Oliveira, L.A.; Fernandes, O.C.; Jesus, M.A.;Bentes, J.L.S.; Andrade, S.L.; Souza, A.Q.L.; Santos, C. *Diversidade Microbiana da Amazônia 2015*. Editora INPA.

Some hydnoid (Basidiomycetes) mushrooms of the Brazilian Amazon

Bastos VIS¹, Jesus MA^{1,2}; Santos Borel JF¹

¹Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, AM. E-mail: vbastae@gmail.com, ²ranna@inpa.gov.br;

Abstract

The general characters of this fungal group are the presence of odontioid hymenium, hymenophore, and pileate or resupinate. Generally, the hydnoid species are ectomycorrhizal, as they are important functional components of forest ecosystems. In some cultures (e.g. Hydnum repandum) it holds nutritional importance. The objective is to report the occurrence of hydnoid mushrooms for the Amazon Rainforest. The collections were made in the following sites: Adolpho Ducke Forest Reserve, Tropical Silviculture Experimental Station, Uatumã Biological Reserve in the State of Amazonas, and Viruá National Park located in the State of Roraima. Besides the aforementioned sites few collections were made in the urban area of Manaus. All the specimens are deposited in the Herbarium of the National Institute of Amazon Research-INPA. A total of 21 specimens of Amazon hydnoid mushrooms were identified: Climacodon sp (1), Dentipellis sp (1), Hydnodon thelephorum (11), Pseudohydnum gelatinosum (1), Stecchericium hydneum (1), S. seriatum (2), S. ochraceum (1), Trechispora gillesii (2), Trechispora sp. (1). Due to the economic and ecological importance of these macrofungi, the study contributed to the expansion of knowledge about hydnoid diversity in the Amazon region. Comments and illustrations are given for most of the species.

Key words: Ectomycorrhizal fungi, Northern Brazil, South America, Taxonomy

Introduction

The hydnoid fungi are characterized by their pileate basidiomes, usually stiptate, centrally attached. Colors range from white to yellow, and orange to brown. The "teeth", or "spines", reach up to 3 mm. The hyphal system varies from monomitic to dimitic, consisting of generative and skeletal hyphae. The generative hyphae are thin-walled, branched, contain septa, and clamp connections. The skeletal hyphae are mostly thick-walled, and branched with septae. Basidiospores are hyaline, being globose or subglobose to ellipsoid, with a smooth or verrucose surface (Chevall., 1826).

Many hydnoid fungi are wood-inhabiting species, but some of them are believed to be ectomycorrhizas, forming mutually beneficial relationships with the roots of living trees and other plants, (e.g., species of *Hydnellum* P. Karst.). Most of the basidiomes typically occur on the ground, or in leaf litter in woodland (Dai, 2010).

Some species (e.g. *Hydnum repandum* L.) are well known edible fungi and are commercially collected and marketed in Europe and France. It is often referred to as "pied de mouton" in France (Roberts and Evans, 2011). In North America, the related *H. umbilicatum* Peck is also known to have a sweet flavor and commercially collected, sometimes under the name "sweet tooth".

Stipitate hydnoid fungi have been shown to be particularly sensitive to nitrogen deposition as an indicator of eutrophication (Vesterholt *et al.*, 2000; Arnolds, 2003). Others (e.g. *Steccherinum ochraceum*) have gone through several studies about its biotechnological potential for the pulping industry (Chernykh *et al.*, 2008).

Therefore, the main objective of this present contribution is to report the occurrence of hydnoid fungi for the Amazon Rainforest and, thereby, show this fungi group's potential to develop future biotechnological studies in northern Brazil.

Materials and Methods

This study represents a few materials collected during the last 18 years by Dr^a. Maria Aparecida de Jesus and collaborators as a part of ongoing projects to search for biodiversity in the Amazon rainforest "Biodiversity Research Program– PPBio" (INPA/ MCTI) and a sustainable forest management project in the Brazilian Amazon "Amazon woods" (INCT–MCTI/ FAPEAM). The collections were made in the per quadrant sampling effort methodology (RAPELD), using a grid of 30 (250×40 m) lots same as the ones employed by (PPBio/MCTI/INPA).

