas y con RAPD's. Por su parte, Borovička et al. (2010) estimaron las relaciones filogenéticas en el complejo de *P. cyanescens* en Europa.

A pesar de la importancia y popularidad de *Psilocybe*, no se han ejecutado estudios filogenéticos que incluyan un amplio muestreo del grupo y las clasificaciones existentes no se han evaluado en un contexto filogenético. Por ello, en el presente trabajo se incluyen 41 especies de *Deconica* y 45 de *Psilocybe*, varias de regiones tropicales que no se consideraron en trabajos previos.

OBJETIVOS

Objetivo general

Dilucidar las relaciones infragenéricas en *Psilocybe* s.l. y contribuir a la taxonomía del género.

Objetivos particulares

1. Analizar la posición filogenética de *Psilocybe* s.s. y *Deconica* con secuencias de las regiones ITS, LSU y *rpb1* de ADN.
2. Estimar, con base en secuencias de ADN, las relaciones filogenéticas de *Psilocybe* s.s.
3. Realizar análisis filogenéticos en *Deconica* con base en secuencias de ADN.
4. Evaluar la pertinencia de las clasificaciones tradicionales de *Deconica* y *Psilocybe* s.s. a partir de los resultados de los análisis filogenéticos.
5. Detectar las homologías morfológicas en los grupos infragenéricos de *Deconica* y *Psilocybe* s.s.

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Type studies in *Psilocybe sensu lato* (Agaricales, Strophariaceae).
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Type studies of *Psilocybe* sensu lato (Strophariaceae, Agaricales)

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A revision of 40 types from 10 herbaria is presented to contribute to the taxonomic understanding of *Psilocybe* and *Deconica*. In all cases, descriptions of the types were completed, with characters not previously included such as the presence of pileocystidia, subhymenium type, and arrangement of the hyphae from pileus trama. We found that *P. chiapanensis* and *P. subyungensis* are synonyms of *P. yungensis*, *P. bipleurocystidiata* of *P. subtropicalis*, *P. subacutipilea* of *P. mexicana*, *P. ochreatea* of *D. montana*, and *P. overeemii* is a synonym of *D. neocaledonica*. Also, five species that were formerly considered to be bluing actually correspond to the nonhallucinogenic genus *Deconica*: *Psilocybe aureicystidiata*, *P. goniospora*, *P. neocaledonica*, *P. ochreatea*, and *P. overeemii*.

Keywords: holotypes, isotypes, nomenclature, protologues, taxonomy

Psilocybe (Fr.) P. Kumm. and *Deconica* (W.G. Sm.) P. Karst. (Strophariaceae, Agaricales, Basidiomycota) are two independent genera according to the results of Moncalvo *et al.* (2002) and Matheny *et al.* (2006). On the basis of these results, Redhead *et al.* (2007) proposed conserving the generic name *Psilocybe* for the hallucinogenic mushrooms and using *Deconica* for the nonhallucinogenic. The most comprehensive studies on *Psilocybe* s.l. (i.e., including *Deconica*) are the monograph of the section *Caerulescentes* (Singer & Smith 1958b), the world monograph of *Psilocybe* and its supplement (Guzmán 1983, 1995), the contributions of Singer (1986), and Noordeloos (2011), in this case, who treated *Deconica* and *Psilocybe* independently. Infrageneric classifications were made considering *Psilocybe* in a broad sense.

As a part of a broader study that includes a phylogenetic analysis of *Psilocybe* and *Deconica*, here we present a revision of 40 types. The aim of this work is to better understand the species on the basis of additional information that was not mentioned in the protologues and contribute to the taxonomic understanding of these genera.

Materials and Methods

Type and additional specimens from 10 herbaria (ENCB, FH, IB, IBUG, K, LPS, NY, QUE, TENN, and XAL) were studied, and material was mounted in 3% KOH or 3% Congo red to observe micromorphology. Patent blue V (1%), a specific dye that stains the chrysocystidia content blue (Jahnke 1984), was used to confirm their presence. Names for microscopic structures were based on those of Vellinga (1988) and Cléménçon (2012). Subhymenium descriptions followed those of Bas (1969). Structures were measured and drawn using a 100× oil-immersion objective on Zeiss K7 or Zeiss Axioskop 40 microscopes and photographed using the Axioskop 40 with Axio Vision 4 software.

In *Psilocybe* the basidiospores have two views, a frontal view with or without angles and a lateral view always without angles. The spore shape in lateral view was determined according

to the Q ratio (length/width ratio, Bas 1969). In angular spores, the shape in frontal view was named according to Vellinga (1988), and Vellinga & Noordeloos (1999). For 20 randomly selected basidiospores, length, width (lateral view), and breadth (frontal view) were measured without including the apiculus. Basidiospore wall was measured from photograph in the Axio Vision 4 software. We distinguish three wall thicknesses: thin (0.30-0.50 μm), slightly thick (0.55-0.80 μm), and thick (0.85-1.90 μm), following Ramírez-Cruz *et al.* (2013). Sterigmata were included in basidia measurements. The arrangement of the trama in the pileus and hymenophore in radial sections was described.

Results and Discussion

Forty type specimens were studied; only additional information omitted in the protologue and subsequent publications is considered here. Taxa are presented alphabetically by the basionym; the accepted name is given in bold. The study includes species that formerly were placed in the hallucinogenic sections proposed by Guzmán (1983, 1995); of these, five currently correspond to the nonbluing genus *Deconica*. Six names are proposed in synonymy.

Basidiospore shape in frontal view has been an important character in the infrageneric classification of the genera *Psilocybe* and *Deconica* (Guzmán 1983, 1995). Many terms have been applied to the specific shapes, but they were used inconsistently and sometimes with different meanings. Here, we used the following for frontal views: hexagonal and subhexagonal (Figs. 2, 6), rhomboid and subrhomboid (Figs. 3, 7, 9), and without angles (Fig. 5). Rhomboid and hexagonal basidiospores can be present in the same specimen (Fig. 8). In lateral view, the basidiospores always lack angles (Fig. 4).

We observed that the basidiospore wall of some species has at least two layers when viewed with a light microscope, in agreement with Singer & Smith (1958a: 263) and Singer (1986: 566), who described the basidiospore wall as a “complex wall” when more than one layer can be seen. Ruch & Motta (1987) pointed out that the basidiospores of *P. cubensis*, observed with TEM, have a thick wall with three distinct layers (ectosporium, episporium, and endosporium). With light microscopy, on rare occasion, the three layers can be seen (Fig. 1). However, in most species, only two layers—the episporium and endosporium described by Singer (1986)—can be observed (Fig. 2). In some basidiospores, the layers were difficult to observe with light microscopy, and just a thin one was evident (Fig. 3).

On the basis of cytoplasmic content, two kinds of cystidia are present in *Deconica*, chrysocystidia and leptocystidia (see Cléménçon 2012: 218, 238 for definitions). Chrysocystidia are either hyaline with refringent content, that is sometimes granular or crystal-like (Fig. 24) similar to that observed in *Pholiota*, or have homogeneous content, that sometimes fills the entire cystidium, hyaline, never yellowish in KOH (Fig. 23). Singer (1986) had already noticed that the content of some chrysocystidia in *Psilocybe* sect. *Chrysocystidiatae* is not yellowish in KOH. Leptocystidia are hyaline, thin-walled, and sometimes with a refringent apex (Fig. 19). In *Psilocybe* there are also two kinds of cystidia: leptocystidia and another type that probably corresponds to some type of deutero-cystidia (Cléménçon 2012) but not chrysocystidia, because the contents do not stain with Patent blue V. Guzmán (2012) used the term pseudocystidia for this type of cystidia, but only for those borne from the hymenophoral trama, not from the subhymenium, to differentiate them from the typical pleurocystidia (leptocystidia) in *Psilocybe*. We prefer not to use this term for *Psilocybe* because the concept of pseudocystidia, in the sense

of Cléménçon (2012), is different. Deuterocystidia in *Psilocybe* (Figs. 20–22) are present in some species, such as *P. banderillensis* Guzmán, *P. brasiliensis* Guzmán, *P. subtropicalis* Guzmán, *P. ovoideocystidiata* Guzmán & Gaines, and *P. zapotecorum* Heim. Deuterocystidial content is not always present or is not pigmented (Fig. 20). At least with light microscopy, elucidating whether they are deuterocystidia or leptocystidia with pigmented vacuoles is difficult.

In this work, we describe the following features of the cystidia: size, shape, colour content, and origin. As already mentioned, the content of the cystidia of *Psilocybe* can be difficult to discern; however, in *Deconica* the chrysocystidia are recognizable with the aid of Patent blue V (Fig. 24). Pileocystidia and caulocystidia are also important structures that we have added to descriptions for relevant species.

According to Vellinga (1988), the pileipellis is a cortical layer of the pileus, and the subpellis is the layer beneath the pileipellis. In *Psilocybe* and *Deconica*, the pileipellis is a true ixocutis (Figs. 10, 13a) or a subgelatinized cutis (Fig. 11). The subpellis is occasionally differentiated; when present, it can be distinguished by the strongly pigment-encrusted hyphae (Fig. 13b). The arrangement of the hyphae in the pileus trama and subpellis can be interwoven (Fig. 14) or radial, i.e., the hyphae are radially arranged, resembling isodiametric cells when transversely cut in radial section (Figs. 12, 13c, 15) or as hyphae in tangential section. The pileus trama is never subcellular; such an interpretation from radial sections has been an erroneous one. Singer (1986) and Singer & Smith (1958b) did not include measurements of the pileus trama in their descriptions; they only described the subpellis, which they called “hypodermium”. On the other hand, Guzmán (1983, 1995) considered the pileus trama as context and sometimes under “hypodermium”, given the diameter of the hyphae. Here, we point out that the arrangement of the hyphae in the pileus trama is an important distinguishing feature at the species level in both genera. Furthermore, we observe that there are three types of subhymenium: cellular, ramose-inflated, and ramose present both in *Deconica* and *Psilocybe* (Figs. 16–18).

Guzmán *et al.* (2005: 159) observed the presence of yellowish-brown mycelium, composed of “setaceous” hyphae, on the base of the stipe. We consider these hyphae as an important character in some species of the *Cordisporae* and *Brunneocystidiatae* sections. In the present work, we have found them in the basal mycelium in *P. cabiensis* Guzmán, M. Torres & Ram.-Guill. and *P. yungensis* Singer & A.H. Sm., both belonging to sect. *Cordisporae*.

Taxonomy

Psilocybe acutipilea (Speg.) Guzmán, Mycotaxon 7(2): 226. 1978. – Figs. 26–28.

Basionym. – *Deconica acutipilea* Speg., Bol. Acad. Nac. Cienc., Córdoba 11(4): 381. 1889.

B a s i d i o s p o r e s 8.4–10.4 (11.2) × 5.6–7.2 × 5.6–6.4 μm, Q = 1.3–1.6, ellipsoid, subrhomboid, and hexagonal in frontal view, Q = 1.5–1.7 (1.8), ellipsoid and elongate in lateral view, yellowish brown, wall thick (0.9–1.2 μm thick), with two layers in light microscopy, with germ pore. – **B a s i d i a** 20–26 × 7–8 μm, clavate, some flexuose, tetrasporic, sterigmata 3–4 μm long, hyaline to yellowish, wall thin. – **P l e u r o c y s t i d i a** not observed. – **C h e i l o c y s t i d i a** 16–27 × 4.5–7 μm, narrowly lageniform, flexuose, neck sometimes bifurcated, apex obtuse, refractive, hyaline, wall thin. – **H y m e n o p h o r a l t r a m a** subregular, hyphae 3–5.5 μm diameter, yellowish to yellowish brown in mass, wall thin. – **P i l e u s t r a m a** radial, hyphae 4–8 μm diameter, yellowish to yellowish brown in mass. –

Pileipellis an ixocutis, 27–45 µm wide, hyphae 1.5–3 µm diameter, hyaline, wall thin. – Stipe basal mycelium white, hyphae hyaline, wall thin. – Clamp connections at all hyphal septa.

M a t e r i a l e x a m i n e d . – BRAZIL, São Paulo State, Apiahy, Oct 1881, *leg.* C. Spegazzini 1536 (LPS 38307, holotype).

R e m a r k s . – The type specimen is contaminated with molds, so it was difficult to observe all features such as pleurocystidia, subhymenium, pileocystidia, and caulocystidia. Guzmán (1978, 1983) described subrhomboid basidiospores in frontal view; however, we observe that they can be ellipsoid and hexagonal, as well as subrhomboid, and in some spores the angles are not well defined. Furthermore, we found that the pileipellis is an ixocutis, and we describe the basidia characters. As Guzmán (1978, 1983) pointed out, *P. acutipilea* could be a synonym of *P. mexicana* R. Heim, due to the similar size and shape of the basidiospores, basidia, and cheilocystidia, although the poor condition of the type specimen makes the pleurocystidia difficult to observe and does not permit us to confirm synonymy.

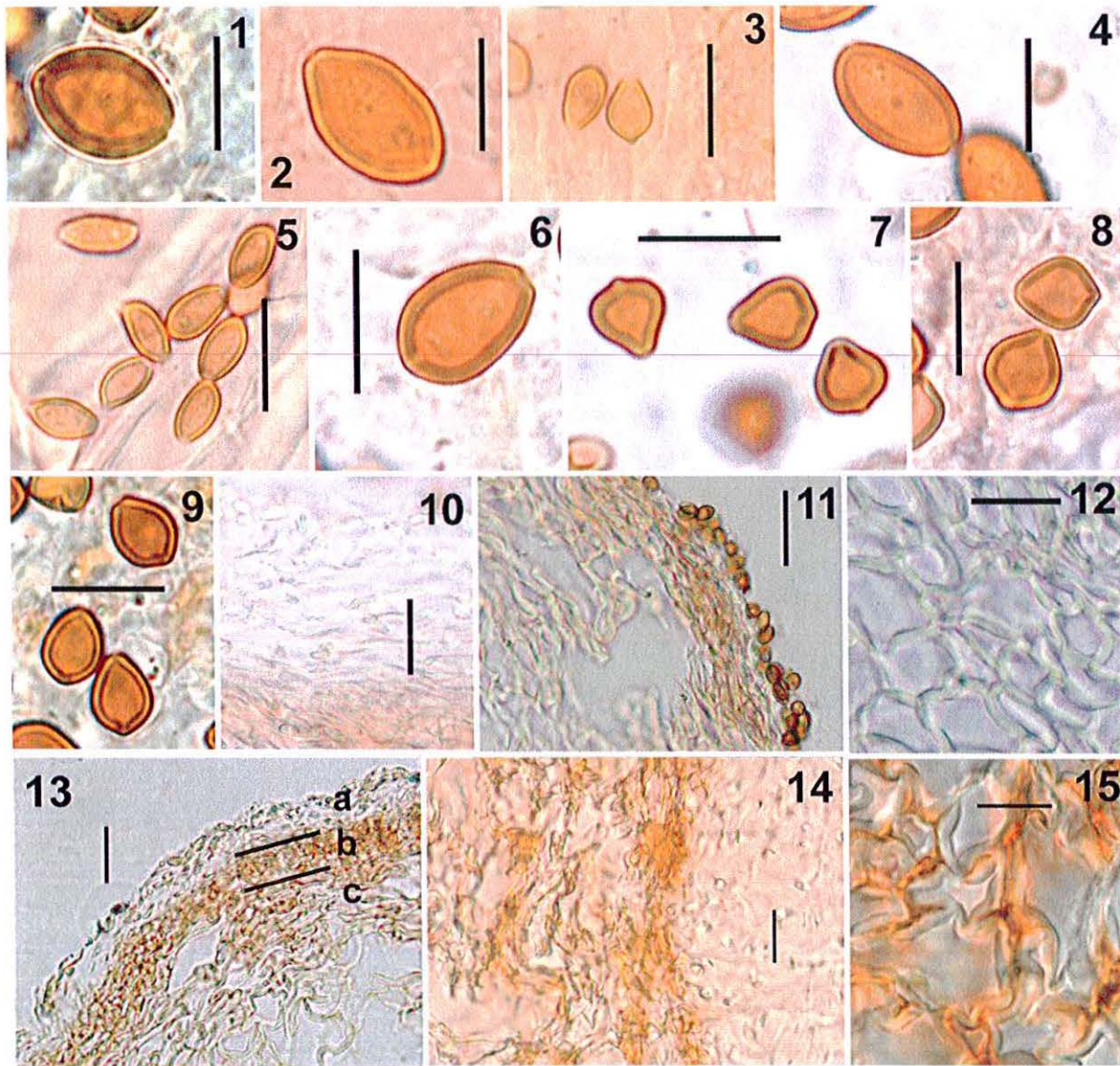
Psilocybe aquamarina (Pegler) Guzmán, *Bibl. Mycol.* 159: 109. 1995. – Figs. 29–31.

Basionym. – *Stropharia aquamarina* Pegler, *Kew Bull. Addit., Ser. 6:* 462. 1977.

B a s i d i o s p o r e s 8.8–11.2 (12.4) × 6.4–8.8 × 4.8–6.4 µm, Q = 1.1–1.5 (1.6), subrhomboid and hexagonal in frontal view, Q = 1.5–1.8 (2), ellipsoid and elongate in lateral view, yellowish brown, wall thick (1–1.5 µm thick), with two layers in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** approximately 28 × 8 µm, subfusiform-flexuose, apex obtuse, hyaline, wall thin, very scanty. – **C h e i l o c y s t i d i a** 16–29 × 7–13 µm, subfusiform-flexuose, pyriform, utriform, and lageniform with short neck, wall thin; embedded in a subgelatinized layer. – **S u b h y m e n i u m** apparently ramose. – **P i l e i p e l l i s** an ixocutis, 27–45 µm wide, hyphae 1.5–3 µm diameter, hyaline, wall thin. – **S t i p i t i p e l l i s** a cutis, hyphae 2.5–13.5 µm diameter, yellowish, wall thin. – **S t i p e b a s a l m y c e l i u m** white, hyphae hyaline, wall thin.

M a t e r i a l e x a m i n e d . – KENYA, Central Province, South Nyeri District, S side of Mt. Kenya, Castle Forest Station, near Thiba River, on the ground at the edge of the forest, alt. 1981 m, 2 Apr 1968, *leg.* D.N. Pegler 370 [(K(M) 160812, holotype)].

R e m a r k s . – An important character of this species is the subgelatinized lamellar edge in which cheilocystidia are embedded, already described by Pegler (1977). We found pleurocystidia, but they were very difficult to observe and measure. Cheilocystidia are slightly wider than those cited by Pegler (1977), 7.5–11 µm wide. Guzmán (1995) made the combination in *Psilocybe* based on the blue tones in the annulus and in some parts of the stipe. A similar species is *P. subaeruginascens* Höhn., differing in its smaller basidiospores (see this species later). It also resembles *P. magnispora* E. Horak, Guzmán & Desjardin in the gelatinized lamellar edge; however, in *P. magnispora* the basidiospores are also smaller (species referred to below).



Figs. 1–15. Microscopic structures: 1. Basidiospore wall with three layers in frontal view, *Psilocybe cubensis*. 2. Hexagonal basidiospore, wall with two layers in frontal view, *P. cubensis*. 3. Subrhomboid basidiospore, wall with one layer in frontal view, *P. zapotecorum*. 4. Basidiospores without angles in lateral view, *P. hispanica*. 5. Basidiospores without angles in frontal and lateral view, *P. zapotecorum*. 6. Hexagonal basidiospore in frontal view, *P. gallaeciae*. 7. Rhomboid and subrhomboid basidiospores in frontal view, *P. yungensis*. 8. Hexagonal (below) and rhomboid (above) basidiospores in frontal view, *P. aquamarina*. 9. Subrhomboid basidiospores in frontal view, *P. subtropicalis*. 10. Ixocutis, *P. caerulescens*. 11. Subgelatinized pileipellis, *P. thaizapoteca*. 12. Pileus trama radial, *P. caerulescens*. 13. Pileipellis and pileus trama: a, ixocutis; b, subpellis; and c, radial pileus trama; *P. caerulescens*. 14. Ixocutis (right side) and interwoven pileus trama (left side), *P. hispanica*. 15. Pileus trama radial, encrusted pigment in the wall, *P. multicellularis*. Bars 10 μm in 1–10, 12, 15; 20 μm in 11, 13–14.

Psilocybe atlantis Guzmán, Hanlin & C. White, Mycotaxon 86: 180. 2003. – Figs. 32, 33.

Basidiospores 8.8–10 (12) × (5.6) 6–7.2 × 5.6–6.4 μm, Q = 1.2–1.6, rhomboid and hexagonal in frontal view, sometimes angles not well defined, Q = 1.3–1.8, ellipsoid and elongate in lateral view, yellowish brown, wall 0.8–1.0 μm thick, with two layers in light microscopy, with germ pore. – **Subhymenium** ramose, with some pigmented hyphae ascending to the hymenium. – **Pileus trama** radial, hyphae 6.5–27 μm diameter, yellowish to yellowish brown in mass, wall 0.4–0.8 μm thick. – **Pileipellis** an ixocutis, 10–20 μm wide, hyphae 1.5–4 μm diameter, hyaline, wall thin. – **Pileocystidia** absent. – **Caulocystidia** 16–41 × 4–9.5 μm, narrowly lageniform, occasionally branched, hyaline, wall less than 0.8 μm thick, apex obtuse and refractive, in clusters throughout stipe. – **Stipe basal mycelium** white, hyphae 3–5.5 μm diameter, hyaline, wall thin.

Material examined. – USA, Georgia, Fulton Co., North of Atlanta, 7 Aug 2000, *leg.* Lingchi s.n. (XAL, holotype).

Remarks. – A distinctive character in this species is the branched cheilocystidia (see Guzmán *et al.* 2003), unlike those of *P. mexicana*, which are not strongly branched. It is similar to *P. mexicana* in the size and shape of the basidiospores, “7–10 (11.2) (12) × 4.8–7.2 × 4.8–7 μm”, according to Guzmán (1983), but differs in the width of the pleurocystidia, 7–9 (12) μm (5–8 μm wide in *P. mexicana*). Furthermore, the caulocystidia of *P. atlantis* are always lageniform, but in the type of *P. mexicana*, they vary from globose to cylindrical (data not shown). See discussion under *P. samuiensis*.

Psilocybe bipleurocystidiata E. Horak & Guzmán, in Guzmán, Horak, Halling & Ramírez-Guillén, Sydowia 61(2): 216. 2009. – Figs. 34–37.

Synonym. – *Psilocybe subtropicalis* Guzmán, Biblioth. Mycol. 159: 107. 1995.

Basidiospores 6.4–8.8 × 5.6–7.2 (8.0) × 4.8–5.6 μm, Q = 1.3–1.4, subrhomboid and subhexagonal in frontal view, Q = 1.2–1.5, broadly ellipsoid and ellipsoid in lateral view, yellowish brown, wall 0.7–1 μm thick, with two-layers in light microscopy, with germ pore. – **Pleurocystidia** as leptocystidia 11–18.5 × 4–6.5 μm, narrowly lageniform and lageniform with short neck, apex obtuse, refractive, hyaline, wall thin, originating from the subhymenium. – **Pleurocystidia** as deutero-cystidia 31–40 × 4.5–9.5 μm, narrowly utriform, sometimes flexuose, hyaline, wall thin, originating from the hymenophoral trama. – **Pileus trama** radial, hyphae 4–28 μm diameter, yellowish to yellowish brown in mass, wall 0.5–0.8 μm thick and pigment-encrusted. – **Subpellis** undifferentiated. – **Pileipellis** an ixocutis, 10–20 μm wide, hyphae 1.5–4 μm diameter, hyaline, wall thin. – **Pileocystidia** 10–25 × 4–11 μm, as modified hyphae, with lateral inflated regions or with very short appendices, or lateral branches, hyaline, wall thin, on a subgelatinized pileipellis.

Material examined. – COSTA RICA, Punta Arenas, Monte Verde Cloud Forest Reserve, alt. 1650 m, 25 Jul 1986, *leg.* R. Singer 14484 (XAL, isotype).

Remarks. – Guzmán *et al.* (2009) recognized two types of pleurocystidia in *P. bipleurocystidiata*; here we complement those observations with the origin of each kind of

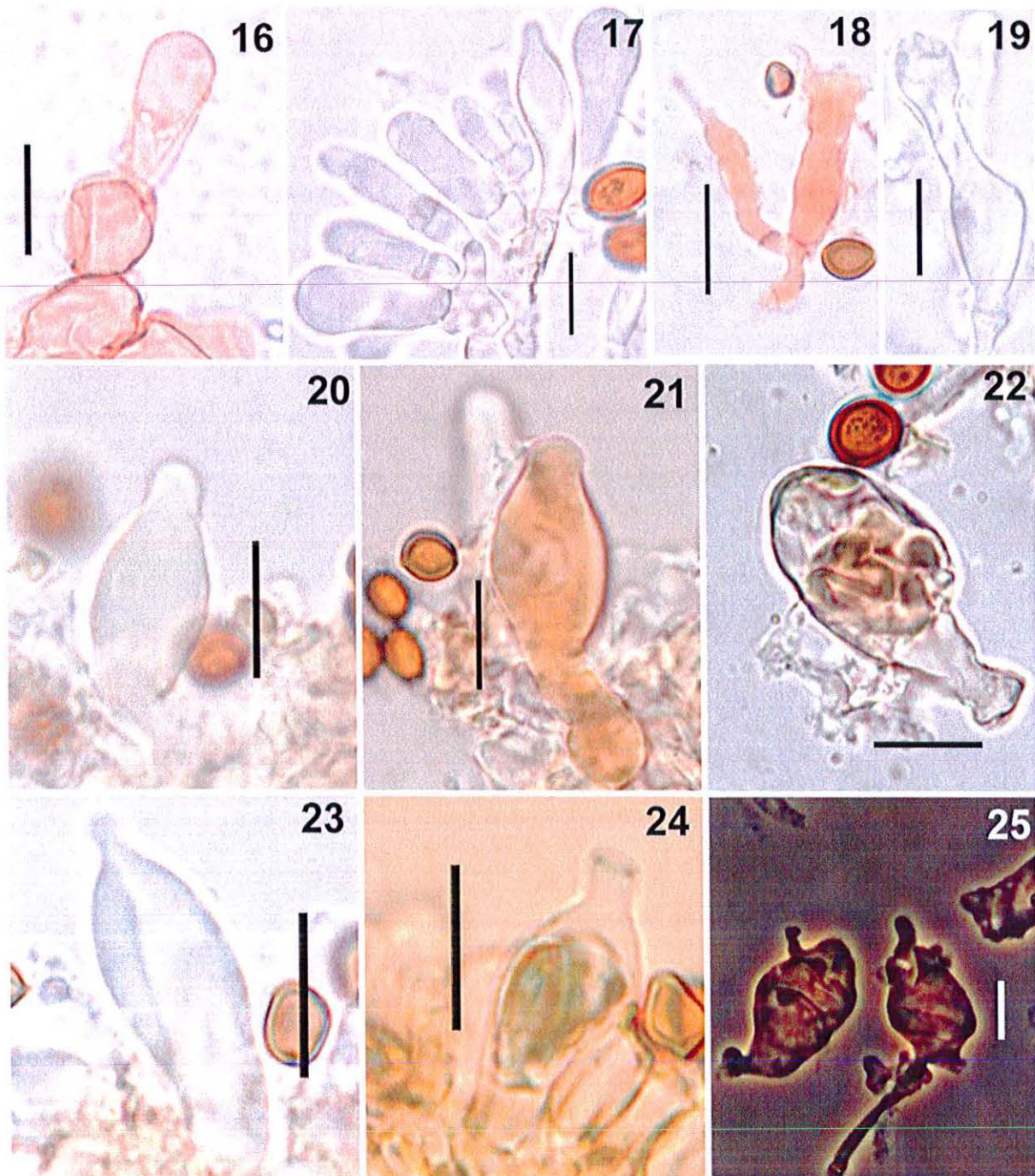
cystidia. An additional character is the presence of pileocystidia that were not described in the protologue. We propose this species as a synonym of *P. subtropicalis* (Guzmán 1995) because of the agreement of the macro- and micromorphologic features (see this species later); for example, both species have two types of pleurocystidia and branched cheilocystidia, but pileocystidia are only seen in *P. bipileurocystidiata*. The presence of pileocystidia is not enough to consider them as separate species because this structure may or may not be present in the pileipellis or sometimes they are not noticed. For example, a new species from Japan, *P. capitulata* Har. Tak., was described by Takahashi (2011) based on the presence of pileocystidia; however, for us this species corresponds to *P. cubensis* because the remaining features of the Japanese species agree with it. We have found pileocystidia in some specimens of *P. cubensis*, e.g., I. Ruvacalba-Gómez 3 (IBUG) and María Sabina s.n. 8-VII-1969 (PC).

Psilocybe brasiliensis Guzmán, Mycotaxon 7(2): 234. 1978. – Figs. 38–41.

B a s i d i o s p o r e s 5.6–7.2 × (4.0) 4.4–6.4 × 4.0–4.8 μm, Q = 1–1.2 (1.4), subrhomboid and subhexagonal in frontal view, Q = 1.2–1.5, broadly ellipsoid and ellipsoid in lateral view, yellowish brown, wall 0.8–1 μm thick, with two layers in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** as leptocystidia 14.5–21 × 3–5 μm, narrowly lageniform, apex obtuse, hyaline, wall thin, originating from the subhymenium. – **P l e u r o c y s t i d i a** as deutero-cystidia 24–37.5 × 10.5–13 (14.5) μm, broadly fusiform, apex mucronate or rostrate, sometimes with long neck or bifurcate, hyaline, wall thin, originating from the hymenophoral trama. – **C h e i l o c y s t i d i a** 17–25 × (3) 4–6.5 μm, subfusiform and lageniform, flexuose, branched, with two or three necks, apex 0.8–1 μm wide, obtuse, refractive, hyaline, wall thin. – **P i l e u s t r a m a** radial, hyphae yellowish to yellowish brown in mass, wall 0.5–0.8 μm thick, with encrusted pigment. – **P i l e i p e l l i s** an ixocutis, 12–16 μm wide, hyphae 2.5–4 μm diameter, hyaline, wall thin. – **P i l e o c y s t i d i a** absent. – **C a u l o c y s t i d i a** 11–35 × 3–6.5 μm, fusiform and lageniform, flexuose or with central constriction, with obtuse apex, hyaline, wall thin, in groups near to apex of the stipe. – **S t i p e b a s a l m y c e l i u m** white, hyphae hyaline, wall thin.

M a t e r i a l e x a m i n e d . – BRAZIL, São Paulo State, 10 km E of Campos de Jordão, Forestal Park of State, near to Sapucaí-guaçu River, gregarious in grassy soil in a forest of *Araucaria*, *Podocarpus*, and some arboreal dicotyledons, 9 Mar 1971, leg. G. Guzmán 8920 (XAL, isotype).

R e m a r k s . – Guzmán (1978, 1983) described only one kind of pleurocystidia 22–29 × 8.8–12 μm, ventricose, fusoid or sublageniform, which corresponds with deutero-cystidia, originating from the hymenophoral trama. This species was only found in the mixed ombrophilous forest of South Brazil, and it has not been collected again although there have been studies on *Psilocybe* in this vegetation type (e.g., Silva *et al.* 2012).



Figs. 16–25. Microscopic structures: 16. Cellular subhymenium and basidiole, *Psilocybe thaiduplicatocystidiata*. 17. Ramose-inflated subhymenium, basidioles, and pleurocystidium, *P. makarorae*. 18. Ramose subhymenium, *P. yungensis*. 19. Leptocystidium, *P. ochreatea*. 20. Deuterocystidium, *P. banderillensis*. 21. Deuterocystidium with homogeneous content, *P. banderillensis*. 22. Deuterocystidium with brown inclusion, *P. ovoideocystidiata*. 23. Chrysocystidium in KOH, *Deconica aureicystidiata*. 24. Chrysocystidium in Patent blue V, *D. overeemii*. 25. Pileocystidia, *P. neoxalapensis* (photo using 40× phase contrast objective). Bar 10 μm in 16–24; 20 μm in 25.

Psilocybe cabiensis Guzmán, M. Torres & Ram.-Guill., in Guzmán, Ramírez-Guillén & Torres, Int. J. Medic. Mushrooms 6(1): 86. 2004. – Figs. 42–43.

B a s i d i o s p o r e s 5.6–7.2 (8.0) × 4.0–4.8 × 3.2–4.4 μm, Q = 1.5–1.8, subrhomboid and ovoid in frontal view, Q = 1.5–1.8, ellipsoid and elongate in lateral view, yellowish brown, wall 0.4–0.5 μm thick, with one layer in light microscopy, with germ pore. – **S u b h y m e n i u m** ramose. – **H y m e n o p h o r a l t r a m a** subregular, hyphae 4–6.5 μm diameter, yellowish, wall 0.3–0.5 μm thick. – **P i l e u s t r a m a** radial, hyphae yellowish to yellowish brown in mass, wall 0.5–0.8 μm thick, with encrusted pigment. – **P i l e o c y s t i d i a** 12–21.5 × 3–5 μm, narrowly lageniform and few clavate, hyaline, wall thin, embedded in the ixocutis. – **S t i p i t i p e l l i s** a cutis, hyphae 3–5 μm diameter, yellowish brown, wall up to 0.5 μm thick. – **C a u l o c y s t i d i a** not found. – **S t i p e b a s a l m y c e l i u m** yellowish, hyphae setaceous, 2–3 μm diameter, yellowish brown, wall up to 0.5 μm thick.

M a t e r i a l e x a m i n e d. – COLOMBIA, Chocó, Quibdó region, SE of Quibdó, Pacurita, 24 Sep 2002, leg. G. Guzmán 35331 (XAL, Isotype).

R e m a r k s. – In *P. cabiensis* the pileocystidia are immersed in the ixocutis, the arrangement of the pileus trama is radial, and setaceous hyphae are present in the basal mycelium of the stipe. These characters were not previously mentioned by Guzmán *et al.* (2004a). Furthermore, we point out in the present paper that the subhymenium is ramose and not cellular.

Psilocybe chiapanensis Guzmán, Biblioth. Mycol. 159: 102. 1995. – Fig. 44.

Synonym. – *Psilocybe yungensis* Singer & A.H. Sm., Mycologia 50(1): 142. 1958.

C h e i l o c y s t i d i a 9–32 × 4–8 μm, widely lageniform with short neck, sometimes branched, clavate, narrowly utriform, and cylindrical-flexuose, few rostrate. – **S u b h y m e n i u m** ramose. – **H y m e n o p h o r a l t r a m a** subregular, hyphae 3–7 μm diameter, yellowish, wall up to 0.8 μm thick. – **P i l e u s t r a m a** radial, hyphae 4–24 μm diameter, yellowish to yellowish brown in mass, wall thick (up to 1.6 μm thick), with yellowish brown, strongly encrusted pigment. – **P i l e i p e l l i s** an ixocutis, up to 21 μm wide, hyphae 1.5–3 μm diameter, hyaline, wall thin.

M a t e r i a l e x a m i n e d. – MEXICO, Chiapas, region of Tapachula, Municipio of Unión Juárez, Ejido Santo Domingo, 4 Oct 1993, leg. G. Guzmán 30379 (XAL, holotype).

R e m a r k s. – The additional features presented here are the subhymenium type, pileus trama arrangement, and ixocutis thickness. The *Psilocybe chiapanensis* holotype agrees very well with *P. yungensis*; the conical to campanulate pileus, frequently acutely papillate or mamiform, and the very narrow lamellae are typical macroscopic characteristics of *P. yungensis*, according to Heim (1958), Singer & Smith (1958b), and Guzmán (1983). Also, the micromorphological characters, shape and size of the basidiospores and pleurocystidia and shape of the cheilocystidia, are very similar in the two species. Guzmán (1995, p. 102) described *P. chiapanensis* with “pleurocystidia 11–14.5 (–16) × (4–) 5–6.5 (–7) μm, common, hyaline, ventricose rostrate, with a short apex”, and two types of cheilocystidia: “a) as the pleurocystidia, (10.5–) 12–16 (–17.5) × 5.5–6.5 μm, hyaline”, and “b) (16–) 17–22.5 × 5–8 μm, ventricose, regular or irregularly in shape, hyaline”. Both types of cheilocystidia fit well with those observed

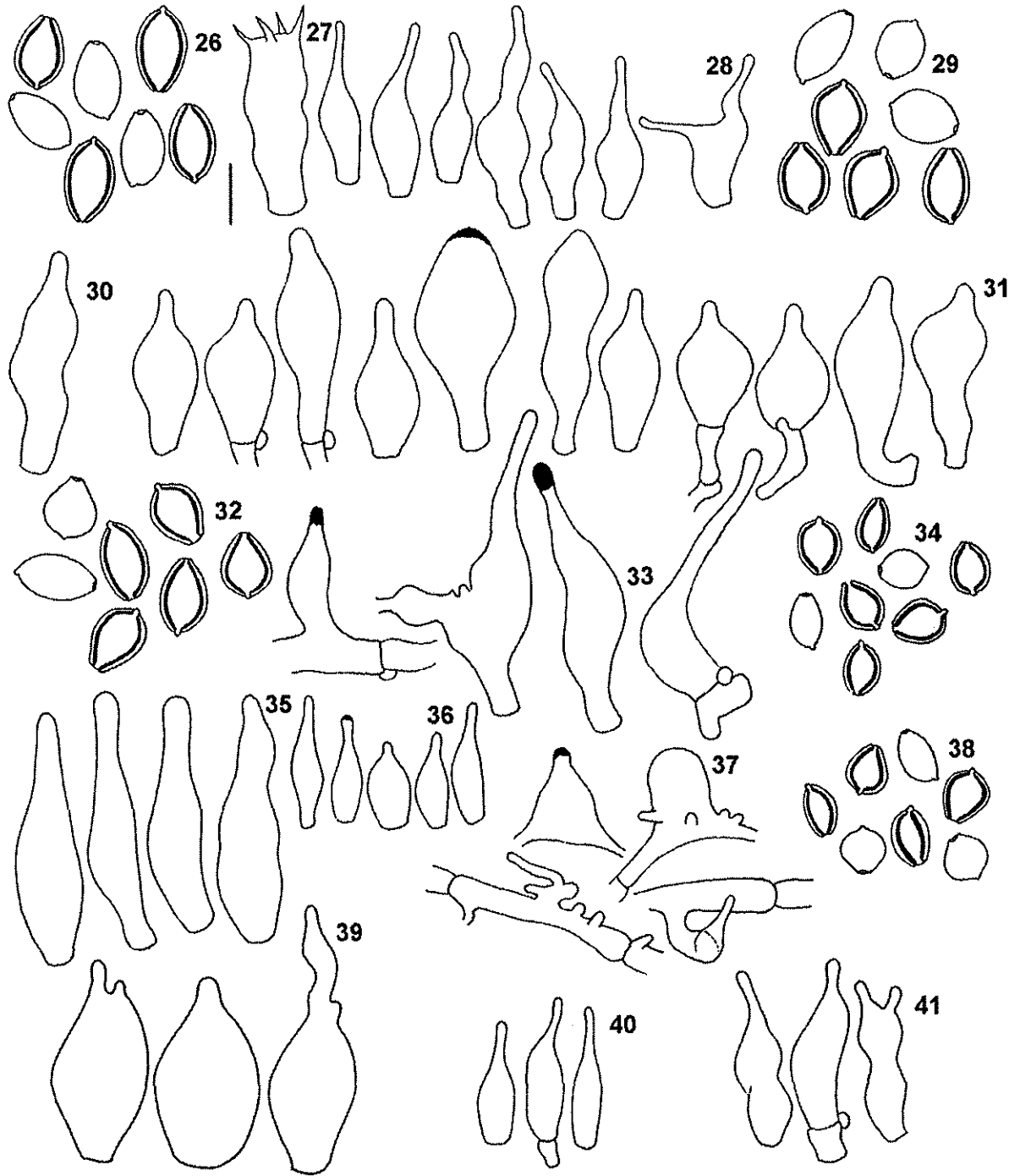
in the type of *P. yungensis* (see this species below). Guzmán (1995) pointed out that this species is similar to *P. yungensis* and *P. subyungensis* Guzmán in pileus shape, basidiospore size, and lignicolous habitat, but differs in having more abundant pleurocystidia (scanty in the last two species) and in the size and variability of the cheilocystidia. We found in the present study that *P. subyungensis* is synonymous with *P. yungensis* (see discussion below) and that the pleurocystidia are common to both species. We observed for *P. chiapanensis* that cheilocystidia are larger than those described by Guzmán (1995). Although Guzmán (1995) divided the cheilocystidia into two types, we decided to consider all the variation as one type, according to the previous description of *P. yungensis* by Singer & Smith (1958b, p. 274), who considered them as “exceptionally variable: some clavate, some ventricose and with capitate to subcapitate apex, some ventricose above and constricted in the middle, others ventricose-fusoid and broadest in mid-portion, some ampullaceous, some capitate-cylindric, some subulate-sublanceolate, all hyaline, narrowest portions 2.7 µm or more”.

Psilocybe eximia E. Horak & Desjardin, Sydowia 58(1): 28. 2006. – Figs. 45–49.

Basidiospores 4.8–5.6 × 4.8–5.6 × 3.2–4.2 µm, Q = 1–1.16, rhomboid and subrhomboid in frontal view, Q = 1.2–1.6, ellipsoid in lateral view, yellowish brown, wall 0.8–1 µm thick, with two-layers, occasionally three layers can be observed in light microscopy, with germ pore. – **Pleurocystidia** 12–16 × 3–5 µm, widely lageniform, lageniform, and fusiform, with short neck, apex refringent, sometimes with two apices, hyaline, wall thin. – **Cheilocystidia** 24–44 × 12–18.5 µm, spheropedunculate, cylindrical, and clavate, hyaline, wall thin with yellowish brown, irregularly encrusted pigment. – **Pileus trama** radial, hyphae 4–40 µm diameter, with some short and globose elements, yellowish to yellowish brown in mass, wall thick (0.8–1.7 µm thick) with encrusted pigment. – **Subpellis** undifferentiated. – **Pileipellis** an ixocutis, 25–35 µm wide, hyphae 3–5 µm diameter, hyaline, wall thin. – **Pileocystidia** 14–26 × 4–7 µm, clavate and cylindrical, hyaline, wall thin. – **Stipitipellis** hyphae 3–10 µm diameter, wall 0.5–1.4 µm thick. – **Caulocystidia** 37–40 × 13–14 µm, cylindrical and clavate, wall thin with yellowish brown, encrusted pigment.

Material examined. – INDONESIA, Java, Mt. Halimun Salak National Park, near Cikaniki Field Station, alt. 1000–1100 m, 10 Jan 1999, *leg.* E. Horak 7326 (XAL, isotype), alt. 1000 m, 9 Jan 2001, *leg.* D. Desjardin DED 7207 (XAL); 11 Jan 2001, *leg.* D. Desjardin 7234 (XAL).

Remarks. – *Psilocybe eximia* was described without pleurocystidia by Horak & Desjardin (2006); however, we found small lageniform pleurocystidia, although in specimen DED 7207 they are larger and sometimes have two apices. Furthermore, Horak & Desjardin (2006) pointed out that globose or ovoid cells compose the subpellis; however, we do not consider the subpellis to be differentiated because we interpret those cells as part of the pileus trama. The arrangement of the pileus trama is radial, with the hyphae nearest to the pileipellis cylindrical, from 4–10 µm diameter, and the cells nearest to the lamellae globose, up to 40 µm diameter, with a trama that is, at least in part, cellular in tangential and radial sections. All the hyphae of the pileus trama are pigment-encrusted, so it is not possible to clearly separate them into two layers.



Figs. 26–41. Microscopic structures: 26–28. *Psilocybe acutipilea* (Holotype). 26. Basidiospores. 27. Basidium. 28. Cheilocystidia. 29–31. *P. aquamarina* (Holotype). 29. Basidiospores. 30. Pleurocystidium. 31. Cheilocystidia. 32–33. *P. atlantis* (Holotype). 32. Basidiospores. 33. Caulocystidia. 34–37. *P. bipleurocystidiata* (Isotype). 34. Basidiospores. 35. Deuterocystidia. 36. Leptocystidia. 37. Pileocystidia. 38–41. *P. brasiliensis* (Isotype). 38. Basidiospores. 39. Deuterocystidia. 40. Leptocystidia. 41. Caulocystidia. Bar 8 μ m.

