Neofavolus subpurpurascens comb. nov., with new records from the Neotropics

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Abstract

*Polyporus subpurpurascens* is a rare Neotropical species characterized by deep purplish pilear surface and radially elongated pores. This study analyzed Neotropical specimens from Argentina, Bolivia and Brazil by applying detailed morphological examination and phylogenetic analyses. We conclude that *P. subpurpurascens* is a species of *Neofavolus*, and *N. subpurpurascens* is proposed as a new combination. New records from Argentina and Bolivia, a detailed description, and photographs of the species are included.

Introduction

*Favolus* E.M. Fries (1828: 44) and *Neofavolus* Sotome & T. Hatt. in Sotome et al. (2013: 249) are white rot polypore genera with similar annual flabelliform basidiomes that are laterally stipitate without black crust on the stipe surface. Microscopically, they are characterized by a dimitic hyphal system with skeletal-binding hyphae and hyaline thin-walled cylindrical basidiospores (Sotome et al. 2013). A glabrous pilear surface and a cutis pileipellis with agglutinated hyaline to brown generative hyphae differentiate *Neofavolus* from *Favolus*. In contrast, the pileipellis in *Favolus* is composed of non-agglutinated hyaline hyphae.

Originally, *Neofavolus* was exclusively described as having poroid basidiomes and being distributed in temperate areas. *Neofavolus mikawae* (Lloyd) Sotome & T. Hatt. in Sotome et al. (2013: 251) and *N. cremeoalbidus* Sotome & T. Hatt. in Sotome et al. (2013: 250) have angular pores and *N. alveolaris* (DC.) Sotome & T. Hatt. in Sotome et al. (2013: 250) have radially elongated pores. However, *N. suavissimus* (Fr.) J.S. Seelan, Justo & Hibbett in Seelan et al. (2015: 468), a subporoid lamellate species, was recently included in the genus *Neofavolus* (Seelan et al. 2015).

*Polyporus subpurpurascens* (Murrill) Ryvarden (1985: 181) is a rare species described from Jamaica (Murrill 1907), characterized by a deep purple pilear surface. *Polyporus* is a polypheletic genus (Sotome et al. 2008, Krüger et al. 2006, Sotome et al. 2011, Dai et al. 2014, Seelan et al. 2015) and the phylogenetic relationships of *P. subpurpurascens* remain unknown. We aim to perform accurate morphological and molecular analyses of specimens identified as *P. subpurpurascens* from Argentina, Bolivia and Brazil in order to access their phylogenetic position.

Material and Methods

Specimens

Specimens of several herbaria (ICN, LIL and CORD) collected from Northwest Argentina, Bolivia and South Brazil were included in this study. Freehand cross sections of dried materials mounted in Melzer’s reagent, 5% KOH and/or 1% phloxine, lactophenol, cresyl blue and/or cotton blue –(CB) were observed under the microscope.

DNA extraction, PCR amplification and sequencing

DNA was extracted from the dried specimens using CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) and FH plant DNA kit II (Demeter Biotech Co., Ltd., Beijing, China),
following the manufacturers protocols. Primer pairs ITS4/ITS5 (White et al. 1990) and LR0R/LR7 (Vilgalys & Hester 1990) were used to amplify the nrITS and nrLSU regions, respectively, by a qualitative simplex polymerase chain reaction. The polymerase chain reaction (PCR) protocol for ITS was the following: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 94 °C for 40 s, 54 °C for 45 s, and 72 °C for 1 min, and final extension of 72 °C for 10 min. The PCR procedure for 28S was the following: initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 1.5 min, and final extension of 72 °C for 10 min. The PCR products were purified and sequenced with the same primers at Beijing Genomics Institute in China.

