



Taxonomy and phylogeny of *Pluteus glaucotinctus* sensu lato (Agaricales, Basidiomycota), a multicontinental species complex

NELSON MENOLLI JR.^{1,2*}, ALFREDO JUSTO³, PEDRO ARRILLAGA⁴, C. K. PRADEEP⁵, ANDREW M. MINNIS⁶ & MARINA CAPELARI²

¹Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Campus São Paulo, CCT / Biologia, Rua Pedro Vicente 625, 01109-010 São Paulo, SP, Brazil

²Núcleo de Pesquisa em Micologia, Instituto de Botânica, Caixa Postal 68041, 04045-972 São Paulo, SP, Brazil

³Biology Department, Clark University, 950 Main St., Worcester, MA 01610, USA

⁴Sociedad de Ciencias Aranzadi, Departamento de Micología, Alto de Zorroaga 11, San Sebastián 20014, Spain

⁵Jawaharlal Nehru Tropical Botanic Garden & Research Institute, Palode, Trivandrum, Kerala 695562, India

⁶Center for Forest Mycology Research, USDA-US Forest Service, One Gifford Pinchot Dr., Madison, WI 53726, USA

*Corresponding author: menolljr@yahoo.com.br

Abstract

In order to better understand species delimitation in the *Pluteus glaucotinctus* species complex, we present a detailed study based on morphological and DNA sequence (nrITS + *tefl*) data. *Pluteus glaucotinctus* sensu stricto is known only from the type collection (Democratic Republic of the Congo), which is re-examined here. Four other species are recognized in this complex and described herein as new: *P. izurun* from Spain, *P. meridionalis* from Brazil, *P. padanilus* from India and *P. thoenii* from Africa. Phylogenetic analyses based on nrITS + *tefl* sequence data support the recognition of all species in this complex.

Key words: nrITS, Pluteaceae, section *Pluteus*, species delimitation, *tefl*

Introduction

The genus *Pluteus* Fr. (Pluteaceae, Agaricales) comprises ca. 300 species with a circumglobal distribution (Kirk *et al.* 2008) and is very common in forested areas from boreal to tropical regions. The genus is found primarily in association with well-rotted wood and it is characterized by free lamellae, absence of a volva, pinkish spore print, inamyloid basidiospores and inverse hymenophoral trama (Singer 1986). Recent molecular and morphological studies (Minnis *et al.* 2006; Corriol & Moreau 2007; Menolli *et al.* 2010; Vizzini & Ercole 2011; Justo *et al.* 2011a, b) found *Pluteus* to include some annulate species previously placed in the genus *Chaemaeota* (W.G. Sm.) Earle and also have typically supported the traditional infrageneric subdivision into three sections: *Pluteus*, *Celluloderma* Fayod and *Hispidoderma* Fayod.

According to the infrageneric classification of *Pluteus* proposed by Singer (1959, 1986) and as clarified by DNA sequence data (Justo *et al.* 2011a, b), *Pluteus* sect. *Pluteus* includes species with a pileipellis as a cutis, with metuloid pleurocystidia, as well as species with indistinct metuloids [*Pluteus albostipitatus* (Dennis) Singer] or with non-metuloid pleurocystidia (*Pluteus glaucotinctus*).

Justo *et al.* (2011b) recognized sixteen well-supported clades in *Pluteus* sect. *Pluteus*, most of which are represented by species complexes with apparent intercontinental distributions. Many of these have been investigated (Justo *et al.* 2014) and others require further molecular and morphological studies to better understand species delimitation, diversity, and biogeography in the genus. In the present article we critically examine the widespread species complex around *P. glaucotinctus* using morphological approaches and molecular phylogenies based on two loci, the nuclear ribosomal internal transcribed spacers region (nrITS) and translation-elongation factor 1-alpha (*tefl* a single-copy, protein-coding gene).

Basidiospores [30/1/1] 6.5–8.5(–9.0) × 5.5–7.5 µm (Q = 1.10–1.17; Qm = 1.15; Lm = 7.7 µm; Wm = 6.7 µm), subglobose to broadly ellipsoid, inamyloid, hyaline, smooth, thick-walled. *Pleurocystidia* 55–85 × 18.0–32 µm; ovoid, clavate or oblong, with rounded apices, colorless, thin-walled. *Cheilocystidia* 53–94 × 12.0–15.0 µm; narrowly utriform, cylindrical, lageniform, flexuous, colorless, thin-walled. *Pileipellis* a cutis; terminal elements 70–105 × 10.0–20 µm; cylindrical or tapering towards apex; colorless or with brown pigments; thin-walled. *Stiptipellis* a cutis; hyphae 5.0–15.0 µm wide; colorless; thin-walled. *Clamp-connections* not observed.

Etymology:—*thoenii* in honor of the collector of the holotype, Daniel Thoen, and for his contribution to the knowledge of fungi from tropical Africa.