Psilocybe farinacea Rick ex Guzmán, Mycotaxon 7(2): 241. 1978. – Figs. 50–52.

Basidiospores 7.2–9.6 (11.2) × 4.9–7.2 (8.0) × 4.8–6.4 μm, Q = 1.1–1.4, rhomboid and hexagonal, in frontal view, Q = 1.3–1.6, broadly ellipsoid in lateral view, yellowish brown, wall thick (1 μm thick), with two layers in light microscopy, with germ pore. – **Pleurocystidia** 16–19 × 5–6 μm, utriform and narrowly lageniform with short neck, some flexuose, apex obtuse, refractive, hyaline, wall thin, scarce. – **Cheilocystidia** 19–24 × 5–6.5 μm, narrowly lageniform with long neck, sometimes bifurcate, apex obtuse, refractive, hyaline, wall thin. – **Pileus trama** radial, hyphae 4–24 μm diameter, yellowish to yellowish brown in mass, wall 0.5–0.8 μm thick, with encrusted pigment.

Material examined. – BRAZIL, São Leopoldo, leg. J. Rick (Lloyd Herb., 27598 BPI, holotype).

Remarks. – The type material is contaminated with molds. We observed pleurocystidia, but they were scarce and difficult to see. This species is very similar to *P. mexicana*; however, it is difficult to determine with certainty whether they are the same species because of the bad condition of the type specimen of *P. farinacea*. Guzmán (1978) mentioned that it is similar to *P. caerulescens*, but the last one has smaller basidiospores.

Psilocybe gallaeciae Guzmán & M.L. Castro, Bol. Soc. Micol. Madrid 27: 185. 2003. – Figs. 6, 53–56.

Basidiospores 8.4–10.4 × (6.4) 7.2–8.0 × 6.4–7.2 μm, Q = 1.2–1.6, subrhomboid, hexagonal, and ellipsoid in frontal view, angles of some spores not well defined, Q = 1.5–1.8 (2), ellipsoid and elongate in lateral view, yellowish brown, wall 0.8–1.2 μm thick, with two layers in light microscope, with germ pore. – **Subhymenium** ramose. – **Pileus trama** radial, hyphae 9.5–20 μm diameter, yellowish, wall up to 0.5 μm thick, with encrusted pigment. – **Subpellis** undifferentiated. – **Pileocystidia** approximately 16–22 × 4–5.5 μm, lageniform, apex obtuse, hyaline, wall thin. – **Stipitipellis** a cutis, hyphae 4–16 μm diameter, yellowish to yellowish brown in mass, wall up to 0.5 μm thick. – **Caulocystidia** 25–28 × 4.5–7.5 μm, lageniform, apex obtuse, hyaline to yellowish, wall thin, present in upper part of stipe.

Material examined. – SPAIN, Galicia, La Coruña, Caaverior, Monfero, La Coruña, gregarious in soil, 23 Nov 1997, leg. J. Comezana “C” (XAL, holotype); Pontevedra, Vigo, 5 Nov 1999, leg. F. Valeiras “B” (XAL).

Remarks. – *Psilocybe gallaeciae* is macromorphologically similar to *P. mexicana*, as Guzmán & Castro (2003) already noted, and probably correspond to the same taxon. The basis that Guzmán & Castro (2003) used to distinguish them, the presence of pseudorhiza and pleurocystidia in *P. gallaeciae*, is weak in light of new observations in the present study. Guzmán (1983) mentioned “pleurocystidia absent or few similar to the cheilocystidia” for *P. mexicana*; however, data on the type and of several specimens that we will publish elsewhere has clearly revealed that this species has pleurocystidia. On the other hand, the type of *P. mexicana* was not properly collected, so we think that the pseudorhiza was left in the soil, based on the observations of an authentic specimen collected by Heim and other recently collected specimens,

all with pseudorhiza, that fit very well with the concept of *P. mexicana*. Currently, the reasons to consider *P. gallaeciae* different from *P. mexicana* are basidiospore size and pileocystidial shape. Although, in the type of *P. gallaeciae* the basidiospores are slightly smaller (see dimensions earlier), in the additional studied specimen we found spores of $8.8\text{--}11.2 \times 6.4\text{--}8 \times 5.6\text{--}6.8 \mu\text{m}$, and Guzmán & Castro (2003) cited $9.5\text{--}11 (13) \times 7\text{--}7.5 (9) \mu\text{m}$. In *P. mexicana* the basidiospores are slightly shorter, with the majority $7\text{--}10 \mu\text{m}$ long, rarely up to 11 or $12 \mu\text{m}$, and also narrower up to $7.2 \mu\text{m}$ wide. Finally, the pileocystidia in *P. mexicana* are globose, cylindrical, and sometimes not well differentiated. On the other hand, *P. gallaeciae* grows in grasslands in Spain, and *P. mexicana* can be found in Mexico and Guatemala. With the available information, we cannot make a decision yet about their synonymy.

Psilocybe goniospora (Berk. & Broome) Singer, Sydowia 15(1–6): 70. 1962 (1961). – Figs. 57–59.

Synonym. – *Deconica goniospora* (Berk. & Broome) Singer, Sydowia 9(1–6): 404. 1955.

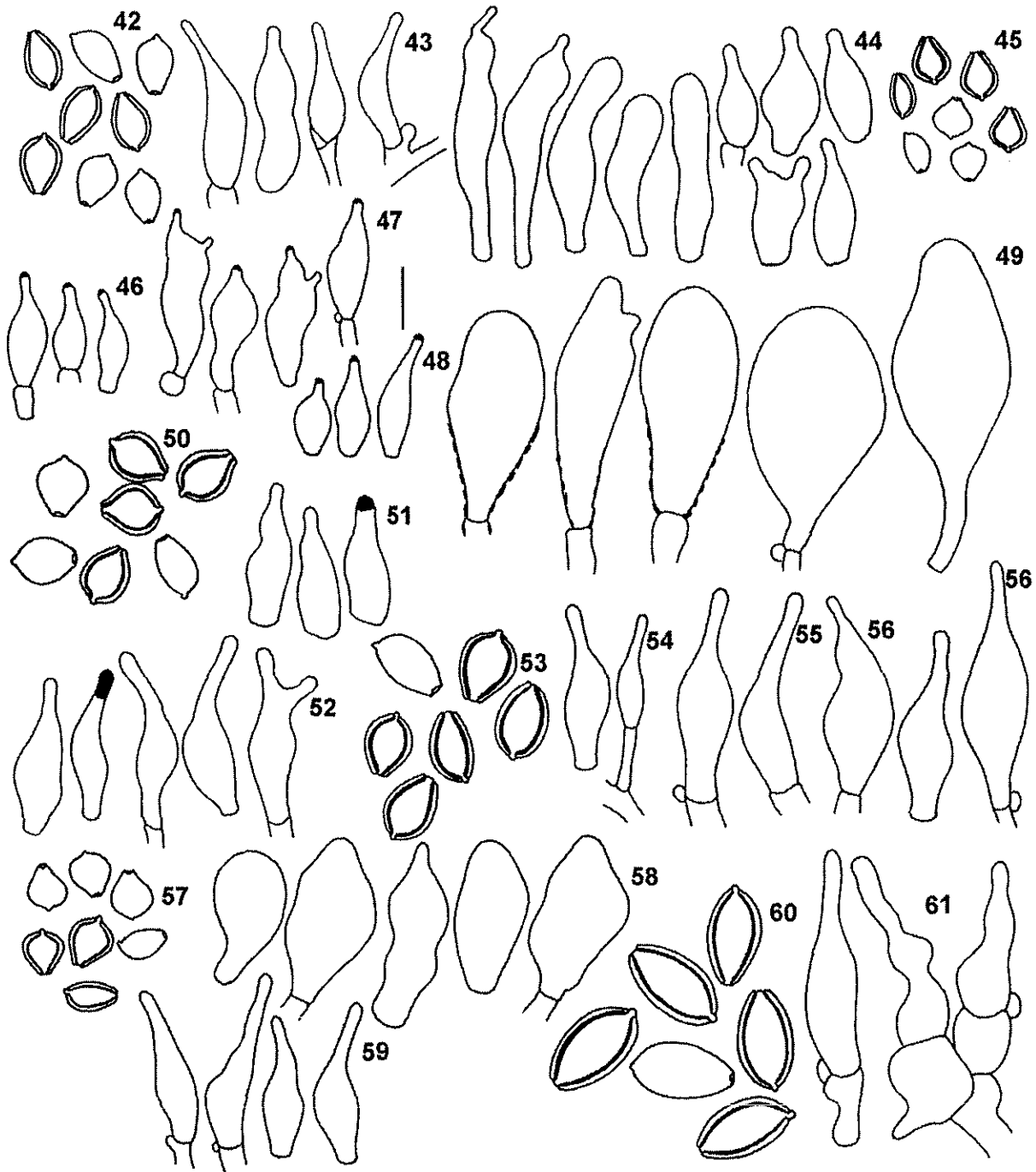
Basionym. – *Agaricus goniosporus* Berk. & Broome, J. Linn. Soc., Bot. 11(56): 541. 1871.

Synonym. – *Flammula goniospora* (Berk. & Broome) Sacc., Syll. Fung. 5: 827. 1887.

Basidiospores $4.8\text{--}5.6 (6.4) \times 4.4\text{--}5.6 \times 3.2\text{--}4.0 \mu\text{m}$, $Q = 1\text{--}1.1$, rhomboid and subrhomboid, few hexagonal in frontal view, $Q = 1.3\text{--}1.7$, ellipsoid in lateral view, yellowish brown, wall $0.5\text{--}0.7 \mu\text{m}$ thick, with two-layers in light microscopy, with germ pore. – **Pleurocystidia** $15\text{--}24 \times 6.5\text{--}11 \mu\text{m}$ broadly fusiform, ellipsoid, cylindrical, fusiform, sometimes mucronate, hyaline, with refractive content in KOH, wall thin. – **Cheilocystidia** $14\text{--}23 \times 5\text{--}6.5 \mu\text{m}$, narrowly lageniform, hyaline, wall thin. – **Subhymenium** ramose, hyphae $2.5\text{--}4 \mu\text{m}$ diameter. – **Pileustrama** radial, hyphae $8\text{--}12 \mu\text{m}$ diameter, yellowish. – **Pileipellis** a subgelatinized cutis, hyphae yellowish, wall thin. – **Stipitipellis** a cutis, hyphae $2.5\text{--}4 \mu\text{m}$ diameter, yellowish, wall up to $0.5 \mu\text{m}$ thick. – **Caulocystidia** approximately $15.5 \times 6 \mu\text{m}$, present, fusiform, some mucronate. – **Stipe basal mycelium** whitish yellow, hyphae hyaline, wall thin.

Material examined. – SRI LANKA, Peradeniya, Nov 1868, leg. M.J. Berkeley 835 [K(M) 160814, isotype].

Remarks. – The isotype consists of seven basidiomata and two pieces of a basidioma, all glued on a piece of paper. We observed with light microscopy that the basidiospore walls have two layers, like the “complex wall” previously reported by Pegler (1987: 411). The subhymenium is ramose, instead of pseudoparenchymatose. We observed the presence of caulocystidia, but the stipe was contaminated with mold, and they were difficult to measure. Pegler (1987) described the pleurocystidia with a refractive content distributed in all the cystidia, but not yellowish as in typical chrysocystidia belonging to *Hypholoma* or *Stropharia*. The content of pleurocystidia of *D. goniospora* in KOH looks similar to that observed in *D. neocaledonica* (see below), *D. thailandensis* (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán, and *D. umbrina* (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán (unpublished data). On the basis of the new observations in the present study, as well as the descriptions of Pegler (1987) and Guzmán (1983), we concluded that this species does not belong to *Psilocybe sensu stricto*, but to *Deconica*. Recently, Horak *et al.* (2009) considered this taxon to be in the bluing *Psilocybe* sect. *Neocaledonicae*. Our ongoing phylogenetic study of members of this



Figs. 42–61. Microscopic structures: 42–44. *Psilocybe cabiensis* (Isotype). 42. Basidiospores. 43. Pileocystidia. 44. *P. chiapanensis* (Holotype), cheilocystidia. 45–49. *P. eximia*. 45. Basidiospores (Isotype). 46. Pleurocystidia (Isotype). 47. Pleurocystidia (DED 7207). 48. Pleurocystidia (DED 7234). 49. Cheilocystidia (Isotype). 50–52. *P. farinacea* (Syntype). 50. Basidiospores. 51. Pleurocystidia. 52. Cheilocystidia. 53–56. *P. gallaeciae* (Holotype). 53. Basidiospores. 54. Pileocystidia. 55. Caulocystidia (Holotype). 56. Caulocystidia (F. Valeiras “B”). 57–59. *P. gonispora* (Isotype). 57. Basidiospores. 58. Pleurocystidia. 59. Cheilocystidia. 60–61. *P. hispanica* (Holotype). 60. Basidiospores. 61. Caulocystidia. Bar 8 μ m.

section (10 taxa) reveals that species of *Psilocybe s. str.* and *Deconica* surprisingly belong in this section (data will be published elsewhere).

Psilocybe hispanica Guzmán, Doc. Mycol. 29(116): 42. 2000. – Figs. 4,14, 60–61.

Basidiospores 13.6–14.4 (15.2) × 6.4–8 (8.8) μm, Q = 1.6–2 (2.5), elongate in frontal view and lateral view, few cylindrical, fusiform, yellowish brown, wall 0.8 μm thick, with two layers in light microscopy, with germ pore. – **Subhymenium** ramose-inflated. – **Pileus trama** interwoven, hyphae 2.5–13 μm diameter, yellowish, wall thin. – **Subpellis** differentiated, hyphae with yellowish brown strongly encrusted pigment. **Pileocystidia** absent. – **Stipitipellis a cutis**, hyphae 4.5–13 μm diameter, yellowish, wall up to 0.5 μm thick. – **Caulocystidia** 25.5–35 × 4.5–8 μm, narrowly lageniform, flexuose, apex obtuse, hyaline, wall thin, in the upper part of the stipe.

Material examined. – SPAIN, Province of Aragón, N of Huesca, Pirineos region, Autumn 1995, alt. 2300 m, alpine meadow, on dung, *leg.* I. Seral-Bozal s.n. (XAL, holotype).

Remarks. – This species is similar to *P. semilanceata* (Fr.) P. Kumm. and *P. liniformans* Guzmán & Bas. The following features are not mentioned in the original description viz. the radial arrangement of the pileus trama, the subhymenium type, and presence of caulocystidia.

Psilocybe isabelae Guzmán, in Guzmán, Ramírez-Guillén, Tapia & Navarro, Acta Bot. Mex. 49: 39. 1999. – Figs 62–63.

Basidiospores 6.4–8.0 × 5.6–6.4 × 4.8–5.2 μm, Q = 1.1–1.4, subrhomboid and subhexagonal in frontal view, Q = 1.3–1.6, ellipsoid and elongate in lateral view, yellowish brown, wall 0.8–1 μm thick, with two layers in light microscopy, with germ pore. – **Pleurocystidia** 12.5–18.5 × 4–5.5 μm, narrowly lageniform, apex obtuse, refractive, hyaline, wall thin. – **Subhymenium** ramose. – **Pileus trama** radial, hyphae 5–24 μm diameter, yellowish to yellowish orange in mass, wall thin with encrusted pigment. – **Pileipellis** a thin subgelatinized cutis, hyphae up to 6.5 μm diameter, yellowish, wall thin. – **Pileocystidia** absent. – **Caulocystidia** 9–26 × 6–11 μm, narrowly lageniform, fusiform or as modified hyphae, occasionally branched, only in the apical part of stipe.

Material examined. – MEXICO, Veracruz, SW of Xico, 15 Sep 1998, *leg.* G. Guzmán 32466 (XAL, holotype).

Remarks. – The original description of Guzmán *et al.* (1999) is complemented with additional features, such as the arrangement of the pileus trama and the presence of caulocystidia. Further, we observed with light microscopy that the basidiospore walls have two layers. This species is similar to *P. subtropicalis* in all microscopic features except that *P. subtropicalis* has two kinds of pleurocystidia. Because the description of *P. isabelae* was based on one specimen, it is not possible to know for sure whether the specimen manifested all intraspecific variation that may be present within the taxon or whether other specimens may present two kinds of pleurocystidia. So, until more specimens are gathered and reviewed, we treat *P. isabelae* as an independent taxon.

Psilocybe jaliscana Guzmán, Doc. Mycol. 29(116): 46. 2000, nom. inval. – Figs. 64–66.

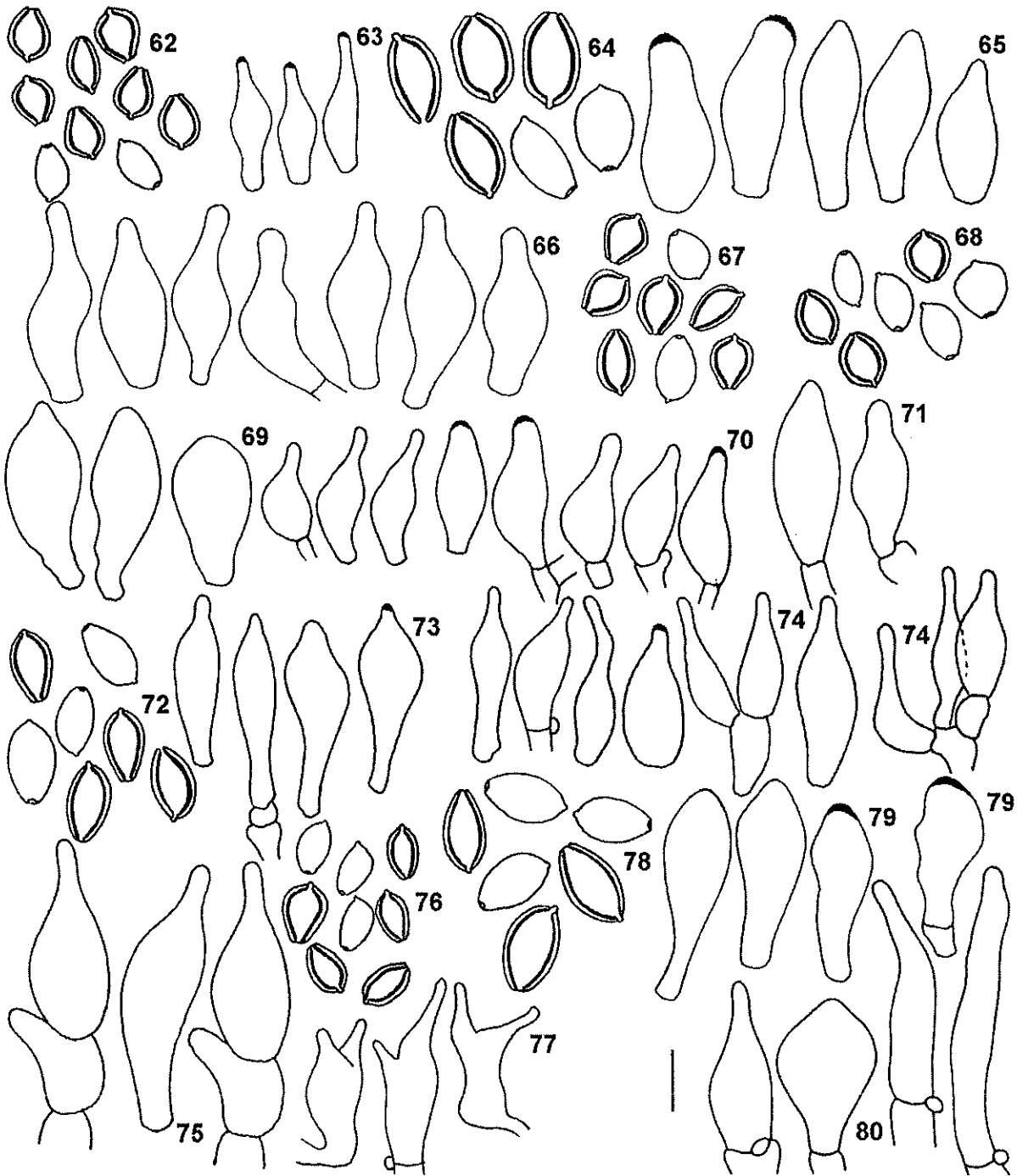
B a s i d i o s p o r e s 10.0–13.0 (15.0) × 7.2–8.8 × 6.4–8.0 μm, Q = 1.2–1.7, hexagonal, sometimes angles not well defined in frontal view, Q = 1.4–1.7, ellipsoid and elongate in lateral view, yellowish brown, wall thick (1–1.4 μm thick), with two layers in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** 22–31 × 8.8–9.6 μm, utriform, fusiform, and clavate, apex obtuse, refractive, wall thin. – **C h e i l o c y s t i d i a** 20–28 × 5.5–8 μm, utriform and lageniform, apex obtuse, refractive, hyaline, wall thin. – **P i l e u s t r a m a** interwoven, hyphae 3–14.5 μm diameter, yellowish. – **P i l e i p e l l i s** an ixocutis, 20–30 μm wide, hyphae 1.5–3 μm diameter, yellowish, wall thin. – **P i l e o c y s t i d i a** absent. – **S t i p i t i p e l l i s** a cutis, hyphae 2.5–5.5 μm diameter, yellowish, wall < 0.5 μm thick. – **C a u l o c y s t i d i a** not observed. – **S t i p e b a s a l m y c e l i u m** hyphae hyaline, wall thin.

M a t e r i a l e x a m i n e d . – MEXICO, Jalisco, Municipality of Mazamitla, near Mazamitla town, 7 Sep 1996, in *Pinus-Quercus* forest with subtropical influence, solitary on soil, leg. G. Guzmán 31693 (IBUG, isotype).

R e m a r k s . – Additional information not included in the protologue is the arrangement of the pileus trama and presence of gelatinized pileipellis. Guzmán (2000, p. 46) described the basidiospores in frontal view as “ellipsoid to obscurely subhexagonal or subrhomboidal”, but we did not observe subrhomboid basidiospores. Also, Guzmán (2000) cited pleurocystidia as ventricose-submucronate to subrostrate, but the drawings represent cylindrical, clavate, and fusiform as in our observations. We also found shorter pleurocystidia. From our study of the isotype, we concluded that *Psilocybe jaliscana* is very similar to *P. subcubensis* in macro- and micromorphological features. According to Guzmán (1983, p. 249–250), *P. subcubensis* Guzmán has basidiospores “(9.9–) 11–13 (–14) × 7.7–8.8 × 6.6–7.1 μm, subhexagonal in frontal view, subellipsoid in lateral view, thick walled (0.5–1 μm)”, pleurocystidia “14–22 × 8–12 μm, hyaline, scarce, thin walled, ventricose or subpyriform, submucronate”, and cheilocystidia “16–25 (–36) × 6–9 μm, hyaline, abundant, forming a sterile band, thin walled, fusoid-ventricose or sublageniform, with short neck, sometimes with subglobose heads”. In broad outline, *P. jaliscana* could fit within the variation of *P. subcubensis*. Both species differ mainly in the absence of a well-developed membranous annulus in *P. jaliscana* and in their habitat. *Psilocybe subcubensis* grows in dung and sometimes in soils, but *P. jaliscana* is only known from the type specimen growing in soil, so it is very poorly known. In our opinion, *P. jaliscana* is a synonym of *P. subcubensis*.

Psilocybe keralensis K.A. Thomas, Manim. & Guzmán, in Thomas, Manimohan, Guzmán, Tapia & Ramírez-Guillén, Mycotaxon 83: 196. 2002. – Fig. 67.

B a s i d i o s p o r e s 7.2–8.4 × (4.8) 5.6–6.4 × 4.8–5.6 μm, Q = 1.2–1.4, subrhomboid and subhexagonal in frontal view, Q = 1.3–1.7, ellipsoid and elongate in lateral view, yellowish brown, wall 0.8 μm thick, with two layers in light microscopy, with germ pore. – **S u b h y m e n i u m** ramose-inflated, hyphae yellowish, wall thin. – **H y m e n o p h o r a l t r a m a** subregular, hyphae 6.5–23 μm diameter, yellowish, wall thin. – **P i l e u s t r a m a**



Figs. 62–80. Microscopic structures: 62–63. *Psilocybe isabelae* (Holotype). 62. Basidiospores. 63. Pleurocystidia. 64–66. *P. jaliscana* (Isotype). 64. Basidiospores. 65. Pleurocystidia. 66. Cheilocystidia. 67. *P. keralensis* (Isotype), basidiospores. 68–71. *P. magnispora* (Isotype). 68. Basidiospores. 69. Pleurocystidia. 70. Cheilocystidia. 71. Caulocystidia. 72–75. *P. makarorae* (Holotype). 72. Basidiospores. 73. Pleurocystidia. 74. Cheilocystidia. 75. Caulocystidia. 76–77. *P. meridionalis* (Holotype). 76. Basidiospores. 77. Caulocystidia. 78–80. *P. mescaleroensis* (Holotype). 78. Basidiospores. 79. Pleurocystidia. 80. Caulocystidia. Bar 8 μ m.

radial, hyphae 5.5–25.5 μm diameter, yellowish, wall thin. – *Pileipellis* an ixocutis, 9–10.5 μm wide, hyphae 1.5–3 μm diameter, hyaline, wall thin.

Material examined. – INDIA, Kerala State, Wayanad District, Ponkuzhy, 21 Jul 1999, *leg.* K.A. Thomas T319a (XAL, isotype).

Remarks. – Additional taxonomic features not described by Thomas *et al.* (2002) are shown here, such as the arrangement of the pileus trama and the type of subhymenium. Thomas *et al.* (2002) described the basidiospores as slightly lenticular, ovo-ellipsoid to subrhomboid in frontal view. Although the authors placed this species in sect. *Cordisporae*, *P. keralensis* is actually more similar to species of sect. *Mexicanae*, e.g., *P. atlantis*, *P. mexicana*, and *P. samuiensis*.

Psilocybe magnispora E. Horak, Guzmán & Desjardin, *Sydowia* 61(1): 25. 2009. – Figs. 68–71.

Basidiospores 6.4–8.0 \times 5.2–6.4 (7.2) \times 4.8–5.6 μm , Q = 1.1–1.25, subrhomboid and hexagonal in frontal view, Q = 1.3–1.5, ellipsoid in lateral view, yellowish brown, wall thick (0.8–1 μm thick), with two or three layers in light microscopy, with germ pore. – *Pleurocystidia* 19–31 \times 9.5–12 μm , ovoid, clavate, and fusiform, sometimes mucronate, hyaline, wall thin, without reaction to Patent blue V. – *Cheilocystidia* 13.5–20 \times 5–7 (10) μm , lageniform, with a short neck, apex obtuse with a refractive cap, hyaline, wall thin, embedded in a gelatinized layer at the edge of the lamellae. – *Pileus trama* interwoven, hyphae 7–20 μm diameter, hyaline, wall thin. – *Pileipellis* an ixocutis, 24–32 μm thick, hyphae 1.6–4 μm diameter, hyaline, wall thin. – *Caulocystidia* 21.5–32 \times 6.5–9.5 μm , utriform and subfusiform, yellowish, wall thin.

Material examined. – THAILAND, Kha Yai National Park, Pong Chang, Princess Trail, in tropical montane broadleaf forest, 10 Jul 2002, *leg.* E. Horak 10171 (XAL, isotype).

Remarks. – Important information is that this species has bluing basidiomata and lacks chrysocystidia. Horak *et al.* (2009) and Guzmán *et al.* (2012) considered *P. magnispora* as similar to *P. thailandensis*, although the two species are very different in macro- and micromorphological features. *Psilocybe thailandensis* actually belongs to the genus *Deconica* because it is not bluing (Ramírez-Cruz *et al.* 2012). Cheilocystidia in *P. magnispora* are embedded in a gelatinized layer at the edge of the lamellae as in *P. aquamarina* and *P. thaiaerugineomaculans* Guzmán, Karunarathna & Ram.-Guill. (Guzmán *et al.* 2012). However, it differs in having smaller basidiospores, 8.8–11.2 (12.4) \times 6.4–8.8 \times 4.8–6.4 μm in *P. aquamarina* and 8.8–10.5 (11.7) \times 6.4–8 \times 5.6–7.2 μm in *P. thaiaerugineomaculans*. Horak *et al.* (2009) described the pleurocystidial content as refringent; we observed that the content is opaque, hyaline to yellowish brown and did not react with Patent blue V, so they cannot be considered as true chrysocystidia. According to Horak *et al.* (2009), this species resembles the Indonesian *P. aureicystidiata* E. Horak & Desjardin, based on the annulated basidiomata, but this species also belongs to *Deconica* (see below).

Psilocybe makarorae P.R. Johnst. & P.K. Buchanan, N. Z. J. Bot. 33(3): 382. 1995. – Figs. 17, 72–75.

Basidiospores 8.8–9.6 × 4.8–6.4 × 5.6–6 µm, Q = 1.3–1.5 (1.8), ellipsoid, subrhomboid, and hexagonal in frontal view, angles not well defined in some spores, Q = 1.4–1.7, ellipsoid and elongate in lateral view, yellowish brown, wall 0.8–1 µm thick, with two layers in light microscopy, with germ pore. – **Pleurocystidia** 19–29 × 5–8 (9) µm, fusiform, some clavate-mucronate, hyaline, wall thin. – **Cheilocystidia** 16–25.5 × 5–8 µm, narrowly lageniform to lageniform and utriform, some fusiform-mucronate, hyaline, some with granulose yellowish content, wall thin. – **Subhymenium** ramose-inflated, wall with yellowish brown, encrusted pigment. – **Pileus trama** radial with some zones with interwoven hyphae, 6–16 µm diameter, hyaline, yellowish in mass, wall thin. – **Subpellis** differentiated, hyphae 2.5–12 µm diameter, hyaline, wall with encrusted pigment. – **Pileipellis** an ixocutis, 6–11 µm wide, hyphae 2.5–4 µm diameter, hyaline, wall thin. – **Stipitipellis** with hyphae 2–5 µm diameter, hyaline, wall slightly thick. – **Caulocystidia** 22.5–34.5 × 6.5–9.5 µm, narrowly lageniform, lageniform, and fusiform, hyaline, wall thin.

Material examined. – NEW ZEALAND, Otago Lakes, Haast Pass, vic. Makarora, Blue Pools Track, on rotten *Nothophagus* wood, leg. P.R. Johnston, B.P. Segedin & R.H. Petersen, 16 May 1990, (PDD 57396, holotype). Southland, Catlins, 5 km north of Papatowai, Table Hill Reserve, leg. P.R. Johnston, T. May & G.S. Ridley, 9 May 1995 (PDD 646666). West coast, Fox Glacier Westland, Westland National Park, 73 km south of Fox Glacier, leg. R.H. Petersen (Field # TFB7069) (TENN 054116).

Remarks. – In the protologue, Johnston & Buchanan (1995) described the pleurocystidia as similar to cheilocystidia, but according to our examination they are different. Additional specimens studied here show somewhat larger caulocystidia 25.5–50 × 4–9 µm, cylindrical, widely lageniform, lageniform, or fusiform. Johnston & Buchanan (1995) pointed out that, based on the basidiospore shape and bluing reaction, this species belongs to sect. *Mexicanae* of Guzmán (1983). However, the shape of the pleurocystidia and cheilocystidia and the macroscopic features are closer to those of *P. cyanescens* Wakef. from sect. *Semilanceatae* of Guzmán (1995). *Psilocybe makarorae* and *P. cyanescens* differ in basidiospore size and shape.

Psilocybe meridionalis Guzmán, Ram.-Guill. & Guzm.-Dáv., in Guzmán, Guzmán-Dávalos, Ramírez-Guillén & Sánchez-Jácome, Mycotaxon 103: 28. 2008. – Figs. 76–77.

Basidiospores 6.4–8.0 (8.8) × 4.8–5.6 × 4.0–4.8 µm, Q = 1.3–1.6, subrhomboid and subhexagonal in frontal view, angles not well defined, Q = 1.4–1.8, ellipsoid and elongate in lateral view, yellowish brown, wall 0.8–1 µm thick, with two layers in light microscopy, with germ pore. – **Pileus trama** radial to the lamellae, interwoven to the pileipellis, hyphae 4–16 µm diameter, yellowish to yellowish brown in mass, wall thin. – **Pileipellis** a subgelatinized cutis, 12–16 µm wide, hyphae 1.5–4 µm diameter, hyaline, wall thin. – **Pileocystidia** absent. – **Stipitipellis** a cutis, hyphae 1.5–4 µm diameter, yellowish, wall up to 0.5 µm thick, in the basal part of the stipe with yellowish brown hyphae, some capitate. – **Caulocystidia** 17.5–24 × 5.5–8 µm, lageniform-flexuose, bifurcate,

hyaline, wall thin, in the apical part of stipe. – Stipe basal mycelium white, hyphae hyaline, wall thin.

Material examined. – MEXICO, Jalisco, Sierra de Cacoma, Municipality of Autlán, Neverías, alt. 2200 m, *Pinus–Quercus* forest, 27 Sep 2005, leg. M.R. Sánchez-Jácome 1163 (IBUG, holotype).

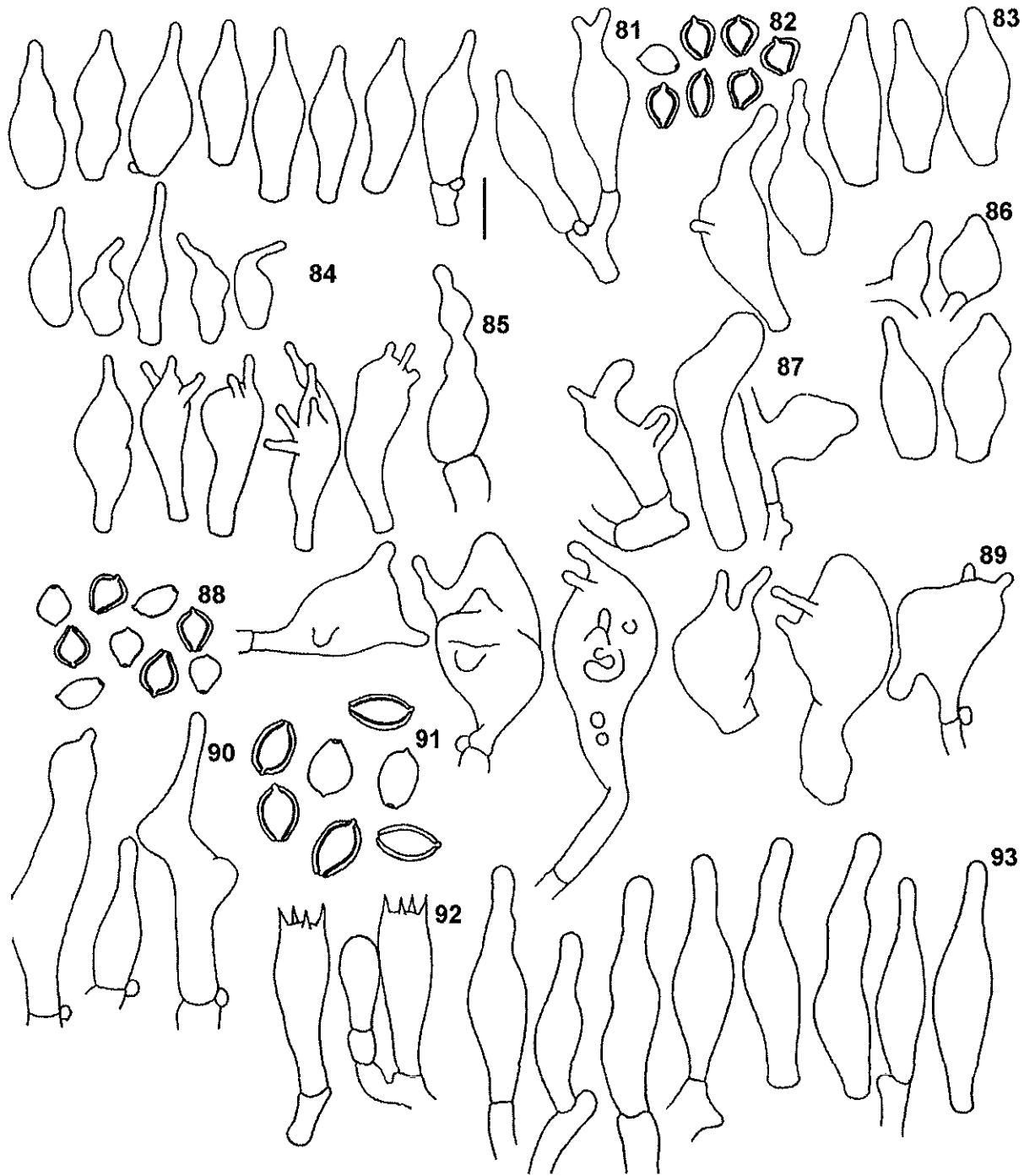
Remarks. – We present the arrangement of the pileus trama, the presence of caulocystidia, and subgelatinized pileipellis. Also, we point out that the basidiospores are hexagonal besides subrhomboid, with a complex wall. Another important observation is that the yellowish brown hyphae of the base of the stipe differ from the setaceous hyphae present in the basal mycelium of some species of sect. *Cordisporae*, e.g., *P. mesophylla* Guzmán, J. Q. Jacobs & Escalona, *P. neoxalapensis* Guzmán, Ram.-Guill. & Halling, *P. fagicola* R. Heim & Cailleux (Guzmán *et al.* 2005). *Psilocybe meridionalis* is very similar to *P. thaicordispora* (see this species below) from Thailand, recently described by Guzmán *et al.* (2012).

Psilocybe mescaleroensis Guzmán, Walstad, Gándara & Ram.-Guill., Mycotaxon 99: 225. 2007. – Figs. 78–81.

Basidiospores 9.6–11.2 (12.0) × (5.6) 6.4–7.2 μm, Q = 1.4–1.6 (1.8), ellipsoid and elongate in frontal and lateral views, fusiform, yellowish brown, wall 0.6–0.8 μm thick, with two layers in light microscopy, with germ pore. – **Pleurocystidia** 20–28 × (5.5) 8–9.5 μm, clavate and fusiform, some irregular flexuose, apex refractive, hyaline, wall thin. – **Cheilocystidia** 17.5–33 × 5.5–8 μm, lageniform and fusiform, flexuose, apex obtuse, occasionally bifurcate, wall thin. – **Subhymenium** ramose-inflated, yellowish. – **Pileus trama** radial, hyphae 4.5–12 μm diameter, yellowish, wall thin. – **Pileipellis** an ixocutis, 80 μm wide, hyphae 2.5–5.5 μm diameter, hyaline, wall thin. – **Pileocystidia** absent. – **Stipitipellis** a cutis, hyphae 5.5–8 μm diameter, yellowish, wall up to 0.5 μm thick. – **Caulocystidia** 22.5–40 × 4–11 μm, lageniform, clavate and cylindrical, flexuose, apex obtuse, hyaline, wall thin.

Material examined. – USA, New Mexico, Sacramento, Aug 2007, leg. L.F. Walstad s.n. (XAL, holotype).

Remarks. – Guzmán *et al.* (2007b) described the basidiospores as subrhomboid and did not mention the presence of pleurocystidia, but they are scarce and very different from cheilocystidia. We also describe the arrangement of the pileus trama and the presence of caulocystidia. Although Guzmán *et al.* (2007b) placed *P. mescaleroensis* in sect. *Stuntzii* for the submembranaceous annulus, we think this species is more closely related to those of sect. *Semilanceatae* sensu Guzmán (1995) or *Cyanescens* in the sense of Guzmán (1983). It is similar to *P. hopii* Guzmán & J. Greene, except *P. hopii* lacks an annulus and has two types of cheilocystidia.



Figs. 81–93. Microscopic structures: **81.** *Psilocybe mescaleroensis* (Holotype), cheilocystidia. **82–87.** *P. mesophylla* (Holotype). **82.** Basidiospores. **83.** Deuterocystidia. **84.** Leptocystidia. **85.** Cheilocystidia. **86.** Pileocystidia. **87.** Caulocystidia. **88–90.** *P. neoxalapensis* (Holotype). **88.** Basidiospores. **89.** Pileocystidia. **90.** Caulocystidia. **91–93.** *P. ochreata* (Holotype). **91.** Basidiospores. **92.** Basidia, basidiole, and subhymenium. **93.** Cheilocystidia. Bar 8 μm .

Psilocybe mesophylla Guzmán, J.Q. Jacobs & Escalona, in Guzmán, Escalona, Ramírez-Guillén & Jacobs, *Int. J. Med. Mush.* 6(3): 276. 2004. – Figs. 82–87.

Basidiospores (4.8) 5.2–6.4 (7.2) × 4.8–5.6 (6.4) × 3.2–4.8 μm, Q = 1–1.16, rhomboid and subrhomboid in frontal view, Q = 1.1–1.5 (1.7), broadly ellipsoid and ellipsoid in lateral view, yellowish brown, wall 0.5–1 μm thick, with two layers in light microscopy, with germ pore, some with two pores. – **Pleurocystidia** as leptocystidia 12–21 × 3–5.5 (7) μm, lageniform and lageniform-flexuose, always hyaline, wall thin. – **Pleurocystidia** as deuteroecystidia 16–34.5 × 6.5–9.5 μm, lageniform and utriform, some with flexuose neck, apex obtuse, hyaline or yellowish grey, wall thin. – **Cheilocystidia** 18.5–29.5 × 4.5–8 μm, fusiform, ventricose, and clavate, with two to five short and narrow, mostly apical branches, few moniliform with a wide base, hyaline to yellowish grey, wall thin. – **Subhymenium** ramose. – **Pileus trama** radial, hyphae 9.5–18.5 μm diameter, yellowish to yellowish brown in mass, wall thick (0.8–1 μm thick) with encrusted pigment. – **Subpellis** differentiated, hyphae 2.4–12 μm diameter, hyaline, wall with encrusted pigment. – **Pileipellis** a subgelatinized cutis, hyphae 2–3 μm diameter, yellowish, wall thin. – **Pileocystidia** 8–20 × 4–10.5 μm, pyriform, globose, conical, and utriform, hyaline, wall thin. – **Caulocystidia** 8–22.5 (41) × 4.5–8 μm, cylindrical and as modified hyphae, sometimes branched, hyaline, wall thin.

Material examined. – MEXICO, Oaxaca, Tuxtepec, near Llano Grande, on orangish red clay soil without vegetation outside of a mesophytic forest, 5 Jul 1980, *leg.* J. Jacobs 159 (XAL, holotype).

Remarks. – Here we describe subhymenium, pileus trama, pileocystidia, and two types of cystidia on the side of the lamellae. Guzmán *et al.* (2004b) found only lageniform caulocystidia; we record them here as highly variable in shape.

Psilocybe neoxalapensis Guzmán, Ram.-Guill. & Halling, in Guzmán, Horak, Halling & Ramírez-Guillén, *Sydowia* 61(2): 220. 2009. – Figs. 25, 88–90.

Synonym. – *Psilocybe novoxalapensis* Guzmán & J.Q. Jacobs, in Guzmán, Jacobs, Ramírez-Guillén, Murrieta & Gándara, *J. Microbiol.* 43(2): 159. 2005, *nom. nud.*

Basidiospores 4.4–5.6 × 4.0–4.8 × 3.2–4.0 (4.4) μm, Q = 1–1.2, rhomboid in frontal view, Q = 1.1–1.3, broadly ellipsoid in lateral view, yellowish brown, wall 0.5–0.8 thick, with two layers in light microscopy, with germ pore. – **Subhymenium** ramose. – **Pileus trama** radial, hyphae 8–36 μm diameter, yellowish to yellowish brown in mass, wall 0.8 μm thick, with encrusted pigment. – **Subpellis** differentiated, hyphae 4–11 μm diameter, yellowish brown, wall thin with strongly encrusted pigment. – **Pileipellis** a subgelatinized layer, hyphae 1.5–4 μm diameter, hyaline. – **Pileocystidia** 24–34.5 × 8.5–15 μm, clavate, as modified hyphae, with short and narrow branches, wall thin, arranged in groups. – **Stipitipellis** with hyphae 5.5–13 μm diameter, yellowish, wall up to 0.5 μm thick. – **Caulocystidia** 19–25 × 4–9 μm, narrowly lageniform, cylindrical flexuose, sometimes apex mucronate, hyaline, some yellowish, wall thin, in groups in all the stipe.

