Cultural studies of *Psilocybe sensu lato* species (Fungi, Agaricales, Strophariaceae)

Silva PS\(^1\)*, Guzmán-Dávalos L\(^2\) and Silveira RMB\(^1\)

\(^1\)Universidade Federal do Rio Grande do Sul, Avenida Bento Gonçalves 9500, CP 91501-970, Porto Alegre, Rio Grande do Sul, Brazil

\(^2\)Universidad de Guadalajara, Apartado Postal 1-139, Zapopan, Jalisco, 45101, Mexico

*Corresponding author: Paula Santos da Silva – e-mail: pulisant@yahoo.com.br
Abstract

Searching for additional taxonomic characters, cultural characteristics of five strains belonging to five different species of *Psilocybe* s.l. were studied. A strain of *Stropharia venusta* was included for comparison. Some macro- and micromorphological characteristics, such as growth rate, mat texture, mycelial cystidia and some hyphae modifications may be useful as taxonomic characters. Cultural characters of *Deconica horizontalis*, *D. neorhombispora* and *S. venusta* are described for the first time.

Key words – *Deconica* – mycelial cystidia – strains
Introduction

Fungal cultural features have been applied for several authors especially for characterization of fungi of economic and biotechnological interest, including wood-rotting, edible and medicinal fungi (Stalpers 1978, Buchalo 1988, Stamets 2000). Nobles (1965) stated that some morphological characters of vegetative mycelium may be helpful for taxonomic purposes, and since then, several works have corroborated this idea (Jacobsson 1989, Molitoris et al. 1996, Fausto-Guerra et al. 2002, Buchalo et al. 2011).

Formerly, *Psilocybe* (Fr.) P. Kumm. included psychotropic and non-psychotropic species, but recently *Psilocybe* *s.str.* and *Deconica* (W. G. Sm.) P. Karst. have been segregated, due the presence of hallucinogenic alkaloids in the first and the absence in the second (Moncalvo et al. 2002). *Psilocybe* *s.l.* comprises saprophytic species, growing on soil, decayed wood, logs, stems, and manure (Singer 1986). Some works have already described cultural characters of *Psilocybe* *s.l.* species (Gilmore 1926, Heim et al. 1957, Heim et al. 1963, Watling 1971, Dubovoy & Herrera 1967, Dubovoy & Herrera 1968, Buchalo et al. 2009). Walther & Weiß (2008) characterized several Strophariaceae species in culture, including four *Psilocybe* *s.l.* strains, providing taxonomically relevant characters.

In this work we present descriptions of five species belonging to *Psilocybe* *s.l.*, four belonging to *Deconica* and one belonging to *Psilocybe*, with macro- and micromorphological data, aiming to complement the traditional taxonomy based on basidiome features.

Material and Methods
Five *Psilocybe* s.l. strains were used: *Deconica coprophila* (Bull.) P. Karst. (P.S. Silva 202/09), *D. horizontalis* (Bull.) Noordel. (P.S. Silva 253/10), *D. neorhombispora* (Guzmán) P.S. Silva & Ram.-Cruz (P.S. Silva 238/09), *Deconica* sp. (P.S. Silva 235/09), and *Psilocybe cubensis* (Earle) Singer (P.S. Silva 200/09). *Stropharia venusta* P.S. Silva, Cortez & R.M. Silveira (P.S. Silva 265/12) was included for comparison. All collections are from Brazil. Fruit bodies from which the strains were obtained are deposited at herbarium ICN, from Universidade Federal do Rio Grande do Sul, and its corresponding strains are deposited at “Laboratório de Micologia Molecular” at the same university.

For morphological characterization, the strains were grown on Difco Malt Extract Agar (MEA) and incubated at 25°C in complete darkness, following procedures indicated by Nobles (1965). The parameters observed were those described by Nobles (1965), with some modifications. Growth rate was evaluated as the percentage of the Petri dish covered in one week (seven days) after inoculating the plate in the center. The macro- and micromorphological characterizations were made every week, during six weeks. Micromorphological observations were made in light microscope, from material mounted in 3% potassium hydroxide (KOH) and 1% fluoxine.