Distribution:—Africa: Known only from Democratic Republic of the Congo.

Specimen examined:—DEMOCRATIC REPUBLIC OF THE CONGO. Haut-Katanga: Kipopo, 19 December 1972, *Thoen 5546* (BR!; nrITS: HM562132; *tef1*: KJ010051).

Notes:—Despite the lack of macro-morphological data from fresh collections of *P. thoenii*, we consider the DNA sequences, the shape of the basidiospores (Qm = 1.15) and the size of the cheilocystidia (up to 94 µm long) as sufficiently distinctive characters to propose this new species. *Pluteus thoenii* has been up until now only represented by the holotype, which represents a single herbarium collection from more than 50 years ago that was previously identified by Horak & Heinemann (1978) as *P. glaucotinctus*.

Acknowledgments

The authors thank the curator of BR for managing the loans of the specimens from Africa and Luiz Antonio S. Ramos for assistance with formatting the plates. N. Menolli Jr. and M. Capelari thank Fernanda Karstedt and L.A.S. Ramos for collecting some specimens of *P. meridionalis*; Francisco Kuhar for the suggestion of the name *P. meridionalis*, the “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP grants 04/04319-2, 09/53272-2) and the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq fellowships) for financial support. A. Justo acknowledges support from the National Science Foundation grant DEB 0933081. A.M. Minnis expresses gratitude for support from Daniel L. Lindner and U.S. Forest Service funds.

References

- Bas, C. (1969) Morphology and subdivision of *Amanita* and a monograph on its section *Lepidella*. *Persoonia* 5: 285–579.
- Corriol, G. & Moreau, P.A. (2007) *Agaricus (Annularia) fenzlii* redécouvert dans les Pyrénées. Notes sur le genre *Chamaeota* en Europe. *Persoonia* 19: 233–250.
- Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113–118.
<http://dx.doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Homola, R.L. (1972) Section *Celluloderma* of the genus *Pluteus* in North America. *Mycologia* 64: 1211–1247.
<http://dx.doi.org/10.2307/3757960>
- Horak, E. (1977) Neue zairische Arten aus der Gattung *Pluteus* Fr. *Bulletin du Jardin Botanique National de Belgique* 47: 87–89.
<http://dx.doi.org/10.2307/3667984>
- Horak, E. & Heinemann, P. (1978) *Flore illustrée des champignons d’Afrique centrale 6: Pluteus & Volvariella (compléments)*. National Botanical Garden of Belgium, Meise.
- Justo, A., Vizzini, A., Minnis, A.M., Menolli Jr, N., Capelari, M., Rodríguez, O., Malysheva, E., Contu, M., Ghignone, S. & Hibbett, D. S. (2011a) Phylogeny of *Pluteaceae* (*Agaricales*, *Basidiomycota*): taxonomy and character evolution. *Fungal Biology* 115: 1–20.
<http://dx.doi.org/10.1016/j.funbio.2010.09.012>
- Justo, A., Minnis, A.M., Ghignone, S., Menolli Jr, N., Capelari, M., Rodríguez, O., Malysheva, E., Contu, M. & Vizzini, A. (2011b) Species recognition in *Pluteus* and *Volvopluteus* (*Pluteaceae*, *Agaricales*): morphology, geography and phylogeny. *Mycological Progress* 10: 453–479.
<http://dx.doi.org/10.1007/s11557-010-0716-z>
- Justo, A., Caballero, A., Muñoz, G., Minnis, A.M. & Malysheva, E. (2011c) Taxonomy of *Pluteus eugraptus* and morphologically similar taxa. *Mycologia* 103: 646–655.
<http://dx.doi.org/10.3852/10-280>
- Justo, A., Malysheva, E., Bulyonkova, T., Vellinga, E.C., Cobian, G., Nguyen, N., Minnis, A.M. & Hibbett, D.S. (2014) Molecular