Material examined. – MEXICO, Veracruz, Banderilla, cerro La Martinica, in subtropical or montane cloud forest, Jul 1983, *leg.* A. López 2186 (XAL, holotype).

Remarks. – We complement the recent re-description of this species (Guzmán *et al.*, 2009) with data on the pileocystidia, subhymenium, and pileus trama. The subpellis is not cellular but has radial hyphae. As far as we know, only *P. eximia* has a pileus trama with inflated hyphae, but the subpellis is never cellular in *Psilocybe* neither in *Deconica*.

Psilocybe ochreata (Berk. & Broome) E. Horak, in Guzmán, Beih. Nova Hedwigia 74: 138. 1983. – Fig. 19, 91–93.

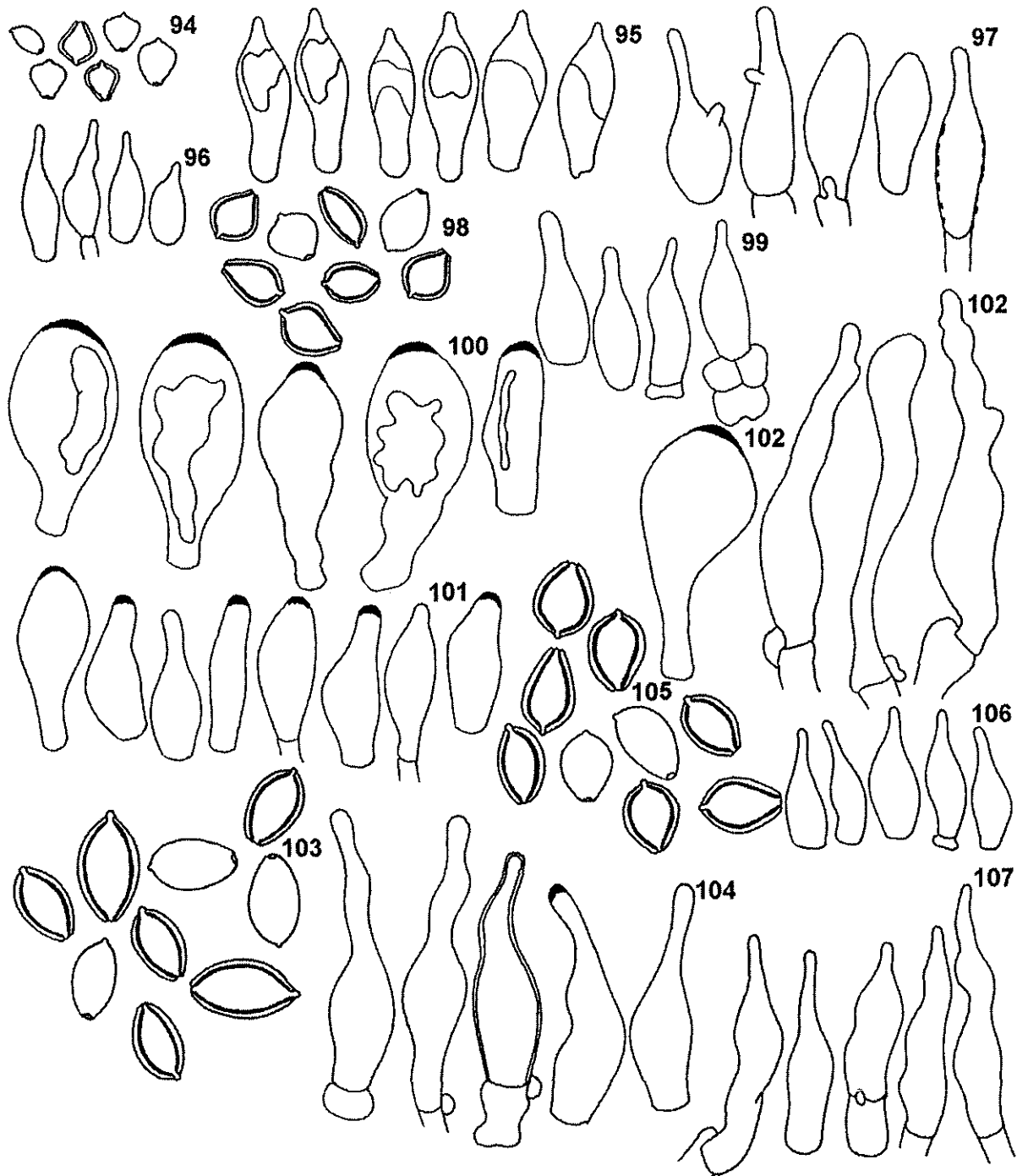
Basonym. – *Agaricus ochreatus* Berk. & Broome, J. Linn. Soc., Bot. 11(56): 555, 1871.

Synonym. – *Deconica montana* (Pers.) P.D. Orton, Trans. Br. Mycol. Soc. 43(2): 175. 1960.

Basidiospores 7.2–8.8 × 4.2–5.6 (6.4) × 4.8 μm, Q = 1.2–1.5, rhomboid and hexagonal in frontal view, Q = 1.5–1.8, subellipsoid in lateral view, yellowish brown, wall 0.5–0.8 μm thick, with two layers in light microscopy, with germ pore. – **Basidia** 21.5–32 × 6.5–8 μm, cylindrical or clavate, tetrasporic, few monosporic, sterigmata 3–5 μm long, hyaline, wall thin. – **Pleurocystidia** absent. – **Cheilocystidia** 19–36 × 5.5–8 μm, utriform and narrowly lageniform, some flexuose, hyaline, wall thin, apex 4–7 μm wide, obtuse to subcapitate, refractive, hyaline. – **Subhymenium** ramose-inflated, with some pigmented hyphae ascending to the hymenium. – **Hymenophoral trama** subregular, hyphae 4.5–8 μm diameter, yellowish, wall thin. – **Pileus trama** radial, hyphae 6.5–8 μm diameter, yellowish, wall thin. – **Subpellis** differentiated, hyphae 4–11 μm diameter, yellowish brown, wall thin with strongly encrusted pigment. – **Pileipellis** an ixocutis, hyphae hyaline, wall thin. – **Pileocystidia** absent. – **Caulocystidia** not observed. – **Clamp connections** present.

Material examined. – SRI LANKA, Peranediya, on dead wood, *leg.* C.E. Broome 835 [281] [K(M) 160813, holotype].

Remarks. – This species was described by Berkeley & Broome (1871) only on the basis of macroscopic features. Horak (in Guzmán, 1983) proposed the combination in *Psilocybe* and described the basidiospores. Later, Pegler (1987) provided additional data on its micromorphology. We agree with Pegler's and Horak's herbarium notes that there are two species in the specimen labelled as the type of *P. ochreata*. The type specimen consists of six basidiomata or fragments glued on paper and several basidiomata in an envelope. Pegler assigned numbers (1–7) to the glued basidiomata, but not for those in the envelope. According to Horak's and Pegler's notes, basidiomata 1, 3, and 5 belong to *P. ochreata*, and 2, 4, and 6 correspond to *P. goniospora*. We checked the basidiomata contained in the envelope, separated them, and numbered them as 8 and 9. The basidiomata corresponding to number 8 belong to *P. goniospora* (= *Deconica goniospora*), and those for number 9 belong to *P. ochreata*. Our description here is based only on basidiomata 1, 3, 5, and 9. We found basidia and cheilocystidia larger than those described by Pegler (1987). For us, *Psilocybe ochreata* agrees well with *Deconica montana* in the size and shape of the basidiospores, absence of pleurocystidia, the size and shape of cheilocystidia, and the pigmented hyphae ascending to the subhymenium, according to the characteristics for *D. montana* described by Guzmán (1983), who studied the type and other specimens. The only difference between *P. ochreata* and *D. montana* is the latter's wider cheilocystidial apex (2–3.5 μm wide). Therefore, we propose that these species are synonyms.



Figs. 94–107. Microscopic structures: 94–97. *Psilocybe overeemii* (Isotype). 94. Basidiospores. 95. Chrysocystidia. 96. Cheilocystidia. 97. Caulocystidia. 98–102. *P. ovoideocystidiata* (Holotype). 98. Basidiospores. 99. Leptocystidia and cellular subhymenium. 100. Deuterocystidia. 101. Cheilocystidia. 102. Caulocystidia. 103–104. *P. quebecensis* (Holotype). 103. Basidiospores. 104. Caulocystidia. 105–107. *P. samuiensis* (Isotype). 105. Basidiospores. 106. Pleurocystidia. 107. Caulocystidia. Bar 8 μ m.

The type specimen of *P. ochreata* was described on rotten wood, unlike soil-growing *D. montana*, but the basidiomata are mixed. Some probably grew in soil, and those belonging to *P. goniospora* grew on rotten wood. *Psilocybe magica* Svrček (nonhallucinogenic) is very similar to *D. montana*, according to Guzmán *et al.* (2008b), only differing in size, shape, and apical width of the cheilocystidia—variable from sublageniform or tibiiform to flexuose-cylindrical, or moniliform, with apex 2–5 (8) μm wide in *P. magica*.

Psilocybe overeemii E. Horak & Desjardin, *Sydowia* 58(1): 30. 2006. – Figs. 24, 94–97.

Synonym. – *Psilocybe neocaledonica* Guzmán & E. Horak, *Sydowia* 31(1–6): 53. 1979 (1978).

Synonym. – *Deconica neocaledonica* (E. Horak & Desjardin) Ram.-Cruz & Guzmán (see below).

B a s i d i o s p o r e s 5.0–6.0 (7.0) \times 5.0–6.0 \times 3.5–4.0 μm , Q = 1–1.2, rhomboid and subrhomboid in frontal view, Q = 1.3–1.7, ellipsoid in lateral view, yellowish brown, wall 0.4–0.6 μm , with two layers in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** as chrysocystidia, 18–30 \times 9–11 μm , fusiform-mucronate, hyaline, with irregular hyaline content in KOH, which stains blue with Patent blue V. – **C h e i l o c y s t i d i a** 18.5–18.5 \times 3.2–5 μm , narrowly lageniform, hyaline, wall thin. – **S u b h y m e n i u m** ramose-inflated. – **P i l e u s t r a m a** radial, hyphae 4–17 μm diameter, yellowish to yellowish brown in mass, wall 0.5–0.8 μm thick, with encrusted pigment. – **P i l e i p e l l i s** a thin ixocutis, less than 8 μm thick, hyphae 2.5–3 μm diameter, hyaline with encrusted pigment. – **P i l e o c y s t i d i a** not observed. – **C a u l o c y s t i d i a** 17.5–27 \times 5.5–8.5 μm , narrowly lageniform, subfusiform, and clavate, few with a short lateral branch, some with encrusted pigment from the base to the middle.

M a t e r i a l e x a m i n e d . – INDONESIA, Java, Mt. Halimun Salak National Park, alt. 1200 m, near Cikaniki Field Station, 8 Jan 1999, *leg.* E. Horak 7311 (XAL, isotype).

R e m a r k s . – From the study of the type, we are proposing this species as a synonym under *D. neocaledonica*, described as *Psilocybe* from New Caledonia by Guzmán & Horak (1978). The main reasons for the synonymy are the similar size and shape of their basidiospores, chrysocystidia, and cheilocystidia and the same types of subhymenium and pileipellis (see characteristics of *D. neocaledonica* below). Macromorphologically, they have a convex to campanulate pileus, covered with concolorous fibrillose squamules, a stipe covered with concolorous or whitish remnant fibrils from the veil, but lacking a distinct cortinate zone or annulus. Both species grow on rotten wood in tropical forests. *Psilocybe overeemii* is related to *P. aureicystidiata* according to Horak & Desjardin (2006), but can be separated mainly by the fibrillose, squamulose veil remnants present on *P. overeemii*.

Psilocybe ovoideocystidiata Guzmán & Gaines, *Int. J. Med. Mush.* 9(1): 75–77. 2007. – Figs. 22, 98–102.

B a s i d i o s p o r e s 6.4–9.6 \times 5.6–7.2 \times 4.4–6.4 μm , Q = 1.17–1.5 (1.7), subrhomboid, rhomboid, and hexagonal in frontal view, Q = 1.3–1.8 (2), ellipsoid and elongate in lateral view, yellowish brown, wall 0.5–0.6 μm thick, with two layers in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** as leptocystidia 17.5–21 \times 4.5–7 μm , lageniform, hyaline, wall thin,

originating from the subhymenium. – *Pleurocystidia* as deuterocystidia $21.5\text{--}32 \times 7\text{--}16 \mu\text{m}$, clavate and subfusiform, wall thin, with a brown or yellowish brown inclusion, negative to Patent blue V, some of them originating from the subhymenium and others from the hymenophoral trama. – *Cheilocystidia* $16\text{--}24 \times 5.5\text{--}8 \mu\text{m}$, lageniform with short neck, cylindrical, and clavate, some mucronate, wall thin, hyaline, some with yellowish green content, more evident in the apex. – *Subhymenium* ramose-inflated and cellular. – *Pileustrama* interwoven toward the pileipellis and radial to the hymenophoral trama, hyphae $8\text{--}18.5 \mu\text{m}$ diameter, yellowish, wall thin. – *Pileipellis* an ixocutis, $10\text{--}15 \mu\text{m}$ wide, hyphae $3\text{--}5 \mu\text{m}$ diameter, hyaline, wall thin. – *Pileocystidia* absent. – *Stipitipellis* a cutis, hyphae $4.5\text{--}16 \mu\text{m}$ diameter, yellowish, wall up to $0.5 \mu\text{m}$ thick. – *Caulocystidia* $24\text{--}45.5 \times 8\text{--}14.5 \mu\text{m}$, lageniform with a flexuose or submoniliform neck, utriform, cylindrical, and clavate, apex obtuse or mucronate, hyaline, wall thin, in groups in the upper part of the stipe.

Material examined. – USA, Montgomery Co., Pennsylvania, East of Evansburg, Evansburg State Park, alt. 67 m, 5 Jun 2005, *leg.* R.V. Gaines 51-b (XAL, holotype).

Remarks. – Guzmán *et al.* (2007a) separated the cheilocystidia into two types according to their shape. Initially, we thought that deuterocystidia could be chrysocystidia due to the coloured inclusion; however, they do not react with Patent blue V, so they are deuterocystidia following Clémenton's (2012) definition, but not chrysocystidia. Pleurocystidia with the same shape but without inclusion were observed in *P. magnispora* (see under this species).

Psilocybe quebecensis Ola'h & R. Heim, C. r. hebd. Séanc. Acad. Sci., Paris, Sér. D 264: 1601. 1967. – Figs. 103–104.

Basidiospores (10.4) $11.2\text{--}13.0 \times (5.2) 6.4\text{--}8.0 \times 6.4\text{--}8.0 \mu\text{m}$, $Q = 1.4\text{--}1.8$, ellipsoid and elongate, few fusiform in frontal and lateral views, yellowish brown, wall thick ($1 \mu\text{m}$ thick), with two layers in light microscopy, with germ pore. – *Subhymenium* ramose-inflated, yellowish. – *Pileustrama* radial, hyphae $6.5\text{--}24 \mu\text{m}$ diameter, yellowish, wall thin. – *Pileipellis* an ixocutis, $16\text{--}38.5 \mu\text{m}$ wide, hyphae $2.5\text{--}5 \mu\text{m}$ diameter, hyaline, wall thin. – *Pileocystidia* absent. – *Stipitipellis* a cutis, hyphae $2.5\text{--}14.5 \mu\text{m}$ diameter, yellowish, wall thin or up to $0.5 \mu\text{m}$ thick. – *Caulocystidia* $27\text{--}43 \times 6.5\text{--}9 \mu\text{m}$, lageniform, few cylindrical, apex obtuse, hyaline, sometimes with yellowish content, wall thin to thick. – *Stipe basal mycelium* white, hyphae hyaline, wall thin.

Material examined. – CANADA, Province of Quebec, Jacques Cartier River Valley, *leg.* R. Heim & G.M. Ola'h 092 (QUE, holotype).

Remarks. – We complement the description of Ola'h & Heim (1967) and Guzmán (1983); the subhymenium is not cellular, the arrangement of the pileus trama is reported, and we observed caulocystidia. According to Guzmán (1978), this species is close to *P. aztecorum* R. Heim, with both in sect. *Aztecorum* Guzmán.

Psilocybe samuiensis Guzmán, Bandala & J.W. Allen, Mycotaxon 46: 156. 1993. – Figs. 105–107.

Basidiospores 9.6–12.0 × 6.4–8.4 × 5.6–7.2 μm, Q = 1.2–1.6, hexagonal and subrhomboid in frontal view, Q = 1.4–1.8, ellipsoid and elongate in lateral view, yellowish brown, wall thick (1–1.2 μm thick), with two layers in light microscopy, with germ pore. – **Pleurocystidia** 14.5–22 × 4–6.5 μm, narrowly lageniform and lageniform, with short neck, hyaline, wall thin. – **Pileus trama** radial, hyphae 9–16 μm diameter, yellowish to yellowish brown in mass, wall thin. – **Pileipellis** an ixocutis, 12 μm wide, hyphae 2–3 μm diameter, hyaline, wall thin. – **Pileocystidia** absent. – **Stipitipellis** a cutis, hyphae 2.5–7 μm diameter, yellowish, wall up to 0.5 μm thick. – **Caulocystidia** 20–33.5 × 4.5–8 μm, narrowly lageniform and cylindrical-flexuose, sometimes branched, hyaline, wall thin, in groups in upper and middle parts of stipe. – **Stipe basal mycelium** white, hyphae hyaline.

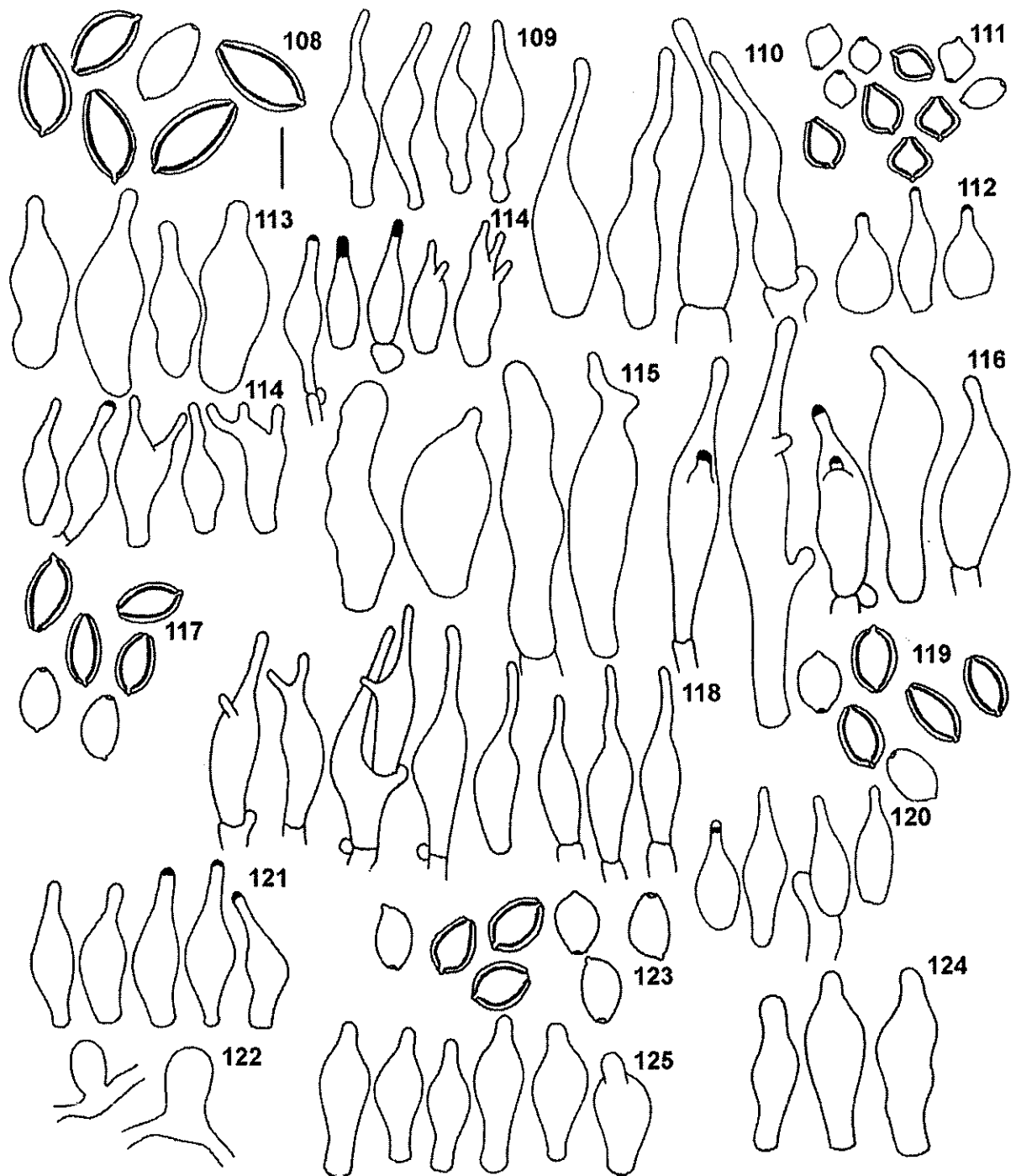
Material examined. – THAILAND, 2 km west to Ban Hua Thanon, Ko Samui, gregarious to scattered in rice paddies, 8 Aug 1991, leg. J. Allen F (XAL, holotype).

Remarks. – Additional features not described by Guzmán *et al.* (1993), such as the arrangement of the pileus trama and the presence of caulocystidia, are provided here. The basidiospores are hexagonal and subrhomboid. This species is very similar to *P. mexicana*; however, *P. mexicana* has shorter basidiospores 8–10 (12) μm long. Another similar species is *P. atlantis*, with smaller spores and strongly branched cheilocystidia, as discussed already.

Psilocybe serbica M.M. Moser & E. Horak, Z. Pilzk. 34(3–4): 138. 1968 (1969). – Figs. 108–110.

Basidiospores 8.0–12.0 (14.4) × 6.4–7.2 μm, Q = 1.4–1.8 (2), ellipsoid and elongate, few cylindrical, fusiform in frontal and lateral views, yellowish brown, wall thick (up to 1 μm thick), with two layers in light microscopy, with germ pore. – **Pleurocystidia** 23–27 × 4.5–6.5 μm, narrowly lageniform, pedicellate, apex obtuse, refractive, hyaline, wall thin, common. – **Subhymenium** ramose-inflated, yellowish. – **Pileus trama** radial, hyphae 10.5–19 μm diameter, yellowish, wall 0.5–0.8 μm thick. – **Pileipellis** an ixocutis, 48–56 μm wide, hyphae 2.5–5 μm diameter, hyaline, wall thin. – **Pileocystidia** absent. – **Stipitipellis** a cutis, hyphae 2.5–14.5 μm diameter, yellowish, wall thin or up to 0.5 μm. – **Caulocystidia** 30.5–46.5 × 4.5–8 μm, narrowly lageniform, some flexuose, apex obtuse, hyaline, wall thin. – **Stipe basal mycelium** white to yellowish, hyphae hyaline.

Material examined. – SERBIA, bei Mitrovats, Tara, auf Erde oder faulendem Laub and modrigen Holzstückchen unter Buchen (*Fagus sylvatica*) in Mischwald (mit *Abies* gemischt), 11 Oct 1963, leg. M.M. Moser 63/727 (IB, holotype).



Figs. 108–125. Microscopic structures: **108–110.** *Psilocybe serbica* (Holotype). **108.** Basidiospores. **109.** Pleurocystidia. **110.** Caulocystidia. **111–116.** *P. singularis* (Holotype). **111.** Basidiospores. **112.** Leptocystidia. **113.** Deuterocystidia. **114.** Cheilocystidia. **115.** Pileocystidia. **116.** Caulocystidia. **117–118.** *P. stuntzii* (Isotype). **117.** Basidiospores. **118.** Cheilocystidia. **119–122.** *P. subacutipilea* (Isotype). **119.** Basidiospores. **120.** Pleurocystidia. **121.** Cheilocystidia. **122.** Pileocystidia. **123–125.** *P. subaeruginascens* (Holotype). **123.** Basidiospores. **124.** Pleurocystidia. **125.** Cheilocystidia. Bar 8 μm .

Remarks. – Moser & Horak (1968) reported basidiospores of $9\text{--}11$ (12) \times $5.5\text{--}6.5$ (7) μm . Guzmán (1983) considered the pleurocystidia as rare or absent, although they were commonly observed in the present study. We report here a complex basidiospore wall, the subhymenium type, the arrangement of the pileus trama, and abundant caulocystidia.

Psilocybe singularis Guzmán, Escalona & J.Q. Jacobs, in Guzmán, Escalona, Ramírez-Guillén & Jacobs, *Int. J. Med. Mush.* 6(3): 283. 2004. – Figs 111–116.

Basidiospores $4.8\text{--}6.4$ (7.2) \times $4.0\text{--}5.6$ (6.4) \times $3.2\text{--}4.0$ μm , $Q = 1.1\text{--}1.2$, rhomboid and subrhomboid in frontal view, $Q = 1.2\text{--}1.5$, ellipsoid and broadly ellipsoid in lateral view, yellowish brown, wall thick ($0.8\text{--}1$ μm), with two layers in light microscopy, with germ pore, some with two pores. – **Pleurocystidia** as leptocystidia $12\text{--}16 \times 4\text{--}8$ μm , lageniform and narrowly lageniform, with short neck, hyaline, wall thin, originating from the subhymenium. – **Pleurocystidia** as deuteroecystidia $16.5\text{--}27 \times 6.5\text{--}9.5$ μm , utriform, and lageniform with short neck, apex obtuse, yellowish grey, wall thin, originating from the hymenophoral trama. – **Cheilocystidia** $11\text{--}19 \times 4\text{--}7$ μm , narrowly lageniform, some branched with two or three necks, hyaline, wall thin. – **Subhymenium** ramose. – **Pileus trama** radial, hyphae $1.5\text{--}20$ μm diameter, yellowish to yellowish brown in mass, wall thick ($0.8\text{--}2$ μm thick) with encrusted pigment. – **Subpellis** differentiated, hyphae $3\text{--}7$ μm diameter, yellowish, wall thick with yellowish brown, encrusted pigment. – **Pileipellis** an ixocutis, $13\text{--}20$ μm thick, hyphae $1.5\text{--}3$ μm diameter, hyaline, wall thin, in some parts with groups of erect cystidia. – **Pileocystidia** $26.5\text{--}41.5 \times 6.5\text{--}15$ μm , cylindrical-flexuose, clavate, narrowly clavate, sometimes mucronate or with a neck, hyaline, wall thin, in groups. – **Caulocystidia** $21.5\text{--}53.5 \times 5\text{--}9$ μm , narrowly lageniform, apex subcapitate, sometimes with one or two additional lateral necks also with subcapitate apex, in groups near the stipe apex.

Material examined. – MEXICO, Oaxaca, Tuxtepec, near Llano Grande, 5 Jul 1980, *leg.* J.Q. Jacobs 158 (XAL, holotype).

Remarks. – We found that there are two types of pleurocystidia, the ones mentioned by Guzmán *et al.* (2004b), here called deuteroecystidia and leptocystidia. To the original description, we also add the presence of pileocystidia in groups, type of subhymenium, arrangement of the pileus trama, and presence of caulocystidia.

Psilocybe stuntzii Guzmán & J. Ott, *Mycologia* 68(6): 1261. 1976 (1977). – Figs. 117, 118.

Basidiospores $8.0\text{--}9.6 \times 5.6\text{--}6.4 \times 5.6\text{--}6.4$ μm , $Q = 1.3\text{--}1.5$ (1.7), ellipsoid and elongate, subfusiform, few widely ellipsoid in frontal view, $Q = 1.4\text{--}1.7$, ellipsoid and elongate in lateral view, yellowish brown, wall 0.8 μm thick, with two layers in light microscopy, with germ pore. – **Cheilocystidia** $20.5\text{--}33.5 \times 4\text{--}6.5$ μm , narrowly lageniform, sometimes apex bifurcated or with a short lateral branch, apex obtuse, refractive, hyaline. – **Subhymenium** ramose-inflated. – **Pileus trama** radial, interwoven near the

pileipellis, hyphae 4.5–25.5 μm diameter, yellowish, wall thin. – *Pileipellis* an ixocutis, 64–72 μm wide, hyphae 1.5–3 μm diameter, hyaline, wall thin. – *Pileocystidia* absent. – *Caulocystidia* not observed.

Material examined. – USA, Washington, Seattle, University of Washington, campus, *leg.* D. Stuntz s.n. Autumn 1973 (XAL, isotype).

Remarks. – In the isotype, the pileipellis is a thick ixocutis instead of a “thin pellicle of filamentous more or less subgelatinized hyphae” according to Guzmán & Ott (1976, p. 1263).

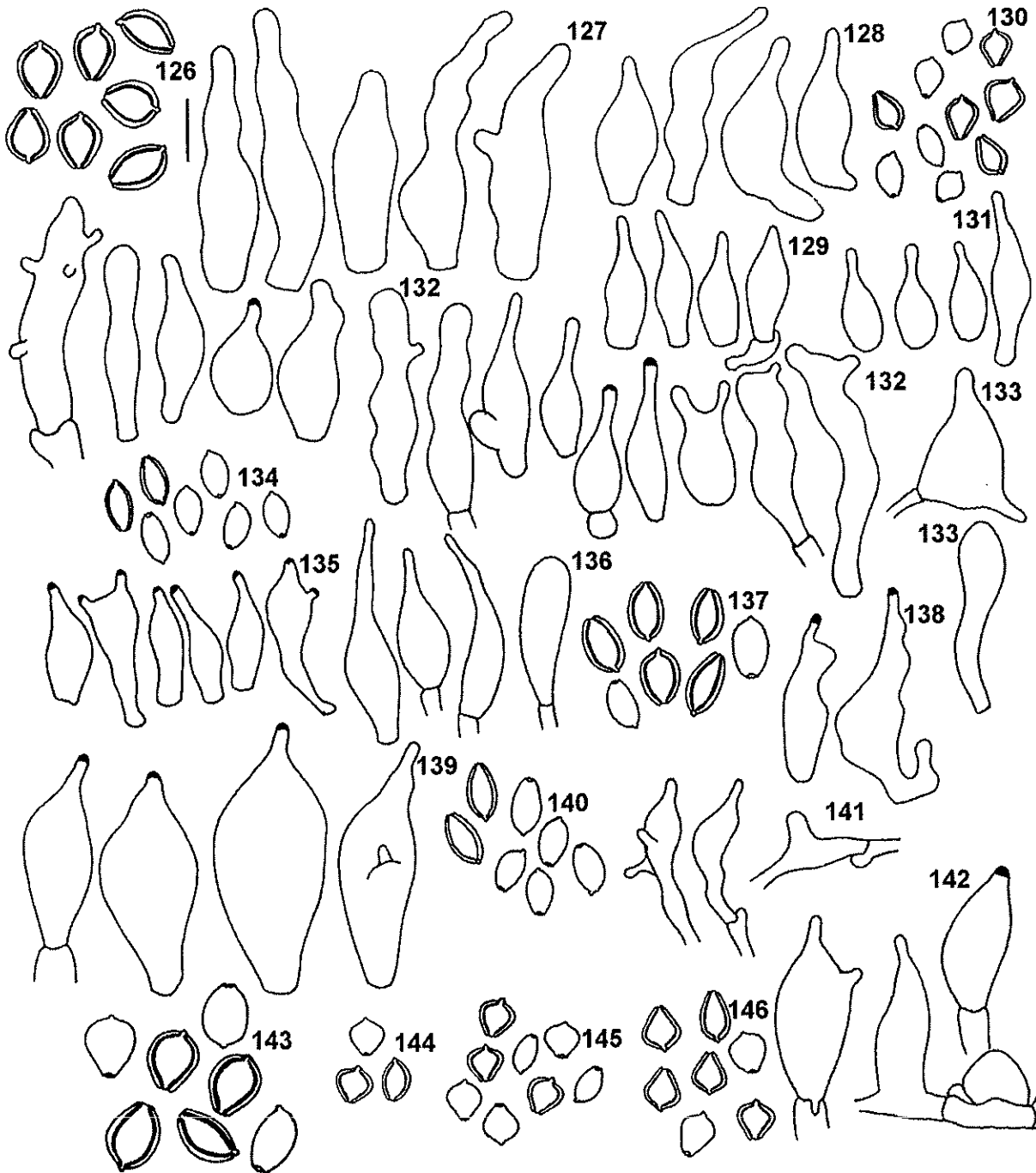
Psilocybe subacutipilea Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez, *Mycotaxon* 51: 230. 1994. – Figs. 119–122.

Synonym. – *Psilocybe mexicana* R. Heim, *Revue Mycol.*, Paris 22: 77. 1957.

Basidiospores 7.2–9.6 \times 5.6–6.4 \times 5.6–6.4 μm , $Q = 1.3$ – 1.6 , hexagonal in frontal view, ellipsoid in lateral view, yellowish brown, wall thick (1 μm thick), with two layers in light microscopy, with germ pore. – *Pleurocystidia* 12.5–20 \times 4.5–7 μm , narrowly lageniform, with short neck, hyaline, wall thin, common. – *Cheilocystidia* 14.5–22.5 \times 4–7 μm , lageniform, apex obtuse, few subcapitate, hyaline, wall thin. – *Subhymenium* ramose-inflated. – *Pileus trama* radial, hyphae 3–13 μm diameter, yellowish to yellowish brown in mass, wall thin. – *Pileipellis* an ixocutis, 24 μm wide, hyphae 1.5–3 μm diameter, hyaline, wall thin. – *Pileocystidia* approximately 8–13 \times 5–6.5 μm , globose and subglobose, as lateral hyphae inflations, hyaline, wall thin. – *Stipitipellis* a cutis, hyphae 1.5–9 μm diameter, yellowish, wall up to 0.5 μm thick. – *Caulocystidia* approximately 24–26 \times 5–6 μm , lageniform, hyaline, wall thin.

Material examined. – COLOMBIA, Department of Antioquia, Municipio Porce, near the road from Medellín to Amalfi, zone of Puente Gabino, 31 Aug 1990, *leg.* G. Guzmán 29561-B (XAL, isotype).

Remarks. – We found here that pleurocystidia, not mentioned in Guzmán *et al.* (1994), are common, also observed were larger cheilocystidia, the arrangement of the pileus trama, and the presence of modified hyphae in the pileipellis, although difficult to see and measure. This species was described from Colombia, and based on the present revision, we propose its synonymy with *P. mexicana*. All micro- and macroscopic features agree very well with the holotype of *P. mexicana* and additional studied specimens. The description of *P. subacutipilea* was based only on one specimen (Guzmán 29561-B), which lacks pseudorhiza, which was probably lost during collection.



Figs. 126–146. Microscopic structures. **126–129.** *Psilocybe subtropicalis* (Holotype). **126.** Basidiospores. **127.** Deuterocystidia. **128.** Caulocystidia. **129.** Leptocystidia. **130–133.** *P. subyungensis* (Holotype) **130.** Basidiospores. **131.** Pleurocystidia. **132.** Cheilocystidia. **133.** Pileocystidia. **134–136.** *P. taiwanensis* (Isotype). **134.** Basidiospores. **135.** Pleurocystidia. **136.** Caulocystidia. **137–139.** *P. thaicordispora* (Isotype). **137.** Basidiospores. **138.** Caulocystidia (G. Guzmán 38346). **139.** Caulocystidia (Isotype). **140–142.** *P. thaizapoteca* (Holotype). **140.** Basidiospores. **141.** Pileocystidia. **142.** Caulocystidia. **143.** *P. wayanadensis* (Holotype), basidiospores. **144–146.** *P. yungensis*. **144.** Basidiospores (Holotype). **145.** Basidiospores (Syntype of *P. isauri*). **146.** Basidiospores (G. Heredia s.n.). Bar 8 µm.

Psilocybe subaeruginascens Höhn., Sitzungsber. Kaiserl. Akad. Wiss. Wien Math.-Naturwiss. Kl. Abt. I, 123: 78. 1914. – Figs. 123–125.

B a s i d i o s p o r e s 7.2–9.0 × 4.8–6.4 × 4.4–5.6 μm, Q = 1.3–1.5, hexagonal in frontal view, Q = 1.4–1.8, ellipsoid and elongate, some amygdaliform in lateral view, yellowish brown, wall thick (1–1.3 μm thick), with two layers in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** 17.5–24 × 8–11 μm, fusiform with short and wide neck and utriform, hyaline to yellowish, wall thin, common. – **C h e i l o c y s t i d i a** 14.5–21.5 × 5–8 μm, lageniform and fusiform, with short neck, apex obtuse, hyaline, wall thin. – **S u b h y m e n i u m** not observed. – **P i l e u s t r a m a** interwoven, hyphae 6–16 μm diameter, yellowish, wall thin. – **P i l e i p e l l i s** an ixocutis, 16 μm wide, hyphae 2.5–4 μm diameter, hyaline, wall thin.

M a t e r i a l e x a m i n e d . – INDONESIA, Java, Buitenzorg, 1907, *leg.* F. Höhnel 3942A (FH, holotype).

R e m a r k s . – The type specimen consists of fragmented basidiomata and broken fragments of stipes and pilei. The basidiospores were originally described by Höhnel (1914) as 10 × 7 × 5 μm, rhomboid and rhomboid-limoniform in frontal view, slightly depressed in lateral view, and cystidia absent. Singer & Smith (1958b) later described the Höhnel species with basidiospores 7.7–10 (11.5) × 7–7.8 × 6–7 μm, ovoid to subrhomboid, pleurocystidia 25–33 × 9–12 μm, cheilocystidia 20–33 × 6–9 μm, and pileipellis not gelatinized. We modify the description of the species with characteristics of the pleurocystidia, cheilocystidia, and pileipellis. Horak & Desjardin (2006) studied this specimen and a recent collection, considered as a topotype. According to Horak & Desjardin (2006), the topotype has slightly larger basidiospores, pleurocystidia absent, and a nongelatinized cutis, so it probably does not correspond to this species. *Psilocybe subaeruginascens* resembles *P. magnispora* and *P. aquamarina*; however, the gelatinized layer present in the lamellar edge of these two species, and distinct size of basidiospores, larger in *P. aquamarina* and smaller in *P. magnispora*, separate them. *Psilocybe subaeruginascens* has only been known from Asia.

Psilocybe subtropicalis Guzmán, Biblioth. Mycol. 159: 107. 1995. – Figs. 126–129.

S y n o n y m . – *Psilocybe bipleurocystidiata* E. Horak & Guzmán, in Guzmán, Horak, Halling & Ramírez-Guillén, Sydowia 61(2): 216. 2009.

B a s i d i o s p o r e s 6.4–8.0 × 5.6–6.4 (7.2) × 4.4–4.8 μm, Q = 1.14–1.28, subhexagonal and subrhomboid in frontal view, Q = 1.3–1.5, ellipsoid in lateral view, yellowish brown, wall thick (0.8–1 μm thick), with two layers in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** as leptocystidia 14.5–20 × 4–5.5 μm, lageniform and fusiform, apex obtuse, hyaline, wall thin, originating from the subhymenium. – **P l e u r o c y s t i d i a** as deutero-cystidia 26.5–39 (41.5) × (6.5) 7–8 μm, narrowly utriform, cylindrical-flexuose, sublageniform, neck long and flexuose, hyaline, wall thin, originating from the hymenophoral trama. – **S u b h y m e n i u m** ramose-inflated. – **H y m e n o p h o r a l t r a m a** subregular, hyphae 5.5–21.5 μm diameter, yellowish, wall 0.8 μm thick, with encrusted pigment. – **P i l e u s t r a m a** radial, hyphae 17.5–34.5 μm diameter, yellowish to yellowish brown in mass, wall 0.5–0.8 μm, with encrusted pigment. – **P i l e i p e l l i s** an ixocutis, 20–24 μm wide,

hyphae 2.5–5.5 μm diameter, hyaline, wall thin. – Pileocystidia absent. – Stipitipellis a cutis, hyphae 5.5–11 μm diameter, yellowish, wall up to 0.8 μm thick. – Caulocystidia 19–25 \times 5.5–9 μm , fusiform, lageniform, flexuose, with obtuse apex, hyaline, wall thin, in groups only in the upper part of the stipe. – Stipe basal mycelium white, hyphae 2.5–4 μm diameter, hyaline, wall thin.

Material examined. – MEXICO, Veracruz, old road Xalapa-Coatepec, km 2.5, Ecological Park Francisco Javier Clavijero, 6 Oct 1986, leg. L. Montoya 910 (XAL, holotype).

Remarks. – The basidiospores are subhexagonal besides subrhomboid. We found deuteroconidia originating from the hymenophoral trama in spite of Guzmán (1995) only mentioning hyaline conidia that correspond to leptocystidia. The subhymenium is ramose-inflated instead of cellular, the arrangement of the pileus trama is mentioned, and caulocystidia were found in the upper part of the stipe. We synonymized *P. bipleurocystidiata* (see above) with this species, because they agree well in all features but the thickness of the ixocutis (wider in *P. subtropicalis* than in *P. bipleurocystidiata*).

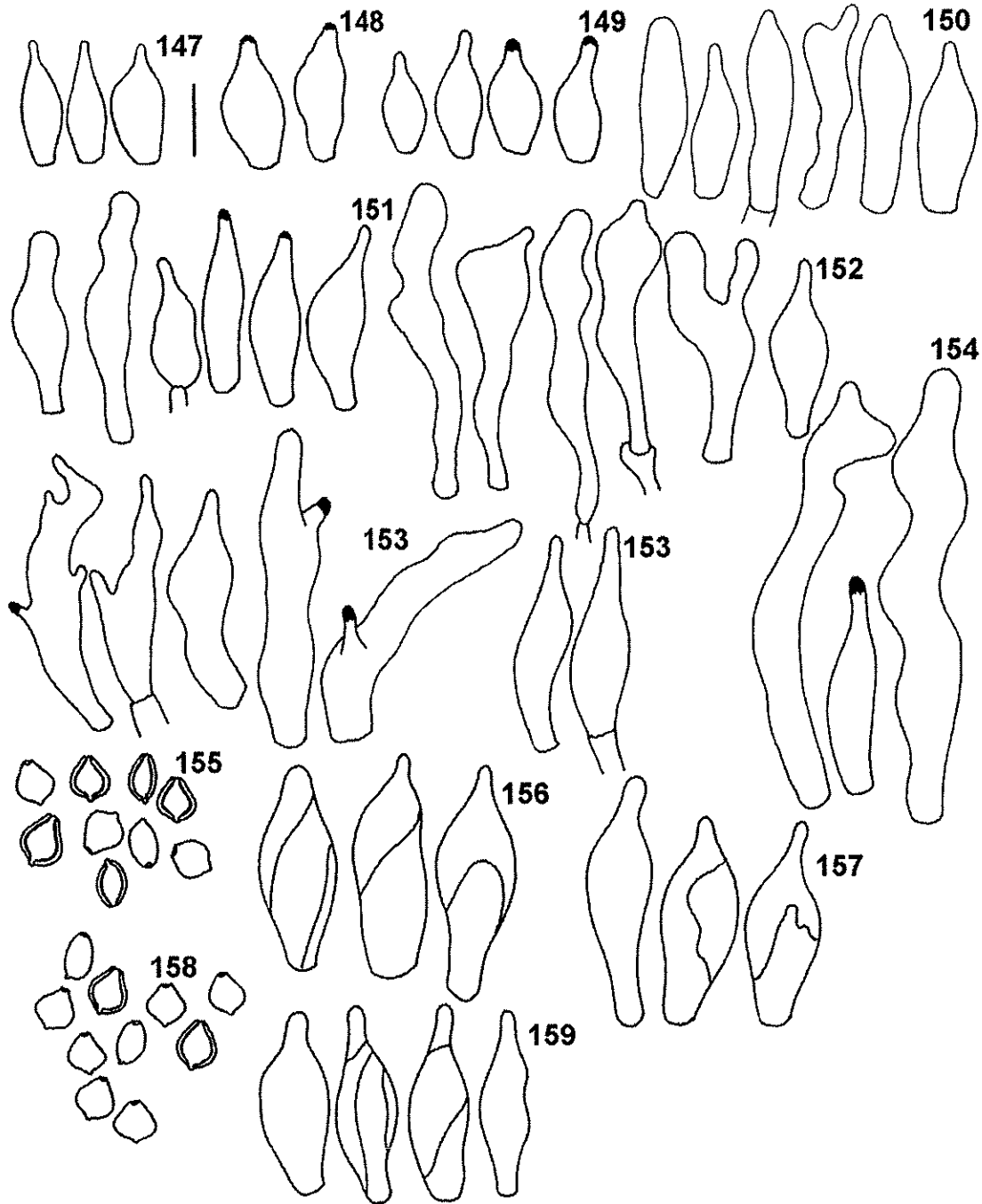
Psilocybe subyungensis Guzmán, Mycotaxon 7(2): 249. 1978. – Figs. 130–133.