**Results**

**Strains descriptions**

*Deconica coprophila* (Bull.) P. Karst. (Figs 1, 7–17).
Macromorphological characters: 43% of plate covered by the 7th day (plates not covered in the six weeks, reaching 88% in the last week); mat downy to floccose in the first two weeks, becoming woolly in the third week, white; advancing zone superficial; margin uneven; odor earthy; reverse unchanged.

Micromorphological characters: Aerial mycelium: hyphae 1.5-6 µm diam., hyaline, sometimes with granulose refringent contents, thin and thick-walled (the thick-walled hyphae have refringent walls and non-staining lumen), branched, septate, with clamp connections or septae simple, with short branches, anastomoses present, sarco-hyphae present, as inflated hyphae with granulose refringent contents, some hyphae with a capitate apex; arthrospores and chlamydospores absent.

Advancing zone: hyphae 1-4 µm diam., hyaline, sometimes with granulose contents, thin or thick-walled, branched, septate, with clamp connections, with short branches, anastomoses present, flexuose hyphae abundant; chlamydospores present (rare, observed in one isolate), globose, thin or thick-walled, with granulose contents, intercalar.


Material studied: strain 001-09, vegetative isolation from spore print of basidiome; growing on dung (Brazil, Rio Grande do Sul, Bagé, 21 March 2009, P.S. Silva 202/09 ICN 154231).

Deconica horizontalis (Bull.) Noordel.

(Figs 2, 18–28).

Macromorphological characters: 100% of plate covered by the 7th day; mat downy to woolly, with some dense spots as from the second week, white; advancing zone superficial; margin uneven; odor strongly as antiseptic; reverse unchanged.
Micromorphological characters: Aerial mycelium: hyphae 1-7 µm diam., hyaline, frequently presenting oily contents, thin and thick-walled, branched, septate, with clamp connections or septae simple, multiple clamps present, with short branches, anastomoses present; arthrospores absent, chlamydospores present in one isolate, ellipsoid, thin-walled, with granulose contents. Advancing zone: hyphae 1.5-6 µm diam., hyaline, with oily contents, thin or thick-walled, branched, septate, with clamp connections, multiple clamps present, anastomoses present, innumerous short branches and flexuose hyphae; arthrospores and chlamydospores absent.


Material studied: strain 002-10, vegetative isolation from spore print of basidiome; growing on decayed wood (Brazil, Rio Grande do Sul, Santa Maria, 14 May 2010, P.S. Silva 253/10 ICN 154677).

Deconica neorhombispora (Guzmán) P.S. Silva & Ram.-Cruz (Figs 3, 29–37).

Macromorphological characters: 35% of plate covered by the 7th day (plates not completely covered during the six weeks, reaching 77% in the last week); mat downy, white; advancing zone superficial; margin uneven; odor musty; reverse unchanged.

Micromorphological characters: Aerial mycelium: hyphae 2-6.5 µm diam., hyaline or with granulose contents, thin or thick-walled, some hyphae with irregularly thick and refractive walls, and some with crystals on the walls, branched, septate, with clamp connections, but also septae simple, with multiple clamps, with innumerous short branches, forming a plectenchyma, anastomoses present, some isolate presenting cystidia, digitate, thick-walled; chlamydospores present, globose to ellipsoid, thin or thick-walled, terminal or
intercalar. Advancing zone: hyphae 2-5 µm diam., hyaline, with or without oily contents, thin-walled, branched, septate, with clamps connections or septae simple, multiple clamps present, with anastomoses, flexuose hyphae abundant; chlamydospores present (in two isolates), globose to ellipsoid, thin-walled, with or without granulose contents, terminal or intercalar.


Material studied: strain 003-09, vegetative isolation from spore print of basidiome, growing on decayed wood (Brazil, Rio Grande do Sul, Morrinhos do Sul, 4 October 2009, P.S. Silva 238/09 ICN 154351).