Phylogenetic analyses

Sequences were manually edited using Geneious v.11.1.4 (http://www.geneious.com, Kearse et al. 2012). The generated sequences, including related sequences downloaded from GenBank (Table 1), were aligned using Mafft v.7 (Katoh & Standley 2013). Besides, the Q-INS-I strategy was adopted for nrITS and G-INS-i, for nrLSU. The alignments were manually examined and adjusted with MEGA 6 (Tamura et al. 2013). We used a combined dataset with nrITS and nrLSU. The dataset was divided into four data partitions: ITS1, 5.8S, ITS2 and LSU. The best-fit model of nucleotide evolution for each partition was selected according to the Akaike Information Criterion and using jModelTest2 v.1.6 (Darriba et al. 2012; Guindon & Gascuel, 2003) as available in CIPRES Science Gateway 3.1 (Miller et al. 2010). The final alignments were submitted to TreeBASE (submission ID: 22972). Both Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic analyses were carried out. BI was conducted using MrBayes 3.2.6 (as available in CIPRES Science Gateway) and implementing two independent runs, each with four chains and starting from random trees. The runs produced 20,000,000 generations and trees were sampled every 1000th generation. Twenty five percent of the sampled trees were discarded as burn-in, while the rest were used for calculating a 50% majority consensus tree and Bayesian Posterior Probabilities (BPP). ML trees were obtained using RAxML v.8.1.4 (Stamatakis 2014) from CIPRES Science Gateway. The analysis first involved 100 ML searches, each one starting from a randomized stepwise-addition parsimony tree in a GTRGAMMA model with no proportion of invariant sites and all the other parameters estimated by the software. We provided a partition file to force RAxML software to search for a separate evolution model in each dataset. Bootstrap support values (BS) were obtained with multi-parametric bootstrapping replicates under the same model, allowing the program halts bootstrapping automatically by the autoMRE option. A node was considered to be strongly supported if it showed a BPP 0.95 and/or BS 90%, while moderate support was considered when BPP < 0.95 and/or BS < 90%. Based on previous studies, Trametes conchifer (Schwein.) Pilát was used as outgroup (Zhou & Cui 2018).

**TABLE 1.** List of species, specimen-voucher information, geographic origin, and GenBank accession numbers of sequences used in the phylogenetic analyses in this study.

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**Results**

**Phylogenetic analyses**

The dataset contains 44 sequences and 2060 characters, including gaps. In total, 397 of them are parsimony informative; 1536, constant; and 474, variable. Additionally, TIM3+I+G, TPM2+I, HKY+G, and GTR+I+G were the evolutionary models selected for ITS1, 5.8S, ITS2 and LSU, respectively. The topology of the BI and ML analyses did not reveal any inconsistency in the supported clades, as is shown in the BI tree (Fig 1).

The phylogenetic analyses retrieved _Neofavolus_ and _Favolus_ as strongly-supported clades (BPP=1, BS =94, and BPP=0.99, BS=93, respectively). Furthermore, six lineages in the Neofavolus clade were also retrieved. Four of those lineages contained specimens of _N. alveolaris_ (BPP=0.99, BS=98), _N. cremealbidus_ (BPP=1, BS =100), _N. mikawai_ (BPP=1, BS = 100), and _N. suavissimus_ (BPP=1, BS =100). Besides, there was a lineage composed of American specimens of a not-yet-described species of _Neofavolus_ (BPP=1, BS =96) that has already been detected (Seelan _et al._ 2015). Finally, a strongly-supported lineage (BPP=1, BS=100) composed of the six specimens collected in Argentina and Brazil was identified as _P. subpurpurascens_.

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**Note:**

- **Species**
- **Voucher**
- **Origin**
- **Genbank accession numbers**
  - nrITS
  - nrLSU
- **References**
Both phylogenetic and morphological analyses revealed that the specimens previously identified as *P. subpurpurascens* belong to the *Neofavolus* clade. As a result, we propose the following new combination:

**Neofavolus subpurpurascens** (Murril) Palacio & Robledo *comb. nov.*

MycoBank MB 826917


**Description:**—Basidiome annual, fragile, centrally to laterally stipitate, solitary or clustered in small groups (2–3 basidiomes); pileus circular to reniform, centrally depressed, up to 2 cm diam., 0.1 cm thick, glabrous, azonate, light purplish, slightly tessullate when living, purplish ochraceous and tessellate when dry; stipe up to 2 cm long., 0.3 diam., light yellowish brown to purplish, with reddish dyes, more purplish and thicker towards the base, with a whitish attachment disc, smooth to longitudinally rugose when dry; margin acute, incurvated when dry; context thin (0.2 mm thick) to absent. Pore surface light brown yellowish, pores 1–2/mm, angular, hexagonal, radially elongated, decurrent, with hyphal pegs. Hyphal system dimitic: generative hyphae clamped, hyaline, thin walled, 3–6 µm diam., skeletobinding hyphae arboriform, hyaline to yellowish, thick walled, with a wide lumen 3–6 µm diam. (in the principal stalk) or 2–3 µm diam. (in the branches), IKI–. Hyphal pegs 23–26 µm diam., composed of generative hyphae. Pileipellis present as a cutis composed of light brown, parallel, agglutinated, thick-walled generative hyphae, distinct from the contextual hyphae, which are hyaline, interwoven and non-agglutinated (Fig. 2). Basidies 18–22 × 6–8 µm, 4-sterigmate, clavate. Basidiospores 9–12 × 2.5–4 µm, Q = 3.2, n = 100/5, narrowly cylindrical, with a slightly suprapapillar depression, smooth, thin walled, hyaline, guttulate, IKI–.
Geographical distribution:—*Neofavolus subpurpurascens* is a rare species, described from Jamaica and also recorded in Brazil (Coelho & Silveira 2014). This is the first record from Argentina and Bolivia.