Characters of the collection sites

Adolpho Ducke Forest Reserve ($2^{\circ}59'38.5''S 59^{\circ}55'05.8''W$) with collections performed in January 2006. The forest reserve is surrounded by the urban area of Manaus, Amazonas, which is composed of a typical Amazon Rainforest. Average temperature in Manaus is 26.0°C/ 78.8°F (maximum 39.0°C/ 102.2°F and minimum 19.0°C/ 66.2°F). The vegetation has uniform canopy with an average height of 32

meters. The topography consists of plateaus crossed by streams. The soils are composed of argillaceous oxisols, and are sandier with increasing inclination and decreasing altitude, see (Oliveira *et al.*, 2008).

The Tropical Silviculture Experimental Station is located 60 km from the city of Manaus (2°35'21"S 60°06'55"W). The collections were performed during 2014. The vegetation corresponds to the dense upland forest, with floristic composition of high trees up to 12–15 meters tall, and 7–12 meter tall small trees and shrubs. The soil is composed of very oxisol acidic, clayey soil, with high aluminum content. The climate is equatorial, with abundant rainfall and well distributed all year round. There is a short dry season, where the lowest rainfall (August) gets less than 100 mm, and a rainy season with monthly precipitation exceeding 290 mm (Jardim and Hosokawa, 1986).

Uatumã Biological Reserve is located in the municipality of São Sebastião do Uatumã, in the State of Amazonas (1°27'10.03"S 59°20'28.601"W) with collections performed in 2009. The reserve holds a large variety of environments composed mostly of rainforest, with trees reaching up to 50 meters. The soil is rocky, with high concentration of nutrients. It is also very hilly and has two distinct habitats, the high and dry plateaus and the humid lowlands with many streams. The vegetation type of the open areas is composed of vines, bamboo, or grasses. Other formations are the open and closed white sand forests, very dense covered of shrubs and climbing plants (Zuquim *et al.*, 2008).

Viruá National Park is located in the municipality of Caracaraí, in the State of Roraima (1°38'57.4"N 61°08'51.7"W) with collections made between 2008/09. The vegetation is an extensive mosaic of complex, seasonally flooded, forested and not forested systems (Mendonça 2011). The climate is equatorial (hot and humid), with a short dry season, and higher rainfall in autumn. In its southern part, the area comprises a wide and nearly flat surface, with predominately sandy, poorly drained soils and lots of ponds. In its northern part, there are residual hills. Along the west extension, bounded by the Branco River, there is occurrence of alluvial flood plains, which is also observed in the southern portion (Rossetti *et al.*, 2012).

Additionally, few collections were performed in 2015 in the urban area of Manaus by collaborators during a project of fungi associated with trees and shrubs in public roads.

Macro- and microscopic examinations

The hydnoid specimens were analyzed according to standard techniques as described by (Martin, 1934) used light microscopy, samples were mounted in 5% potassium hydroxide (KOH), Melzer's reagent (IKI), and 0.1% Cotton Blue (CB) in 60% lactic acid to determine cyanophily. All the specimens were deposited at the Herbarium of the National Institute of Amazon Research (INPA). Substrates were not identified properly due to their high stage of rotting. IH indicates the herbarium collection number.

Results and Discussion

A total of 21 specimens of Amazon hydnoid mushrooms were identified in the Brazilian Amazon. Substrate preference and collection sites are shown below (Table 1).

Таха	Family	Substrate	Collection site	Total
Climacodon sp 1	Phanerochaetaceae	Ft	PARNA	1
Dentipellis sp 1	Hericiaceae	Fb	ZFII	1
Hydnodon thelephorum (Lév.) Banker	Hydnodontaceae	Rt, Ft	ZFII, RDS	11
Pseudohydnum gelatinosum (Scop.) P.	Exidiaceae	Ft	INPA	1
Karst.				
Steccherinum hydneum Rick: Maas Geest.	Phanerochaetaceae	Lt	UB	1
Steccherinum seriatum (Lloyd) Maas Geest.	Phanerochaetaceae	Ft	ZFII	2
Steccherinum ochraceum (Pers. ex J.F.	Phanerochaetaceae	Fb	ZFII	1
Gmel.) Gray				
Trechispora gillesii (Maas Geest.) Liberta	Hydnodontaceae	Rt, Lt	RFAD, UB	2
Trechispora sp 1	Hydnodontaceae	Lt	RFAD	1
Total				21

Table 1. Occurrence of hydnoid fungi in the Brazilian Amazon

Substrate: Fb. fallen tree branch, Ft. fallen tree trunk, Lt. live tree, Rt. root. Collection sites: PARNA. Viruá National Park, RDS. Uatumã Forest Reserve, RFAD. Adolpho Ducke forest reserve, INPA. National Institute of Amazon Research, UB. Urban area, ZFII. Tropical Silviculture Experimental Station.