- phylogeny and phylogeography of Holarctic species of *Pluteus* section *Pluteus* (Agaricales: Pluteaceae), with description of twelve new species. *Phytotaxa* 180: 1–85.
<http://dx.doi.org/10.11646/phytotaxa.180.1.1>
- Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.
<http://dx.doi.org/10.1093/bib/bbn013>
- Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J. A. (Eds.) (2008) *Ainsworth & Bisby's dictionary of the fungi, 10th edn.* CAB International, Wallingford.
- Küppers, H. (1979) *Atlas de los colores.* Editorial Blume, Barcelona.
- Maddison, D.R. & Maddison, W.P. (2002) *MacClade4: analysis of phylogeny and character evolution.* Sinauer Associates, Sunderland.
- Martinez, D., Challacombe, J., Morgenstern, I., Hibbett, D., Schmoll, M., Kubicek, C. P., Ferreira, P., Ruiz-Duenas, F. J., Martinez, A.T., Kersten, P., Hammel, K.E., Vanden Wymelenberg, A., Gaskell, J., Lindquist, E., Sabat, G., Splinter BonDurant, S., Larrondo, L.F., Canessa, P., Vicuna, R., Yadav, J., Doddapaneni, H., Subramanian, V., Pisabarro, A.G., Lavín, J.L., Oguiza, J.A., Master, E., Henrissat, B., Coutinho, P.M., Harris, P., Magnuson, J.K., Baker, S.E., Bruno, K., Kenealy, W., Hoegger, P.J., Kues, U., Ramiaiy, P., Lucas, S., Salamov, A., Shapiro, H., Tu, H., Chee, C.L., Misra, M., Xie, G., Teter, S., Yaver, D., James, T., Mokrejs, M., Pospisek, M., Grigoriev, I.V., Brettin, T., Rokhsar, D., Berka, R. & Cullen, D. (2009) Genome, transcriptome, and secretome analysis of wood decay fungus *Postia placenta* supports unique mechanisms of lignocellulose conversion. *Proceedings of the National Academy of Sciences* 106: 1954–1959.
<http://dx.doi.org/10.1073/pnas.0809575106>
- Menolli Jr, N., Asai, T. & Capelari, M. (2010) Records and new species of *Pluteus* from Brazil based on morphological and molecular data. *Mycology* 1: 130–153.
<http://dx.doi.org/10.1080/21501203.2010.493531>
- Menolli Jr, N., de Meijer, A.A.R. & Capelari, M. (2014) The genus *Pluteus* (Pluteaceae, Agaricales) from the state of Paraná, Brazil. *Nova Hedwigia* (in press).
- Minnis, A.M., Sundberg, W.J., Methven, A.S., Sipes, S.D. & Nickrent, D.L. (2006) Annulate *Pluteus* species: a study of the genus *Chamaeota* in the United States. *Mycotaxon* 96: 31–39.
- Minnis, A.M. & Sundberg, W.J. (2010) *Pluteus* section *Celluloderma* in the U.S.A. *North American Fungi* 5: 1–107.
<http://dx.doi.org/10.2509/naf2010.005.001>
- Munsell Color (Ed.) (2009) *Munsell Soil-Color Charts.* X-Rite, Grand Rapids.
- Rehner, S.A. & Buckley, E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence from cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98, 2005.
<http://dx.doi.org/10.3852/mycologia.97.1.84>
- Rodríguez, O., & Guzmán-Dávalos, L. (2001) Clave dicotómica de las especies del género *Pluteus* Fr. (Pluteaceae) conocidas de La región de Nueva Galicia y algunas áreas aledañas, Mexico. *Acta Botanica Mexicana* 57: 23–36.
- Saupe, S.G. (1981) Occurrence of psilocibin/psilocin in *Pluteus salicinus* (Pluteaceae). *Mycologia* 73: 781–784.
<http://dx.doi.org/10.2307/3759505>
- Singer, R. (1959) Monographs of South American Basidiomycetes, especially those of the east slope of the Andes and Brazil. 1. The genus *Pluteus* in South America. *Lloydia* 21: 195–299.
- Singer, R. (1962) Monographs of South American Basidiomycetes, especially those of the east slope of the Andes and Brazil. 4. *Inocybe* in the Amazon region, with a Supplement to part 1 (*Pluteus* in South America). *Sydowia* 15: 112–132.
- Singer, R. (1969) Mycoflora Australis. *Beiheft zur Nova Hedwigia* 29: 1–405.
- Singer, R. (1986) *The Agaricales in modern taxonomy.* 4th edn. Koeltz Scientific Books, Koenigstein.
- Stijve, T., & Bonnard, J. (1986) Psilocybine et urée dans le genre *Pluteus*. *Mycologia Helvetica* 2: 123–130.
- Stijve, T., & de Meijer, A.A.R. (1993) Macromycetes from the state of Paraná, Brazil, 4. The psychoactive species. *Arquivos de Biologia e Tecnologia* 36: 313–329.
- Thiers, B. (2014) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/ih/> (accessed July 2014).
- Vizzini, A. & Ercole, E. (2011) A new annulate *Pluteus* variety from Italy. *Mycologia* 103: 904–911.
<http://dx.doi.org/10.3852/10-382>
- White, T.J., Bruns, T., Lee, S.S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In: Innis, M. A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) PCR Protocols: a guide to methods and applications.* Academic Press, New York, pp. 315–322.
<http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Yelle, D.J., Ralph, J., Lu, F., & Hammel, K. (2008) Evidence for cleavage of lignin by a brown rot basidiomycete. *Environmental Microbiology* 10: 1844–1849.
<http://dx.doi.org/10.1111/j.1462-2920.2008.01605.x>