Remarks:—*Neofavolus subpurpurascens* is a species recognized by its purplish basidiomes, compared to other *Neofavolus* taxa, which are white to cream or brownish (Sotome et al. 2013; Seelan et al. 2015). The specimens under study match the macro and microscopical descriptions based on the type collection provided by Ryvarden (1985) and (Coelho & Silveira 2014). Also we examined type material through NY virtual herbarium. Additionally, we found that the pilear surface is a cutis composed of light brown, parallel, agglutinated, thick-walled generative hyphae (Fig. 2c). Specimens:—ARGENTINA. Jujuy: Ledesma, Parque Nacional Calilegua, Mesada de las Colmenas, La Cascada trail, on dead twig, 1170 m, 23°42’1.5”S, 64°51’56.8”W, 6 March 2005, Robledo 383 (CORD); Robledo 385 (CORD); Robledo 390 (CORD);—BOLIVIA. La Paz: Nor Yungas, Rio Yariza, 23 February 1956, Singer B1346 (LIL);—BRAZIL. Rio Grande do Sul: Santa Maria, Seminários São José, 23 March 2007, Coelho & Cortez 624–1 (ICN); Coelho & Cortez 624–2 (ICN).

**FIGURE 2.** Morphological characteristics of *N. subpurpurascens*. a. pore surface detail. (Robledo383). b. pilear surface detail (Robledo390). c. pileipellis detail (CG624–1), cutis composed of light brown, parallel, agglutinated, thick-walled generative hyphae. d basidiospores (CG624–1). Scale bar: a,b=1cm; c,d=10 µm.

Discussion

*Neofavolus subpurpurascens* is the only neotropical species of *Neofavolus* and a rare species that has been collected few times in South America. Other *Neofavolus* species have been found in temperate regions (Sotome et al. 2013) but not in the tropics. Specifically, *Neofavolus alveolaris* and *N. suavissimus* are known in the Northern Hemisphere (North America, Europe and Asia), *N. mikawae* was recorded in China and Japan, and *N. cremeoalbidus* is restricted to temperate areas in Japan (Sotome et al. 2013).
Previously, *N. subpurpurascens* was mentioned as a member of the morphological group of Favolus (Ryvarden & Iturriaga 2003, Drechsler-Santos *et al.* 2008) and also suggested as a species of *Favolus* (Sotome *et al.* 2013, Coelho & Silveira 2014). However, none of those previous studies considered the pilear surface or the phylogenetic position of these species. In this study, we discovered that the pilear surface of *N. subpurpurascens* is a cutis composed of hyphae distinct from the contextual hyphae, as in other *Neofavolus* *spp.* (Sotome *et al.* 2013).

Therefore, the pilear surface is a useful and consistent feature to recognize *Neofavolus* species. Furthermore, the morphology of this surface has been highlighted as a strong feature in the classification of some polypore groups (Costa-Rezende 2016, Torres-Torres & Guzmán-Dávalos 2012). However, this surface in *Polyporus s.l* is scarcely described. *Neofavolus* is the single genus in the *Polyporus s.l*. genera whose pilear surface has been accurately examined and compared. Considering the complexity of *Polyporus s.l*. species, pilear surface morphology is a recommended feature to validate and compare genera and species.

Since *Polyporus s.l.* is a polyphyletic and morphologically heterogeneous group, accurate morphological examination (e.g. Sotome *et al.* 2013, Palacio *et al.* 2017) may support the identification of morphological patterns.

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References


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https://doi.org/10.11646/phytotaxa.244.2.1


https://doi.org/10.1007/s13225-013-0248-3


https://doi.org/10.1038/nmeth.2109


https://doi.org/10.1080/10635150390235520


https://doi.org/10.1270/tax.606003


https://doi.org/10.1093/molbev/mst010


https://doi.org/10.1093/bioinformatics/bts199