Synonym. – *Psilocybe yungensis* Singer & A.H. Sm., Mycologia 50(1): 142. 1958.

Basidiospores 4.8–5.6 (6.4) \times 4.0–4.4 (6.4) \times 3.2–3.6 μm , Q = 1.09–1.2, rhomboid and subrhomboid in frontal view, Q = 1.2, ellipsoid in lateral view, yellowish brown, wall 0.5–0.6 μm thick, with two layers in light microscopy, with germ pore. – Pleurocystidia 13–21.5 \times 5–9 μm , lageniform and narrowly lageniform, wall thin, hyaline. – Cheilocystidia 13.5–40 \times 5–9 μm , very variable in shape, cylindrical-flexuose, narrowly lageniform, and lageniform, sometimes branched or with short apical or lateral appendices, hyaline, wall thin. – Pileus trama radial, hyphae 8–24 μm diam., hyaline, wall thick (0.8–2.4 μm thick) with irregular encrusted pigment. – Subpellis undifferentiated. – Stipe basal mycelium yellowish, with setaceous hyphae 1.5–4 μm diameter, yellowish brown, wall thick (0.5–0.9 μm thick). Pileipellis a subgelatinous cutis 10–12 μm wide, hyphae 2–3 μm diameter. Pileocystidia approximately 17–24 \times 5.6–9.5 μm , cylindrical or variable in shape.

Material examined. – VENEZUELA, Vicinity of El Arado, 12 km SW of Makarao, Edo. Miranda, leg. K.P. Dumont VE6363 (NY, holotype).

Remarks. – We observed larger pleurocystidia and cheilocystidia than those of 8–11 \times 3.8–5.5 μm , and 15.5–25 \times (5.5) 7.7–12 μm , respectively, described by Guzmán (1978, 1983). For many years, *P. subyungensis* and *P. yungensis* were considered as independent species, based only on the branched cheilocystidia observed in *P. subyungensis*. However, in the present study, we found branched cheilocystidia in the type of *P. yungensis* and in several specimens determined as *P. yungensis*. Both *P. yungensis* and *P. subyungensis* have a conical to campanulate pileus, frequently acutely papillate or mamiform, and very narrow lamellae, typical macroscopic characteristics of *P. yungensis* according to Heim (1958), Singer & Smith (1958b), and Guzmán (1983). We observed pileocystidia but difficult to observe and measure. Pileocystidia were not observed in the types of *P. yungensis* and *P. chiapanensis*; however, this character is not enough to separate these species.



Figs. 147–159. Microscopic structures: **147–154.** *Psilocybe yungensis*. **147.** Pleurocystidia (Holotype). **148.** Pleurocystidia (Syntype of *P. isauri*). **149.** Pleurocystidia (G. Heredia s.n.). **150.** Cheilocystidia (Holotype). **151.** Cheilocystidia (Syntype of *P. isauri*). **152.** Cheilocystidia (G. Heredia s.n.). **153.** Cheilocystidia (M. A. Gómez 2467). **154.** Caulocystidia (M. A. Gómez 2467). **155–157.** *Deconica aureicystidiata* (Isotype). **155.** Basidiospores. **156.** Chrysocystidia. **157.** Chrysocystidia (E. Horak 7310). **158–159.** *D. neocaledonica* (Isotype). **158.** Basidiospores. **159.** Chrysocystidia. Bar 8 μm .

Psilocybe taiwanensis Zhu L. Yang & Guzmán, Sydowia 62(2): 185. 2010. – Figs. 134–136.

B a s i d i o s p o r e s 4.8–6.4 (7.0) × 3.2–4.0 × 3.2–3.6 μm, Q = 1.2–1.6 (2.0), ellipsoid to elongate, ovoid with slightly defined angles in frontal view, Q = 1.5–1.8, ellipsoid in lateral view, yellowish brown, wall 0.4–0.5 (0.6) μm thick, with one layer in light microscopy, with germ pore. – **P l e u r o c y s t i d i a** 13.5–21.5 (32) × 4–8 (10.5) μm, narrowly lageniform, fusiform, with short neck, some with two necks, apex obtuse, refractive, hyaline, wall thin. – **S u b h y m e n i u m** ramose-inflated. – **P i l e u s t r a m a** radial, hyphae 5–21.5 μm diameter, yellowish to yellowish brown in mass, wall thin. – **C a u l o c y s t i d i a** 20–40 × 5–7 μm, narrowly lageniform, clavate, as modified hyphae, apex obtuse, hyaline, wall.

M a t e r i a l e x a m i n e d . – CHINA, Taiwan Province, Nantou Country, Shitou, 12 Apr 2006, *leg.* Z.L. Yang 4637 (XAL, isotype).

R e m a r k s . – Additional features, not mentioned in the protologue, such as type of subhymenium, arrangement of the pileus trama, and size of caulocystidia are indicated. Furthermore, we extend the size range of pleurocystidia, cited as 15–20 × 5.5–6.5 (7) μm (Guzmán & Yang 2010). This species is very similar to the recently described *P. thaizapoteca* Guzmán (Guzmán *et al.* 2012), but a remarkable feature to separate them is the persistent annulus in *P. taiwanensis* as opposed to a poorly developed one in *P. thaizapoteca*, present only as fibrillar remnants. In addition, *P. thaizapoteca* has strongly branched cheilocystidia. The interpretation of the basidiospore shape and wall thickness moved Guzmán & Yang (2010) to place *P. taiwanensis* in sect. *Stuntzae*, but we think that it actually belongs to sect. *Zapotecorum* because of the size, shape, and wall thickness of the basidiospores and the size and shape of the pleurocystidia and cheilocystidia. The basidiomata features also resemble *P. zapotecorum* or *P. angustipleurocystidiata* Guzmán (both in sect. *Zapotecorum*).

Psilocybe thaicordispora Guzmán, Ram.-Guill. & Karunarathna, in Guzmán, Ramírez-Guillén, Hyde & Karunarathna, Mycotaxon 119: 72. 2012. – Fig. 137–139.

B a s i d i o s p o r e s 6.4–8.0 × 4.8–5.6 (7.2) × 4.8–5.6 μm, Q = 1.1–1.5, hexagonal and subrhomboid, sometimes angles not well defined in frontal view, Q = 1.3–1.5 (1.8), ellipsoid and few elongate in lateral view, yellowish brown, wall 0.8–1 μm thick, with two layers in light microscopy, with germ pore. – **P i l e u s t r a m a** radial, hyphae 7–20 μm diameter, yellowish to yellowish brown in mass, wall up to 0.5 μm thick. – **S u b p e l l i s** undifferentiated. – **S t i p i t i p e l l i s** hyphae 4–13 μm diameter, yellowish, wall 0.4–0.9 μm thick. – **C a u l o c y s t i d i a** 24–35 × 9–16 μm, lageniform-flexuose, broadly fusiform, apex mucronate to rostrate, refractive, hyaline, wall thin.

M a t e r i a l e x a m i n e d . – THAILAND, Chiang Mai Prov., approximately 100 km from Chiang Mai, near Huai Nam Dang National Park, (19° 16' N, 98° 16' E), alt. 1500 m, rainforest dominated by *Pinus kesiya*, 12 Jul 2010, *leg.* G. Guzmán 38349 (XAL, isotype), *leg.* G. Guzmán 38346 (XAL).

R e m a r k s . – Additional features presented here are the caulocystidia, very similar to cheilocystidia in shape but larger, the arrangement of the pileus trama, and the undifferentiated subpellis. This species is very similar to *P. meridionalis* described by Guzmán *et al.* (2008a) from Mexico (see above); the well-developed veil, which leaves a membranous annulus on the

stipe in *P. meridionalis* but is poorly developed in *P. thaicordispora*, distinguishes them. The rest of the macroscopic features, such as the conical to campanulate pileus and the stipe slightly scaly toward the base with a subbulbose base, are very similar. Cheilocystidia in *P. meridionalis* were cited as $5\text{--}28 \times 3\text{--}6.5$ (8) μm (Guzmán *et al.* 2008a) and in *P. thaicordispora* as $12\text{--}20$ (28) \times $(4.5) 5\text{--}7$ (8) μm (Guzmán *et al.* 2012); however, in the isotype of *P. thaicordisporae*, we found cheilocystidia that were $5\text{--}8$ (10) μm wide, making the cystidia slightly wider in this species. The caulocystidia are also larger in *P. thaicordispora*, as opposed to $17.5\text{--}24 \times 5.5\text{--}8$ μm in *P. meridionalis* (Guzmán *et al.* 2008a).

Psilocybe thaizapoteca Guzmán, Karunarathna & Ram.-Guill. in Guzmán, Ramírez-Guillén, Hyde & Karunarathna, Mycotaxon 119: 77. 2012. – Figs. 11, 140–142.

B a s i d i o s p o r e s $5.6\text{--}7.2 \times 3.2\text{--}4.0 \times 3.2\text{--}4.8$ μm , $Q = 1.4\text{--}1.7$ (1.8), ellipsoid to slightly subrhomboid in frontal view, $Q = 1.2\text{--}1.7$ (2), ellipsoid and some elongate in lateral view, yellowish brown, wall $0.5\text{--}0.7$ μm , with one layer in light microscopy, with germ pore. – **S u b h y m e n i u m** ramose-inflated, sometimes seems cellular. – **P i l e u s t r a m a** radial, hyphae $4\text{--}30$ μm diam., yellowish, wall 0.8 μm thick. – **P i l e i p e l l i s** a subgelatinized layer 10 μm wide, hyphae $1.6\text{--}4$ μm diameter. – **P i l e o c y s t i d i a** $12\text{--}21 \times 6.5\text{--}9$ μm , lageniform, sometimes with lateral short and narrow branches, and cylindrical as lateral branches of the hyphae, hyaline, wall thin. – **C a u l o c y s t i d i a** $16\text{--}23 \times 5\text{--}9.5$ μm , fusiform and conical, apex mucronate to rostrate, hyaline, wall thin, in the upper part of the stipe.

M a t e r i a l e x a m i n e d . – THAILAND, Chiang Mai Prov., close to Huai nam Dang National Park ($19^\circ 16' \text{N}$, $98^\circ 16' \text{E}$), alt. 1500 m, rainforest dominated by *Pinus kesiya*, 12 Jul 2010, *leg.* G. Guzmán 38342 (XAL, holotype).

R e m a r k s . – New data for this species are the presence of pileocystidia and caulocystidia, the arrangement of the pileus trama, the subgelatinized pileipellis, and the type of subhymenium. *Psilocybe thaizapoteca* is very similar to *P. taiwanensis* in nearly all the features, except *P. taiwanensis* has a persistent annulus and weakly branched cheilocystidia. As noted by Guzmán *et al.* (2012), this species is a member of sect. *Zapotecorum* and is very similar to *P. angustipleurocystidiata*.

Psilocybe wayanadensis K.A. Thomas, Manim. & Guzmán, in Thomas, Manimohan, Guzmán, Tapia & Ramírez-Guillén, Mycotaxon 83: 198. 2002. – Fig. 143.

B a s i d i o s p o r e s $8.0\text{--}8.8 \times 5.6\text{--}7.2 \times 4.2\text{--}5.6$ μm , $Q = 1.16\text{--}1.3$, subhexagonal and subrhomboid in frontal view, $Q = 1.4\text{--}1.8$, ellipsoid in lateral view, yellowish brown, wall thick ($0.8\text{--}1.2$ μm thick), with two layers in light microscopy, with germ pore. – **S u b h y m e n i u m** ramose. – **P i l e u s t r a m a** radial, hyphae $3\text{--}13$ μm diameter, yellowish, wall thin. – **P i l e i p e l l i s** a subgelatinized cutis, $8\text{--}10.5$ μm wide, hyphae $1.5\text{--}2.5$ μm diameter, yellowish, wall thin. – **P i l e o c y s t i d i a** absent.

M a t e r i a l e x a m i n e d . – INDIA, Kerala State, Wayanad District, Muthanga, 21 Jul 1999, *leg.* K.A. Thomas T-320a (XAL, holotype).

Remarks. – We add a subhexagonal basidiospore to the shape described in the protologue and subgelatinized nature of the cutis, and pileus trama and subhymenium arrangement. This species is very similar to *P. subaeruginascens* and *P. magnispora* (see above), in the size and shape of the basidiospores, pleurocystidia, and cheilocystidia; *P. magnispora* also has a gelatinized lamellar edge that makes it more similar to *P. wayanadensis*. Thomas *et al.* (2002) described basidiospores of $8.0\text{--}9.5$ (11) \times $6.5\text{--}7$ (8) \times $5.5\text{--}6.0$ (6.5) μm in the holotype, for which we observed smaller basidiospores.

Psilocybe yungensis Singer & A.H. Sm., *Mycologia* 50(1): 142. 1958. – Figs. 144–154.

Synonyms: – *Psilocybe yungensis* var. *diconica* Singer & A.H. Sm., *Mycologia* 50(1): 142. 1958.

– *Psilocybe isauri* Singer, *Sydowia* 12(1–6): 237. 1959 (1958).

– *Psilocybe acutissima* R. Heim, *Revue Mycol.*, Paris 24: 106. 1959.

– *Psilocybe subyungensis* Guzmán, *Mycotaxon* 7(2): 249. 1978.

– *Psilocybe chiapanensis* Guzmán, *Biblioth. Mycol.* 159: 102. 1995.

Basidiospores (4.5) $5.0\text{--}6.0 \times 4.5\text{--}5 \times 3.5\text{--}4.0$ μm , $Q = 1.1\text{--}1.2$ (1.4), rhomboid and subrhomboid in frontal view, $Q = 1.2\text{--}1.5$, broadly ellipsoid and ellipsoid in lateral view, yellowish brown, wall $0.5\text{--}0.8$ μm thick, with two layers in light microscopy, with germ pore, some with two pores. – **Pleurocystidia** $15\text{--}21 \times 5\text{--}8$ μm , lageniform with short neck, apex obtuse, some refractive, hyaline, wall thin. – **Cheilocystidia** $14\text{--}32 \times 4\text{--}7$ (8) μm , clavate, cylindrical-flexuose, lageniform, hyaline, wall thin. – **Subhymenium** ramose-inflated and sometimes ramose. – **Pileus trama** radial, hyphae $5\text{--}16$ μm diameter, yellowish to yellowish brown in mass, wall $0.5\text{--}0.8$ μm thick, with encrusted pigment; with lactiferous hyphae. – **Pileipellis** an ixocutis, hyphae $3\text{--}5$ μm diameter, hyaline, wall thin. – **Pileocystidia** not observed. – **Stipe basal mycelium** yellow with setaceous hyphae $3\text{--}5$ μm in diameter, wall thick ($0.5\text{--}0.8$ μm).

Material examined. – BOLIVIA, La Paz, Nor-Yungas, Cataratas San Juan, alt. 2400 m, on very rotten, woody detritus in a shady place among mosses and hepatics, 28 Jan 1956, *leg.* R. Singer B-648 (MICH, isotype). MEXICO, Oaxaca, east of Huautla de Jiménez, rancharía Agua Fierro, alt. 1600 m, 13 Jul 1959, *leg.* G. Guzmán 1128-B (XAL, syntype of *Psilocybe isauri*); Jalisco, Municipality of Cuautitlán, Sierra de Manantlán, 8 km to northwest of Las Joyas, 18 Aug 1982, *leg.* G. Nieves 138; Tamaulipas, Municipality of Gómez Farías, Reserva de la Biosfera El Cielo, rancho El Cielo, Summer 1987, *leg.* G. Heredia s.n. (as *P. yungensis* in IBUG), 22 Jul 1988, *leg.* V.M. Bandala-Muñoz 1428; Veracruz, Municipality of San Andrés Tlalnahuayocan, Agüita Fría, alt. 1520 m, 8 Jul 2007, *leg.* M. A. Gómez 2467 (IBUG); Municipality of San Andrés Tuxtla, Reserva de la Biosfera Los Tuxtlas, slopes of volcano San Martín Tuxtla, alt. 900 m, 30 Oct 2010, *leg.* A. Cortés-Pérez 549.

Remarks. – In the protologue, pleurocystidia, pileus trama, and subhymenium were not described. Subsequent work (Singer & Smith 1958b; Heim 1958; Guzmán 1983) mentioned size and shape of pleurocystidia and the subhymenium type. We contribute here the presence of caulocystidia and setaceous hyphae in the basal mycelium of the stipe. Studies of the type of *P. yungensis*, *P. isauri*, and additional specimens helped us to clarify the concept of *P. yungensis* and to understand the variation in this taxon. The variability of the cheilocystidia is notable: $13\text{--}40$ (53) \times $4.8\text{--}8$ μm , cylindrical, flexuose, clavate, lageniform, sometimes branched. Caulocystidia were observed in Heredia s.n., Bandala-Muñoz 1428, Gómez 2467, Nieves 138, and Cortés-Pérez 549, being $24\text{--}53.5 \times 5\text{--}9.5$ μm , narrowly lageniform and cylindrical-flexuose, hyaline to yellowish, with lateral branches, in the middle and upper part of the stipe, but were not observed in the type specimen.

New combinations in *Deconica*

Deconica aureicystidiata (E. Horak & Desjardin) Ram.-Cruz & Guzmán, **comb. nov.** – Figs. 23, 155–157.

MycoBank no.: MB 803997

Basionym. – *Psilocybe aureicystidiata* E. Horak & Desjardin, *Sydowia* 58(1): 32. 2006.

Basidiospores 4.0–5.6 × 4.4–5.6 × 3.2–4.0 μm, Q = 1.1–1.2 (1.6) subrhomboid in frontal view, Q = 1.2–1.5 (1.6), subellipsoid in lateral view, yellowish brown, wall 0.5–0.9 μm thick, with two layers in light microscopy, with germ pore. – **Pleurocystidia** as chrysocystidia, 17–29 × 6.5–9.5 μm, clavate to broadly fusiform, apex mucronate or rostrate, hyaline, with a hyaline content in KOH and blue in Patent blue V, wall thin. – **Subhymenium** ramose-inflated. – **Pileus trama** radial, hyphae 4–8 μm diameter, yellowish to yellowish brown in mass, wall thin with encrusted pigment. – **Subpellis** undifferentiated. – **Pileipellis** an ixocutis, 8–10 μm wide, hyphae 2.5–3 μm diameter, hyaline, wall thin.

Material examined. – INDONESIA, Java, Cibodas, trail to Mt. Gedeh, between entrance and waterfalls, 10 Jan 1998, *leg.* E. Horak 7310 (XAL, isotype); Java, Mt. Halimun Salak National Park, Cikaniki Field Station, 8 Jan 1999, *leg.* E. & A. Horak 7310 (XAL).

Remarks. – New observations such as arrangement of the pileus trama, type of subhymenium, and presence of a gelatinized cutis are added to the original description. We confirmed the presence of chrysocystidia with the aid of Patent blue V. Although this species was described by Horak & Desjardin (2006) as lacking a bluing reaction, it had been placed in the bluing *Psilocybe* sect. *Neocaledonicae* (Guzmán 2004). It is separated from *D. neocaledonica* (see below) mainly by the presence of annulus in *D. aureicystidiata*. This species is similar to *D. thailandensis* described by Horak *et al.* (2009) in microscopic features such as size and shape of basidiospores, chrysocystidia, and cheilocystidia, as well as in the fibrillose, membranous annulus.

Deconica neocaledonica (Guzmán & E. Horak) V. Ram.-Cruz & Guzmán, **comb. nov.** – Figs. 7, 158–159.

MycoBank no.: MB 803998

Basionym. – *Psilocybe neocaledonica* Guzmán & E. Horak, *Sydowia* 31(1–6): 53. 1979 (1978).

Synonyms: – *Hypholoma neocaledonicum* (Guzmán & E. Horak) Guzmán, *Doc. Mycol.* 29(114): 66. 1999.

– *Naematoloma neocaledonicum* (Guzmán & E. Horak) Guzmán [as '*neocaledonica*'], *Mycotaxon* 12(1): 236. 1980.

– *Psilocybe overeemii* E. Horak & Desjardin, *Sydowia* 58(1): 30. 2006.

Basidiospores 5.0–5.5 (6.0) × 5.0–6.0 × 3.5–4.0 μm, Q = 1–1.2, subrhomboid and rhomboid in frontal view, Q = 1.3–1.7, ellipsoid and elongate in lateral view, yellowish brown, wall 0.4–0.8 μm thick, with two layers in light microscopy, with germ pore. – **Pleurocystidia** as chrysocystidia, 18–30 × 9–11 μm, fusiform, apex mucronate or rostrate, hyaline in KOH, with content that turns blue in Patent blue V, wall thin. – **Subhymenium** ramose-inflated. – **Pileus trama** radial, hyphae 4–17 μm diameter, yellowish to yellowish brown in mass, wall thin with encrusted pigment. – **Subpellis**

undifferentiated. – Pileipellis an ixocutis, thin (less than 8 µm wide), hyphae 2.5–3 µm in diameter, hyaline, wall thin with encrusted pigment. – Pleocystidia not observed. – Caulocystidia not observed.

Material examined. – NEW CALEDONIA, Mt. Mou, N of Paita, 22 Feb 1977, alt. 1000 m, leg. E. Horak 77–161 (ENCB, isotype of *Psilocybe neocaledonica*).

Remarks. – Additional features are the following: nonbluing mushroom, pileipellis a gelatinized layer, and the presence of chrysocystidia on the side of lamellae. Guzmán & Horak (1978) mentioned the possibility that this species had hallucinogenic properties for its similarity to the hallucinogenic species of the *Psilocybe* stirp *Yungensis*. However, this species differs in its macro- and micromorphological characteristics (see description of *P. yungensis* above). Also, Guzmán & Horak (1978) considered it similar to the bluing *P. naematoliformis* Guzmán, but in a recent type study of *P. naematoliformis*, we observed that the pleurocystidia are hyaline or have a yellowish brown pigment in the vacuoles or sometimes filling the whole cystidium but not reacting with Patent blue V. This species is the type of the sect. *Neocaledonicae* (Guzmán 2004), considered a section with species having bluing basidiomata (Guzmán 2004; Horak *et al.* 2009). A similar species is *D. aureicystidiata*, but differs by the annulate basidioma and larger pleurocystidia that are more variable in shape in *D. aureicystidiata* (see this species above).

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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 multi-gene phylogeny for *Psilocybe*, *Deconica*, and other exemplars of the families Hymenogastraceae and Strophariaceae *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 at least on two occasions in the Strophariaceae *s.str.*, including in a novel lineage of *Deconica*.

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

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 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 as divine mushrooms (Wasson 1957). Their traditional use was rediscovered in Mexico in the 1950s among the Mazatecs of Oaxaca (Heim 1956a, Wasson and Wasson 1957). Furthermore, other Mexican indigenous people, e.g., Chatinos, Chinantecs, Mixes, Nahuas, and Zapotecs, also used them for ceremonial purposes (Heim 1956b, 1957a, 1957b; 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 ethnomycology, taxonomy, and chemistry of these mushrooms have been published (e.g., Singer 1958; Singer and Smith 1958a, 1958b; Guzmán 1959, 1978a, 1978b, 1978c; 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 others 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 s.str.*, Fig. 1), whereas the name *Deconica* is applied to those former species of *Psilocybe* and *Melanotus* 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 U.S.A. possession of psilocybin-containing mushrooms is illegal, and in Mexico psilocin and psilocybin are forbidden under the “Ley General de Salud” (Health General 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. (2007a) 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 above-mentioned characteristics. However, Singer did not consider spore shape and content color of cystidia as 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. 1h-i, 2i, 3a-b), rhomboid to subrhomboid (Figs. 1j, 2f-h), or without angles (Figs. 1g, 2e). In lateral view angles are not apparent. Some species of *Deconica* and *Psilocybe* feature unusually shaped basidiospores that are narrower in profile than in frontal view. Such an unusual spore shape was referred 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 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 multi-gene phylogeny of *Psilocybe s.l.* Our objectives are to: 1) provide an overview of the family-level classification of *Psilocybe* and *Deconica*, 2) resolve infra-generic phylogenetic relationships within *Psilocybe* and *Deconica* and evaluate previous morphological based classifications; 3) analyze the evolution of their unusual basidiospore shape and spore wall-thickness in both clades; and 4) 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. (2007a) 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 type specimens recently collected 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 E.Z.N.A.® Fungal DNA Kit (Omega Bio-Tek Inc., Norcross, USA). The DNA extracts were undiluted or diluted (1:10, 1:100) for PCR reactions.

Polymerase chain reaction (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

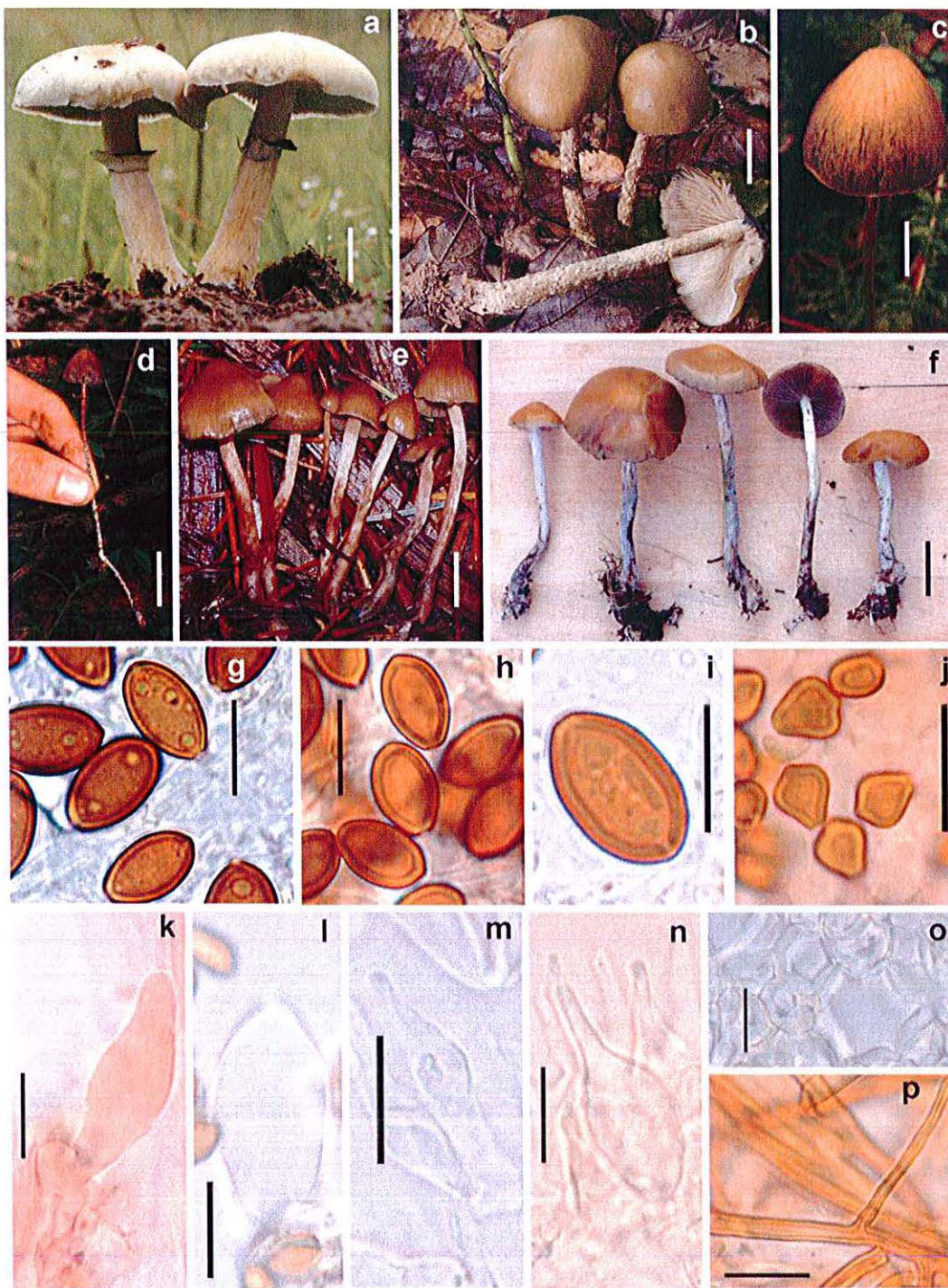


Fig. 1 Macro and micromorphologic features of *Psilocybe*: a-f Basidiomata; a *P. cubensis* (photo: F. Landeros); b *P. zapotecorum* (photo: E. Fanti); c-d *P. fagicola* (photos: M.A. Gómez); e *Psilocybe* sp. (photo: M. No-Line); f *P. subaeruginosa* (photo: P.B. Matheny); g basidiospores without angles, *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 20 mm in a, 15 mm in b, 3 mm in c, 10 mm en d, 25 mm en e, 30 mm in f

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 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, int2F/Ps-Ex3R, were used to amplify shorter fragments.

DNA amplification was done with two protocols. In the first one, each 20 μ L of PCR reaction contained 11.85 μ L water, 2 μ L 10X Taq reaction buffer, 1 μ L of 50 mM MgCl₂, 1 μ L of 5 mM dNTP, 2 μ L Bovine Serum Albumin (BSA), 0.5 μ L of each 10 μ M primer, 0.15 μ L of Taq polymerase (5U/ μ L), and 1 μ L of DNA template to amplify ITS and LSU. Similarly, on the second protocol, each 24 μ L of PCR reaction contained 14.875 μ L water, 5 μ L 5X Taq reaction buffer, 0.5 μ L of 5 mM dNTP, 1.25 μ L of each 10 μ M primer, 0.125 μ L of Taq polymerase (5U/ μ L), and 2 μ L of DNA template to amplify *rpb1*. PCR reactions were performed in Swift MaxPro (ESCO, Portland, USA) and Techne TC-312 thermocyclers (Bibby Scientific Limited, Staffordshire, United Kingdom). 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, then 34 cycles of denaturing at 95 C for 1 min, annealing at 56 C for 45 s, extension at 72 C for 2 min, final extension step of 72 C for 10 min, and refrigerated at 4 C. The *rpb1* 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, USA) using a 100 bp DNA size marker, and then stained in an ethidium bromide solution (20 μ L/500 mL, from a 10 mg/mL stock solution). PCR products were cleaned using Illustra GFX columns (GE Healthcare, Little Chalfont, United Kingdom) or PCR Purification Kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocols, and in some cases with the enzymatic method USB® Exo-sap-IT® (Affymetrix, Santa Clara, USA).

Sequencing reactions were performed with BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) in 20 μ L or 10 μ L 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, United Kingdom), and 18 μ L of formamide was added. Sequences were visualized by capillary electrophoresis on an ABI-Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, USA) at the Institute of Botany, University of Guadalajara, or in ABI 3730 (Applied Biosystems, Foster City, USA) 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, MI, 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 ten million generations with trees sampled every 1 000 generations. The standard deviation of the split frequencies was examined to confirm that independent runs had converged to similar tree scores. The first 2 501 trees were burned, and posterior probabilities (PP) were calculated from a consensus of the remaining 7 500 trees from two runs. This analysis was repeated twice. In ML analyses, 1 000 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.) Qué. 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 1 250× magnifications. Structures were photographed through Axio Vision 4 software on the Zeiss Axioskop 40.

Character evolution

We studied the evolution of basidiospore shape in frontal view, basidiospore wall thickness, and presence/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), 1 000 trees from the posterior distribution were analyzed. Trees were re-rooted and dichotomized in Mesquite 2.5 (Maddison and Maddison 2011).

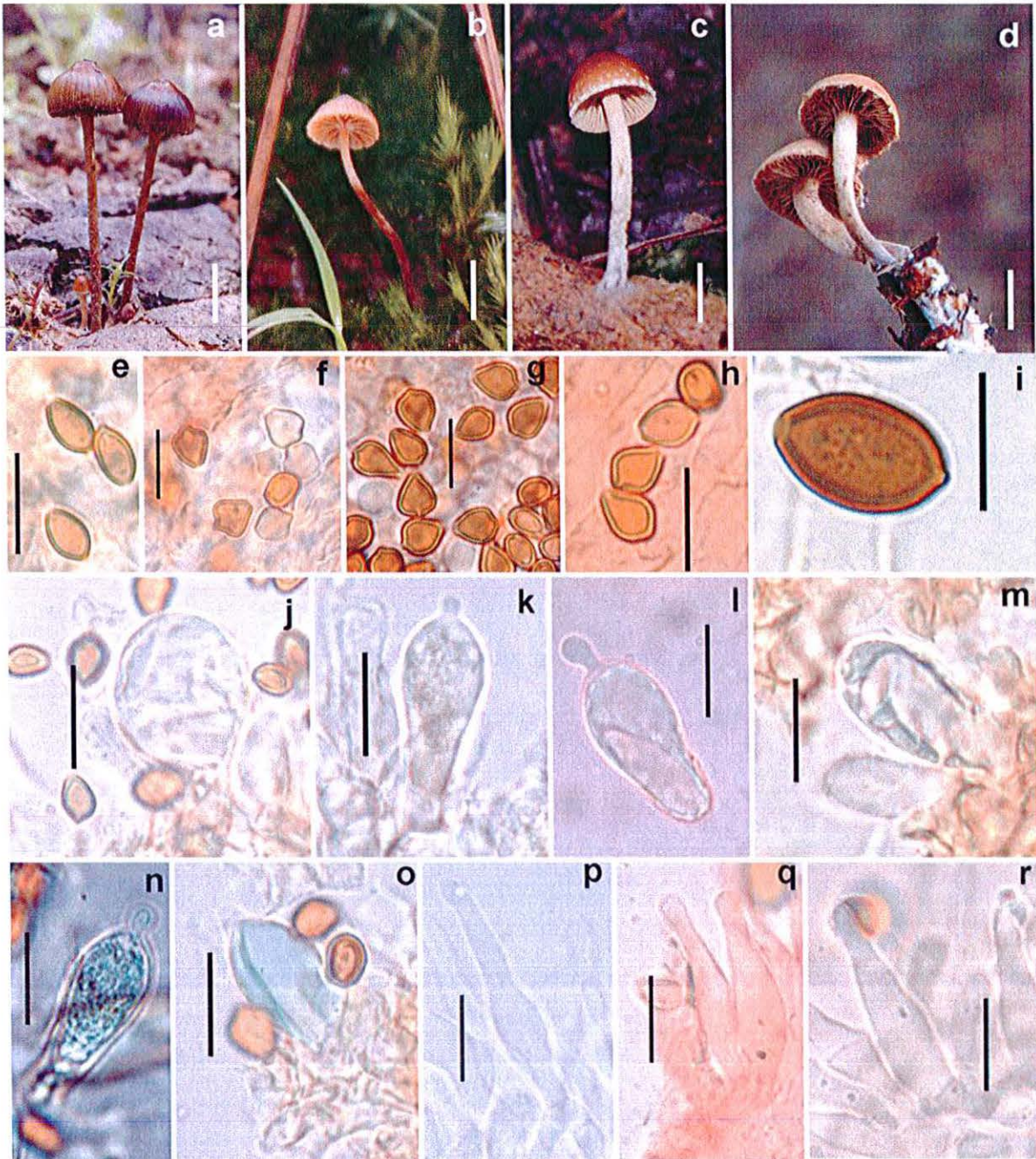


Fig. 2 Macro and micromorphologic features of *Deconica*: a-d Basidiomata a *D. coprophila* (photo: L. Guzmán-Dávalos); b *D. aff. montana* (photo: V. Ramírez-Cruz); c *Deconica* sp. (photo: V. Ramírez-Cruz); d *Deconica* sp. (photo: C. Braaten); e basidiospore without angles, *Deconica* sp. (CCB45); f rhomboid and subrhomboid basidiospores, *D. umbrina*; g subrhomboid basidiospores, *Deconica* sp. (PBM2790); h subrhomboid basidiospores, *Deconica* sp.; i hexagonal basidiospore, *D. coprophila*; j-l chryso- and cheilocystidia in KOH; j *D. subbrunneocystidiata*; k *Deconica* sp. (CCB45); l *D. aureicystidiata*; m-o chryso- and cheilocystidia in patent blue V; m *D. thailandensis*; n *Deconica* sp. (CCB45); o *D. aureicystidiata*; p cheilocystidia (leptocystidia) in KOH, *Deconica* sp. (TFB6422); q cheilocystidia in Congo red, *D. montana*; r cheilocystidia in KOH, *Deconica* sp. Scale bars 10 mm in a, 6 mm in b-c, 7 mm in d

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 >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 ten million generations, with a sampling frequency every one thousand 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 is interpreted as positive evidence, BF up to 10 as strong evidence, and BF >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. 1g, 2e), or (1) angled (rhomboid to subrhomboid or hexagonal) (Figs. 1h-j, 2f-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. 1j), and (2) thick-walled (0.85-1.89 μm) (Figs. 1i, 2i, 3a-b). In the Agaricales, Garnica et al. (2007) split the basidiospore wall thickness into two classes corresponding to whether or not 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 4 900 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, the 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 since 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-H). *Psilocybe* contains two main clades, each one subdivided in 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. 1b, 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 µm) pigmented hyphae from the pileus trama (Fig. 1o) and hymenophoral trama; and basal mycelium of the stipe composed by setoid hyphae (Fig. 1p). 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. 1b), 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 6-8(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* that was recently described from Thailand (Guzmán et al. 2012).

Clade B groups *P. caerulipes*, *P. hispanica*, *P. pelliculosa*, *P. stuntzii*, and *Psilocybe* sp. (Fig. 1e). 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. 1f) (*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. 1a), *P. subcubensis*, *P. thaiaerugineomaculans*, 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.

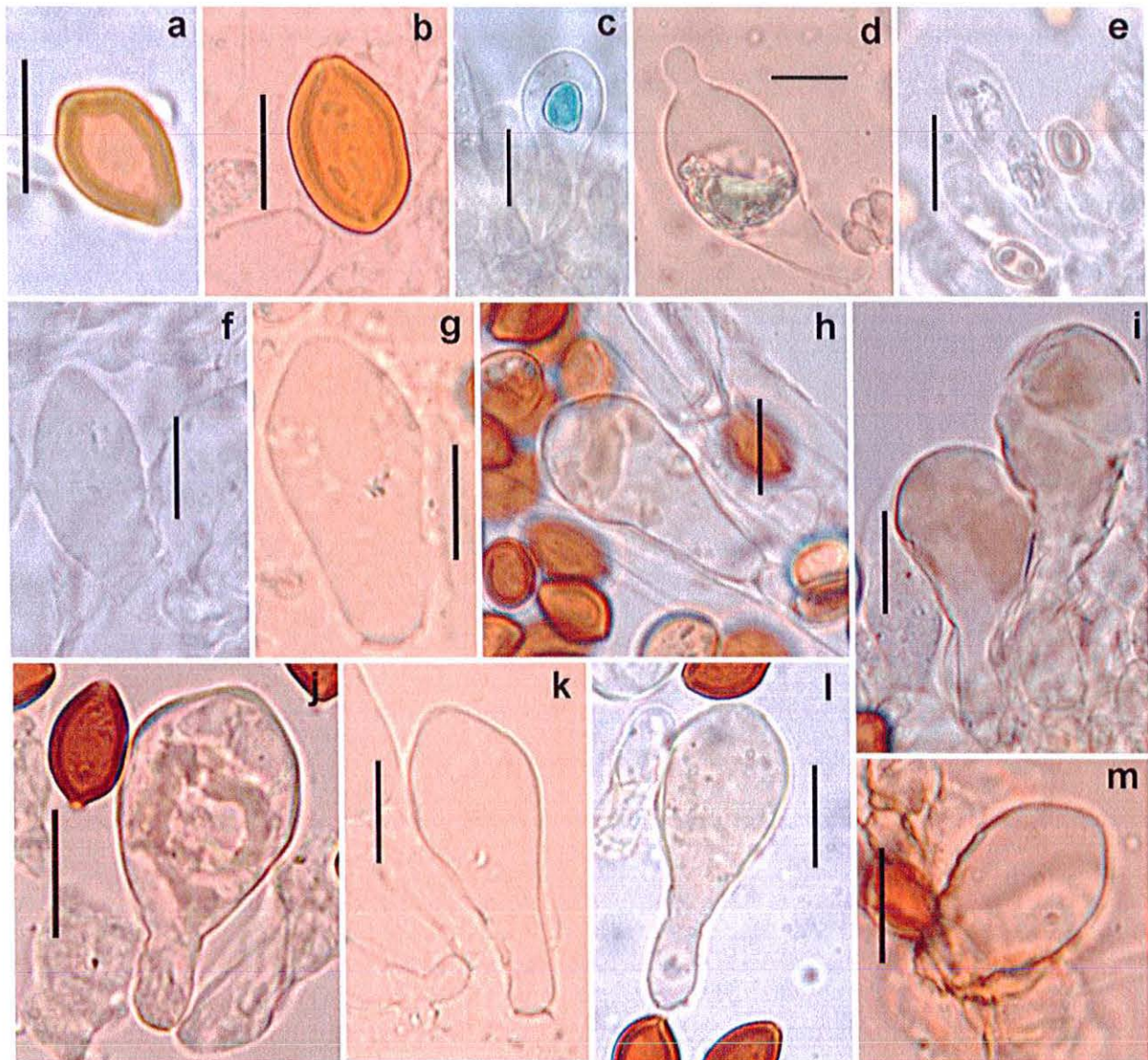


Fig. 3 Micromorphologic features of *Strophariaceae*: a-b Hexagonal basidiospores with three layers under light microscope, a *Psilocybe caerulescens*; b *P. cubensis*; c-d chrysocystidia in patent blue V, c *Hypholoma fasciculare*, d *Hypholoma* sp.; e chrysocystidium in KOH, *Pholiota* aff. *gummosa*; 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*

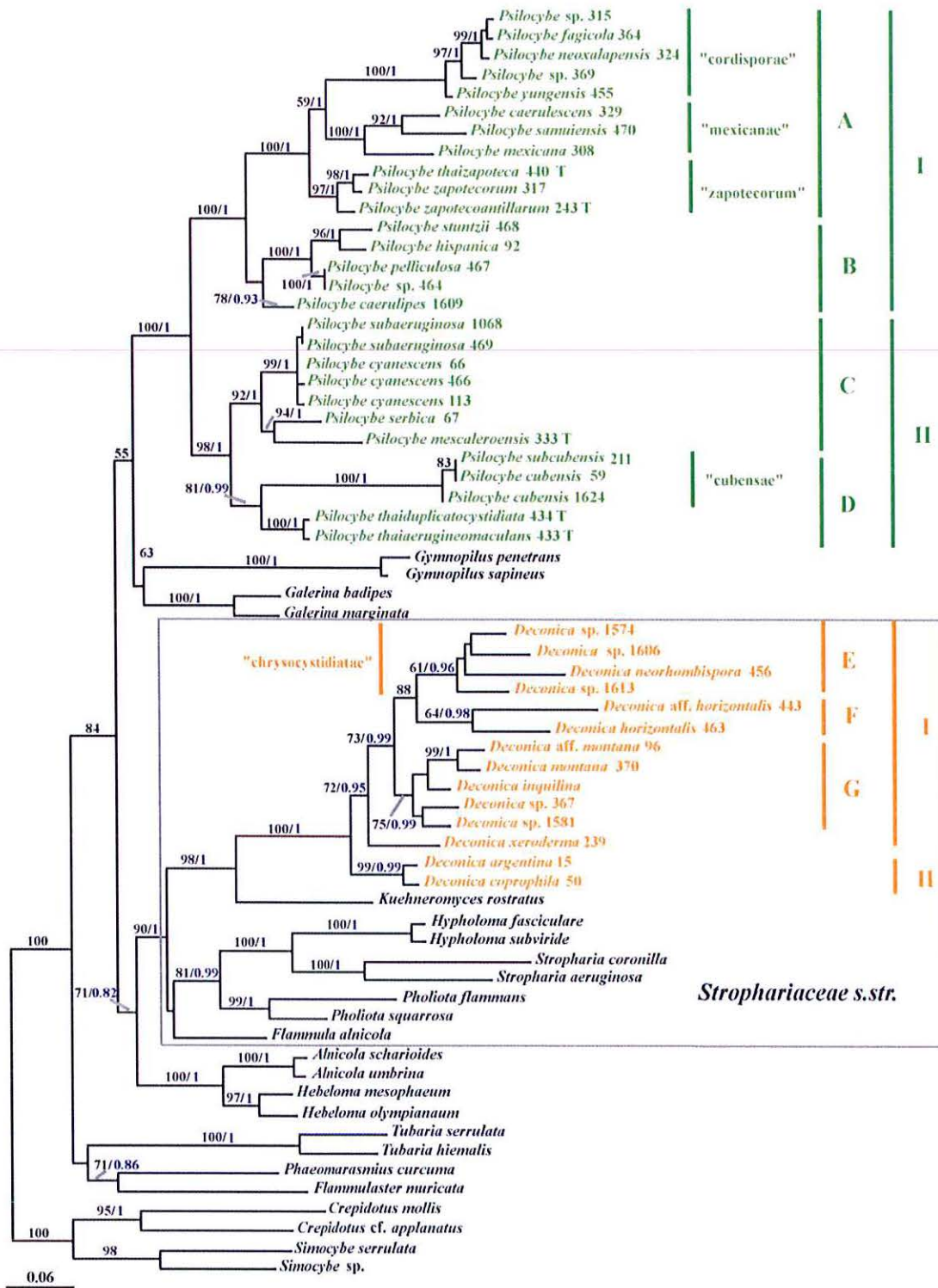


Fig. 4 Maximum likelihood tree with average branch lengths from the combined analyses of 5.8S, partial nLSU rDNA, and *rpb1* dataset of *Psilocybe* and *Deconica*. Maximum likelihood bootstrap support and posterior probability obtained from the Bayesian inference are indicated over the branches. Branch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

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 is described as bluing in sect. *Brunneocystidiatae* (Silva et al. 2007)] and three undetermined species, each annotated as *Deconica* sp. (Fig. 2d). 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. 2j-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* Pat. 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. 2b), *D. inquilina*, and two undetermined species (Figs. 2c, g- 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, 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 2a), both in sect. *Coprophilae sensu* Guzmán (1983, 1995) or sect. *Merdariae sensu* Singer (1986). They are characterized by the 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 to 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 are seen, and only in few cases was it possible to detect three layers (Figs. 3a-b). In *Deconica*, two types of cystidia can be observed according to their contents: leptocystidia (euplasmatic cystidia) (Fig. 2p-r) and chrysocystidia (deuteroplasmatic cystidia) (Figs. 2j-o). Chrysocystidia are entirely absent from species of *Psilocybe s.str.* Species of *Psilocybe* do possess leptocystidia (Figs. 1m-n) and a second type of deuteroplasmatic cystidia that do not stain with Patent blue V (Figs. 1k-l, 3f-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 runs. Table 4 shows the fifteen 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, chrysocystidia evolved independently 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 subject of much debate. Guzmán (1983) and Singer (1986) considered *Psilocybe s.l.* in the Strophariaceae. Singer (1986) recognized two subfamilies in Strophariaceae: Stropharioideae and Pholiotoideae based on the basidiospore morphology and spore print color. In Stropharioideae 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 to distinguish the genus *Stropharia* (Cortez 2008a, 2008b; 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 as a key character to distinguish *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 separate clades “/psychodelia” and “/psilocybe”, respectively. Additionally, this analysis recovered the “/stropharioid” 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 “stropharioid” 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, *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.