Deconica sp.

(Figs 4, 38–45).

Macromorphological characters: 68% of plate covered by the 7th day, with plates completely covered as from the second week; mat downy, white, with concentric zones; advancing zone superficial; margin uneven; odor fruity; reverse unchanged.

Micromorphological characters: Aerial mycelium: hyphae 2-6 µm diam., hyaline, with oily contents, thin or thick-walled, with refringent walls, branched, septate, with clamp connections, multiple clamps present, with short branches and nodules, forming a plectenchyma, anastomoses present; arthrospores and chlamydospores absent. Advancing zone: hyphae 2.5-5 µm diam., hyaline, branched, septate, with clamp connections or septae simple, multiple clamps present, with short branches and nodules, flexuose hyphae abundant; chlamydospores present (rare, in one isolate), globose to ellipsoid, thin-walled, with granulose contents.

**Material studied:** strain 004-09, vegetative isolation from spore print of basidiome, growing among mosses (Brazil, Rio Grande do Sul, Derrubadas, 17 September 2009, P.S. Silva 235/09 ICN 154348).

**Note:** This species is similar to *D. inquilina* (Fr.) Romagn. from which differs by smaller basidiospores and cheilocystidia, and the presence of pleurocystidia.

*Psilocybe cubensis* (Earle) Singer

(Figs 5, 46–53, 58–61).

**Macromorphological characters:** 64% of plate covered by the 7th day, with plates completely covered in two weeks; mat downy to woolly, with some dense spots as from the third week, white; presence of rhizomorphs in some isolates; advancing zone superficial; margin uneven; odor as “fungus”; reverse unchanged.

**Micromorphological characters:** Aerial mycelium: hyphae 2-4 µm diam., hyaline, thin or thick-walled (some hyphae are thick-walled with refringent walls and non-staining lumen), branched, septate, with clamp connections, sarco-hyphae present, as inflated, thin-walled, with granulose contents hyphae, anastomoses present; chlamydospores present, globose, thin or thick-walled, with granulose contents, intercalar. Advancing zone: hyphae 2-4 µm diam., hyaline, thin or thick-walled (refringent thick walls with non-staining lumen), branched, septate, with clamp connections, sarco-hyphae present, anastomoses present, and flexuose hyphae abundant; arthrospores and chlamydospores absent.

**Species code:** 3. (7). (16). (25). (26). 32. (34). 36. 38. 42. 53. 56

**Material studied:** strain 005-09, vegetative isolation from spore print of basidiome, growing on dung (Brazil, Rio Grande do Sul, Bagé, 21 March 2009, P.S. Silva 200/09 ICN 154229).
Stropharia venusta P.S. Silva, Cortez & R.M. Silveira

(Figs 6, 54–57, 62–63).

**Macromorphological characters:** 21% of plates covered by the 7th day, with plates completely covered in the 6th week; mat cottony, white; advancing zone superficial; margin uneven; odor earthy; reverse unchanged.

**Micromorphological characters:** Aerial mycelium: hyphae 2-4 μm diam., hyaline, thin or thick-walled (the thick walls are refractive, with non-staining lumen), branched, septate, clamp connections not observed, anastomoses present, acanthocytes present; arthrospores and chlamydospores absent. Advancing zone: hyphae 1-3 μm diam., hyaline, thin or thick-walled hyphae with non-staining lumen, branched, septate, clamp connections not observed, with flexuose hyphae, acanthocytes present; chlamydospores present, globose to ellipsoid, thin or thick-walled, with or without granulose contents, terminal and intercalar.

**Species code:** 6. (7). (25). (26). (32). (34). 36. 38. 46. 51. 54

**Material studied:** strain 006-09, vegetative isolation from spore print of basidiole, growing on decayed wood (Brazil, Rio Grande do Sul, São Francisco de Paula, 29 May 2009, P.S. Silva 265/12 ICN).