Among the studied species, the largest number of specimens is *H. thelephorum* most likely due to the high sampling effort performed in ZF II, which compared to the others, possibly shows that the number of species in the Amazon is low, demonstrating the need for a greater sampling effort. Likewise, most of the species studied were

described on soil corroborating with (Cifuentes *et al.*, 2005) who reported these species in the tropical and subtropical forests.

In Regards to *S. ochraceum*, its potential lignocellulolytic has been heavily studied and some different forms of laccase with unusual properties have been isolated from its culture (Chernykh, *et al.*, 2008).

Taxonomy

Hydnodon thelephorum

Fig. 1–2

Description: Basidiome piliate, about 2–6 cm (wide), pale orange to orange brown, surface smooth to rimose at center, margin curved downward, imbricate. Hymenophore tuberculate with cylindrical spines, 0.5 to 1 mm (long), smaller and scattered toward margin, orange-brown to cream colored. Stipe, 2–3.5 × 3–5 cm, central portion subcylindrical to flattened, covered with short yellowish to cream colored spines, surface longitudinally rugose. Hyphal system monomitic, thin walled generative hyphae, 1.5–3 μ m (wide), frequently simple clamped and scanty inflated. Cystidia absent. Basidia subclavate, 19(–25)–30 × 4.5–7.5 μ m, sterigmata 2–5 μ m (long), hyaline, with basal clamps. Basidiospores ellipsoid to subglobose, 4–5 × 2.5–5 μ m, verrucose, in KOH 5% ornamentation cyanophilic.

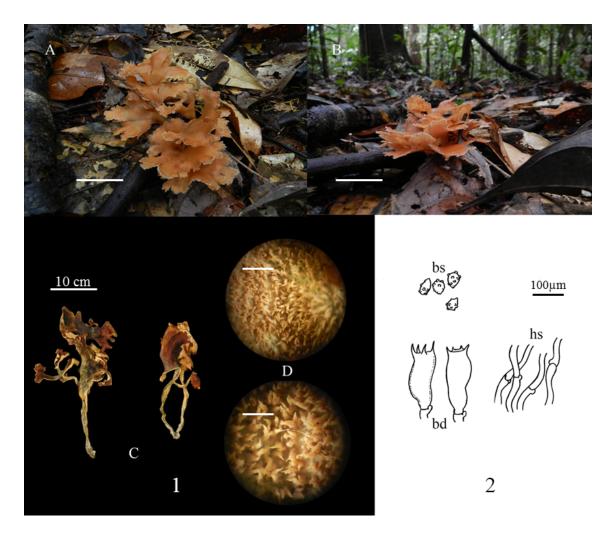


Figure 1–2. *H. thelephorum* in different perspectives. A–B. Aspect of the fresh piliate basidiome on the substrate, C. Dry hymenophore, D. Hymenophore tuberculate with cylindrical spines. bs. Basidiospores, bd. Basidia clavate with basal clamps, hs. Hyphal system (generative hyphae). Bar 100 μ m (2); 1mm (D); 10 mm (A, B, C).

Known distribution: This species was previously described for the Amazon region by (Bononi, 1981). Also known to be restricted to the Neotropic zone (Cifuentes *et al.*, 2005).

Material examined: Brazil, Amazonas, Manaus, Tropical Silviculture Experimental Station, on soil/root or fallen tree trunk, 23 Apr. 2014, 11 Apr. 2013, 12 Apr. 2013, 23 Apr. 2014, 12 Apr. 2013, 23 Apr. 2014, 24 Apr. 2014, 23 Apr. 2014, 24 Apr. 2014, VIS Bastos and MA Jesus (IH 8.217, 8.261, 8.412, 8.746, 10.526, 10.527, 10.528, 10.529, 10.530); Uatumã Biological Reserve, on a tree root, 26 May 2009, MA Jesus (IH 5285).