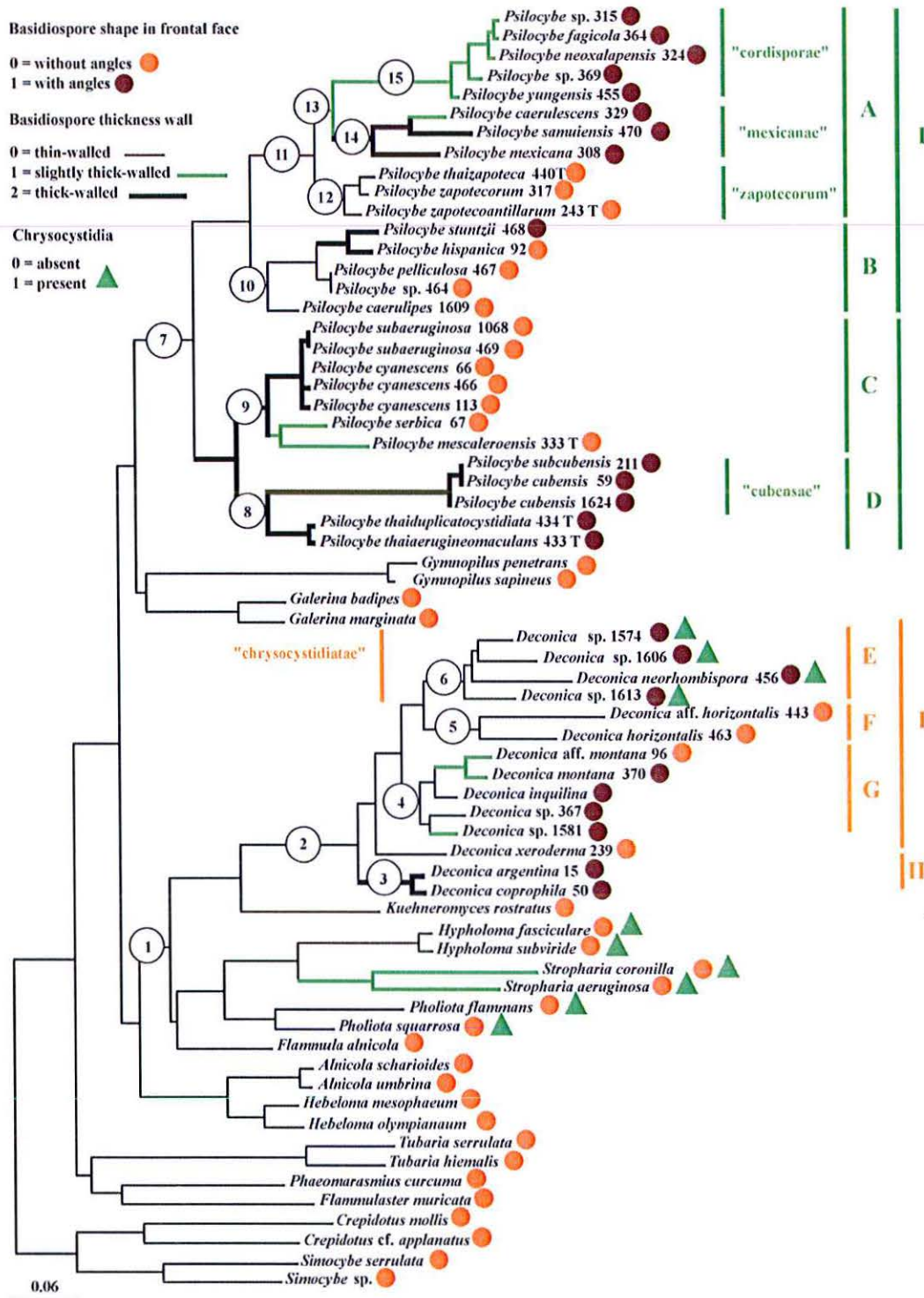


Fig. 5 Characters traced in a phylogram obtained with the Bayesian inference with 5.8S, partial LSU rDNA, and *rpb1* dataset of *Psilocybe* and *Deconica*. Numbers indicate the nodes

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 “/stropharioid”. 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. 2a), *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* a non-stipitate genus in Strophariaceae. Noordeloos (2011), likely following Moncalvo et al. (2002), transferred species of *Melanotus* to *Deconica*. Our analyses re-affirm that *Melanotus* is nested within *Deconica* (Fig. 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* (A-D, Fig. 4), 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. thaaerugineomaculans*, *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 to the above-mentioned 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. 2007b) has been found in the U.S.A. We suspect that *P. cyanescens* and *P. subaeruginosa* (sect. *Subaeruginascens*, Fig. 1f) 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 (Fig. 3f-m).

Members of sect. *Stuntzae* (Guzmán 1983; Guzmán et al. 2007b, 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, Guzman & Desjardin and *P. umbrina* E. Horak, Guzman & Desjardin were considered as bluing and placed in sect. *Neocaledonicae* (Horak et al. 2009; Guzmán et al. 2012) but 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 Kausrud 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 a 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*. Cléménçon (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* (Fig. 3c-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 (1987). 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 (Fig. 2j-k, 2n) 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. 3e). 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 (Fig. 2l-m, 2o), *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 Cléménçon (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 (Fig. 1m-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. 1k-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 different content 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”. Though 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. thiaerugineomaculans* (sect. *Stuntzae*), and *P. thaiduplicatocystidiata* (sect. *Cordisporae*), contrary to *Hypholoma* (Fig. 3c-d) and *Deconica* (Fig. 2m-o), none of them showed any reaction (Fig. 3g, i, k, m). Therefore,

our observations confirmed that chrysocystidia are present in *Deconica* (clade E, Fig. 5), 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, since 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 was 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 or subsections. 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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Table 1. Infrageneric classification of *Psilocybe sensu lato* (sections represented in this work are indicated with *, and hallucinogenic sections in bold)

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

Table 2. Table 2. Specimens of *Deconica*, *Psilocybe*, and out-groups used in this study (sequences produced for this work in bold).

Specimen -DNA number	Species name	Section	Herbarium and specimen voucher	Collector, number	Date of collection	Country	Accession numbers		
		1. Guzmán (1983, 1995, 2004) and Guzmán et al. (2007a) 2. Singer (1986) 3. Noordeloos (2011)					ITS	LSU	RBP1
	INGROUP								
	Hymenogastraceae								
Ps-329	<i>Psilocybe caerulescens</i> Murrill	1. <i>Cordisporae</i> 2. <i>Caerulescentes</i> , stirp <i>Caerulescens</i>	IBUG	L.J. Franco-Galván 1	31 August 2005	Mexico	KC669281	KC669317	KC669342
UT 1609	<i>P. caerulipes</i> (Peck) Sacc.	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Caerulipes</i>	TENN- 064502	SAT09-216-06	8 August 2009	USA	KC669282	—	KC669343
Ps-59	<i>P. cubensis</i> Earle (Singer)	1. <i>Cubensae</i> 2. <i>Caerulescentes</i> , stirp <i>Cubensis</i> 3. <i>Caerulescentes</i> , stirp <i>Caerulescens</i>	XAL	V. Ramírez-Cruz 87	1 July 2004	Mexico	KC669283	KC669318	KC669344
UT 1524	<i>P. cubensis</i>	1. <i>Cubensae</i> 2. <i>Caerulescentes</i> , stirp <i>Cubensis</i> 3. <i>Caerulescentes</i> , stirp <i>Caerulescens</i>	TENN- 051528	RHP5203	7 May 1992	Costa Rica	KC669284	—	KC669345
Ps-66	<i>P. cyanescens</i> Wakef.	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , Stirp <i>Cyanescens</i> 3. <i>Psilocybe</i> , stirp <i>Psilocybe</i>	XAL	J. Workman & P. Werner s.n.	Without date	USA	KC669285	KC669319	KC669346
Ps-113	<i>P. cyanescens</i>	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i> 3. <i>Psilocybe</i> , stirp <i>Psilocybe</i>	F-1021111	Ower 2157	Without date	USA	KC669286	KC669320	KC669347
Ps-466	<i>P. cyanescens</i>	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i> 3. <i>Psilocybe</i> , stirp <i>Psilocybe</i>	IBUG	S. Chornick	November 2011	USA	KC669287	KC669321	KC669348
Ps-364	<i>P. fagicola</i> R. Heim & Cailleux	1. <i>Cordisporae</i>	IBUG	M.A. Gómez 22731	July 2010	Mexico	KC669288	KC669322	KC669349
Ps-92	<i>P. hispanica</i> Guzmán	1. <i>Semilanceatae</i> 3. <i>Psilocybe</i> , stirp	XAL	R. Fernández-Sasia s.n.	15 January 2005	Spain	KC669289	KC669323	KC669350

			<i>Psilocybe</i>						
Ps-333	<i>P. mescaleroensis</i> Guzmán, Walstad, E. Gándara & Ram.-Guill.	1. <i>Stuntzae</i>	XAL	L. Field Walstad s.n., Holotype	August 2005	USA	KC669290	KC669324	KC669351
Ps-308	<i>P. mexicana</i> R. Heim	1. <i>Mexicanae</i> 2. <i>Caerulescentes</i> , stirp <i>Mexicanae</i>	IBUG	M.R. Sánchez-Jácome 1038	30 June 2002	Mexico	KC669291	KC669325	KC669352
Ps-324	<i>P. neoxalapensis</i> Guzmán, Ram.-Guill. & Halling	1. <i>Cordisporae</i>	XAL	M.A. Gómez 1883	5 September 2007	Mexico	—	KC669326	KC669353
Ps-467	<i>P. pelliculosa</i> (A.H. Sm.) Singer & A.H. Sm.	1. <i>Semilanceatae</i>	IBUG	S. Chronick s.n.	December 2011	USA	KC669292	—	KC669354
Ps-470	<i>P. samuiensis</i> Guzmán, Bandala & J.W. Allen	1. <i>Mexicanae</i>	XAL	Sihanonth & J. Allen	18 June 2004	Thailand	KC669293	—	KC669355
Ps-67	<i>P. serbica</i> M.M. Moser & E. Horak	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i> 3. <i>Caerulescentes</i> , stirp <i>Serbica</i>	WU-4448	I. Krisai s.n.	5 November 1987	Austria	KC669294	KC669327	KC669356
Ps-468	<i>P. stuntzii</i> Guzmán & J. Ott	1. <i>Stuntzae</i> 3. <i>Caerulescentes</i> , stirp <i>Cyanescens</i>	IBUG	S. Chornick	October 2011	USA	KC669295	—	KC669357
Ps-459	<i>P. subaeruginosa</i> Cleland	1. <i>Subaeruginosae</i>	PDD	L. Taylor	16 May 2008	New Zealand	KC669296	—	KC669358
UT 1608	<i>P. subaeruginosa</i>	1. <i>Subaeruginosae</i>	TENN-065481	PBM 3218	9 June 2009	Australia	KC669278	—	KC669359
Ps-211	<i>P. subcubensis</i> Guzmán	1. <i>Cubensae</i> 2. <i>Caerulescentes</i> , stirp <i>Cubensis</i>	XAL	G. Guzmán 35102	8 October 2001	Nepal	KC669297	KC669328	KC669360
Ps-434	<i>P. thaiiaerugineomaculans</i> Guzmán, Karunarathna & Ram.-Guill.	1. <i>Stuntzae</i>	XAL	S.C. Karunarathna NTS-121, Holotype	27 July 2010	Thailand	KC669298	—	KC669361
Ps-433	<i>P. thaiduplicatocystidiata</i> Guzmán, Karunarathna & Ram.-Guill.	1. <i>Cordisporae</i>	XAL	S.C. Karunarathna NTS-120, Isotype	27 July 2010	Thailand	KC669299	KC669329	KC669362
Ps-440	<i>P. thaizapoteca</i> Guzmán, Karunarathna & Ram.-Guill.	1. <i>Zapotecorum</i>	XAL	G. Guzmán 38342, Holotype	12 July 2010	Thailand	KC669300	—	KC669363
Ps-455	<i>P. yungensis</i> Singer	1. <i>Cordisporae</i>	XAL	A. Cortés-Pérez 549	30 October 2010	Mexico	KC669301	KC669330	KC669364
Ps-243	<i>P. zapotecoantillarum</i> Guzmán & T.J. Baroni & Lodge	1. <i>Zapotecorum</i>	XAL	S. Cantrell & Salgado s.n., Isotype	23 May 2000	Puerto Rico	KC669302	KC669331	KC669365
Ps-317	<i>P. zapotecorum</i> R. Heim	1. <i>Zapotecorum</i> 2. <i>Caerulescentes</i> , stirp <i>Caerulescens</i>	IBUG	V. Ramírez-Cruz 1094	30 July 2009	Mexico	KC669303	KC669332	KC669366
Ps-315	<i>Psilocybe</i> sp.	1. <i>Cordisporae</i>	IBUG	V. Ramírez-Cruz 551	25 August 2006	Mexico	KC669304	KC669333	KC669367

Ps-369	<i>Psilocybe</i> sp.	1. <i>Cordisporae</i>	IBUG	V. Ramírez-Cruz 1328	12 August 2010	Mexico	KC669305	KC669334	KC669368
Ps-464	<i>Psilocybe</i> sp.	1. <i>Semilanceatae</i>	IBUG	M. No-Line	19 December 2011	USA	KC669306	KC669335	KC669369
<i>Strophariaceae s. str.</i>									
Ps-15	<i>Deconica argentina</i> Speg.	1. <i>Coprophilae</i> 2. <i>Merdariae</i>	XAL	M. Contu s.n.	7 October 2002	Italy	KC669307	KC999956	KC669370
Ps-50	<i>D. coprophila</i> (Bull.) P. Karst.	1. <i>Coprophilae</i> 2. and 3. <i>Merdariae</i>	XAL	V. Ramírez-Cruz 114	1 July 2004	Mexico	KC669308	KC669336	KC669371
Ps-463	<i>D. horizontalis</i> (Bull.) Noordel.	3. <i>Melanotus</i>	ICN-154677	P.S. Silva 253/10	14 May 2010	Brazil	KC669309	KC669337	KC669372
Ps-443	<i>D. aff. horizontalis</i>	3. <i>Melanotus</i>	IBUG	V. Ramírez-Cruz 1520	23 July 2011	Costa Rica	KC669310	KC669338	KC669373
	<i>D. inquilina</i> (Fr.) Romagn.	1. <i>Psilocybe</i> 3. <i>Deconica</i> , subsect. <i>Inquilinae</i>	GLM-51242				—	DQ071689	DQ067969
Ps-370	<i>D. montana</i> (Pers.) P.D. Orton	1. <i>Psilocybe</i> 3. <i>Deconica</i> , subsect. <i>Inquilinae</i>	IBUG	V. Ramírez-Cruz 1323	12 August 2012	Mexico	KC669311	—	KC669374
Ps-96	<i>Deconica</i> aff. <i>montana</i>	1. <i>Psilocybe</i> 3. <i>Deconica</i> , subsect. <i>Inquilinae</i>	XAL	J. Trappe 10065	12 July 1986	USA	—	—	KC669375
Ps-456	<i>D. neorhombispora</i> nom. prov.* = <i>Psilocybe</i> <i>subbrunneocystidiata</i> P.S. Silva & Guzmán**	1. <i>Neocaledonicae</i> * 1. <i>Brunneocystidiatae</i> **	XAL	A. Cortés-Pérez 739	12 October 2011	Mexico	—	KC669339	KC669376
Ps-239	<i>D. xeroderma</i> (Huijsman) Noordel.	1. <i>Psilocybe</i> 3. <i>Deconica</i> , subsect. <i>Deconica</i>	WU	Oswald s.n.	24 August 2004	Austria	KC669312	KC669340	KC669377
Ps-367	<i>Deconica</i> sp.	1. <i>Psilocybe</i>	IBUG	V. Ramírez-Cruz 1269	17 July 2010	Mexico	—	KC669341	KC669378
UT 1574	<i>Deconica</i> sp.		TENN- 062238	TFB12591	11 August 2005	USA	KC669313	—	KC669379
UT 1606	<i>Deconica</i> sp.		TENN- 067047	PBM3781	3 March 2012	Australia	KC669314	—	KC669380
UT 1613	<i>Deconica</i> sp.		TENN- 067013	CCB45	19 July 2012	USA	KC669315	—	KC669381
UT 1581	<i>Deconica</i> sp.	1. <i>Psilocybe</i>	TENN- 062588	PBM 2790	23 July 2006	USA	KC669316	—	KC669382
OUTGROUP									
<i>Crepidotaceae</i>									
	<i>Crepidotus applanatus</i> (Pers.) P. Kumm.		WTU	P.B. Matheny 717			DQ202273	AY380406	AY333303

<i>Crepidotus mollis</i> (Schaeff.) Staute	TUB-011566				—	DQ071698	DQ067977
<i>Simocybe serrulata</i> (Murrill) Singer	CUW	PBM 2536		USA	DQ494696	AY745706	DQ447940
<i>Simocybe</i> sp.	TENN- 062784	PBM3031	31 August 2008	USA	—	—	KC669280
<i>Gymnopileae</i>							
<i>Gymnopilus penetrans</i> (Fr.) Murrill	GLM-45929			Germany	—	AY207208	DQ068014
<i>Gymnopilus sapineus</i> (Fr.) Murrill	WTU	PBM 1541			—	AY380362	AY351789
<i>Hymenogastraceae</i>							
<i>Alnicola escharioides</i> (Fr.) Romagn.	WTU	PBM 1719		USA	—	—	AH013186
<i>Alnicola solecina</i> var. <i>umbrina</i> Singer	TU-110280	L. Tedersoo	16 September 2010	Estonia	—	JN938854	JQ014106
<i>Flammula alnicola</i> (Fr.) P. Kumm.	CUW	P.B. Matheny 2608			DQ486703	DQ457666	DQ447900
<i>Galerina badipes</i> (Pers.) Kühner	GLM-45922			Germany	—	AY207201	DQ067975
<i>Galerina marginata</i> (Batsch) Kühner	CUW	PBM-2518		USA	DQ192182	DQ457669	DQ447901
<i>Hebeloma mesophaeum</i> (Pers.) Quéf.	TUB-011577				—	DQ071690	DQ067971
<i>Hebeloma olympianum</i> A.H. Sm., V.S. Evenson & Mitchel	UTC	BK 21-Nov-98-20			—	AY038310	AF389532
<i>Strophariaceae s. str.</i>							
<i>Hypholoma fasciculare</i> (Huds.) P. Kumm.	WTU	PBM 1844			—	AY380409	AY351829
<i>Hypholoma subviride</i> (Berk. & M.A. Curtis) Krieglst.	TENN- 062712	P.B. Matheny 2954	24 July 2008	USA	—	—	KC669279
<i>Kuehneromyces rostratus</i> Singer & A.H. Smith	CUW	P.B. Matheny 2703		USA	—	DQ457684	DQ447918
<i>Pholiota flammans</i> (Bastch) P. Kumm.	TUB-011573				—	DQ071688	DQ067973
<i>Pholiota squarrosa</i> (Oeder) P. Kumm.	CUW	PBM 2735			DQ494683	DQ470818	DQ447931
<i>Stropharia coronilla</i> (Bull.) Quéf.	GLM-46074				—	DQ071687	DQ067966
<i>Stropharia aeruginosa</i> (Curtis) Quéf.	TUB-012151				—	DQ071686	DQ067967
<i>Tubarieae</i>							
<i>Flammulaster muricatus</i> (Fr.) Watling	TUB-012150				—	DQ071740	DQ068012

<i>Phaeomarasmium</i> <i>curcuma</i> (Berk. & M.A. Curtis) Singer	WTU	JFA11323		—	AY038329	AF389551
<i>Tubaria hiemalis</i> Romagn. ex Bon	GLM-46038		Germany	—	AY207311	DQ067966
<i>Tubaria serrulata</i> (Cleland) Bougher & Matheny			Australia	DQ182507	DQ156128	DQ447930

Table 3. Frequencies and likelihoods of models sampled during the RJ-MCMC analyses. The frequency and likelihood of the best-fit model are in bold.

Character	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 ($q_{10} = 0$)	
	Frequency	-logL	Frequency	-logL	Frequency	-logL	Frequency	-logL	Frequency	-logL
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

Table 4. The fifteen most prevalent models of character state transition for basidiospore wall thickenss (0, thin-walled; 1, slightly thick-walled, and 2, thick-walled) sampled during RJ-MCMC. Rate parameters and the number of times each model was sampled are shown.

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

Table 5. Table 5. Probabilities of ancestral state reconstructions for presence/absence of chrysocystidia and basidiospore shape (without angles or with angles). Bayes factors are in bold (*positive evidence, ** strong evidence, and *** very strong evidence).

Character	Probability of reconstructed ancestral states		Harmonic mean of likelihood when fixed at a state (-log L)		Bayes factor
	0	1	0	1	
Chrysocystidia					
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					
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***

Table 6. Table 6. Ancestral state reconstructions of character basidiospore wall thickness. Asterisk and positive sign denote positive, strong, or very strong evidence (BF) against the other states (* first state, + second state).

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

Capítulo IV. Ramírez-Cruz V, Guzmán G, Villalobos-Arámbula AR, Rodríguez A, Matheny PB, Guzmán-Dávalos L. 2013. Infrageneric relationships of the hallucinogenic genus *Psilocybe* based on the rDNA ITS region. Mycologia

**Infrageneric relationships of the hallucinogenic genus *Psilocybe* (Agaricales)
based on the rDNA ITS region**

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Abstract: The genus *Psilocybe* is a diverse group that contains the hallucinogenic mushrooms, recognized by their traditional and recreational uses. We use a phylogenetic approach to elucidate the infrageneric relationships in this group. DNA sequences from 62 specimens were used, including 15 types. Nine clades were recovered and four are congruent with the traditional sections. Our results show that morphological characters are highly homoplastic; however, some of them could be helpful at infrageneric levels.

Key words: sect. *Cubensae*, sect. *Cordisporae*, sect. *Cyanescens*, sect. *Mexicanae*, molecular systematics, taxonomy, tropics, sect. *Zapotecorum*

INTRODUCTION

The genus *Psilocybe* (FIGS. 1–9) encompasses exclusively the hallucinogenic mushrooms (Redhead et al. 2007; Norvell 2010), known by their psychotropic properties and their traditional and recreational uses (Ott 1993; Guzmán 2008). The presence of hallucinogenic compounds, mainly psilocybin, is the character that separates the genus from others in the Agaricales (Moncalvo et al. 2002). Nevertheless, psilocybin is also found in another phylogenetically unrelated genera, *Gymnopilus* P. Karst., *Inocybe* (Fr.) Fr. s.s., *Panaeolina* Maire, and *Pluteus* Fr. (Stamets 1996; Wurst et al. 2002). *Psilocybe* is widely distributed, with approximately 144 species, being their highest diversity in the tropics (Guzmán 2005). They are saprobes and grow in several substrates as soil, dung, stem, leaves, or rotting wood (Guzmán 1983; Singer 1986). Basidiomata varies from robust to delicate; pileus brown to yellowish brown tones or reddish brown; lamellae adnexed to adnate, brownish, gray violet, or brownish violaceous; stipe not viscid, whitish, yellowish or brownish, or concolorous with the pileus, central, glabrous or fibrillose, usually hollow; sometimes with a pseudorhiza; veil varies from weakly to strongly developed, in this case forming a membranous permanent annulus (Guzmán 1983; Singer 1986). With molecular data, it was found that not only gill basidiomata are included in the genus; furthermore, there are secotioid fungi as *Psilocybe nova-zelandiae* (Borovička et al. 2010).

The current circumscription of *Psilocybe* to the family Strophariaceae is doubtful. Matheny et al. (2006) have shown that it does not belong to this family in the sense of Singer (1986). The most robust hypothesis suggests the genus belongs to Hymenogastraceae and is closely related to Cortinariaceae (Matheny et al. 2006). Another hypothesis shows low support for a sister relationship between *Psilocybe* and the /stropharioid clade (Moncalvo et al. 2002).

The monophyly of *Psilocybe* has already been tested (Moncalvo et al. 2002; Matheny et al. 2006; Ramírez-Cruz et al. 2013), but still little is known about the infrageneric relationships. A general approach about the species kinships was made, including 28 taxa of *Psilocybe* and obtaining three groups (Ramírez-Cruz et al. 2013). Traditionally, the species boundaries and infrageneric units have been based on morphological data, and three proposals exist (TABLE I). Important gross morphological characters used to separate species include fruit body features, basidiospores shape and wall thickness, cystidia attributes, among others. Thus, the goals of this work are 1) elucidate the infrageneric relationships in *Psilocybe* using sequences from ITS region (ITS1, 5.8S, and ITS2), and 2) evaluate the traditional proposals in the genus.

MATERIALS AND METHODS

Taxon sampling

For the ingroup, we sampled 62 specimens and 45 taxa, including 15 type specimens of *Psilocybe*. Taxon sampling incorporated species representing 11 out of 12 sections of Guzmán (1983, 1995) and Guzmán et al. (2007a). One of the two sections of Singer (1986) was included in the analysis, and two of three sections of Noordeloos (2011) were included (TABLE II). Two sequences of *Gymnopilus* were used as outgroup.

Micromorphological analyses

All the specimens included in this work were morphologically studied to check the determination, except those that their sequences were obtained from GenBank. The specimens are deposited at the following herbaria IBUG, NY, OSC, PDD, PRM, TENN, UBC, WU, and XAL. Microscopic characteristics were observed from dried material mounted in 3% potassium hydroxide (KOH), and in some cases Congo red was used. Microscopic structure measures and drawings were made using a 100× oil-immersion objective, in Zeiss K7 or Zeiss Axioskop 40 microscopes. Structures were photographed through Axio Vision 4 software in a Zeiss Axioskop 40 microscope.

DNA extraction, PCR, and sequencing

DNA was extracted from small pieces (*ca.* 4 mg) of the pileus, using one of the following procedures: Doyle and Doyle (1987), Aljanabi and Martinez (1997), or the Omega Bio-Tek fungal DNA Extraction Kit. DNA extracts were undiluted or diluted (1:10, 1:100) for PCR reactions. Polymerase Chain Reaction (PCR) was performed to amplify the internal transcribed spacer 1 (ITS1), the 5.8S rDNA gene, and the internal transcribed spacer 2 (ITS2) following the protocols indicated by Guzmán-Dávalos et al. (2003).



FIG 1–8. Macromorphology of *Psilocybe*. 1. *P. semilanceata* (photo: A. Rockefeller). 2. *P. cyanescens* (photo: A. Rockefeller). 3–4. *P. mexicana* (photos: A. Cortés-Pérez). 5. *P. caerulescens* (photo: A. Cortés-Pérez). 6–7. *P. zapotecorum* (photos: 6. A. Cortés-Pérez, 7. V. Ramírez-Cruz). 8. *P. cyanofibrillosa* (photo: B. Fogg). 9. *P. yungensis* (photo: A. Cortés-Pérez).

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). PCR products were cleaned using Illustra GFX columns (GE Healthcare) or Qiagen PCR Purification Kit following the manufacturer's

protocols, and in some cases with the enzymatic method Exo-sap-IT© (USB Corporation). Sequencing reactions were performed with BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in 20 µL or 10 µL final volume, following the manufacturer's protocol and using the same primers as in the DNA amplification. Sequencing reactions were purified with AutoSeq™ G-50 column (Amersham Biosciences), and finally 18 µL of formamide were 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 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, MI, USA). Sequence assembly and alignment were carried out with MacClade 4.08 (Maddison and Maddison 2000). Trees were obtained using Maximum Parsimony (MP) in PAUP* 4.0b10 (Swofford 2002), Bayesian inference with MrBayes 3.1 (Ronquist and Huelsenbeck 2003), and Maximum Likelihood (ML) in RAxML 7.4.2 (Stamatakis 2006).

For the MP analyses, heuristic searches were conducted with stepwise addition, random addition sequence with 1 000 replicates, tree-bisection-reconnection as branch swapping algorithm, and branches collapsed if maximum branch length was zero. Support for nodes recovered was obtained from 1 000 bootstrap (BSP) replications, with the same parameters as above but with 10 random addition replicates. For the Bayesian analyses, the most likely model of evolution was determined using jModeltest 3.7 with the Akaike criterion (Posada and Crandall 1998). The Bayesian analysis was run for two million generations with trees sampled every 100th generations. The analyses performance was evaluated using Tracer v.1.4.1 (Rambaut 2009) to check the convergence between chains of runs of Mr. Bayes. The first 2 500 trees, representing the burn-in phase, were discarded and posterior probabilities (PP) were calculated from a consensus of the remaining 7 500 trees. In ML analyses, 1 000 rapid bootstrap inferences were performed with all free model parameters estimated by RAxML using a GTRGAMMAI (Stamatakis 2006) model and empirical base frequencies. The trees were visualized in PAUP* 4.0b10 or in FigTree v1.4.1 (Rambaut 2011). Additionally, the amount of genetic variation within and among some species was assessed in terms of absolute nucleotide differences, and pairwise genetic distances were calculated in PAUP* 4.0b10.

RESULTS

DNA sequence data and phylogenetic analyses

In this study, 34 new sequences were generated 23 were retrieved from a previous work (Ramírez-Cruz et al. 2013), and seven were obtained from GenBank. The 64 sequences represented 45 taxa (TABLE II). The alignment of the ITS sequences resulted in 619 nucleotide positions, after the inclusion of gaps and removing both ends. Ambiguously aligned regions were excluded. At the end, 413 characters were included and 61 of them were parsimoniously informative. The regions excluded were the following: 82–192, 233–239, 446–456, 478–486, 495–507, 535–550, and 581–619. The best-fit model of molecular evolution was the TVM+I+G for ITS sequences (Posada and Crandall 1998).

The tree topology recovered by MP, ML, and Bayesian inference was almost the same. Some exceptions are shown and discussed below. Phylogenetic relationships inferred are shown in

FIGS. 10 and 11. In the MP analyses, 636 most parsimonious trees of 133 steps were recovered, with CI=0.5982 excluding non-informative characters and RC=0.5721.

Infrageneric groups

Nine well-supported clades were recognized in *Psilocybe* (FIG. 10). However, not all the relationships among these clades were resolved. Clade “cyanescens” contains *P. allenii*, *P. azurescens*, *P. cyanescens*, *P. makarorae*, and *P. subaeruginosa*. In this group, the species have middle sized basidiomata growing in rotten wood, basidiospores without angles and thick-walled, and pleurocystidia present.

Clade “cubensae” agrees with sect. *Cubensae* of Guzmán (1983, 1995). It includes *P. cubensis* and *P. subcubensis*, which are coprophilous species, widely distributed in tropical and subtropical habitats of the world. They produce a robust basidiomata, with a membranous ring and have large (up 17.5 µm long), hexagonal thick-walled basidiospores. The pleurocystidia are clavate or fusiform, and the cheilocystidia narrowly lageniform to lageniform.

Clade I groups *P. magnispora* and *P. ovoideocystidiata*. However, it was only supported by PP. *Psilocybe magnispora* is from Thailand where it grows in dung and *P. ovoideocystidiata* has been found in the USA on wood or wood debris. Their morphological features are similar to clade “cubensae”, for they produce a robust basidiomata, hexagonal thick-walled basidiospores, and lageniform cheilocystidia. Though, they lack annulate basidiomata, with smaller (6–10 µm) basidiospores, and have ovoid to clavate or sometimes broadly fusiform pleurocystidia (Guzmán et al. 2007b, 2012).

Clade II contains *P. hopii* and *P. mescaleroensis*. Both grow in rich soils of the temperate zones in the USA. Their basidiomata are middle sized, the basidiospores have not angles, from 10–13 µm long, with thick-wall, and have pleurocystidia. Meanwhile, clade III groups *P. serbica* var. *serbica* and *P. serbica* var. *arcana*, both European taxa. The macromorphology is similar to those species recovered in clade “cyanescens” and II. *Psilocybe serbica* was considered in the *Cyanescens* complex by Krieglsteiner (1984) together with *P. cyanescens*. This clade agrees with the stirp *Serbica* of Noordeloos (2011).

Clade IV includes *P. fimetaria*, *P. pelliculosa*, and one undetermined taxon. These fungi grow in temperate zones in the USA and Europe, and have basidiospores without angles, thick to thin-walled, and sometimes with pleurocystidia. Clade “mexicanae” is composed by *P. cabiensis*, *P. gallaeciae*, *P. mexicana* (FIGS. 3 and 4), *P. pileocystidiata* and *P. subhoogshagenii*. It represents a lineage of tropical and subtropical places. This group has ovoid, rhomboid, and hexagonal, 6–12 µm long, thin or slightly thick-walled [0.5–0.8 (–1 µm)] basidiospores. *Psilocybe cabiensis* and *P. subhoogshagenii* were described from Colombia and are sister taxa. Further, *P. mexicana* and *P. pileocystidiata* grow in Mexico, and *P. gallaeciae* is just known from Galicia, Spain. The last one grows in grassland and gardens, sharing a similar habitat with *P. mexicana* that grows in tropical meadows in several regions of Mexico and Guatemala.

Clade “cordisporae” includes *P. eximia*, *P. fagicola*, *P. yungensis* (FIG. 9), and two undetermined species. In this clade, the basidiomata are small, with conical to papillate pilei. The basidiospores are rhomboid to subrhomboid, small and thick-walled. They show small and lageniform pleurocystidia, thick-walled hyphae from the pileus trama and hymenophoral trama, and setaceous hyphae in the basal mycelium of the stipe. *Psilocybe eximia* is from Papua New Guinea and the

remaining taxa grow in tropical and subtropical forests in Mexico. This clade fits well in the sect. *Cordisporae* (Guzmán 1983), according to the diagnostic features.

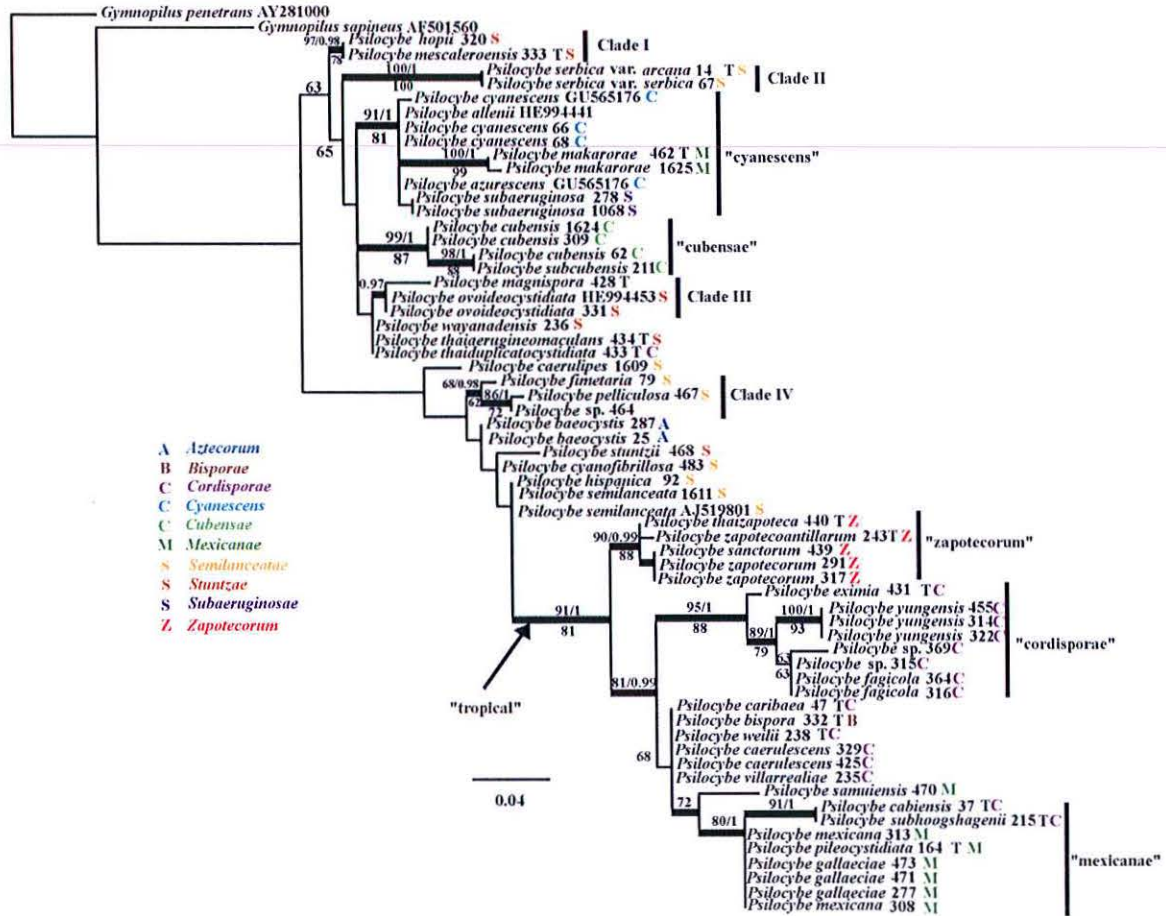


FIG 10. ML tree with average branch lengths from ITS rDNA sequences of *Psilocybe*. Maximum likelihood bootstrap support and posterior probability obtained from the Bayesian inference are indicated over the branches, bootstrap support obtained with MP is indicated below branches. Branch lengths are scaled to the expected number of nucleotide substitutions per site. Taxonomic types are indicated by a "T" by the end of the species name. Highly supported clades are with thickened branches. The traditional sections of Guzmán (1983) and Guzmán et al. (2007a) are indicated by colors.

Clade "zapotecorum" groups four species of the traditional sect. *Zapotecorum*: *P. sanctorum*, *P. thazapoteca*, *P. zapotecoantillarum*, and *P. zapotecorum* (FIGS. 6–7). They are characterized by robust to delicate basidiomata, small thin-walled basidiospores, and the presence of pleurocystidia. They grow in tropical and subtropical regions. *Psilocybe sanctorum* was described from Mexico; *P. zapotecorum* is known from Argentina, Brazil, Chile, Colombia, Mexico, and Peru; *P.*

zapotecoantillarum was described from Puerto Rico, and *P. thaizapoteca* from Thailand. This clade matches the diagnostic features provided by Guzmán (1983) to the sect. *Zapotecorum*.

Higher clades in Psilocybe

There is only one supported clade, here designated as the “tropical” clade. It contains clades “mexicanae”, “cordisporae”, “zapotecorum”, and *P. caerulescens* (FIG. 5) and its synonyms (*P. bispora*, *P. caribaea*, *P. villarrealii*, and *P. weilli*). It includes tropical and subtropical species with small basidiospores up to 11 µm long. *Psilocybe caerulescens* is macromorphologically very variable that is why several specimens were described under different names, but the molecular data and the micromorphological features place them as synonyms. *Psilocybe caerulescens* grows in subtropical places in the USA (Alabama, Georgia, and South Caroline), Mexico, and South America.

ITS intra and interspecific variation

Within *Psilocybe*, the genetic variation is low, ranging from 0 to 0.54% (TABLE III). For instance, there is no infraspecific variation in *P. mexicana* and the two ITS sequences of *P. makarorae* differ in 0.54 %. Some examples about the interspecific variation are shown in TABLES IV-IX. Based on sequence similarity and morphological data some synonyms were confirmed and others were found. *Psilocybe bispora*, *P. caribaea*, *P. villarrealii*, and *P. weilii*, all correspond to *P. caerulescens* because their sequences are identical (TABLE IV) and their macro and micromorphological features are very similar. Pertinent synonymization will be published elsewhere. Besides, *P. pileocystidiata* is a synonym of *P. mexicana* (TABLE V). Based only on morphology, Guzmán (2012) recently proposed the synonymy of *P. sanctorum* with *P. zapotecorum*, so it is ratified here (TABLE VI).

The intraspecific variation in *P. fagicola* and in *P. yungensis* is 0.2 % and 0.25%, respectively (TABLE III), but the interspecific variation between them is 2.9 % (TABLE VII). However, this does not occur in all clades, for example the relationships of *P. mexicana* and *P. gallaeciae* were not resolved due to the low divergence (0.37 %) between the two sequences, very low compared with the interspecific variation among other species. Other example is *P. cubensis* and *P. subcubensis*, species traditionally separated by morphological variations that have low DNA intraspecific variation (TABLE VIII).

DISCUSSION

The results are incongruent with traditional sections proposed by different authors. Diagnostic characters to some sections agree very well with the taxa recovered in natural groups. That is the case of clades “cubensae”, “cordisporae”, and “zapotecorum”, which correspond with the sections *Cordisporae*, *Cubensae*, and *Zapotecorum* (Guzmán 1983). In other cases, the diagnostic features of sect. *Mexicanae* agrees in part with clade “mexicanae” and some of the characteristics of sect. *Cyanescens* (Guzmán 1983) correspond with clade “cyanescens”. In the sense of Noordeloos (2011) stirp *Serbica* also was recovered. Below, supported clades are compared and discussed with the original description of the section that each one corresponds.