**Discussion**

The macromorphology of the cultures studied present some remarkable differences. Deconica neorhombispora and D. coprophila present the mat margin irregular and slow growth, while D. horizontalis, Deconica sp. and P. cubensis have their mat margin regular...
and faster growth. *Stropharia venusta* presents a different mat texture, being cottony, not translucent, while the *Psilocybe s.l.* studied strains are all downy or woolly, and translucent.

In basidiome *in situ*, a bluing coloration can be observed, with material bruising or aging, as result of alkaloids oxidation, and it has been used for differing *Psilocybe* species from *Deconica*. This coloration change can be also observed in cultures, but it was not observed during the six weeks in *P. cubensis*, although the authors have observed this reaction in older strains of this species.

Some micromorphological characters observed, such as presence of anastomoses on hyphae, are rather constant in all studied strains, as well as in other genera (Buchalo et al. 2011), and probably do not represent taxonomic relevance. Although just one studied species presented crystals on some hyphae walls, these are also considered no valuable characters, once that oxalic acid is naturally formed as metabolite of the Krebs cycle (Molitoris et al. 1996).

Size and shape of arthrospores and chlamydospores have been considered helpful for species identification in some genera (Fausto-Guerra et al. 2002). In the present work, the strains did not present arthrospores in the six weeks, but they did present chlamydospores. However, these structures are very similar among the studied species, regarding their size, shape and wall thickness, giving no clue for delimitating species. Meanwhile, they may present some value in larger sampling, for delimitating species group.

Mycelial cystidia have been observed just in *D. neorhombispora* and seem to be rather characteristic, utriform, with a narrow or large base, and thick-walled. This can be a good specific character, as have been already discussed by Nobles (1965), who observed mycelial cystidia in some species of her study. Walther & Weiβ (2008) also observed cyanophilous mycelial cystidia in *Hypholoma* and *Stropharia* species, but there are few
records of these structures in fungal culture studies. Additionally, sarco-hyphae seem to be uncommon in mycelial studies. Kirtk et al. (2001) characterized as sarco-hyphae long, inflated hyphae. Following this concept we concluded that the structures observed here may be included in this classification and provide a relevant taxonomic character.

Gilmore (1926) observed arthrospores in *D. coprophila*, but these were not observed in the studied strain. It is probable that the presence or absence of these structures are variable among different strains. Likewise, Buchalo et al. (2009) observed arthroconidia and crystals on hyphae in *P. cubensis*, which were not observed in the studied strain in this work.

The presence of acanthocytes in *Stropharia* mycelium in culture has been first observed by Farr (1980) and recently by Walther & Weiß (2008), and these structures are considered a good generic character (Cortez & Silveira 2007, Noordeloos 2011). *Stropharia venusta* presents abundant acanthocytes in mycelium. Acanthocytes are spine shape structures, with refringent thick walls, and are known to function as nematode-trapping (Luo et al. 2006).

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Fig. 1. *Deconica coprophila*. Mycelial growth during the six weeks (1\textsuperscript{st} to 6\textsuperscript{th} week from left to right).
Fig. 2. *Deconica horizontalis*. Mycelial growth during the six weeks.
Fig. 3. *Deconica neorhombispora*. Mycelial growth during the six weeks.
Fig. 4. *Deconica* sp. Mycelial growth during the six weeks.
Fig. 5. *Psilocybe cubensis*. Mycelial growth during the six weeks.
Fig. 6. *Stropharia venusta*. Mycelial growth during the six weeks.
Figs. 7-28. 7-17: *Deconica coprophila*. 7-8: hyphae with short branches, 9: intercalar chlamydospore, 10: sarco-hypha, 11: flexuose hypha, 12-13: thick-walled hyphae with anastomose, 14: septate inflate and fibulate hypha, 15: fibulate thin-walled hypha and
fibulate hypha, 41: intercalar chlamydospore, 42: flexuose hypha, 43: hypha with anastomose and short branch, 44: hypha with branches, 45: anastomose. Scale bar = 10 μm.

Scale bar = 10 µm.