Remarks: *H. thelephorum* is distinguished among the stipitate hydnoid fungi because of the pale orange to red color, gelatinous basidiome, and the basidiospores

with cyanophilic warts. (Coker and Beers, 1951). The specimens described here were mostly found on soil close to tree roots, corroborating with (Bononi, 1981; Cifuentes *et al.*, 2005; Sobestiansky, 2005).

Pseudohydnum gelatinosum

Fig. 3–4

Description: Basidiome spatulate to fan-shaped; pileus $3-4 \times 2-5$ cm, upper surface smooth, white to greyish-white when fresh, pale grey to somewhat dark brown when dry; odontioid hymenophore surface, provided with dense pale grey to light brown conic spines, $2-12 \times 1-2$ mm, with 3–6 spines per centimetre. Cystidia absent. Probasidia globose, $45-50 \times 3-7.5$ µm, divided into four cells by longitudinal septa, with 2–4 cylindrical sterigmata $3-5 \times 1.5-2$ µm. Basidiospores subglobose to ellipsoid, $6-7(-7.5)-8 \times 4.2-5$ µm, hyaline, smooth, inamiloides and indextrinoides.

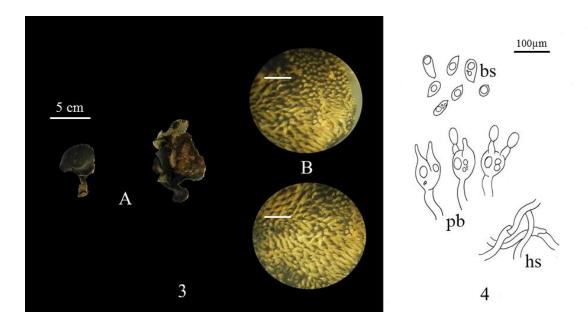


Figure 3. Macro and microstructures. A. Aspect of the dry piliate basidiome, B. Dry odontioid hymenophore. bs. Basidiospores, pb. Protobasidia, hs. Hyphal system (generative hyphae). Bar 100 μ m (4); 1mm (B); 5 cm (A).

Known distribution: It is distributed worldwide (Kirk *et al.*, 2008). In Brazil, it is reported to the State of Pará (Bononi, 1981).

Material examined: Brazil, Amazonas, Manaus, National Institute of Amazon Research, on a fallen tree trunk, 05 Mar 2015, MA Jesus (IH 10.051).

Remarks: This species is characterized by its hydnoid hymenophore and gelatinous consistency (Lowy, 1971).

Steccherinum hydneum

Description: Basidiome annual, effused reflexed, usually $5-8 \times 2-4$ cm, when dries easily separable from substrate; odontioid hymenophore, dense spines, brownish to cream colored, subulate, flattened, straight to somewhat flexuous, 2–3 mm (long). Hyphal system dimitic; generative hyphae with clamps, thin-walled; skeletal hyphae thick-walled, $1.5-5 \mu m$ (wide), branched, somewhat interwoven. Cystidia clavate, heavily encrusted, $25-35 \times 3.5-5 \mu m$, thick-walled, no basal clamps. Basidia clavate, $15-20 \times 4.5-5 \mu m$, with basal clamps, usually with 2–4 sterigmata. Basidiospores subglobose, $5-(5.5)-6 \times 3-(3.5-) 4.5 \mu m$, hyaline, thin-walled, smooth, uninucleate.

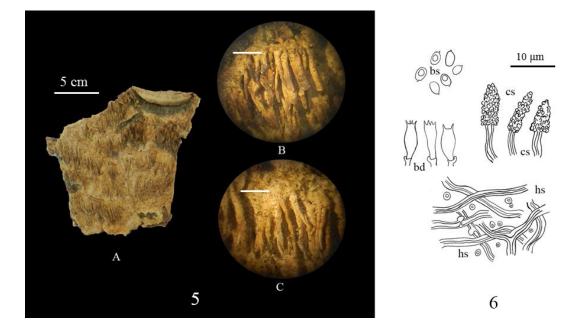


Figure 5–6. Macro and microstructures. A. Aspect of the annual basidiome, B–C. hymenophore odontioid with dense spines. bs. Basidiospores, bd. Basidia clavate, cs. Cystidia encrusted, hs. Hyphal system (generative hyphae and skeletal hyphae). Bar 10 μ m (6); 1mm (B, C); 5 cm (A).