Sect. *Cordisporae* in the sense of Guzmán (1983) is the biggest section in *Psilocybe*, and was described with rhomboid basidiospores up to 8 µm long, presence of pleurocystidia, but inconspicuous, with the hyphae of subhymenium and/or subpellis thick-walled hyaline or with yellow brown to orange brown encrusted pigment. They are common in tropical and subtropical

forests. *Psilocybe eximia* from Indonesia is an additional taxon to the ones recovered by Ramírez-Cruz et al. (2013) in their clade “cordisporae”, thus not only neotropical species are grouped here. Some taxa that traditionally were placed in sect. *Cordisporae*, like *P. cabiensis*, *P. caerulescens*, *P. thaiduplicatocystidiata*, and *P. subhooshagenii*, were not recovered here. This was also pointed out in part by Ramírez-Cruz et al. (2013). The absence of these species has sense, since their morphological features, mainly the basidiospores form, do not fit in the diagnosis of sect. *Cordisporae*. Therefore, their placement in this section is an error in the first place.

Sect. *Cubensae* was diagnosed by Guzmán (1983) as “Coprophilous or subcoprophilous bluing species, with subhexagonal thick walled (0.5–1 µm) spores. Annulus well developed”. This group was recovered as monophyletic in this work and by Ramírez-Cruz et al. (2013). The taxa included are very similar in their morphology, being the main differences the basidiospores size, little bigger in *P. cubensis*, as well as the size of the basidiomata. With regard to the ITS sequence similarity, intraspecific variation in *P. cubensis* is 0.37 %. The difference between *P. cubensis* and *P. subcubensis* is 0.54%. It could be possible that they represent only one species instead of two, because the differences are not sharply defined. This clade corresponds to stirp *Caerulescentes*, sect. *Caerulescentes* according to Noordeloos (2011) that includes only *P. cubensis*. Singer (1986) groups other species in his stirp *Cubensis* besides *P. cubensis*. One of them is *P. fimetaria*, sampled here that was not recovered in this clade.

Sect. *Cyanescens* in the sense of Guzmán (1983) was described as having hyaline pleurocystidia and subellipsoid, up to 10 µm long, thick-walled basidiospores. This section is represented in this paper by *P. cyanescens* (FIG. 2) which was recovered in the clade “cyanescens” together with others, that have been considered as part of the *Cyanescens* complex by some authors, e.g., Borovička et al. (2012), excluding *P. makarorae*. We can find some morphological features that group the members of this clade, as the robust basidiomata growing in rotten wood, pileus never papillate, basidiospore lacking angles between 10–13 µm long, and presence of pleurocystidia and cheilocystidia. Based on the micromorphological features, the description of the section should be emended to allow longer basidiospores. These species grow in North America, Europe, Australia, and New Zealand. *Psilocybe makarorae* was considered in sect. *Mexicana* by Johnston and Buchanan (1995). However, in our analyses is not related with species of the clade “mexicanae” and it has the morphological characters mentioned above, so it should be considered in sect. *Cyanescens*. Ramírez-Cruz et al. (2013) obtained a clade with *P. cyanescens*, *P. subaeruginosa*, *P. serbica*, and *P. mescaleroensis*. In the present work *P. serbica* and *P. mescaleroensis* were recovered in different clades. Guzmán (1995) merged sect. *Cyanescens* in sect. *Semilanceata*; this proposal was not supported in these analyses. Singer (1986) besides *P. cyanescens*, included *P. baeocystis* and *P. serbica* in his stirp *Cyanescens*, but these species were not recovered here. Noordeloos (2011) considered the following taxa in stirp *Cyanescens*, sect. *Cyanescens*: *Psilocybe azurescens*, *P. cyanescens*, and *P. stuntzii*, the last one was not recovered in this clade.

Sect. *Mexicanae*, according to Guzmán (1983), has species with basidiospores large, more than 8 µm long, subrhomboid, and with thick wall. Their basidiomata are mycenoid or collybioid, common in subtropical regions, rare in the tropics. In this work, *P. mexicana*, *P. gallaeciae*, and *P. pileocystidiata* from this section, are recovered in clade “mexicanae”, together with *P. cabiensis* and *P. subhooshagenii* (from the traditional sect. *Cordisporae*). The last two species do not agree with the diagnosis of the sect. *Mexicanae*, because they differ in the size, shape, and wall thickness of the basidiospores. The diagnosis of the section should be emended to include all the variation observed in these taxa, proving the clade permanence in future works with more species and DNA regions. Stirp *Mexicanae* of Singer (1986) only includes *P. mexicana*. *Psilocybe samuiensis* from traditional sect. *Mexicanae* (Guzmán et al. 1993) was recovered outside the clade “mexicanae”, but with low support.

Sect. *Zapotecorum* groups subtropical and temperate widespread species with thin-walled, up to 0.5 μm thick, subellipsoid, subrhomboid, or subfusiform basidiospores, and hyaline or brownish pleurocystidia, or absent (Guzmán 1983). All specimens sampled from this section form a natural group, recovered in clade “zapotecorum”, also recovered by Ramírez-Cruz et al. (2013). The morphological features in these species are quite similar. They have small basidiospores, with not well-defined angles or without angles, up to 8 μm long, and thin-walled, pleurocystidia narrow, and cheilocystidia branched. Only *P. zapotecorum* and *P. sanctorum* have two kinds of pleurocystidia, very variable. The macromorphological features of the species contained in this section are variable, and this had led to propose new species that actually correspond to *P. zapotecorum*.

Stirp *Serbica*, sect. *Caerulescentes* of Noordeloos (2011) includes all the varieties of *P. serbica*. Two taxa were sampled in this work and were recovered in clade III. Probably, they represent the same species, because *P. serbica* var. *arcana* was separated of *P. serbica* var. *serbica* by the absence of pleurocystidia; however, we found these cells in the type of *P. serbica* var. *arcana*.

The remaining sections of different authors (TABLE I) do not correspond with any of the clades recovered here. Sect. *Neocaledonicae* (Guzmán 2004) contains a mix of *Deconica* and *Psilocybe* (Ramírez-Cruz et al. 2013), with only two hallucinogenic species, *P. magnispora* and *P. naematoliformis*. Here only *P. magnispora* is represented and resulted together with *P. ovoideocystidiata* with some characters in common, like basidiospore size and cystidia shape. Thus, this section is not natural and cannot be kept in *Psilocybe* because the type species belongs to the genus *Deconica* (Ramírez-Cruz et al. 2014). Sect. *Bisporae* was recently described by Guzmán et al. (2007a) to accommodate *P. bispora*, which resulted a synonym of *P. caerulescens* (see below). The ITS sequences similarity is 100% and the macro and micromorphological features are very similar between these species.

Intraspecific and interspecific variation in Psilocybe

It is known that the ITS region does not vary equally in all lineages. In Basidiomycotina, Hughes et al. (2009) considered 2–3% of divergence between ITS sequences of the same species; however, it varies according to particular groups. For instance, intraspecific variation in *Boletus* is 0.3 % and in *Amanita* 0.9 % (Nilsson et al. 2008). In *Psilocybe* we found low intraspecific ITS variation. The sequence difference among species was also low. In those well-resolved clades as “cordisporae”, it is easy to delimitate species; however, in others it result problematic as in clades “mexicanae” and “cyanescens”. In clade “mexicanae”, *P. mexicana* and *P. gallaeciae* are very similar in morphology and differ in only two bases in the ITS sequences. Thus, these species would correspond to the same taxon or as two varieties because they have few micromorphological differences as the basidiospore size and pileocystidial form, as well as the geographic distribution, *P. gallaeciae* grows in Spain, and *P. mexicana* can be found in Mexico and Guatemala. By contrast, in the *Cyanescens* complex there are more molecular variation, but still the substitution rates are low and the morphology is almost identical, as was pointed out by Borovička et al. (2010, 2012). Ramírez Cruz et al. (2013) suggested that probably *P. cyanescens* is the same species that *P. subaeruginosa*; however, the geographical distribution is different.

A similar pattern to the one discovered by Ramírez-Cruz et al. (2013) was found here, with a well-supported clade named here as “tropical” that groups tropical and subtropical species not included in previous works (e.g., Moncalvo et al. 2002; Matheny et al. 2006). Some of these species have been important for their traditional use in ceremonies by some ethnics groups in Mexico. This approach about evolutionary relationships in *Psilocybe* will help us to reevaluate the characters that had been used in the classification of the genus.

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Table I. Infrageneric classification of hallucinogenic sections in *Psilocybe sensu lato* (sections in bold were sampled in this study).

Guzmán (1983, 1995, 2004) and Guzmán et al. (2007a)	Singer (1986)	Noordeloos (2011)
Sect. <i>Aztecorum</i>	Sect. <i>Caerulescentes</i>	Sect. <i>Atrobrunneae</i>
Sect. <i>Bisporae</i>	Stirp	Sect. <i>Caerulescentes</i>
Sect. <i>Brunneocystidiatae</i>	<i>Caerulescens</i>	Stirp <i>Caerulescentes</i>
Sect. <i>Cordisporae</i>	Stirp <i>Caerulipes</i>	Stirp <i>Cyanescens</i>
Sect. <i>Cubensae</i>	Stirp <i>Cubensis</i>	Stirp <i>Serbica</i>
Sect. <i>Cyanescens</i>	Stirp <i>Cyanescens</i>	Sect. <i>Psilocybe</i>
Sect. <i>Mexicanae</i>	Stirp <i>Mexicanae</i>	Stirp <i>Psilocybe</i>
Sect. <i>Neocaledonicae</i>	Stirp <i>Silvatica</i>	Stirp <i>Puberula</i>
Sect. <i>Semilanceatae</i>	Stirp <i>Yungensis</i>	
Sect. <i>Stuntzae</i>	Sect. <i>Tenaces</i>	
Sect. <i>Subaeruginosae</i>		
Sect. <i>Zapotecorum</i>		

Table II. Species of *Psilocybe* plus the outgroup included in this study. Specimens that provided the sequences used for molecular phylogenetic analysis in this paper are listed in bold.

Species	Section	Specimen-DNA number	Herbarium and specimen voucher	Collector, number	Date of collection	Country	GenBank Number
	1. Guzmán (1983, 1995, 2004) and Guzmán et al. (2007a) 2. Singer (1986) 3. Noordeloos (2010)						
<i>Psilocybe allenii</i> Borov., Rockefeller & P.G. Werner	—	—	PRM 899877	—	—	—	HE994441
<i>P. azurescens</i> Stamets & Gartz	3. <i>Psilocybe</i> , stirp <i>Psilocybe</i>	—	PRM 901020	—	—	—	GU565173
<i>P. baeocystis</i> Singer & A.H. Sm.	1. <i>Aztecorum</i> 2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i>	Ps-25	OSC-51196	Morgan s.n.	11 Jan 1979	USA	X
		Ps-287	UBC-F10235	P. Kroeger 89	28 Nov 1981	Canada	X
<i>P. bispora</i> Guzmán, Franco-Mol. & Ram.-Guill.	1. <i>Bisporae</i>	Ps-332	IBUG	A.E. Franco Molano 1766, holotype	11 Mar 2003	Colombia	X
<i>P. cabiensis</i> Guzmán, M. Torres & Ram.-Guill.	1. <i>Cordisporae</i>	Ps-37	XAL	G. Guzmán 35331, isotype	26 Sep 2002	Colombia	X
<i>P. caerulescens</i> Murrill	1. <i>Cordisporae</i> 2. <i>Caerulescentes</i> , stirp <i>Caerulescens</i>	Ps-329	IBUG	I.J. Franco-Galván 1	31 Aug 2005	Mexico	KC669281
		Ps-425	NY	K.P. Dumont -VE 4145	7 Jul 1972	Venezuela	X
<i>P. caerulipes</i> (Peck) Sacc.	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Caerulipes</i>	UT-1609	TENN-064502	SAT09-216-06	8 Aug 2009	USA	KC669282
<i>P. caribaea</i> Guzmán, T.J. Baroni & Tapia	1. <i>Cordisporae</i>	Ps-47	XAL	T.J. Baroni 7971, isotype	29 Jun 1996		X
<i>P. cubensis</i> Earle (Singer)	1. <i>Cubensae</i> 2. <i>Caerulescentes</i> , stirp <i>Cubensis</i> 3. <i>Caerulescentes</i> , stirp <i>Caerulescentes</i>	Ps-62	XAL	G. Guzmán 31198	8 Jul 1995	USA	X
		Ps-309	IBUG	I. Álvarez-Barajas 3	29 Aug 2005	Mexico	X
		UT-1524	TENN-051528	RHP5203	7 May 1992	Costa Rica	KC669284
<i>P. cyanescens</i> Wakef.	1. <i>Semilanceatae</i>	Ps-66	XAL	J. Workman & P.	Without date	USA	KC669285

	2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i>			Werner s.n.			
	3. <i>Psilocybe</i> , stirp <i>Psilocybe</i>	Ps-68	XAL	S. Voogelbreinder s.n. PRM 902040	Jun 2001	Australia	X
		—	—		—	—	GU565176
<i>P. cyanofibrillosa</i> Guzmán & Stamets	1. <i>Semilanceatae</i>	Ps-483	IBUG	B. Fogg s.n.	Sep 2012	USA	X
<i>P. eximia</i> E. Horak & Desjardin	1. <i>Cordisporae</i>	Ps-431	XAL	E. Horak 7326, isotype	10 Jan1999	Indonesia	X
<i>P. fagicola</i> R. Heim & Cailleux	1. <i>Cordisporae</i>	Ps-316	XAL	M.A. Gómez s.n.	31 Aug 2007	Mexico	X
		Ps-364	IBUG	M.A. Gómez 22731	Jul 2010	Mexico	KC669288
<i>P. fimetaria</i> (P.D. Orton) Watling	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Cubensis</i> 3. <i>Psilocybe</i> , stirp <i>Psilocybe</i>	Ps-79	XAL	M. Contu s.n.	18 Oct1996	Italy	X
<i>P. gallaeciae</i> Guzmán & M.L. Castro	1. <i>Mexicanae</i>	Ps-277	XAL	F. Valeiras "B"	5 Nov1999	Spain	X
		Ps-471	XAL	R. Fernández-Sasia s.n.	28 Jul 2011	Spain	X
		Ps-473	XAL	J. Pérez s.n.	5 Nov 1999	Spain	X
<i>P. hispanica</i> Guzmán	1. <i>Semilanceatae</i> 3. <i>Psilocybe</i> , stirp <i>Psilocybe</i>	Ps-92	XAL	R. Fernández-Sasia s.n.	15 Jan 2005	Spain	KC669289
<i>P. hopii</i> Guzmán & J. Greene	1. <i>Semilanceatae</i>	Ps-320	XAL	J. Greene s.n.	12 Aug 2007	USA	X
<i>P. magnispora</i> E. Horak, Guzmán & Desjardin	1. <i>Neocaledonicae</i>	Ps-428	XAL	E. Horak 10171, isotype	10 Jul 2002	Thailand	X
<i>P. makarorae</i> P.R. Johnst. & P.K. Buchanan	1. <i>Mexicanae</i>	Ps-462	PDD 57396	P.R. Johnston, B.P. Segedin & R.H. Petersen s.n., holotype TFB7096	16 May 1990	New Zealand	X
	1. <i>Mexicanae</i>	UT-1625	TENN-054116		12 May 1994	New Zealand	X
<i>P. mescaleroensis</i> Guzmán, Walstad, E. Gándara & Ram.-Guill.	1. <i>Stuntzae</i>	Ps-333	XAL	L.F. Walstad s.n., holotype	Aug 2005	USA	KC669290
<i>P. mexicana</i> R. Heim	1. <i>Mexicanae</i> 2. <i>Caerulescentes</i> , stirp	Ps-308	IBUG	M.R. Sánchez-Jácome 1038	30 Jun 2002	Mexico	KC669291

	<i>Mexicanae</i>						
<i>P. ovoideocystidiata</i> Guzmán & Gaines	1. <i>Stuntzae</i>	Ps-313	IBUG	A. Cortes-Pérez s.n.	25 Jun 2009	Mexico	X
		Ps-333	XAL	R. V. Gaines 51b	5 Jun 2005	USA	X
<i>P. pelliculosa</i> (A.H. Sm.) Singer & A.H. Sm.	1. <i>Semilanceatae</i> 2. Sect. <i>Caerulescentes</i> , stirp <i>Silvatica</i>	Ps-467	IBUG	S. Chronick s.n.	Dec 2011	USA	HE994453 KC669292
<i>P. pileocystidiata</i> Guzmán & Ram.-Guill.	1. <i>Mexicanae</i>	Ps-164	XAL	F. Ramírez-Guillén 199, holotype	31 Jul 2003	Mexico	X
<i>P. sanctorum</i> Guzmán	1. <i>Zapotecorum</i>	Ps-439	XAL	R. Valenzuela 4073	16 Sep 1994	Mexico	X
<i>P. samuiensis</i> Guzmán, Bandala & J.W. Allen	1. <i>Mexicanae</i>	Ps-470	XAL	Sihanonth & J. Allen	18 Jun 2004	Thailand	KC669293
<i>P. semilanceata</i> (Fr.) P. Kumm.	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i> 3. <i>Caerulescentes</i> , stirp <i>Serbica</i>	UT-1611	TENN-058220	S.A. Redhead, N. Psurtserva	27 Aug 1999	Russia	X
				MA-Fungi 39559			AJ519801
<i>P. serbica</i> var. <i>arcana</i> (Borovička & Hlaváček) Borovička, Oborník & Noordel.	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i> 3. <i>Caerulescentes</i> , stirp <i>Serbica</i>	Ps-14	PRM-895093	J. Borovička and J. Hlaváček, holotype	11 Nov 2000	Czechoslovakia	X
<i>P. serbica</i> M.M. Moser & E. Horak var. <i>serbica</i>	1. <i>Semilanceatae</i> 2. <i>Caerulescentes</i> , stirp <i>Cyanescens</i> 3. <i>Caerulescentes</i> , stirp <i>Serbica</i>	Ps-67	WU-4448	I. Krisai s.n.	5 Nov 1987	Austria	KC669294
<i>P. stuntzii</i> Guzmán & J. Ott	1. <i>Stuntzae</i> 3. <i>Caerulescentes</i> , stirp <i>Cyanescens</i>	Ps-468	IBUG	S. Chornick	October 2011	USA	KC669295
<i>P. subaeruginosa</i> Cleland	1. <i>Subaeruginosae</i>	Ps-278	XAL	Y.S Chang 132	27 Apr 1989	Tasmania	X
		UT-1608	TENN-065481	PBM 3218	9 Jun 2009	Australia	KC669278
<i>P. subcubensis</i> Guzmán	1. <i>Cubensae</i>	Ps-211	XAL	G. Guzmán 35102	8 Oct 2001	Nepal	KC669297
<i>P. subhoogshagenii</i> Guzmán, M. Torres & Ram.-Guill.	1. <i>Cordisporae</i>	Ps-215	XAL	G. Guzmán 35293, holotype	24 Sep 2002	Colombia	X
<i>P. subcubensis</i> Guzmán	1. <i>Cubensae</i>	Ps-211	XAL	G. Guzmán 35102	8 Oct 2001	Nepal	KC669297

<i>P. thalpaerugineomaculans</i> Guzmán, Karunarathna & Ram.-Guill.	1. <i>Stuntziae</i>	Ps-434	XAL	S.C. Karunarathna NTS-121, holotype	27 Jul 2010	Thailand	KC669298
<i>P. thaiduplicatocystidiata</i> Guzmán, Karunarathna & Ram.-Guill.	1. <i>Cordisporae</i>	Ps-433	XAL	S.C. Karunarathna NTS-120, isotype	27 Jul 2010	Thailand	KC669299
<i>P. thazapoteca</i> Guzmán, Karunarathna & Ram.-Guill	1. <i>Zapotecorum</i>	Ps-440	XAL	G. Guzmán 38342, holotype	12 Jul 2010	Thailand	KC669300
<i>P. villarealii</i> Guzmán	1. <i>Cordisporae</i>	Ps-235	XAL	F.A. Castillo s.n.	Sep 2004	Mexico	X
<i>P. wayanadensis</i> K.A. Thomas, Manim. & Guzmán	1. <i>Stuntziae</i>	Ps-236	XAL	K.A. Thomas T-320d	31 Oct 1999	India	X
<i>P. weilii</i> Guzmán, Stamets & F. Tapia	1. <i>Cordisporae</i>	Ps-238	XAL	P. Stamets 951101, holotype	Sep 1995	USA	X
<i>P. yungensis</i> Singer	1. <i>Cordisporae</i> 2. <i>Caerulescentes</i> , stirp <i>Yungensis</i>	Ps-314	XAL	M.A. Gómez 2717	12 Jun 2007	Mexico	X
		Ps-322	XAL	M.A. Gómez 2467	8 Jul 2007	Mexico	X
		Ps-455	XAL	A. Cortés-Pérez 549	30 Oct 2010	Mexico	KC669301
<i>P. zapotecoantillarum</i> Guzmán & T.J. Baroni & Lodge	1. <i>Zapotecorum</i>	Ps-243	XAL	S. Cantrell & Salgado s.n., isotype	23 May 2000	Puerto Rico	KC669302
<i>P. zapotecorum</i> R. Heim	1. <i>Zapotecorum</i> 2. <i>Caerulescentes</i> , stirp <i>Caerulescens</i>	Ps-291	IBUG	F. Castillo 27	20 Oct 2007	Mexico	X
		Ps-317	IBUG	V. Ramírez-Cruz 1094	30 Jul 2009	Mexico	KC669303
<i>Psilocybe</i> sp. 1	1. <i>Cordisporae</i>	Ps-315	IBUG	V. Ramírez-Cruz 551	25 Aug 2006	Mexico	KC669304
<i>Psilocybe</i> sp. 2	1. <i>Cordisporae</i>	Ps-369	IBUG	V. Ramírez-Cruz 1328	12 Aug 2010	Mexico	KC669305
<i>Psilocybe</i> sp. 3	1. <i>Semilanceatae</i>	Ps-464	IBUG	M. No-Line s.n.	19 Dec 2011	USA	KC669306
Outgroup							
<i>Gymnopilus penetrans</i> (Fr.) Murrill			IBUG	L. Guzmán-Dávalos 8223	19 Nov 1999	France	AY281000
<i>Gymnopilus sapineus</i> (Fr.) Murrill			IB 78/225				AF501560

Table III. Intraspecific variation of ITS rDNA sequences in *Psilocybe*.

Taxon	Number of sequences	% of variation
<i>Psilocybe baeocystis</i>	2	0.00
<i>P. caerulescens</i>	2	0.00
<i>P. cubensis</i>	3	0.18
<i>P. cyanescens</i>	2	0.45
<i>P. fagicola</i>	3	0.20
<i>P. gallaeciae</i>	3	0.00
<i>P. makarorae</i>	2	0.54
<i>P. mexicana</i>	3	0.00
<i>P. semilanceata</i>	2	0.00
<i>P. subaeruginosa</i>	2	0.00
<i>P. yungensis</i>	3	0.25
<i>P. zapotecorum</i>	6	0.18

Table IV. ITS rDNA variation in *Psilocybe caerulescens* and its synonyms.

	<i>P. bispora</i> 332	<i>P. caerulescens</i> 329	<i>P. caerulescens</i> 425	<i>P. caribaea</i> 47	<i>P. villarrealii</i> 235	<i>P. weilii</i> 238
<i>P. bispora</i> 332	0	0	0	0	0	0
<i>P. caerulescens</i> 329		0	0	0	0	0
<i>P. caerulescens</i> 425			0	0	0	0
<i>P. caribaea</i> 47				0	0	0
<i>P. villarrealiae</i> 235					0	0
<i>P. weilii</i> 239						0

Table VII. ITS rDNA variation among species of clade "cordisporae".

	<i>P. eximia</i> 431	<i>P. fagicola</i> 316	<i>P. fagicola</i> 364	<i>Psilocybe</i> sp.1 315	<i>Psilocybe</i> sp.2 369	<i>P. yungensis</i> 314	<i>P. yungensis</i> 322	<i>P. yungensis</i> 455
<i>P. eximia</i> 431	0.00	4.37	3.92	4.33	7.08	5.28	5.07	5.47
<i>P. fagicola</i> 316		0.00	0.20	0.00	2.07	2.90	2.92	2.92
<i>P. fagicola</i> 364			0.00	0.19	1.79	2.49	2.49	2.49
<i>Psilocybe</i> sp. 1 315				0.00	1.79	2.69	2.68	2.69
<i>Psilocybe</i> sp. 2 369					0.00	1.77	2.12	1.76
<i>P. yungensis</i> 314						0.00	0.38	0.00
<i>P. yungensis</i> 322							0.00	0.37
<i>P. yungensis</i> 455								0.00

Table VIII. ITS rDNA variation among *Psilocybe cubensis* and *P. subcubensis*.

	<i>P. cubensis</i> 1624	<i>P. cubensis</i> 309	<i>P. cubensis</i> 62	<i>P. subcubensis</i> 211
<i>P. cubensis</i> 1624	0.00	0.00	0.37	0.54
<i>P. cubensis</i> 309		0.00	0.37	0.54
<i>P. cubensis</i> 62			0.00	0.00
<i>P. subcubensis</i> 211				0.00

Table V. ITS rDNA variation among *Psilocybe gallaeciae*, *P. mexicana*, and *P. pileocystidiata*.

	<i>P. mexicana</i> 308	<i>P. mexicana</i> 313	<i>P. pileocystidiata</i>	<i>P. gallaeciae</i> 277	<i>P. gallaeciae</i> 471	<i>P. gallaeciae</i> 473
<i>P. mexicana</i> 308	0.00	0.00	0.00	0.37	0.37	0.38
<i>P. mexicana</i> 313		0.00	0.00	0.38	0.38	0.38
<i>P. pileocystidiata</i>			0.00	0.38	0.38	0.38
<i>P. gallaeciae</i> 277				0.00	0.00	0.00
<i>P. gallaeciae</i> 471					0.00	0.00
<i>P. gallaeciae</i> 473						0.00

Table VI. ITS rDNA variation among species of clade “zapotecorum”.

	<i>P. thaizapoteca</i> 440	<i>P. zapotecoantillarum</i> 243	<i>P. zapotecorum</i> 291	<i>P. zapotecorum</i> 317
<i>P. thaizapoteca</i> 440	0.00	1.15	0.95	0.76
<i>P. zapotecoantillarum</i> 243		0.00	1.33	1.14
<i>P. zapotecorum</i> 291			0.00	0.18
<i>P. zapotecorum</i> 317				0.00

Capítulo V. Ramírez-Cruz V, Guzmán G, Villalobos-Arámbula AR, Matheny PB, Guzmán-Dávalos L. 2013. Phylogenetic inference of the genus *Deconica* based on ITS rDNA sequences with description of a new species with chrysocystidia. *Mycologia*

Phylogenetic inference of the genus *Deconica* based on ITS rDNA sequences
with description of a new species with chrysocystidia

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Abstract: The genus *Deconica*, formerly considered as part of the genus *Psilocybe*, belongs to Strophariaceae. A phylogenetic approach with ITS rDNA sequences is presented, with a more intensive sampling in relation with previous works. The analyses resulted in five well-supported clades in the genus, none of them corresponding with sections previously proposed. A new species is described of sect. *Chrysocystidiatae*.

Keywords: Strophariaceae, taxonomy, molecular systematics, sect. *Chrysocystidiatae*

INTRODUCTION

The genus *Deconica* (W.G. Sm.) P. Karst. (FIGS. 1–8) was described by Smith (1870) as a tribe inside of *Agaricus*, then raised to generic level by Karsten (1879). For long time, it was considered as a good genus (e.g., Singer 1951). Since the sixties (e.g., Singer 1962), it was taken as synonym of *Psilocybe* (Singer 1975, 1986; Guzmán 1986). Recently, the works of Moncalvo et al. (2002), Matheny et al. (2006), and Ramírez Cruz et al. (2013) have shown that *Deconica* and *Psilocybe* are independent genera. 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*), whereas, the name *Deconica* is applied to those former species of *Psilocybe* and *Melanotus* (FIGS. 6–7) that lack hallucinogenic properties. Pertinent combinations in *Deconica* for several non-hallucinogenic *Psilocybe* have already been made (Noordeloos 2009; Cooper 2012; Ramírez-Cruz et al. 2012; Redhead 2012). Macromorphology and micromorphology are very similar in *Deconica* and *Psilocybe*; nevertheless, hallucinogenic compounds are only present in *Psilocybe* (Beug and Bigwood 1981, Gartz et al. 1994; Marcano et al. 1994). The presence of these substances is detected by blue tones when basidiomata are bruised (Guzmán 1983). It is interesting to note that these genera do not have a sister relationship (Matheny et al. 2006; Ramírez-Cruz et al. 2013). As in *Psilocybe*, the species of *Deconica* grow in several substrates as plant debris, rotten wood, and dung; and have mycenoid, collybioid, or omphalioid basidiomata; conical, convex, or hemispherical pileus, with or without umbo, dry or viscid; free to

broadly adnate lamellae, brown to purple brown, and slender stipe (Noordeloos 2011). Guzmán (2005) estimated 277 species in *Psilocybe* s.l., from which 144 are bluing species; thus, there are approximately 133 species in the genus *Deconica*. Noordeloos (2011) in his recent book about Strophariaceae considered 24 species in *Deconica* from Europe.

Traditional classifications in a broad sense are those proposed by Guzmán (1983, 1995), Singer (1986), Watling and Gregory (1987), and Noordeloos (2011). Guzmán (1983, 1995) considered eight non-hallucinogenic sections: *Atrobrunnea*, *Blattariopsidae*, *Coprofilae*, *Merdariae*, *Pratensae*, *Psilocybe*, *Singerianae*, and *Squamosae*. Meanwhile, Singer (1986) included five sections: *Atrobrunnea*, *Chrysocystidiatae*, *Merdariae*, *Psilocybe*, and *Septembres*. Watling and Gregory (1987) mentioned four non-bluing sections: *Merdariae*, *Psilocybe* (*Stirps Montana*, *Inquilina*, and *Bullacea*), *Atrobrunnea*, and *Squamosae*. The most recent infrageneric classification of this genus was made by Noordeloos (2011), who divided *Deconica* in three sections: *Deconica*, *Melanotus*, and *Merdariae*. In sect. *Deconica* there are two subsections, *Deconica* and *Inquilinae*. The classification in the sections or stirps above mentioned have been based in almost the same attributes of the basidiomata, basidiospores, pileipellis, and cystidia, among others. Although, the characters are the same, the interpretation of each author varies according to their criteria.

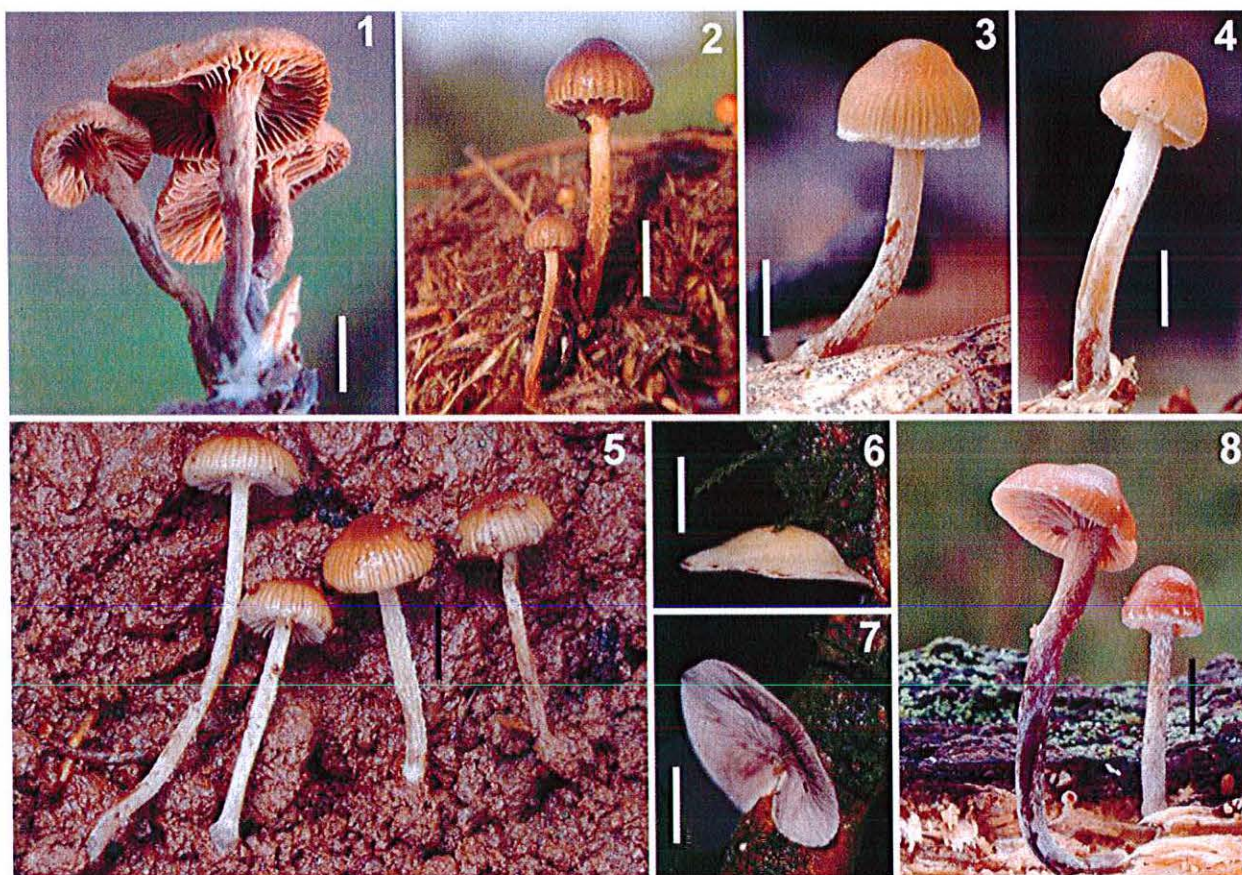


FIG. 1–8. Macromorphology of *Deconica*. 1. *Deconica cokeriana*. (photo: C. Braaten) 2. *D. coprophila* (photo: G. Munguía Lino). 3–4. *Deconica* sp. 2 (photo: L. Guzmán-Dávalos). 5. *D. australiana* (photo: P. Matheny). 6–7. *Deconica* aff. *horizontalis* (photo: V. Ramírez-Cruz). 8. *Deconica* sp. (photo: P. Matheny).

Previous works about relationships in *Deconica* are those by Moncalvo et al. (2002) who include 22 taxa of non-bluing *Psilocybe*. Boekhout et al. (2005) used mating, morphology, and RAPD's in the traditional sect. *Psilocybe* (non-bluing). In a recent work, Ramírez-Cruz et al. (2013) included 14 taxa of *Deconica* and found a clade named “chrysocystidiata”. Based in a more intensive taxon sampling, the aims of this work are 1) resolve infra-generic phylogenetic relationships within *Deconica* and evaluate morphological classifications, and 2) describe a new species with chrysocystidia.

MATERIALS AND METHODS

Taxon sampling

We sampled 46 herbarium specimens (Table I), including species of most sections within *Deconica* (formerly non-bluing *Psilocybe* s.l.). *Kuehneromyces* Singer & A.H. Sm. was used as outgroup based on the work of Matheny et al. (2006) and Ramírez-Cruz et al. (2013), being the sister group of this genera.

Micromorphological analyses

Specimens belonging to non-hallucinogenic sections were studied, including seven types. Types and additional specimens were from the following herbaria: IBUG, ICN, F, TENN, WU, and XAL. Color notes for fresh specimens were taken from Korneup and Wanscher (1978) or Munsell Color (1976). Microscopic characteristics were observed from dried material mounted in 3% potassium hydroxide (KOH) and Congo red. Patent blue V 1% was used to detect chrysocystidia (Jahnke 1984). Microscopic structure measures and drawings were made using a 100× oil-immersion objective, in Zeiss K7 or Zeiss Axioskop 40 microscopes. Structures were photographed through Axio Vision 4 software in a Zeiss Axioskop 40 microscope.

DNA extraction, amplification, and sequencing

DNA was extracted from herbarium specimens, using one of the following procedures: Doyle and Doyle (1987), Aljanabi and Martinez (1997), or the Omega Bio-Tek fungal DNA Extraction Kit. Polymerase Chain Reaction (PCR) was performed to amplify the internal transcribed spacer 1 (ITS1), the 5.8S rDNA gene, the internal transcribed spacer 2 (ITS2), following the protocol indicated by Guzmán-Dávalos et al. (2003). The primer pairs ITS1F/ITS4S, ITS1F/ITS4, ITS1/ITS4, and ITS5/ITS4 were used to amplify the entire ITS. Primers 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). PCR products were cleaned using Illustra GFX columns (GE Healthcare) or Quiagen PCR Purification Kit following the manufacturer's protocols, and in some cases with the enzymatic method Exo-sap-IT[®] (USB Corporation). Sequencing reactions were performed with BigDye[™] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in 20 µL or 10 µL final volume, following the manufacturer's protocol and using the same primers as in the DNA amplification for ITS. Sequencing reactions were purified with AutoSeq[™] G-50 column (Amersham Biosciences), and finally 18 µL of formamide were 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 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, MI, USA). Sequence assembly and alignment were carried out with MacClade 4.08 (Maddison and Maddison 2000). Probabilistic and Maximum Parsimony (MP) analyses were carried out. MP heuristic searches were conducted in PAUP* 4.0b10 (Swofford 2002) with stepwise addition, random addition sequence with 1 000 replicates, tree-bisection-reconnection as branch swapping algorithm, and branches collapsed if maximum branch length is zero. Support for nodes recovered from MP analysis was obtained from 1 000 bootstrap (BSP) replications, with the same parameters as above but with 10 random addition replicates; also the branch swapping was limited to 1 million rearrangements per replicate.

For the Bayesian analyses the most likely model of evolution was determined using jModeltest 3.7 with the Akaike criterion (Posada and Crandall 1998). The Bayesian analysis was run for two million generations with trees sampled every 100th generations in Mr. Bayes 3.2 (Ronquist and Huelsenbeck 2003). The performance of analyses was evaluated using Tracer v.1.4.1 (Rambaut 2009), to check the convergence between chains of runs of Mr. Bayes. The first 2 500 trees, representing the burn-in phase, were discarded and posterior probabilities (PP) were calculated from a consensus of the remaining 7 500 trees. In Maximum Likelihood (ML) analyses, 1 000 rapid bootstrap inferences were performed with all free model parameters estimated by RAxML using a GTRGAMMAI model and empirical base frequencies (Stamatakis 2006). The trees were visualized in PAUP* 4.0b10 or in FigTree v1.4.1 (Rambaut 2011).

RESULTS

DNA sequence data and phylogenetic analyses

We obtained 34 new sequences for the ITS region, 10 were sequenced in a previous work (Ramírez-Cruz et al. 2013), and three sequences were from GenBank (Table I). The alignment of the 47 ITS sequences resulted in 475 nucleotide positions, after removing both ends and ambiguously aligned regions do to gaps in the ITS matrix. From the included characters, 61 were parsimoniously informative. The best-fit model of molecular evolution was the TVM+I+G (Posada and Crandall 1998).

The tree topology (FIG. 9) recovered by MP, ML, and Bayesian inference was almost the same. In the MP analyses 1544 most parsimonious trees of 210 steps were recovered, with CI = 0.4437 excluding non-informative characters and RC = 0.4661.

Clades in Deconica

With the ITS matrix, five clades within *Deconica* are recovered (FIGS. 9) labeled here A-E.

Clade A includes a lineage with chrysocystidia as the synapomorphy, and a reversion to its absence in *Deconica* sp. 1 (481). The clade contains the following taxa: *D. aequatoriae*, *D. cokeriana* (FIG. 1), *D. neorhombispora* (= *D. subbrunneocystidiata*), *D. rhombispora* (misapplied name), *D. thailandensis*, *D. umbrina*, and several undetermined taxa, one of them is described here. All these species have small basidiomata, ellipsoid, rhomboid, or hexagonal, small basidiospores and grow in rotten wood. This lineage is widely distributed and is represented by tropical or subtropical species from Mexico, Brazil, Ecuador, the USA, Thailand, Malaysia, and Australia.

Clade B groups the following species: *D. alpestris*, *D. apeliculosa*, *D. bullacea*, *D. graminicola*, *D. aff. latispora*, *D. aff. smithiana*, *D. subviscida*, *D. xeroderma*, and an undetermined taxon. Small basidiomata,

less than 8 μm long thin-walled (in some cases up to 0.5 μm) basidiospores, absent pleurocystidia, and lageniform cheilocystidia are the characteristics of this clade. These mushrooms grow in several

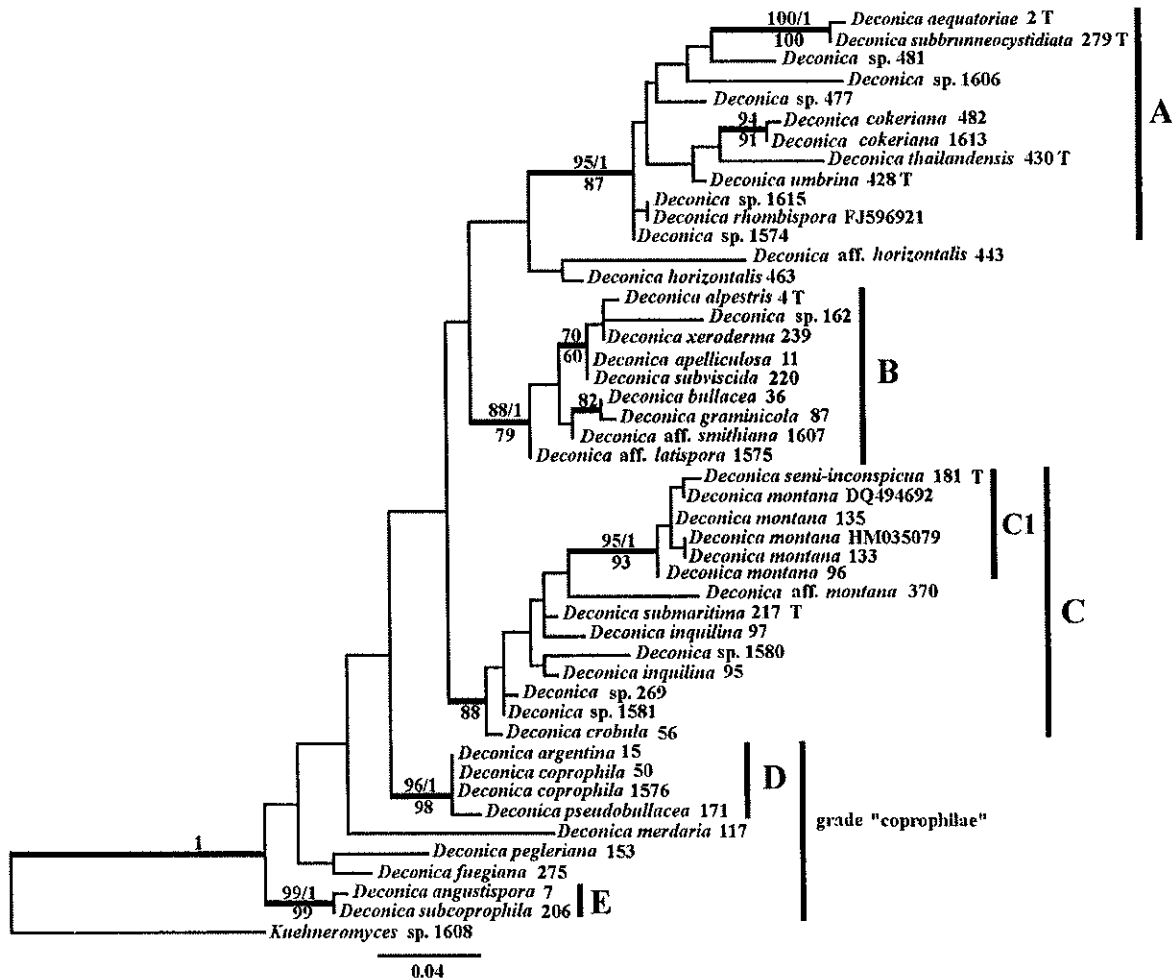


FIG. 9. ML tree with average branch lengths from ITS rDNA sequences of *Deconica*. Maximum likelihood bootstrap support and posterior probability are indicated over the branches, bootstrap support obtained with MP is indicated below branches. Branch lengths are scaled to the expected number of nucleotide substitutions per site. Taxonomic types are indicated by a (T) by the end of the species name. Thickened branches indicate highly supported clades.

substrates as in soil, plant debris, rotten wood, and also in dung, in temperate zones in Europe and the USA; none tropical taxon was recovered here.

Clade C was supported only with MP. Includes clade C1 (composed by *D. montana* and *D. semi-inconspicua*), *D. crobula*, *D. inquilina*, *D. aff. montana*, *D. submaritima*, and three undetermined taxa (FIGS. 3–4, 8). The specimens sampled here are from temperate zones from the USA, Europe, and Mexico. The members of this clade fruit among mosses or on plant debris and are characterized by small

basidiomata, thin to slightly thick-walled basidiospores up to 10 µm long with or without angles, and lageniform cheilocystidia.

Clade D groups *D. argentina*, *D. coprophila* (FIG. 2), and *D. pseudobullacea*. All these species are coprophilous and have basidiospores with angles (hexagonal). It agrees in part with sect. *Coprophilae* in the sense of Guzmán (1983); however *D. pseudobullacea* belongs to sect. *Merdariae* (Guzmán 1983).

Clade E recovered *D. angustispora* and *D. subcoprophila*, both in sect. *Atrobrunnea* according to Guzmán (1983). These species are coprophilous and have large (13–17 µm) and without angles basidiospores.

TAXONOMY

Deconica australiana Ram.-Cruz, Matheny & Guzm.-Dáv., nom. prov.

Mycobank:

FIGS. 5, 11–16, 17–21

Etymology: Referred that it was collected in Australia.

Pileus 8–11 mm diameter, convex to campanulate, without umbo, margin decurved, center and along the striae umber or warm caramel brown (7.5YR 4/4), light brown towards the margin, center fading to light brown (7.5YR 6/4), surface sticky-viscid, margin translucent-striate, shiny, smooth, hygrophanous, without veil remnants; context light brown when moist, not changing color where bruised. Lamellae adnate, broadly attached, or adnate with slight decurrent tooth, broad, subdistant, with few tiers of lamellulae, brown (7.5YR 5/4), edges pallid-fimbriate. Stipe 20–35 × 1 mm, even, terete, more or less fragile, pruinose at extreme apex only, fibrillose or with minute floccose-fibrils below, light yellowish brown to yellowish brown (10YR 6/4-5/4); context solid, strong brown. Odor somewhat acidulous.

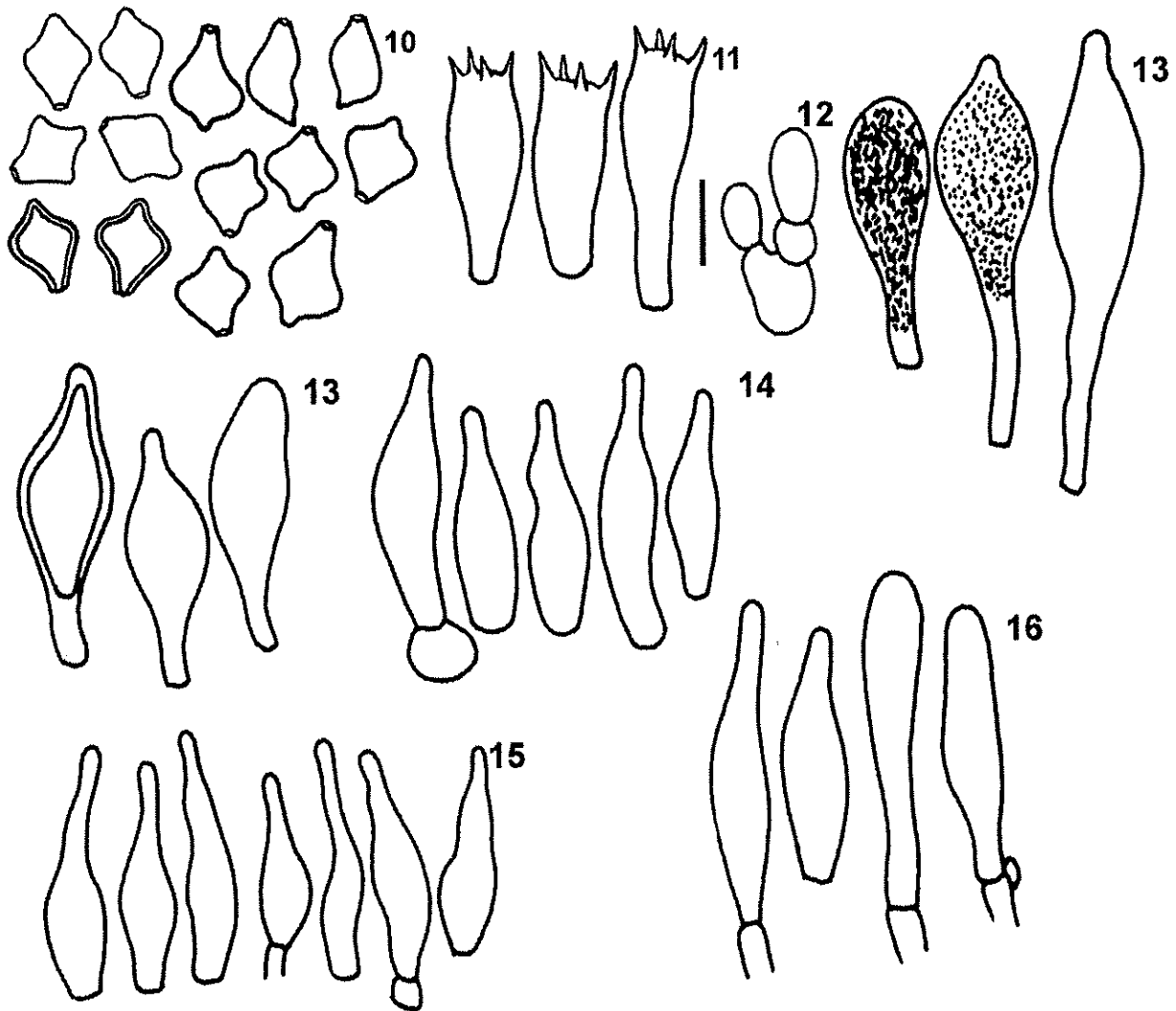
Basidiospores 7.5–9 (11) × 5.5–7.5 × 4–6 µm, Q = (1.06) 1.1–1.4, rhomboid and nodulose in frontal view, Q = 1.3–2.2, ellipsoid and few cylindrical, nodulose in lateral view, thick-walled, up to 0.5 µm thick, one layer in light microscope, with germ pore. Basidia 19–30 × 6–8 (9) µm, cylindrical, clavate, tetrasporic, hyaline, thin-walled. Pleurocystidia type A as chrysocystidia 27–45 × 8–13 µm, clavate to broadly fusiform, apex obtuse, mucronate, or rostrate, hyaline, with hyaline content in KOH, blue in patent blue V, thin-walled. Pleurocystidia type B as leptocystidia, 20–28 × 6–8 µm, narrowly lageniform with short neck, hyaline, thin-walled. Cheilocystidia 18–28 × 4–7 µm, narrowly lageniform with short neck hyaline, thin-walled. Subhymenium cellular, with elements 6–12 µm diameter, hyaline, thin-walled, with yellowish brown encrusted pigment. Hymenophoral trama subregular, hyphae 5–22 µm diameter, hyaline, thin-walled, with yellowish brown encrusted pigment. Pileus trama radial, hyphae 4–20 µm diameter, hyaline, thin-walled, with encrusted pigment. Pileipellis an ixocutis 22–40 µm wide, hyphae 2.5–3 µm diameter, hyaline, thin-walled. Pileocystidia absent. Stipitipellis hyphae 3–10 µm diameter, hyaline, thin-walled with yellowish brown encrusted pigment. Caulocystidia 13–42 × 4–7 µm, cylindrical, narrowly lageniform, hyaline, thin-walled. Clamp connections present.

Habitat and habit: Scattered singly on soil on banks of track in warm temperate rainforest under *Lophostemon confertus*.

Specimen examined: AUSTRALIA. QUEENSLAND, Main Range National Park, Cunninghams Gap, Rainforest Circuit Track, 28° 2' 59", 152° 23' 37.3", 770 m, 3 Mar 2013, P.B. Matheny 3781 (holotype, TENN).

Comments: The basidiospore shape is the most remarkable character in this species. Until now, no taxon in *Deconica* has basidiospores with nodules. This kind of basidiospores is found in other non-related lineages in Agaricales, as in *Panaeolina rhombisperma* Hongo from India (Noordeloos et al. 2007).

Deconica australiana does not fit with any *Deconica* cited from Australia by Grgurinovic (1997). It appears unresolved inside clade A (FIG. 9).



FIGS. 10–16. Micromorphological structures in *Deconica australiana* (holotype). 10. Basidiospores. 11. Basidia. 12. Subhymenium. 13. Pleurocystidia type B. 14. Pleurocystidia type A (chrysocystidia). 15. Cheilocystidia. 16. Caulocystidia. Bar 8 μ m.

DISCUSSION

Clades in Deconica

Phylogenetic relationships were not fully resolved and our clades do not correspond with previous sections proposed in *Deconica*.

Clade A

The presence of chrysocystidia and the rotten wood habitat are the homologies in this clade; however, chrysocystidia were lost in a terminal (*Deconica* sp. 481). The basidiospore shape, a character that was used to separate sections, is homoplastic. This clade would correspond with the sect. *Chrysocystidiatae* of Singer (1986). According to this author, chrysocystidia can lack amorphous yellowish content in KOH, but must have their typical shape (fusiform, ventricose mucronate) differing from *Naematoloma* (= *Hypholoma*) in the hygrophanous pileus. *Deconica aequatoriae*, representative of sect. *Chrysocystidatae*, was sampled in this work and recovered in clade A, but other taxa included by Singer (1986) in his section were not sequenced. However, taxa recovered here agree with the description of the section. This group was also recovered by Ramírez-Cruz et al. (2013) and named “chrysocystidiata”.

The relationships inside the clade A are not fully resolved. *Deconica aequatoriae* and *D. neorhombispora* from Brazil and Mexico, respectively, show a sister relationship. Macro and micromorphology, as well as the ITS sequences, are very similar between these two, but until now we consider them as separate species.

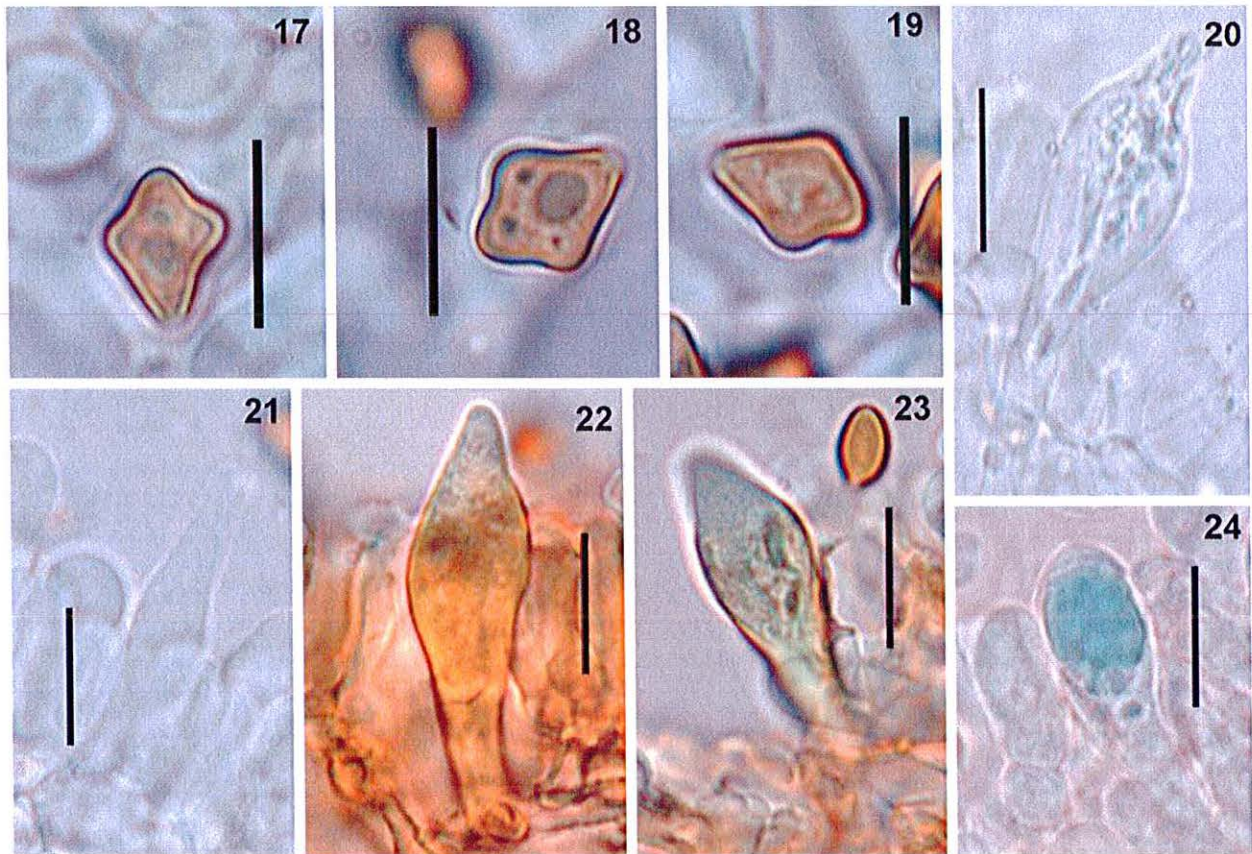
Pleurocystidia in *Deconica* could correspond to chrysocystidia or leptocystidia, as it was pointed out by Ramírez-Cruz et al. (2013). It had been thought that ‘true’ chrysocystidia were not present in the genus by some authors, as Guzmán (1983, 1995), Watling and Gregory (1987), and Noordeloos (2011). Singer (1986) already had considered one section with chrysocystidia in *Psilocybe* s.l., but this idea was not followed by Guzmán (1980, 1983, 1999) who transferred species with chrysocystidia to *Hypholoma*. Later, Guzmán (2004), Horak et al. (2009), and Guzmán et al. (2012) considered chrysocystidia in the bluing sect. *Neocaledonicae*, that resulted in a mix of both genera, *Deconica* and *Psilocybe* s.s. (Ramírez-Cruz et al. 2013). The phylogenetic analyses and taxonomic work made by Ramírez-Cruz et al. (2013) and Silva et al. (2013) confirm that this structure is present only in *Deconica*, not in *Psilocybe*.

For us, Patent blue V (Jahnke 1984) is the key substance to detect chrysocystidia in *Deconica* (FIGS. 20, 23–24) because these cells are different to the typical chrysocystidia in Strophariaceae, i.e., without the yellow content in KOH. Only in few cases, yellowish coloration is observed, like in *D. cokeriana* (FIG. 22). Singer (1986) had already notice that chrysocystidia in *Deconica* were atypical, being their shape the way to recognize them.

Clade B

The majority of mushrooms of clade B has small basidiomata and basidiospores, pleurocystidia absent, and grows in temperate zones in Europe and the USA. Noordeloos (2001) synonymized *P. xeroderma* with *P. alpestris*. We sequenced the type of *D. alpestris* and remain unresolved with respect to *D. xeroderma* and one undetermined taxon. The basidiospore shape is very similar in *D. xeroderma* and *D. alpestris*, the only variation that we can observe is wider cheilocystidia in *D. xeroderma*, but this could be intraspecific variation. Basal to this group are *D. apelliculosa* and *D. subviscida*, both species from Europe and unresolved.

Inside clade B, we can observe a sister relationship between *D. graminicola* and *D. bullacea*, supporting the idea of Watling and Gregory (1987) to consider this group as a stirp in sect. *Psilocybe*. In contrast, other authors have treated *D. graminicola* as synonymous with *D. subviscida* (Guzmán 1983; Noordeloos 2001). Noordeloos (2011) based on a study of types synonymized *D. graminicola* with *D. subviscida* var. *velata*; however, our sample determined as *D. subviscida* is distant to this species.



FIGS. 17–28. Micromorphology in *Deconica*. 17–21. *D. australiana* (holotype). 17–18. Basidiospores in frontal view. 19. Basidiospore in lateral view (holotype). 20. Chrysocystidia in Patent blue V. 21. Pleurocystidia as leptocystidia in KOH. 22. Chrysocystidia in KOH, *D. cokeriana* (T. Kewin s.n.). 23. Chrysocystidia in Patent blue V, *D. cokeriana* (T. Kewin s.n. 29). 24. Chrysocystidia in Patent blue V, *Deconica* sp. 3 (V. Ramírez-Cruz 1849).

Clade C

This clade corresponds to clade G in Ramírez-Cruz et al. (2013), and agrees in part with sect. *Psilocybe* of Guzmán (1983) or sect. *Deconica* of Noordeloos (2011) because *D. subviscida* and *D. xeroderma* are in clade B. The homologies of subclade C1 are the hexagonal to rhomboid basidiospores with thick wall (0.5 to 0.8 μm) and lageniform cheilocystidia. A taxon determined as *D. aff. montana* from temperate zones in Mexico was not grouped here and it is basal to this clade but without support. So, despite its morphology, very similar with *D. montana*, it represents a distinct lineage. There is a complex of several species morphologically very similar to *D. crobula* and *D. inquilina*, which are unresolved and related to clade C1. These species have been considered as varieties of the same species (Høiland 1978) or as separated species (Boekhout et al. 2005; Noordeloos 2011).

Clades D and E

Species with basidiomata growing in dung were not recovered in unique clade; instead the grade “coprophilae”, with two clades (D and E), resulted basal to all species of *Deconica*. This is different to the results found by Ramírez-Cruz et al. (2013), with a clade with two coprophilous species at the base of the *Deconica*.

Clade D groups species with hexagonal basidiospores from two sections according to Guzmán (1983, 1995): *Coprophilae* and *Merdariae*. Singer (1986), Watling and Gregory (1987), and Noordeloos (2011) include all coprophilous species in sect. *Merdariae*. Neither the idea of considering all the species in a unique section nor in two sections is supported. Until now, the morphological features that we can observe in clade D are hexagonal basidiospores and coprophilous habitat.

Clade E groups also coprophilous fungi with larger, without angles basidiospores. The sampled species would correspond to *Psilocybe* sect. *Atrobrunneae* of Guzmán (1983); however, the type of the section, *P. atrobrunnea* (Lasch) Gillet, was not included. Singer (1986) considered in this section *P. atrobrunnea* and *D. fuegiana*; the last species is not in clade E. On the other hand, Noordeloos (2011) placed *P. atrobrunnea* in the genus *Psilocybe* s.s. Probably, this traditional section represent a mix of *Deconica* and *Psilocybe*, and chemical and taxonomic work is necessary to elucidate to which genera correspond these mushrooms. The inclusion of *D. subcoprophila* in sect. *Merdariae* in the sense of Singer (1986) and Noordeloos (2011) is not supported because this taxon was recovered in clade E. Singer (1986) considered that in cases when macromorphology is very similar, the separation in sections by the basidiospore shape had not sense. For this reason, he included *D. subcoprophila* (basidiospores without angles) in sect. *Merdariae* because its basidioma is almost identical to *D. coprophila*. On the other hand, for Guzmán (1983) the basidiospore shape has high taxonomic value.

Deconica sect. *Melanotus* (Noordeloos 2011) was not recovered as a monophyletic group. Nevertheless, Ramírez-Cruz et al. (2013) recovered it as a supported group. Instead here, it is unresolved in relation to A, but without support. This section should be more intensively sampled.

Three sections in the sense of Guzmán (1983, 1995) were not sampled: *Blattariopsidae*, *Singerianae*, and *Squamosae*. Following Singer (1986) classification, sect. *Septembres* was not sampled. Sect. *Squamosae* in the sense of Guzmán (1983) and Watling and Gregory (1987) includes *P. luteonitens* (Fr.) Park.-Rhodes, *P. percevalii* (Berk. & Broome) P.D. Orton, and *P. squamosa* (Pers.) P.D. Orton; the first one belongs to the genus *Stropharia* (Fr.) Quél. (Moncalvo et al. 2002; Cléménçon and Roffler 2003) and the remaining belongs to the genus *Leratiomyces* Bresinsky & Manfr. Binder ex Bridge, Spooner, Beever & D.C. Park (Bridge et al. 2008). Sect. *Blattariopsidae* includes only *P. blattariopsis* (Speg.) Singer, which is in bad condition and was not possible to obtain its DNA sequence.

Moncalvo et al. (2002) pointed out that probably *Hypholoma udum* (Pers.) Quél., *Kuehneromyces*, and *Phaeogalera* Kühner could be inside the clade *psilocybe* (non-hallucinogenic mushrooms), making *Deconica* non-monophyletic, but these relationships were not supported. *Hypholoma udum* was recently transferred to a new genus, *Bogbodia* Redhead (Redhead 2013). Until now, the most robust hypothesis has shown that *Kuehneromyces* is the sister group of *Deconica* (Matheny et al. 2006; Ramírez-Cruz et al. 2013); however, the monophyly of *Kuehneromyces* has not been tested.

Despite the recovered clades here do not fit well with the traditional proposals, we can detect some morphological shared characters at certain levels, in clades A, B, and C, that could be synapomorphies. The inclusion of more DNA regions and specimens from other geographic places, especially from South America and Europe, will improve our understanding on the infrageneric relationships in *Deconica*.

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Table I. Specimens of *Deconica* and out-group used in this study (sequences produced for this work in bold).

Species name	Section		Herbarium and specimen voucher	Collector, number	Date of collection	Country	ITS
	Specimen-DNA number						
	1. Guzmán (1995)						
	2. Singer (1986)						
	3. Watling and Gregory (1986)						
	4. Noordeloos (2010)						
<i>Deconica aequatoriae</i> (Singer) Ram.-Cruz & Guzmán	2. <i>Chrysocystidiatae</i>	Ps-2	F1018172	R. Singer 7425, holotype	14 May 1973	ECUADOR	X
<i>D. alpestris</i> (Singer) Ram.-Cruz & Guzmán	1. <i>Psilocybe</i>	Ps-4	WU784	Singer C9168, holotype	16 Aug 1979	Austria	X
<i>D. angustispora</i> (A. H. Sm.) Ram.-Cruz & Guzmán	1. <i>Atrobrunneae</i>	Ps-7	XAL	J. Trappe 10070	12 Jul 1986	USA	X
<i>D. apeliculosa</i> nom. prov.	1. <i>Pratensae</i>	Ps-11	WU6815	A. Hausknecht s.n.	7 Jun 1987	Austria	X
<i>D. argentina</i> Speg.	1. <i>Coprophilae</i>	Ps-15	XAL	M. Contu s.n.	7 Oct 2002	Italy	KC669307
	2. <i>Merdariae</i>						
<i>D. australiana</i>	2. <i>Chrysocystidiatae</i>	UT 1606	TENN-067047	PBM3781, holotype	3 Mar 2012	Australia	KC669314
<i>D. bullacea</i> (Bull.) Fr.	1. <i>Psilocybe</i>	Ps-36	WU 4849	A. Hausknecht s.n. o Koflac s.n.	June 4, 1989	Austria	X
	3. <i>Bullaceae</i>						
<i>D. cokeriana</i> nom. prov.	2. <i>Chrysocystidiatae</i>	Ps-482	IBUG	T. Kewin s.n. (B)	23 Jul 2012	USA	X
<i>D. cokeriana</i> nom. prov.	2. <i>Chrysocystidiatae</i>	UT 1613	TENN-067013	CCB45	19 Jul 2012	USA	KC669315
<i>D. coprophila</i> (Bull.) P. Karst.	1. <i>Coprophilae</i>	UT-1576	TENN-061255	RHP 13177	8 June 2006	USA	X
	2. and 3. <i>Merdariae</i>						
<i>D. coprophila</i>		Ps-50	XAL	V. Ramírez-Cruz 114	1 Jul 2004	Mexico	KC669308
<i>D. crobula</i> (Fr.) Romagn.	1. <i>Psilocybe</i>	Ps-56	WU11285	A. Hausknecht s.n. (1992-10-20)	20 Oct 1992	Austria	X
	2. <i>Psilocybe</i>						
	4. Sect. <i>Deconica</i> subsect. <i>Inquilinae</i>						
<i>D. fuegiana</i> E. Horak	1. <i>Coprophilae</i>	Ps-275	XAL	I. Kytovouri 95852	30 Aug 1995	Finlandia	X
	2. <i>Merdariae</i>						
<i>D. graminicola</i> P.D. Orton	1. <i>Pratensae</i>	Ps-87	F-1018356	Singer N-7506		USA	X
	3. <i>Bullaceae</i>						
	4. Sect. <i>Deconica</i> subsect. <i>Inquilinae</i>						
<i>D. horizontalis</i> (Bull.) Noordel.	4. <i>Melanotus</i>	Ps-463	ICN-154677	P.S. Silva 253/10	14 May 2010	Brazil	KC669309

<i>D. aff. horizontalis</i>		Ps-434	IBUG	V. Ramírez-Cruz 1520	23 Jul 2011	Costa Rica	KC669310
<i>D. inquilina</i> (Fr.) Romagn.	1. <i>Psilocybe</i> 4. <i>Deconica</i> , subsect. <i>Inquilinae</i>	Ps-95	XAL	M. Contu s.n.	22 Jan 1994	Italy	X
<i>D. inquilina</i>		Ps-97	WU5476	I. Krisai s.n.	24 Jul 1992	Austria	X
<i>D. aff. latispora</i>	1. <i>Psilocybe</i>	UT 1575	TENN-062443	PBM2605	7 Sep 2004	USA	X
<i>D. merdaria</i> (Fr.) Noordel.	1. 2. 4. <i>Merdariae</i>	Ps-117	F-1015258	Singer C-4015	Ago 1964	Gran Bretaña	X
<i>D. montana</i> (Pers.) P.D. Orton	1. <i>Psilocybe</i> 2. <i>Psilocybe</i> 4. <i>Deconica</i> , subsect. <i>Deconica</i>	Ps-133	WU0788	A. Hausknecht s.n.	26 Dec 1979	Austria	X
<i>D. montana</i>		Ps-135	F-1016841	Singer C5470	19 Aug 1971	Switzerlad	X
<i>D. Montana</i>			GENT 3330				HM035079
<i>D. montana</i>		AFTOL-ID 820	WTU	PBM961		USA	DQ494692
<i>D. aff. Montana</i>		Ps 370	IBUG	V. Ramírez-Cruz 1323	12 Aug 2010	Mexico	KC66931
<i>D. aff. Montana</i>		Ps-96	IBUG	J. Trappe 10065	12 Jul 1986	USA	X
<i>D. pegleriana</i> (Guzmán) Ram.-Cruz & Guzmán	1. <i>Merdariae</i>	Ps-153	IBUG	V. Cortez 054/00	13 Dic 2000	Brazil	X
<i>D. pseudobullacea</i> (Petch) Ram.-Cruz & Guzmán	1. <i>Merdariae</i>	Ps-171	XAL	G. Guzmán 35038	24 Sep 2001	Nepal	X
<i>D. semi-inconspicua</i> (Guzmán & Trappe) Ram.- Cruz & Guzmán	—	Ps-181	XAL	J. Trappe 10352, isotype	16 Jul 1988	USA	X
<i>D. aff. smithiana</i>	1. <i>Pratensae</i>	UT 1607	TENN-060416	E.B. Lickey 12358	4 Nov 2004	USA	X
<i>D. subcoprophila</i>	1. <i>Atrobrunnea</i> 2. y 4. <i>Merdariae</i>	Ps-206	XAL	J. Trappe 10180	22 Jul 1987	USA	X
<i>D. submaritima</i> (Guzmán & Contu) Noordel.	1. <i>Psilocybe</i>	Ps-217	XAL	M. Contu, isotipo	3 Dec 2000	Italy	X
<i>D. subvicida</i> Peck	1. <i>Pratensae</i> 2. <i>Psilocybe</i>	Ps-220	WU 4074	W. Koflac s.n .	15 Jun 1986	Austria	X
<i>D. thailandensis</i> (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán	—	Ps-430	XAL	E. Horak 10128, isotype	2 Jun 2002	Thailand	X
<i>D. umbrina</i> (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán	—	Ps-429	XAL	E. Horak 12186, isotype	15 Jan 2004	Malaysia	X
<i>D. xeroderma</i> (Huijsman)	1. <i>Psilocybe</i>	Ps-239	WU25046	Oswald s.n.	24 Aug 2004	Austria	KC669312

Noordel.	2. <i>Psilocybe</i>						
	4. <i>Deconica</i> , subsect.						
	<i>Deconica</i>						
<i>Deconica</i> sp. 1	1. <i>Psilocybe</i>	Ps-162	WU 2959	I. Krisai	5 Aug 1984	Austria	X
<i>Deconica</i> sp. 2	1. <i>Psilocybe</i>	Ps-269	IBUG	L. Guzmán- Dávalos 10070	6 Sep 2006	Mexico	X
<i>Deconica</i> sp. 3	2. <i>Chrysocystidiatae</i>	Ps-477	IBUG	V. Ramírez-Cruz 1849	7 Oct 2012	Mexico	X
<i>Deconica</i> sp. 4	2. <i>Chrysocystidiatae</i>	Ps-481	IBUG	T. Kewin s.n. (A)	23 Jul 2012	USA	X
<i>Deconica</i> sp. 5	2. <i>Chrysocystidiatae</i>	UT 1574	TENN-062238	TFB12591	11 Aug 2005	USA	KC669313
<i>Deconica</i> sp. 6	1. <i>Psilocybe</i>	UT 1580	TENN-053344	TFB6425	8 Nov 1993	USA	X
<i>Deconica</i> sp. 7	1. <i>Psilocybe</i>	UT 1581	TENN-062588	PBM 2790	23 Jul 2006	USA	KC669316
<i>Deconica</i> sp. 8	2. <i>Chrysocystidiatae</i>	UT 1615	TENN-008938	AJS8938	28 Nov 1966	USA	X
<i>Deconica</i> sp. 9 as <i>D.</i> <i>rhombspora</i> en GenBank	2. <i>Chrysocystidiatae</i>			SCM678		USA	FJ596920
<i>Psilocybe</i>	---	Ps-279	XAL	P. Silva 086-04 Isotype	22 May 2004	Brazil	X
<i>subbrunneocystidiata</i> P.S. Silva & Guzmán= <i>Deconica neorhombspora</i>							
OUTGROUP							
<i>Kuehneromyces</i> sp.		UT 1608	TENN050219	RHP 4002	06 Jun 1991	Australia	X

Capítulo VI. Silva PS, Ramírez-Cruz V, Cortés-Pérez A, Guzmán G, Guzmán-Dávalos L, Silveira RMB 2013. *Deconica neorhombispora* (Fungi, Agaricales, Strophariaceae): combination and synonymy. *Sydowia* 65 (2)

Deconica neorhombispora (Agaricales, Strophariaceae): new combination and synonymy

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Silva P. S., Ramírez-Cruz V., Cortés-Pérez A., Guzmán G., Guzmán-Dávalos L. & Silveira R. M. B. (2013) *Deconica neorhombispora* (Agaricales, Strophariaceae): new combination and synonymy. – *Sydowia* XX (X): pp–pp.

Based on the study of type specimens, we conclude that *Psilocybe subbrunneocystidiata* is a synonym of *P. neorhombispora*. Because of its non-bluing nature, it belongs in *Deconica* and is named *Deconica neorhombispora* comb. nov., and it is re-described, discussed, and illustrated.

Keywords: chrysocystidia, non-hallucinogenic mushrooms, tropics.

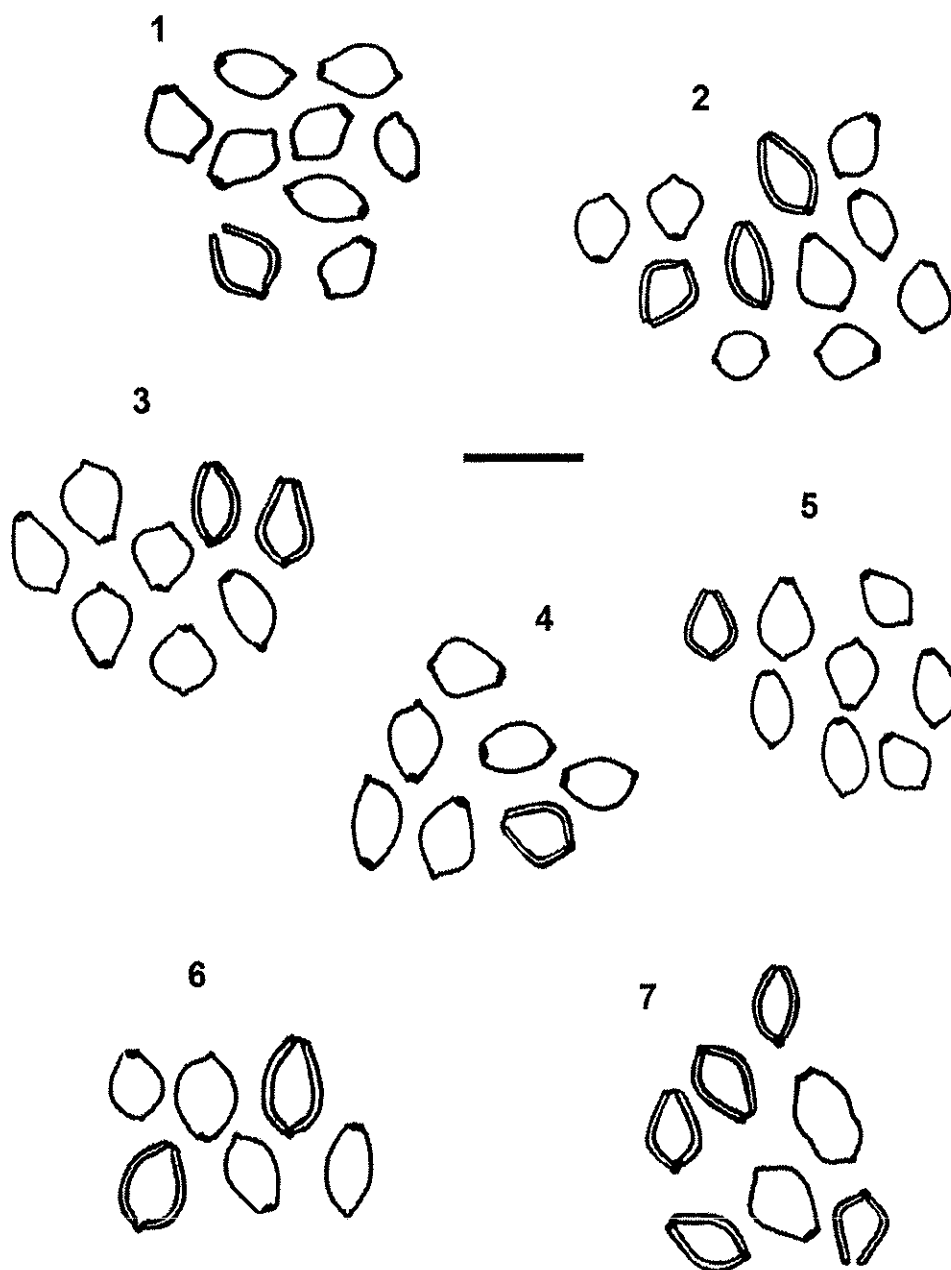
The recently revealed polyphyly of the genus *Psilocybe* (Fr.) P. Kumm. (Moncalvo *et al.* 2002; Matheny *et al.* 2006; Ramírez-Cruz *et al.* 2013), has led to reclassification and combinations in *Deconica* (W.G. Sm.) P. Karst. (Noordeloos 2009; Ramírez-Cruz *et al.* 2012; Cooper 2012; Redhead 2012). *Deconica* is the oldest available name for the non-hallucinogenic species while the generic name *Psilocybe* is available for the psychotropic taxa (Redhead *et al.* 2007).

We studied specimens determined as *Psilocybe subbrunneocystidiata* P. S. Silva & Guzmán, including the type, as well as the type of *P. neorhombispora* Guzmán, and conclude that the types are conspecific hence they are synonymous. As this species belongs to the non-hallucinogenic group, a new combination in *Deconica* was accordingly proposed.

The morphological study of the specimens revealed the presence of chrysocystidia, a characteristic not previously described for *P. subbrunneocystidiata* (Silva *et al.* 2007). However, chrysocystidia were previously observed in *Naematoloma rhombisporum* Guzmán (\equiv *Psilocybe neorhombispora*) (Guzmán 1980, 2004).

Materials and Methods

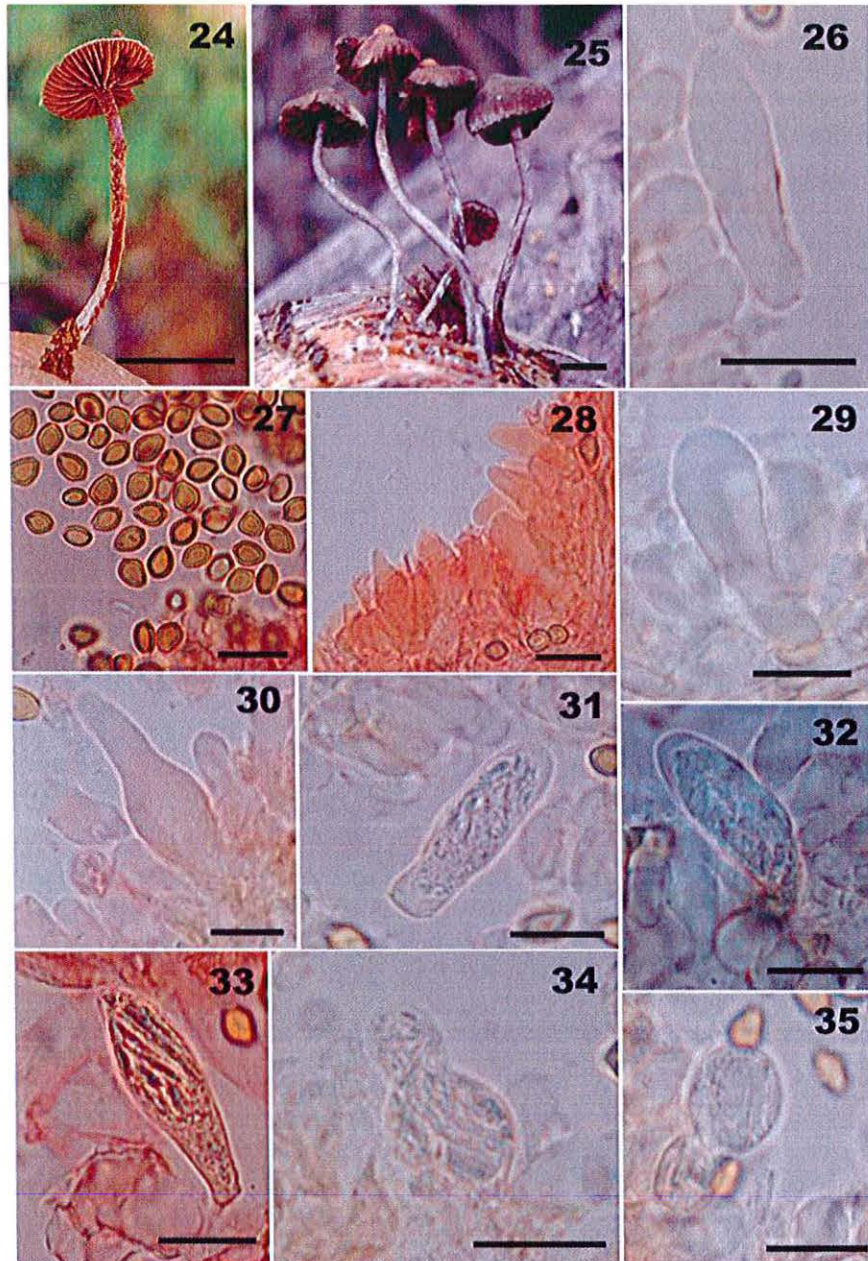
Macroscopic characters were examined following conventional techniques utilized in taxonomic studies of mushrooms. The colour annotations are from Kornerup & Wanscher (1978). For the study of microscopic features, free-hand sections of the basidiome were mounted in 5% potassium hydroxide (KOH) and 1% Congo red solutions. Patent blue V was used to detect chrysocystidia following Jahnke (1984). Basidiospores dimensions indicate length, width (face view), and thickness (side view). The terminology for



Figs. 1—7. Basidiospores of *Deconica neorhombispora*: **1.** Holotype (J. Jacobs 46). **2.** P. S. Silva 086/04. **3.** A. Cortés-Pérez 755. **4.** A. Cortés-Pérez 744. **5.** A. Cortés-Pérez 739. **6.** P. S. Silva 241/09. **7.** P. S. Silva 238/09.



Figs. 8–23. Micromorphological features of *Deconica neorhombispora*: **8–9.** Basidia: **8.** A. Cortés-Pérez 755. **9.** A. Cortés-Pérez 744. **10.** Subhymenium ramose-inflated (A. Cortés-Pérez 744). **11–16.** Pleurocystidia (leptocystidia and chrysocystidia): **11.** Holotype. **12.** P. S. Silva 086/04. **13.** P. S. Silva 238/09. **14.** A. Cortés-Pérez 755. **15.** A. Cortés-Pérez 739. **16.** A. Cortés-Pérez 744. **17–21.** Cheilocystidia: **17.** Holotype. **18.** P. S. Silva 086/04. **19.** A. Cortés-Pérez 755. **20.** A. Cortés-Pérez 744. **21.** A. Cortés-Pérez 739. **22–23.** Caulocystidia: **22.** A. Cortés-Pérez 755. **23.** A. Cortés-Pérez 744. Bar = 8 μ m.



Figs. 24–35. *Deconica neorhombispora*: 24–25. Basidiomata: 24. A. Cortés-Pérez 846B. 25. P.S. Silva 086/04. 26. Pleurocystidium without content, in KOH (A. Cortés-Pérez 744). 27. Basidiospores (P.S. Silva 241/09). 28. Cheilocystidia in Congo red (holotype, J. Jacobs 46). 29. Pleurocystidium without content, in Patent blue V (A. Cortés-Pérez 739). 30. Pleurocystidium without content and basidioles, in Congo red (P.S. Silva 238/09). 31. Chrysocystidium in KOH (A. Cortés-Pérez 744). 32. Chrysocystidium in Patent blue V (A. Cortés-Pérez 739). 33. Chrysocystidium in Congo red (P.S. Silva 241/09). 34. Chrysocystidium in KOH (P.S. Silva 086/04). 35. Chrysocystidium in KOH (A. Cortés-Pérez 755). Bars = 5 mm (24–25) and 10 μ m (26–35). Photograph 24 by A. Cortés-Pérez, photograph 25 by P.S. Silva.

microstructures follows Vellinga (1988) and herbarium acronyms are according to Thiers (2012).

Taxonomy

Deconica neorhombispora (Guzmán) P. S. Silva, Ram.-Cruz & Guzmán, **comb. nov.** – Figs. 1–35.

MycoBank: MB 803061

Basionym. – *Psilocybe neorhombispora* Guzmán, Revista Mexicana de Micología 18: 28. 2004.

Nom. nov. for *Naematoloma rhombisporum* Guzmán, Mycotaxon 12(1): 237. 1980. Holotype.—MEXICO. Oaxaca: near San Bartolomé Ayautla, 20 Jun 1978, *leg.* J. Jacobs 46 (ENCB).

Non *Psilocybe rhombispora* (Britzelm.) Sacc., Sylloge Fungorum 11: 72. 1895, nec *Deconica rhombispora* (Britzelm.) Singer, Lilloa 22: 740. 1951 [1949].

Synonym. – *Psilocybe subbrunneocystidiata* P. S. Silva & Guzmán, Mycotaxon 102: 204. 2007.

Holotype.—BRAZIL. Rio Grande do Sul: Viamão, Parque Estadual de Itapuã, 22 May 2004, *leg.* P. S. Silva 086/04 (ICN 139168).