Known distribution: Brazil, in the State of São Paulo (Bononi 1976; Bononi *et al.*, 1981).

Material examined: Brazil, Amazonas, Manaus, Urban area, on a live tree, 06 Mar 2015, MA Jesus (IH 9.987).

Remarks: The species is very similar to *S. ochraceum*, reported by (Bononi, 1976) by the color of the basidiomes and shape. However, *S. hydneum* has larger basidiospores, and its context is thin and homogeneous whereas the context of *S. ochraceum* is thick and duplex.

Stecchericium seriatum

Fig. 7-8

Description: Basidiome imbricate to effused-reflexed with flabelliform pileus, flat or convex $15-50 \times 10-30$ mm, ranging from light yellow to reddish orange when fresh, whitish to cream-colored when dry, usually in association with algae. Hymenial surface tuberculate with cylindrical spines 3 mm (long). Hyphal system monomitic; generative hyphae bearing clamp connections; skeletal hyphae thick-walled 1.5-2 µm. Cystidia abundant, cylindrical to fusiform, $30-40 \times 10$ µm, thick-walled, rarely with encrustation. Basidia clavate, hyaline $8-15 \times 3-5$ µm, with 2–4 stigmata, up to 1.5 µm. Basidiospores ellipsoid to globose, 2–6 (wide), non-cyanophilous, amyloid.

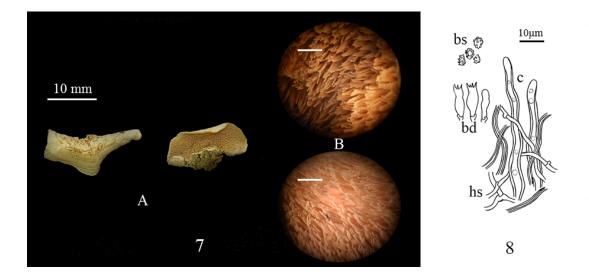


Figure 7–8. Macro and microstructures. A. Aspect of the dry effused-reflexed basidiome, B. hymenophore odontioid with dense spines. bs. Basidiospores, bd. Basidia clavate, cs. Cylindrical cystidia, hs. Hyphal system (generative hyphae and skeletal hyphae). Bar 10 μ m (8); 10 mm (A).

Known distribution: According to (Kirk *et al.*, 2008), the species is widespread in the tropics. It is reported in the State of Santa Catarina, Brazil by (Drechsler-Santos *et al.*, 2008) and for the Amazon region by (Bononi, 1981).

Material examined: Brazil, Amazonas, Manaus, Tropical Silviculture Experimental Station, on dead tree trunks, 23 Apr. and 23 Dec. 2014, MA Jesus (IH 8.548, 10.531).

Remarks: *S. seriatum* can be easily confused as *S. ochraceum* by macro-characters in the field. However, microscopically, *S. seriatum* is strongly amyloid, has finely verrucose basidiospores, and tubular, thick-walled gloeocystidia. In contrast, *S. ochraceum* has non-amyloid, smooth, ellipsoid basidiospores, and apically encrusted skeletocystidia.

Steccherinum ochraceum

Description: Basidiome ressupinate, odontioid, adnate to effused-reflexed, fibrillar velutina margin, ranging from light yellow to reddish orange when fresh, whitish to cream colored when dry; odontioid surface with abundant fimbriate, bleached teeth, about 1 mm (long). Hyphal system dimitic, generative hyphae 2.5–3 μ m (wide), hyaline, thin-walled, fibulate, septate, very branched; subiculum hyphae, about 3–4 μ m (wide). Thick walls are observed on some simple septa. Lamprocystidia cylindrical, 35– 50 × 6–7 μ m, obtuse apex, protruding from the hymenium, some without encrustation. Basidia tubular to suburniform, 18–20 × 4.5–5 μ m, hyaline, with 2–4 sterigmata, about 2 μ m (long). Basidiospore ellipsoid, 4–4.5 × 2–3 μ m, hyaline, thin walled, smooth, inamiloides.