Pileus 5–10 mm, convex or plano-convex to campanulate, slightly umbonate to umbonate, dark brown (8F6) to reddish brown (8E6) after drying, hygrophanous; surface moist, smooth; margin decurved, turning to incurved after drying. – **Context** pale yellowish. – **Lamellae** adnate with decurrent tooth, dark brown (8F6), close, with whitish or concolorous edges. – **Stipe** 10–30 × 1–2 mm, central, cylindrical, dark brown (8F6) to reddish brown (8D6), turning black after drying, with white fibrils throughout the surface, fibrous, hollow. – **Veil** absent. – **Odour** non-distinctive.

Basidiospores 4.0–5.6 (6.4) × 3.2–4.0 × 3.2–4.0 μm (Q = 1–1.5, Q_m = 1.2, n = 47 in face view; Q = 1.1–1.7, Q_m = 1.7, n = 46 in side view), rhomboid to subrhomboid, sometimes hexagonal or ellipsoid in face view, oblong in side view, yellowish brown, wall 0.5–0.7 μm thick, with a conspicuous germ pore, sometimes presenting double germ pore, and few abnormal shaped (these reaching up to 7.2 × 4.0 μm). – **Basidia** 14–25.5 × 4–5.5 (7) μm, cylindrical to narrowly utriform, with or without central constriction, hyaline, bearing four sterigmata. – **Pleurocystidia** 17–36 (42) × 5.5–15 μm, subcylindrical, clavate, narrowly utriform, narrowly lageniform, fusiform, some with a slightly mucronate apex or subcapitate, hyaline or yellowish brown in alkaline solutions, occasionally with irregularly encrusted wall at the base and medium part, some of them as chrysocystidia presenting strongly refringent crystal-like or granulose content with weak reaction with Patent blue V, originated from hymenium and hymenophoral trama. – **Cheilocystidia** (13.5) 16–23 (30) × 3–7 (9) μm, narrowly lageniform, narrowly utriform, subcylindrical, hyaline, thin-walled. – **Hymenophoral trama** subregular, formed by 3–18 μm wide hyphae, wall up to 0.5 μm thick, encrusted with yellowish brown pigment. – **Subhymenium** ramose-inflated, with hyaline elements, occasionally pigment encrusted. – **Pileus trama** radial, composed by 6–16 μm wide hyphae, inflated, wall up to 0.7 μm thick, with yellowish brown pigment encrusted. – **Pileipellis** an ixocutis 8–16 μm wide, with 4–7 μm wide hyphae, hyaline, thin-walled. – **Stipitipellis** composed by 2.5–9 μm wide, parallel hyphae, with yellowish brown pigment encrusted, fibulate, wall up to 0.5 μm thick. –

Caulocystidia (8) 17–40 × 3–8 μm, subcylindrical, lageniform, clavate, hyaline, with yellowish brown pigment encrusted walls. Clamp connections present in all tissues.

Habitat and distribution. – Gregarious, on soil or rotten wood, in tropical and subtropical forests. Known from Mexico and southern Brazil.

Additional material examined. – BRAZIL, Rio Grande do Sul, Viamão, Parque Saint Hillaire, 25 May 2009, *leg.* P. S. Silva 241/09 (ICN 154462); Morrinhos do Sul, 4 October 2009, *leg.* P. S. Silva 238/09 (ICN 154351); MEXICO, Veracruz, Coatepec, 12 October 2011, *leg.* A. Cortés-Pérez 739 (XAL), 744 (XAL); road Teocelo-Coyopolan, Mpio. Ixhahuacán de los Reyes, 15 October 2011, *leg.* A. Cortés-Pérez 755 (XAL), 30 August 2012, *leg.* A. Cortés-Pérez 846B (XAL).

Remarks. – This species is recognized by its small basidioma, absence of an annulus, small, rhomboid basidiospores, and very variable pleurocystidia as chrysocystidia and leptocystidia. *Deconica aequatoriaae* (Singer) Ram.-Cruz & Guzmán in Ramírez-Cruz *et al.* (2012) (Holotype. ECUADOR, Lago Agrio, 14 May 1973, *leg.* R. Singer B7425, F) is a similar species, differing mainly by their larger basidiomata and chrysocystidia 13–29 × 8–9 μm, with homogeneous content, neither granulose nor crystal-like, but with a positive reaction with Patent blue V.

Deconica neorhombispora was first described as *Naematoloma rhombisporum*, from a tropical forest in Mexico (Guzmán 1980). It was placed in *Naematoloma* P. Karst. [= *Hypholoma* (Fr.) P. Kumm.] due to its “hyaline or more frequently with opaque striate or granular contents” pleurocystidia, which Guzmán (1980) considered as chrysocystidia. Later, Guzmán (2004) placed the species in *Psilocybe* sect. *Neocaledonicae* Guzmán (2004) that includes species with bluing basidiomata, based on a set of morphological features (subrhomboid and thick-walled basidiospores, presence of chrysocystidia, non-cellular subpellis and basidiome coloration). However, bluish tones were not recorded in the original description of *Naematoloma rhombisporum* (Guzmán 1980).

Psilocybe subbrunneocystidiata was described from a subtropical forest in southern Brazil (Silva *et al.* 2007), and in a first examination based in one specimen, it was interpreted as having bluing tones on the stipe basis, which had led the authors to consider it as hallucinogenic and therefore classified it in *Psilocybe* sect. *Brunneocystidiatae* Guzmán (1983). This section is characterized by bluing basidiomata with yellowish brown or orange brown cystidia (Guzmán 1983). The observation of additional specimens demonstrated that this species was erroneously considered as hallucinogenic because a bluing reaction or bluish tones were not observed in fresh basidiomata.

Silva *et al.* (2007) noted two distinct types of pleurocystidia in *P. subbrunneocystidiata*, differing mainly in size and shape, being both hyaline to yellowish, but they did not mention if some of them corresponded to chrysocystidia. In the present paper, we found that all studied specimens present both chrysocystidia and leptocystidia as pleurocystidia. However, it is difficult to separate them because there are intermediate states in size and shape. The only difference is the distinctive refringent crystal-like content that stains blue in Patent blue V in chrysocystidia (Figs. 31–35), following the technique described by Jahnke (1984). Leptocystidia do not have content or if they have it is not refringent and does not stain with Patent blue V (Figs. 26, 29 and 30). Chrysocystidia seem to be related to non-hallucinogenic *Psilocybe* sect. *Chrysocystidiatae* Singer (1986), differing from *Hypholoma*, also with chrysocystidia, by having hygrophaneous pilei and basidiomata of different shape.

For some authors (Guzmán 2004, Horak & Desjardin 2006, Horak *et al.* 2009, Guzmán *et al.* 2012), the occurrence of chrysocystidia in *Psilocybe s.l.* is restricted to tropical and subtropical bluing species, in *Psilocybe* sect. *Neocaledonicae*. However, the study of several *Psilocybe* and *Deconica* specimens indicated that true chrysocystidia, considering them as the ones that stain with Patent blue V, only occur in *Deconica* (Ramírez-Cruz *et al.* 2013).

Acknowledgments

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Capítulo VII. Ramírez-Cruz V, Guzmán G, Guzmán-Dávalos L.
2012. New combinations in *Deconica*. *Sydowia* 64(2): 217–219

New combinations in the genus *Deconica* (Fungi, Basidiomycota, Agaricales)

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Ramírez-Cruz V., Guzmán G. & Guzmán-Dávalos L. (2012) New combinations in the genus *Deconica* (Fungi, Basidiomycota, Agaricales). – *Sydowia* 64 (2): 217–219.

Based on phylogenetics studies on *Deconica* and *Psilocybe*, eight new combinations for *Deconica* species previously assigned to *Psilocybe* are proposed.

Keywords: Strophariaceae, non-hallucinogenic mushrooms.

The genus *Psilocybe*, as conceived by Guzman (1983, 1995) appears to be polyphyletic (Moncalvo *et al.* 2002) and should be restricted to the species with hallucinogenic properties. To avoid unwanted nomenclatural consequences, Redhead *et al.* (2007) proposed the change of the type-species from *Agaricus montanus* Pers. into *Psilocybe semilanceata* (Fr.) P. Kumm., a proposal which has been approved (Barrie 2011, McNeill *et al.* 2011, Norvell 2011). Consequently, Noordeloos (2009) reinstalled the generic name *Deconica* for the non-hallucinogenic taxa.

Three species in the present paper, viz. *Psilocybe semi-inconspicua*, *P. thailandensis*, and *P. umbrina* were originally described as hallucinogenic mushrooms without a really observable bluing reaction. Although it is well known that the bluing reaction is not the main evidence to support the presence of psilocybin (e.g. in *P. semilanceata*), chemical tests have not been made in these species, so the conclusions to transfer these species to *Deconica* are based on the study of macro- and micromorphological features. *Psilocybe semi-inconspicua* was placed in sect. *Semilanceatae*; however, the DNA sequence (Ramírez-Cruz *et al.*, unpubl.) and the analysis of macro- and micromorphological characters helped us to conclude that it belongs to *Deconica*. Furthermore, it is very similar to *D. montana* (Pers.) P. D. Orton.

Deconica umbrina and *D. thailandensis* were placed in the bluing sect. *Neocaledonicae* (Horak *et al.* 2009). However, we have observed that this section contains species of *Deconica* and *Psilocybe*, because the main difference between the two genera at this level (sect. *Neocaledonicae*) is the presence of chrysocystidia in *Deconica*, which are present in *D. thailandensis* and *D. um-*

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brina. From the other five species considered in this paper the non-hallucinogenic properties were already known.

New combinations

Deconica aequatoriae (Singer) Ram.-Cruz & Guzmán, **comb. nov.**

MycoBank no.: MB 800723

Basionym. – *Psilocybe aequatoriae* Singer, Nova Hedwigia 29(1–2): 59, 1978 (“1977”).

Deconica alpestris (Singer) Ram.-Cruz & Guzmán, **comb. nov.**

MycoBank no.: MB 800359

Basionym. – *Psilocybe alpestris* Singer, Fieldiana, Bot. 21: 108, 1989.

Deconica angustispora (A. H. Sm.) Ram.-Cruz & Guzmán, **comb. nov.**

MycoBank no.: MB 800724

Basionym. – *Psilocybe angustispora* A. H. Sm., J. Elisha Mitchell Scient. Soc. 62: 193, 1946.

Deconica pegleriana (Guzmán) Ram.-Cruz & Guzmán, **comb. nov.**

MycoBank no.: MB 800725

Basionym. – *Psilocybe pegleriana* Guzmán. Doc. Mycol. 29 (116): 43, 2000.

Deconica pseudobullacea (Petch) Ram.-Cruz & Guzmán, **comb. nov.**

MycoBank no.: MB 800726

Basionym. – *Stropharia pseudobullacea* Petch, Ann. Roy. Bot. Garden (Peradeniya) 9(2): 128, 1924. = *Psilocybe pseudobullacea* (Petch) Pegler, Kew Bull., Add. Ser. 6: 473, 1977.

Deconica semi-inconspicua (Guzmán & Trappe) Ram.-Cruz & Guzmán, **comb. nov.**

MycoBank no.: MB 800727

Basionym. – *Psilocybe semi-inconspicua* Guzmán & Trappe, Int. J. Medic. Mushrooms 7(4): 588, 2005.

This species was considered in the hallucinogenic sect. *Semilanceatae* by Guzmán & Trappe (2005).

Deconica thailandensis (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán, **comb. nov.**

MycoBank no.: MB 800728

Basionym. – *Psilocybe thailandensis* E. Horak, Guzmán & Desjardin, Sydowia 61(1): 30, 2009.

This species was considered in the bluing sect. *Neocaledonicae* by Horak *et al.* (2009).

Deconica umbrina (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán, **comb. nov.**

Mycobank no.: MB 800729

Basionym. – *Psilocybe umbrina* E. Horak, Guzmán & Desjardin, *Sydowia* 61(1): 28, 2009.

This species was considered in the bluing sect. *Neocaledonicae* by Horak *et al.* (2009).

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Capítulo VIII. Discusión general y conclusiones

Capítulo VIII. Discusión general y conclusiones

ESTUDIO TAXONÓMICO

Es importante la determinación correcta del material que se utiliza en los estudios filogenéticos. Por ello, en este trabajo se utilizó como base el estudio de ejemplares tipo de *Psilocybe* s.l. (*Deconica* y *Psilocybe* s.s.) para entender el concepto de las especies y además determinar correctamente los especímenes usados en los análisis filogenéticos mediante su comparación con los tipos. En el Anexo 1 se presentan los 100 ejemplares tipo estudiados y los herbarios en donde se encuentran. De ellos, 15 corresponden a *Deconica* y 83 a *Psilocybe* s.s.; dos especímenes permanecen sin determinar a qué género pertenecen.

Con el estudio de los caracteres micromorfológicos, se ampliaron las descripciones originales con observaciones que no se mencionaron en los protólogos ni en estudios subsecuentes. Las principales características que se añadieron o se corrigieron, ya que su descripción era incorrecta o confusa, son la forma de las basidiosporas y el grosor de su pared en μm ; forma, color del contenido y origen de los cistidios; tipo de subhimenio; tipo de trama del píleo, y tipo de pileipellis, entre los principales. En el caso de la forma de basidiosporas, se describió toda la variación, ya que usualmente sólo se mencionaba la más abundante, y se dio un intervalo para el grosor de la pared, para evitar la subjetividad al utilizar los términos delgada, subgruesa y gruesa. Otro carácter observado, que ya se había señalado por Guzmán et al. (2005), pero sólo en algunas especies de *Psilocybe*, es la presencia de hifas setoides en el micelio basal del estípite.

Como se mencionó en la introducción, *Psilocybe* s.l. agrupa entre 277 y 300 especies (Guzmán 2005; Kirk et al. 2008). Sin embargo, es probable que esta cifra esté sobreestimada, debido a que varios nombres son sinónimos. En este trabajo el estudio de los tipos contribuyó a que se descubrieran 10 sinonimias; de ellas ya se publicaron siete (Ramírez-Cruz et al. 2013a, Silva et al. 2013). Algunos ejemplos inéditos son los siguientes:

Psilocybe caerulescens tiene una gran variación macromorfológica (Guzmán 1983). En consecuencia, varias formas se describieron bajo especies diferentes, pero en realidad son conespecíficas. Tal es el caso de *P. bispora*, *P. caribaea*, *P. villarealii*, *P. subannulata*, *P. weilii* y *P. wrightii* (Guzmán 1978a, 1998; Guzmán et al. 1997, 2003, 2007, 2009) de México, Centro América y Sudamérica. En sus protólogos se mencionó que eran muy semejantes a *P. caerulescens*, pero que diferían por ejemplo en los pleurocistidios y pileocistidios. Estas estructuras no fueron descritas por Murrill (1923) en *P. caerulescens* ni señaladas en estudios posteriores (e.g., Singer y Smith 1958; Guzmán 1983). A través de la revisión del tipo de *P. caerulescens*, se comprobó que los pleurocistidios y pileocistidios están presentes.

En *Psilocybe mexicana* no se consideró la pseudorriza en las descripciones de Heim (1957), Singer y Smith (1958) y Guzmán (1983). Con base en el estudio del tipo y material

adicional, recolectado por R. Heim de la localidad tipo y de otros topotipos, se llegó a la conclusión de que la pseudorriza es común en esta especie y no se recolectó completo el basidioma del holotipo. Por tal motivo, *P. galindoi* (Guzmán 1978b) es sinónimo de *P. mexicana*. Por otra parte, *P. pileocystidiata*, que se describió como nueva por la presencia de pileocistidios (Guzmán et al. 2004), es también *P. mexicana*, porque estas estructuras son comunes en esta especie.

Otras sinonimias que se confirmaron con este trabajo son aquéllas propuestas recientemente por Guzmán (2012) en *P. zapotecorum*, en donde 13 nombres son conespecíficos. En *Deconica* también resultaron algunos sinónimos, como el caso de *P. subbrunneocystidiata* descrita como especie alucinógena (Silva et al. 2007), pero que en realidad corresponde a *Deconica*, por lo que se propuso la combinación bajo el nombre de *D. neorhombispora* (Silva et al. 2013).

Crisocistidios en *Deconica*

Los crisocistidios son células estériles en el himenio. Tradicionalmente, se distinguían por ser vesiculares o fusiformes a veces mucronadas, con contenido agrupado y amarillo en soluciones alcalinas. Cléménçon (2012) aceptó crisocistidios tipo “fasciculare” y tipo “semiovatus”. Éstos se encuentran en linajes separados, el primero en *Hypholoma*, *Pholiota* y *Stropharia*, y el segundo en *Panaeolus*. Los crisocistidios en *Deconica* son semejantes en forma al tipo “fasciculare”, pero su contenido es hialino, no amarillo, granuloso o con cristales y puede estar agrupado o distribuido en toda la célula. Por lo que en este trabajo se siguió la tinción de Jahnke (1984), quien sugirió el Patent blue V para detectar crisocistidios, ya que tiñe el contenido de azul. En *Deconica* se presentan células cuyo contenido se tiñe, por lo que se confirma la presencia de crisocistidios en el género.

Singer (1986) reconoció la importancia de los crisocistidios y propuso a *Psilocybe* s.l. sección *Chrysocystidiatae* (con especies no alucinógenas). Sin embargo, Guzmán (1980, 1983, 1999) consideró a especies con crisocistidios en el género *Naematoloma* (= *Hypholoma*) y posteriormente en *Psilocybe* s.l. sección *Neocaledonicae* (Guzmán 2004). En este trabajo, se comprobó que los crisocistidios sólo están presentes en un grupo de *Deconica* (Ramírez-Cruz et al. 2013a; Silva et al. 2013), y que los cistidios que en *Psilocybe* s.s. se habían considerado como crisocistidios (Horak et al. 2009; Guzmán et al. 2012), corresponden a otro tipo de deuteroquistidios de Cléménçon (2012).

ESTUDIO FILOGENÉTICO

Relaciones filogenéticas en *Psilocybe sensu lato*

Con el propósito de estimar las relaciones de parentesco en *Deconica*, *Psilocybe* y otros géneros de las familias Hymenogastraceae y Strophariaceae (Matheny et al. 2006) se reconstruyó una filogenia con base en secuencias de ADN de las regiones nucleares nLSU-rRNA, 5.8S rRNA y *rpb1*. Los resultados (Ramírez-Cruz et al. 2013b) confirman la monofilia de ambos géneros y su posición taxonómica. A pesar de que casi todas las

relaciones infragenéricas en *Deconica* y *Psilocybe* tuvieron soporte alto; sólo algunos clados corresponden con las secciones o subsecciones previamente propuestas. La reconstrucción ancestral de tres caracteres morfológicos, muestra que la forma de las basidiosporas en vista frontal y el grosor de la pared de las basidiosporas están distribuidos en ambos géneros, pero que los crisocistidios se presentan únicamente en un clado dentro de *Deconica*.

Relaciones filogenéticas en el género *Psilocybe*

Las relaciones infragenéricas en *Psilocybe* obtenidas con base en secuencias de ITS del ADN ribosomal no corresponden a las secciones tradicionales propuestas por Guzmán (1983, 1995), Singer (1986) y Noordeloos (2011). La excepción son las secciones *Cordisporae*, *Cubensae* y *Zapotecorum* (Guzmán 1983), o en parte *Cyanescens* y *Mexicanae* (Guzmán 1983). Es posible identificar algunos caracteres morfológicos compartidos en algunos clados a niveles anidados. Por ejemplo, en el clado “cordisporae” (Fig. 10, Ramírez-Cruz et al. 2014a) las homologías probables son el tamaño pequeño (4–6 μm) y la forma romboide a subromboide de las basidiosporas (forma de corazón y de ahí el nombre de la sección), las hifas de la trama del píleo y trama himenófora con paredes gruesas (hasta 1.6 μm), y el micelio basal del estípite compuesto por hifas setoides. El clado “cubensae” presenta las basidiosporas hexagonales y con pared gruesa. Por su parte, en el clado “zapotecorum” las homologías probables son basidiosporas pequeñas, sin ángulos y con pared delgada. Sin embargo, los clados “cyanescens” y “mexicanae” carecen de características morfológicas únicas. De tal forma que las características diagnósticas de las secciones que corresponden con los clados son homoplasias.

Relaciones filogenéticas en el género *Deconica*

Las relaciones infragenéricas en *Deconica* usando sólo secuencias de ITS del ADN ribosomal, están menos resueltas en comparación con las de *Psilocybe* s.s. El análisis resultó en cinco clados, de los cuales sólo el “chrysocystidiatae” de Ramírez-Cruz et al. (2013b) o clado A de Ramírez-Cruz et al. (2014b) corresponde con la sección *Chrysocystidiatae* de Singer (1986), con los crisocistidios como la homología. Probablemente varios taxa dentro de “chrysocystidiatae” son nuevos, por ahora sólo se describe a *D. australiana* nom. prov. como especie nueva.

Mapeo de caracteres morfológicos en los árboles obtenidos con secuencias de ADN

Entre los caracteres morfológicos en los que se ha basado la clasificación de *Psilocybe* s.l. se encuentran la forma de las basidiosporas y el grosor de su pared. Se llevó a cabo la reconstrucción ancestral de ellos, además de la presencia de crisocistidios, en los árboles obtenidos de los análisis filogenéticos (Fig. 5, Ramírez-Cruz et al. 2013b). A pesar de que la forma de las basidiosporas es un carácter que la mayoría de los autores ha considerado en la clasificación infragenérica, la reconstrucción reveló que este carácter se

distribuye sin un patrón filogenético y sólo en pocos casos, a niveles anidados resultó una homología. Tal fue el caso de los clados “cubensae”, “zapotecorum” y “cordisporae” en *Psilocybe* s.s. De manera similar, el grosor de la pared de las basidiosporas también resultó homoplásico, aunque hay clados que únicamente tienen especies con basidiosporas de paredes gruesas y sus basidiomas son coprófilos en *Deconica* (clado H) y *Psilocybe* (clado “cubensae”). Garnica et al. (2007) afirmaron que el grosor de la pared de las basidiosporas representa una ventaja evolutiva en algunos hongos, ya que ayudan a ser más resistentes a la deshidratación y a la radiación UV. En el caso de los grupos coprófilos en *Deconica* y *Psilocybe*, poseer esporas con pared gruesa les da la ventaja de sobrevivir al tracto digestivo de herbívoros, en comparación con aquéllos que crecen en otros sustratos.

En el futuro el tamaño de las basidiosporas se podría mapear en la estimación filogenética. Antes es necesario un estudio morfométrico para separar intervalos de variación y delimitar estados de carácter. En general, existen clados (Fig. 5, Ramírez-Cruz et al. 2013b) que agrupan especies con basidiosporas grandes o pequeñas. Por otro lado, Guzmán (1979) observó una relación entre la distribución altitudinal de las especies y el tamaño de las basidiosporas. *Psilocybe aztecorum* presenta esporas grandes y crece a elevaciones por encima de 3200 m. En contraste, *P. uxpanapensis* y *P. weldenii* tienen esporas pequeñas y prosperan en elevaciones que oscilan entre 0 y 1000 m.

Los crisocistidios representan la sinapomorfia de un linaje dentro de *Deconica* (clado E). Al considerar un nivel más inclusivo, son homólogos en Strophariaceae, con reversiones a su ausencia en varios clados (Fig. 5, Ramírez-Cruz et al. 2013b). Esto se apoya con los resultados de Matheny et al. (2006), en los que se recupera a Strophariaceae como grupo monofilético, con los géneros *Agrocybe*, *Deconica*, *Hypholoma*, *Kuehneromyces*, *Nivatogastrium*, *Pholiota* y *Stropharia*.

Especie nueva para la ciencia

Con la evidencia morfológica y las secuencias de ITS del ADN ribosomal, se encontró una especie nueva para la ciencia, *Deconica australiana* nom. prov. El material estudiado fue recolectado en Australia y se agrupó en el linaje “chrysocystidiatae”. Presenta basidiosporas romboides con nódulos que es una característica única en *Deconica*, además de dos tipos de pleurocistidios (crisocistidios y leptocistidios). La descripción de esta especie se presenta en Ramírez-Cruz et al. (2014b).

CONCLUSIONES

A pesar de sus similitudes morfológicas, *Deconica* y *Psilocybe* son diferentes y no tienen una relación de grupos hermanos. *Deconica* es parte de la familia Strophariaceae s.s. y su grupo hermano es *Kuehneromyces*. Por otro lado, *Psilocybe* pertenece a Hymenogastraceae (Matheny et al. 2006), y su grupo hermano no está definido. La familia Strophariaceae no es monofilética en el sentido de Singer (1986).

El estudio morfológico permitió detectar sinonimias, añadir caracteres micromorfológicos a las descripciones originales y dar elementos para la codificación de caracteres, es decir, establecer las homologías primarias en ambos géneros. A pesar de que se revisaron varios ejemplares tipo, todavía queda mucho por hacer, considerando el número de especies descritas y el estimado para estos grupos. En *Deconica*, la delimitación de especies es más complicada, ya que existen varias interpretaciones de los nombres utilizados, además algunos caracteres son muy variables, por ejemplo la forma de las basidiosporas.

Las secuencias de ADN son muy útiles para la separación de *Deconica* y *Psilocybe*. En contraste, la mayoría de los caracteres morfológicos observados representan homoplasias. Sin embargo, existen algunos grupos en los que se pueden detectar homologías probables. Tal fue el caso de los clados “cordisporae”, “cubensae” y “zapotecorum” en *Psilocybe*; y en el clado que corresponde a la sección *Chrysocystidiatae* en *Deconica* (Singer 1986).

PERSPECTIVAS

No fue posible revisar ni obtener secuencias de la mayoría de las especies, ya que de muchas sólo se conoce el tipo y éste puede ser un material viejo o en malas condiciones. Para mejorar los resultados obtenidos en este trabajo, se recomienda incrementar el número de taxa y de regiones de ADN. Además obtener más secuencias del gen *rpb1*, ya que se comprobó que tiene información filogenética útil. En el caso de *Deconica*, es necesario ampliar el muestreo a especies europeas y de otros lugares del mundo, así como llevar a cabo estudios morfológicos de especies tropicales, para entender mejor sus relaciones filogenéticas.

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Anexo 1. Especímenes tipo de *Psilocybe* s.l. revisados en este trabajo. Especies de *Psilocybe* que pertenecen a *Deconica*^a y no se han transferido. Especies en proceso de transferirse a *Deconica*^b. Especies dudosas^c.

Basónimo	Nombre actual	Tipo	Herbario
<i>Agaricus goniosporus</i> Berk. & Broome	<i>Psilocybe goniospora</i> (Berk. & Broome) Singer ^b	Isotipo	K
<i>Agaricus ochreatus</i> Berk. & Broome	<i>Psilocybe ochreatea</i> (Berk. & Broome) E. Horak ^b	Holotipo	K
<i>Agaricus plutonius</i> Berk. & M.A. Curtis	<i>Psilocybe plutonia</i> (Berk. & M.A. Curtis) Sacc. ^c	Holotipo	FH
<i>Deconica acutipilea</i> Speg.	<i>Psilocybe acutipilea</i> (Speg.) Guzmán	Holotipo	LPS
<i>Naematoloma rhombisporum</i> Guzmán	<i>Deconica neorhombispora</i> (Guzmán) P.S. Silva, Ram.-Cruz & Guzmán	Holotipo	ENCB
<i>Pholiotella blattariopsis</i> Speg.	<i>Psilocybe blattariposis</i> (Speg.) Singer ^c	Holotipo	LPS
<i>Psilocybe aequatoria</i> Singer	<i>Deconica aequatoria</i> (Singer) Ram.-Cruz & Guzmán	Holotipo	F
<i>Psilocybe aggericola</i> Singer & A.H. Sm.	<i>Psilocybe zapotecorum</i> R. Heim emend. Guzmán	Isotipo	XAL
<i>Psilocybe alpestris</i> Singer	<i>Deconica alpestris</i> (Singer) Ram.-Cruz & Guzmán	Holotipo	WU
<i>Psilocybe antioquiensis</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	<i>Psilocybe antioquiensis</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	Isotipo	XAL
<i>Psilocybe arcana</i> Borovička & Hlaváček	<i>Psilocybe serbica</i> var. <i>arcana</i> (Borovička & Hlaváček) Borovička, Oborník & Noordel.	Isotipo	PRM
<i>Psilocybe atlantis</i> Guzmán, Hanlin & C. White	<i>Psilocybe atlantis</i> Guzmán, Hanlin & C. White	Holotipo	XAL
<i>Psilocybe aucklandiae</i> Guzmán, C.C. King & Bandala	<i>Psilocybe aucklandiae</i> Guzmán, C.C. King & Bandala	Isotipo	XAL
<i>Psilocybe aureicystidiata</i> E.	<i>Deconica aureicystidiata</i> (E. Horak & Desjardin) Ram.-	Isotipo	XAL

Horak & Desjardin	Cruz & Guzmán ^b		
<i>Psilocybe aztecorum</i> R. Heim	<i>Psilocybe aztecorum</i> R. Heim	Holotipo	PC
<i>Psilocybe banderillensis</i> var. <i>paulensis</i> Guzmán & Bononi	<i>Psilocybe paulensis</i> (Guzmán & Bononi) Guzmán	Isotipo	XAL
<i>Psilocybe barrerae</i> Cifuentes & Guzmán	<i>Psilocybe zapotecorum</i> R. Heim emend. Guzmán	Isotipo	FCME
<i>Psilocybe bispora</i> Guzmán, Franco-Mol. & Ram.-Guill.	<i>Psilocybe bispora</i> Guzmán, Franco-Mol. & Ram.-Guill.	Holotipo	XAL
<i>Psilocybe bipleurocystidiata</i> E. Horak & Guzmán	<i>Psilocybe bipleurocystidiata</i> E. Horak & Guzmán	Isotipo	XAL
<i>Psilocybe brasiliensis</i> Guzmán	<i>Psilocybe brasiliensis</i> Guzmán	Isotipo	XAL
<i>Psilocybe bohémica</i> Šebek	<i>Psilocybe serbica</i> var. <i>bohémica</i> (Šebek) Borovička, Oborník & Noordel.	Paratipo	PRM
<i>Psilocybe bolivarii</i> Guzmán	<i>Psilocybe zapotecorum</i> R. Heim emend. Guzmán	Isotipo	XAL
<i>Psilocybe chaconii</i> Guzmán, Escalona & Ram.-Guill.	<i>Psilocybe zapotecorum</i> R. Heim emend. Guzmán	Holotipo	XAL
<i>Psilocybe caerulescens</i> Murrill	<i>Psilocybe caerulescens</i> Murrill	Holotipo	NY
<i>Psilocybe caerulescens</i> var. <i>mazatecorum</i> R. Heim	<i>Psilocybe caerulescens</i> Murrill	Isotipo	XAL
<i>Psilocybe caribaea</i> Guzmán, T.J. Baroni & Tapia	<i>Psilocybe caribaea</i> Guzmán, T.J. Baroni & Tapia	Isotipo	XAL
<i>Psilocybe cabiensis</i> Guzmán, M. Torres & Ram.-Guill.	<i>Psilocybe cabiensis</i> Guzmán, M. Torres & Ram.-Guill.	Isotipo	XAL
<i>Psilocybe chiapanensis</i> Guzmán	<i>Psilocybe chiapanensis</i> Guzmán	Holotipo	XAL
<i>Psilocybe eximia</i> E. Horak & Desjardin	<i>Psilocybe eximia</i> E. Horak & Desjardin	Isotipo	XAL
<i>Psilocybe farinacea</i> Rick ex Guzmán	<i>Psilocybe farinacea</i> Rick ex Guzmán	Sintipo	BPI
<i>Psilocybe gallaeciae</i> Guzmán & M.L. Castro	<i>Psilocybe gallaeciae</i> Guzmán & M.L. Castro	Holotipo	XAL

<i>Psilocybe galindoi</i> Guzmán	<i>Psilocybe galindoi</i> Guzmán	Holotipo	ENCB
<i>Psilocybe guatapensis</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	<i>Psilocybe guatapensis</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	Isotipo	XAL
<i>Psilocybe guilartensis</i> Guzmán, F. Tapia & Nieves- Riv.	<i>Psilocybe guilartensis</i> Guzmán, F. Tapia & Nieves- Riv.	Isotipo	XAL
<i>Psilocybe heimii</i> Guzmán	<i>Psilocybe heimii</i> Guzmán	Isotipo	XAL
<i>Psilocybe heliconiae</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	<i>Psilocybe heliconiae</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	Isotipo	XAL
<i>Psilocybe hispanica</i> Guzmán	<i>Psilocybe hispanica</i> Guzmán	Holotipo	XAL
<i>Psilocybe hoogshagenii</i> var. <i>convexa</i> Guzmán	<i>Psilocybe hoogshagenii</i> var. <i>convexa</i> Guzmán	Holotipo	ENCB
<i>Psilocybe hopii</i> Guzmán & J. Greene	<i>Psilocybe hopii</i> Guzmán & J. Greene	Holotipo	XAL
<i>Psilocybe isabelae</i> Guzmán	<i>Psilocybe isabelae</i> Guzmán	Holotipo	XAL
<i>Psilocybe jaliscana</i> Guzmán	<i>Psilocybe jaliscana</i> Guzmán	Isotipo	IBUG
<i>Psilocybe keralensis</i> K.A. Thomas, Manim. & Guzmán	<i>Psilocybe keralensis</i> K.A. Thomas, Manim. & Guzmán	Isotipo	XAL
<i>Psilocybe laurae</i> Guzmán	<i>Psilocybe laurae</i> Guzmán	Isotipo	IBUG
<i>Psilocybe magnispora</i> E. Horak, Guzmán & Desjardin	<i>Psilocybe magnispora</i> E. Horak, Guzmán & Desjardin	Isotipo	XAL
<i>Psilocybe makarorae</i> P.R. Johnst. & P.K. Buchanan	<i>Psilocybe makarorae</i> P.R. Johnst. & P.K. Buchanan	Holotipo	PDD
<i>Psilocybe meridensis</i> Guzmán	<i>Psilocybe meridensis</i> Guzmán	Isotipo	MER
<i>Psilocybe meridionalis</i> Guzmán, Ram.-Guill. & Guzm.-Dáv.	<i>Psilocybe meridionalis</i> Guzmán, Ram.-Guill. & Guzm.-Dáv.	Holotipo	IBUG
<i>Psilocybe mescaleroensis</i> Guzmán, Walstad, Gándara & Ram.-Guill.	<i>Psilocybe mescaleroensis</i> Guzmán, Walstad, Gándara & Ram.-Guill.	Holotipo	XAL
<i>Psilocybe mesophyla</i> Guzmán, J.Q. Jacobs & Escalona	<i>Psilocybe mesophyla</i> Guzmán, J.Q. Jacobs &	Holotipo	XAL

	Escalona		
<i>Psilocybe mexicana</i> Heim	<i>Psilocybe mexicana</i> Heim	Holotipo	PC
<i>Psilocybe microcystidiata</i> Guzmán & Bononi	<i>Psilocybe microcystidiata</i> Guzmán & Bononi	Isotipo	XAL
<i>Psilocybe moseri</i> Guzmán	<i>Psilocybe moseri</i> Guzmán	Holotipo	XAL
<i>Psilocybe moravica</i> Borovička	<i>Psilocybe serbica</i> var. <i>moravica</i> (Borovička) Borovička, Oborník & Noordel.	Isotipo	XAL
<i>Psilocybe muliercula</i> Singer & A.H. Sm.	<i>Psilocybe muliercula</i> Singer & A.H. Sm.	Isotipo	XAL
<i>Psilocybe naematoliformis</i> Guzmán	<i>Psilocybe naematoliformis</i> Guzmán	Holotipo	ENCB
<i>Psilocybe neocaledonica</i> Guzmán & E. Horak	<i>Psilocybe neocaledonica</i> Guzmán & E. Horak ^b	Isotipo	ENCB
<i>Psilocybe neoxalapensis</i> Guzmán, Ram.-Guill. & Halling	<i>Psilocybe neoxalapensis</i> Guzmán, Ram.-Guill. & Halling	Holotipo	XAL
<i>Psilocybe oaxacana</i> Guzmán, Escalona & J.Q. Jacobs	<i>Psilocybe oaxacana</i> Guzmán, Escalona & J.Q. Jacobs	Holotipo	XAL
<i>Psilocybe overeemii</i> E. Horak & Desjardin	<i>Psilocybe overeemii</i> E. Horak & Desjardin ^b	Isotipo	XAL
<i>Psilocybe ovoideocystidiata</i> Guzmán & Gaines	<i>Psilocybe ovoideocystidiata</i> Guzmán & Gaines	Holotipo	XAL
<i>Psilocybe pileocystidiata</i> Guzmán & Ram.-Guill.	<i>Psilocybe pileocystidiata</i> Guzmán & Ram.-Guill.	Holotipo	XAL
<i>Psilocybe pleurocystidiosa</i> Guzmán	<i>Psilocybe pleurocystidiosa</i> Guzmán	Isotipo	ENCB
<i>Psilocybe portorricensis</i> Guzmán, Nieves-Riv. & F. Tapia	<i>Psilocybe portorricensis</i> Guzmán, Nieves-Riv. & F. Tapia	Holotipo	XAL
<i>Psilocybe quebecensis</i> Ola'h & R. Heim	<i>Psilocybe quebecensis</i> Ola'h & R. Heim	Holotipo	QUE
<i>Psilocybe rickii</i> Guzmán & Cortez	<i>Psilocybe rickii</i> Guzmán & Cortez	Holotipo	FH

<i>Psilocybe rolfsingerii</i> Guzmán & Halling	<i>Psilocybe rolfsingerii</i> Guzmán & Halling ^a	Holotipo	F
<i>Psilocybe sardoa</i> Guzmán & Contu	<i>Psilocybe sardoa</i> Guzmán & Contu	Isotipo	XAL
<i>Psilocybe samuiensis</i> Guzmán, Bandala & J.W. Allen	<i>Psilocybe samuiensis</i> Guzmán, Bandala & J.W. Allen	Holotipo	XAL
<i>Psilocybe semiangustipleurocystidiata</i> Guzmán, Ram.-Guill. & M. Torres	<i>Psilocybe semiangustipleurocystidiata</i> Guzmán, Ram.-Guill. & M. Torres	Holotipo	XAL
<i>Psilocybe semi-inconspicua</i> Guzmán & Trappe	<i>Deconica semi-inconspicua</i> (Guzmán & Trappe) Ram.-Cruz & Guzmán	Isotipo	XAL
<i>Psilocybe serbica</i> M.M. Moser & E. Horak	<i>Psilocybe serbica</i> M.M. Moser & E. Horak	Holotipo	IB
<i>Psilocybe singularis</i> Guzmán, Escalona & J.Q. Jacobs	<i>Psilocybe singularis</i> Guzmán, Escalona & J.Q. Jacobs	Holotipo	XAL
<i>Psilocybe stuntzii</i> Guzmán & J. Ott	<i>Psilocybe stuntzii</i> Guzmán & J. Ott	Isotipo	XAL
<i>Psilocybe subannulata</i> E. Horak & Guzmán	<i>Psilocybe subannulata</i> E. Horak & Guzmán	Holotipo	XAL
<i>Psilocybe subaeruginascens</i> Höhn.	<i>Psilocybe subaeruginascens</i> Höhn.	Holotipo	FH
<i>Psilocybe subacutipilea</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	<i>Psilocybe subacutipilea</i> Guzmán, Saldarr., Pineda, G. García & L.-F. Velázquez	Isotipo	XAL
<i>Psilocybe subbrunneocystidiata</i> P. S. Silva & Guzmán	<i>Deconica neorhombispora</i> (Guzmán) P.S. Silva, Ram.-Cruz & Guzmán	Isotipo	XAL
<i>Psilocybe subheliconia</i> Guzmán, Ram.-Guill. & M. Torres	<i>Psilocybe subheliconia</i> Guzmán, Ram.-Guill. & M. Torres ^a	Isotipo	XAL
<i>Psilocybe subhoogshagenii</i> Guzmán, M. Torres & Ram.-Guill.	<i>Psilocybe subhoogshagenii</i> Guzmán, M. Torres & Ram.-Guill.	Holotipo	XAL
<i>Psilocybe submaritima</i> Contu	<i>Deconica submaritima</i> (Contu	Isotipo	XAL

& Guzmán	& Guzmán) Noordel.		
<i>Psilocybe subtropicalis</i> Guzmán	<i>Psilocybe subtropicalis</i> Guzmán	Holotipo	XAL
<i>Psilocybe subvicida</i> var. <i>velata</i> Noordel. & Verduin	<i>Deconica subviscida</i> var. <i>velata</i> (Noordel. & Verduin) Noordel.	Isotipo	XAL
<i>Psilocybe subyungensis</i> Guzmán	<i>Psilocybe subyungensis</i> Guzmán	Holotipo	NY
<i>Psilocybe taiwanensis</i> Zhu L. Yang & Guzmán	<i>Psilocybe taiwanensis</i> Zhu L. Yang & Guzmán	Isotipo	XAL
<i>Psilocybe thaicordisporae</i> Guzmán, Ram.-Guill. & Karunarathna	<i>Psilocybe thaicordisporae</i> Guzmán, Ram.-Guill. & Karunarathna	Isotipo	XAL
<i>Psilocybe</i> <i>thaiaerugineomaculans</i> Guzmán, Karunaratha & Ram.-Guill.	<i>Psilocybe</i> <i>thaiaerugineomaculans</i> Guzmán, Karunaratha & Ram.-Guill.	Holotipo	XAL
<i>Psilocybe</i> <i>thaiduplicatocystidiata</i> Guzmán, Karunaratha & Ram.-Guill.	<i>Psilocybe</i> <i>thaiduplicatocystidiata</i> Guzmán, Karunaratha & Ram.-Guill.	Isotipo	XAL
<i>Psilocybe thailandensis</i> E. Horak, Guzmán & Desjardin	<i>Deconica thailandensis</i> (E. Horak, Guzmán & Desjardin) Ram.-Cruz & Guzmán	Isotipo	XAL
<i>Psilocybe thaizapoteca</i> Guzmán, Karunarathna & Ram.-Guill.	<i>Psilocybe thaizapoteca</i> Guzmán, Karunarathna & Ram.-Guill	Holotipo	XAL
<i>Psilocybe teofiloi</i> Guzmán & Ram.-Guill. nom. inval.	<i>Psilocybe teofiloi</i> Guzmán & Ram.-Guill. nom. inval.	Holotipo	XAL
<i>Psilocybe umbrina</i> E. Horak, Guzmán & Desjardin	<i>Deconica umbrina</i> (E. Horak, Guzmán & Desjardin) Ram.- Cruz & Guzmán	Isotipo	XAL
<i>Psilocybe uxpanapensis</i> Guzmán	<i>Psilocybe uxpanapensis</i> Guzmán	Holotipo	ENCB
<i>Psilocybe villarrealiae</i> Guzmán	<i>Psilocybe villarrealiae</i> Guzmán	Holotipo	XAL
<i>Psilocybe wayanadensis</i> K.A.	<i>Psilocybe wayanadensis</i> K.A.	Holotipo	XAL

Thomas, Manim. & Guzmán	Thomas, Manim. & Guzmán		
<i>Psilocybe weilii</i> Guzmán, Stamets & F. Tapia	<i>Psilocybe weilii</i> Guzmán, Stamets & F. Tapia	Holotipo	XAL
<i>Psilocybe weldenii</i> Guzmán	<i>Psilocybe weldenii</i> Guzmán	Holotipo	ENCB
<i>Psilocybe yungensis</i> Singer & A.H. Sm.	<i>Psilocybe yungensis</i> Singer & A.H. Sm.	Isotipo	MICH
<i>Psilocybe zapotecoantillarum</i> Guzmán, T.J. Baroni & Lodge	<i>Psilocybe zapotecoantillarum</i> Guzmán, T.J. Baroni & Lodge	Isotipo	XAL
<i>Psilocybe zapotecocaribaea</i> Guzmán, Ram.-Guill. & T.J. Baroni	<i>Psilocybe zapotecocaribaea</i> Guzmán, Ram.-Guill. & T.J. Baroni	Holotipo	K
<i>Stropharia aquamarina</i> Pegler	<i>Psilocybe aquamarina</i> (Pegler) Guzmán	Holotipo	K