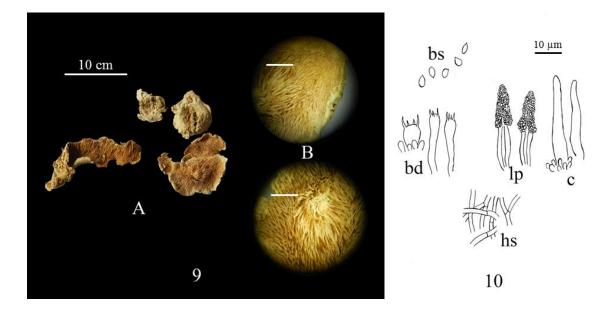


Figure 9-10. Macro and microstructures. A. Aspect of the dry effused-reflexed basidiome, B. Hymenophore odontioid with dense spines. bs. Basidiospores, bd. Basidia subuniform, c. Cystidia, hs. Hyphal system (generative hyphae and skeletal hyphae), lp. Lamprocystidia. Bar 10 μ m (10); 1mm (B); 10 cm (A).

Known distribution: Distributed worldwide (Jülich and Stalpers, 1980).

Material examined: Brazil, Amazonas, Manaus, Tropical Silviculture Experimental Station, on a fallen tree branch, 12 Apr. 2013, VIS Bastos (IH 10.513)

Remarks: *S. ochraceum* seems to be morphologically similar to *S. tenuispinum* Spirin, Zmitr. & Malysheva differing from it by being shorter up to 2 mm (long) and densely arranged (5–7 per mm) pale ochraceous spines.

Trechispora gillesii

Fig. 11–12

Description: Basidiomes piliate $1.5-2 \times 1$ cm, whitish to cream colored, with brown edges when fresh, dark orange to brownish when dried. Stipe ochraceous, up to 1.2-1.5 cm. Hymenophore tuberculate with cylindrical spines, 0.5-1 mm (long), near stipe, smaller and scattered toward margin, cream colored. Hyphal system monomitic; hyphae fibulate and inflated close to the septa. Cystidia absent. Basidia hyaline, clavate, $15-20 \times 5.5-6 \mu m$. Basidiospore hyaline, subglobose, $5-6.6 \times 4.5-6 \mu m$, verrucose, non-amyloid.

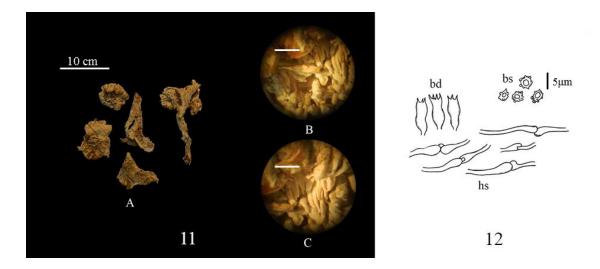


Figure 11–12. Macro and microstructures. A. Aspect of the dry piliate basidiome, B–C. hymenophore tuberculate with cylindrical spines. bs. Basidiospores, bd. Basidia clavate, hs. Hyphal system, hyphae with clamp connections. Bar 5 μ m (12); 1mm (B, C); 10 cm (A).

Known distribution: Little is known about *T. gillesii*, previously reported only in Central Africa. This is the first report of *T. gillesii* in the Brazilian Amazon.

Material examined: Brazil, Amazonas, São Sebastião do Uatumã, Uatumã Biological Reserve, on soil, 27 May 2009, MA Jesus (IH 5.534); Amazonas, Manaus, Adolpho Ducke Forest Reserve, on a live tree, 27 Jan. 2006, MA Jesus (IH 3.078).

Remarks: *T. gillesii* has a particular stipitate-pileate basidiome and has large basidiospores, being one of the few species of the genera with these characters (Julich, 1976).

Conclusion

Due to the economic and ecological importance of these macrofungi, the study contributed to the expansion of knowledge about hydnoid's fungal diversity in the Amazon region. However, a lot of them still remain unknown in the Amazon rain forest.

Acknowledgments

The authors gratefully acknowledge the Brazilian government for funding collections and research by National Council for Scientific and Technological Development (CNPq). The Brazilian Program for Biodiversity Research (PPBio/INPA/MCTI). The sustainable forest management project in the Brazilian Amazon, Amazon wood (INCT-MCTI) and the National Institute for Amazonian Research (INPA) by infrastructure.

References

Arnolds E (2003). De stekelzwammen en pruikzwammen van Nederland en België.. Coolia 46: 1–96.

Bononi VLR (1976). Basidiomicetos hidnoides da micota brasileira. (Tese), doutorado em Ciências na área de Botânica, Instituto de Biociências, Universidade de São Paulo, 314 p.

Bononi VLR (1981). Alguns Basidiomicetos hidnóides da região Amazônica. *Rickia*. 9: 17–30.

Chernykh A, Myasoedova N, Kolomytseva M, Ferraroni M, Briganti F, Scozzafava A, Golovleva L (2008). Laccase isoforms with unusual properties from the basidiomycete *Steccherinum ochraceum* strain 1833. *J. Appl. Microbiol.*, 105: 2065–2075.

Chevallier FF (1826). Flore Générale des Environs de Paris. 1: 1-674.

Cifuentes J, Patiño-Conde V, Villegas M, García-Sandoval R, Valenzuela R (2005). First record of Hydnodon thelephorus from Belize, Dominican Republic, Mexico, with new data on its morphology and distribution. *Mycotaxon* 91: 27–34.

Coker WC, Beers AH (1951). The stipitate Hydnums of the eastern United States. University of North Carolina Press, Chapel Hill, NC. 211 p.

Dai YC (2011). A revised checklist of corticioid and hydnoid fungi in China for 2010. *Mycoscience* 52: 69–79.

Drechsler-Santos ER, Groposo C, Loguercio-Leite C (2008). Additions to the knowledge of lignocellulolytic Basidiomycetes (Fungi) in forests from Santa Catarina State, Brazil. *Mycotaxon* 103: 197–200.

Jardim FCS, Hosokawa RT (1986). Estrutura da floresta equatorial Úmida da Estação Experimental de Silvicultura Tropical do INPA. *Acta Amazonica*, 16/17 (Único): 411–508.

Julich W (1976). Studies in resupinate Basidiomycetes – IV. In Persoonia 8: 431-442.

Jülich W, Stalpers A (1980). The resupinate non–poroid Aphyllophorales of the temperate northern hemisphere. Verhande–lingen der Koninklijke Nederlandse Akademie van Wetenschappen 74: 1–335.

Kirk PM, Canhão PF, Minter DW, Stalpers JA (2008). Dicionário da Fungi (10th ed.). Wallingford, UK: CAB International. 340 p.

Lowy B (1971). Tremellales. Flora Neotropica 6: 153 p.

Martin GW (1934). Three new Heterobasidiomycetes. Mycologia 26: 261–265.

Mendonça BAF (2011). Campinaranas amazônicas. Pedogênese e relações solovegetação. 110p. (Dissertação) Mestrado em solos e nutrição de Plantas. Universidade Federal de Viçosa, Viçosa

Oliveira ML, Baccaro FB, Braga-Neto R, Magnusson WE (2008). Reserva Ducke: A biodiversidade amazônica através de uma grade. Manaus : Áttema Design Editorial, 168 p.

Roberts P, Evans S (2011). The Book of Fungi. University of Chicago Press, Chicago, Illinois, 471 p.

Rossetti DF, Zani H, Cohen MLC, Cremon EH (2012). A Late Pleistocene-Holocene wetland megefan in the Brazilian Amazonia. Journal of Sedimentary Geology 282: 276–293.

Sobestiansky G (2005). Contribution to a macromycete survey of the states of Rio Grande do Sul and Santa Catarina in Brazil. Brazilian Archieves of Biology and Technology 48 (3): 437–457.

33

Vesterholt J, Asman WAH, Christensen M (2000). Nitrogen deposition and decline of fungi on poor and sandy soils. Svampe 42: 53–60.

Whittaker RH (1969). New concepts of Kingdoms of organisms. Sci ence 163: 150–160.

Zuquim G, Costa FRC, Prado J, Tuomisto H (2008). Guia de identificação das samambaias e licófitas da REBIO Uatumã, Amazônia Central. Manaus: Áttema Design Editorial, 321 p.