

Short title: *Neopaxillus dominicanus*

A new *Neopaxillus* species (Agaricomycetes) from the Dominican Republic and the status of *Neopaxillus* within the Agaricales

Alfredo Vizzini<sup>1</sup>

*Dipartimento di Biologia Vegetale, Università di Torino, Viale Mattioli 25, 10125 Torino, Italy*

Claudio Angelini

*Via del Tulipifero 9, 33080 Porcia (PN), Italy*

Enrico Ercole

*Dipartimento di Biologia Vegetale, Università di Torino, Viale Mattioli 25, 10125 Torino, Italy*

**Abstract:** The new species *Neopaxillus dominicanus* is described on the basis of collections from the Dominican Republic. It is distinguished by having a basidiome with decurrent, distant, white lamellae with evident pink-lilac tinges, the non-depressed pileus at maturity and well developed catenulate cheilocystidia. A description, color photographs of fresh basidiomes and line drawings of relevant microscopic traits are provided. *N. dominicanus* is morphologically similar to *Neopaxillus echinospermus*, the type species of the genus. Based on comparative ITS-LSU rDNA gene sequence analyses, *Neopaxillus*, formerly placed in the Boletales, is considered within the Agaricales where it is sister to *Crepidotus* (Crepidotaceae), and *N. dominicanus* is supported as distinct from *N. echinospermus*. Finally, according to our morphological and molecular analyses, two collections of *N. echinospermus* from Mexico are referable to *N. dominicanus*.

**Key words:** Central America, Crepidotoid clade, Mexico, Paxillaceae, Serpulaceae, taxonomy

INTRODUCTION

Singer (1948) described the genus *Neopaxillus* Singer to accommodate a single South American species, *N. echinosporus* Singer, characterized by a *Phylloporus*-like habit, distant and strongly decurrent lamellae, slightly bilateral hymenophoral trama, frequent clamp connections, and globose, echinulate brown spores. Singer (1951), after recognizing *Naucoria echinosperma* Speg. from Brazil (Spegazzini 1889) as a priority synonym of *Neopaxillus echinosporus*, proposed the new combination *Neopaxillus echinospermus* (Speg.) Singer. Three other species of *Neopaxillus* were added: one from Patagonia by Horak (1980) (*N. bryogenus* E. Horak), another from Sri Lanka by Pegler (1986) (*N. reticulatus* [Petch] Pegler) and one more from Puerto Rico by Singer and Lodge (1988) (*N. plumbeus* Singer & Lodge). *Neopaxillus bryogenus* later was transferred to *Galerina* Earle by Horak (1988). Due to its habit, slightly bilateral trama and spore print color, the genus was considered within Paxillaceae Lotsy of the Boletales E.-J. Gilbert (Machol and Singer 1971, Singer 1986, Singer and Lodge 1988). Binder and Hibbett (2006) tentatively placed *Neopaxillus* in the Serpulaceae Jarosch & Bresinsky, without providing subordinal placement.

During surveys of macrofungi in the Dominican Republic (Greater Antilles) collections of a strange paxilloid fungus were recorded and subsequently identified as a novel *Neopaxillus*. The aim of this paper is to fully describe and illustrate the new taxon as well as investigate the phylogenetic position of *Neopaxillus* within the Agaricomycetes.

#### MATERIALS AND METHODS

*Morphology.*—The macromorphological descriptions follow the detailed field notes taken for each collection on fresh material by the second author. The micromorphological descriptions are based on study of herbarium material rehydrated in 5% KOH and stained in Congo red, cresyl blue and Melzer's reagent. Spore size is expressed both as a range and a mean value based on 90 randomly chosen spores from three specimens of three collections stained in Melzer's reagent (spinulae not included). The width of basidia was measured at the widest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. Line drawings were done with the aid of a drawing tube.

These abbreviations are used: Q = the quotient of length and width of the spores in side view; Qm = average quotient; L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae. Color comparisons were made with the Methuen Handbook of Color (Kornerup and Wanscher 1978). Author citations follow the Index Fungorum Authors of Fungal Names (<http://www.indexfungorum.org/authorsoffungalnames.htm>). Herbarium abbreviations are according to Thiers (2011). All the material examined is housed at MCVE (Herbarium del Museo Civico di Storia Naturale, Venezia, Italy). The Latin description of the new species and the new combinations are deposited in MycoBank (<http://www.mycobank.org>).

*DNA extraction, PCR amplification and DNA sequencing.*—Genomic DNA was isolated from 1 mg dried herbarium specimens (TABLE I) using, in parallel, both the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions and a modified 2% CTAB method (Savolainen et al. 1995). Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990, Gardes and Bruns 1993) and primers LR0R/LR6 (Vilgalys and Hester 1990, Vilgalys lab, unpubl <http://www.botany.duke.edu/fungi/mycolab>) for the LSU rDNA amplification. Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in 25 µL reaction mixtures using these final concentrations or total amounts: 5 ng DNA, 1× PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM each primer, 2.5 mM MgCl<sub>2</sub>, 0.25 mM each dNTP, 0.5 unit Taq polymerase (Promega, Milan, Italy). The PCR program was 3 min at 95 C for 1 cycle, 30 s at 94 C, 45 s at 50 C, 2 min at 72 C for 35 cycles and 10 min at 72 C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and visualized by staining with ethidium bromide. The PCR products were purified with the AMPure XP kit (Beckman, Milan, Italy) and sequenced by DiNAMYCODE s.r.l. (Turin, Italy) and Macrogen Inc. (Seoul, Republic of Korea). Sequences were assembled and edited with the phred/phrap/consed software suite and were submitted to GenBank (accession numbers are reported in TABLE I and FIGS. 3, 4), and the alignments and phylogenetic trees are available at TreeBASE ([www.treebase.org](http://www.treebase.org)) under accession number S11583.

*Sequence alignment and phylogenetic analysis.*—Sequences were checked and assembled with Geneious 5.1.6 (Drummond et al. 2009) and compared to those available in the GenBank database (<http://www.ncbi.nlm.nih.gov/Genbank/>) using the BLASTN algorithm. Based on BLASTN results, sequences were selected according to the outcomes of other phylogenetic studies on Agaricales (Aime 1999, 2001; Matheny et al. 2006, 2007; Aime et al. 2005; Petersen et al. 2010; Alvarado et al. 2010).

Two phylogenetic analyses were performed: the first, based on LSU sequences, to focus on the position of *Neopaxillus* species in the Crepidotoid clade (the clade contains families Crepidotaceae [S. Imai] Singer and Inocybaceae Jülich; Matheny 2009, Alvarado et al. 2010); the second to investigate the relationships among *Neopaxillus* species with a combined ITS and LSU sequences dataset. Alignments were generated for each ITS and LSU dataset using MAFFT (Kato et al. 2002) with default conditions for gap openings and gap extension penalties. The two alignments were imported into MEGA 5 (Tamura et al. 2011) for manual adjustment. The best-fit substitution model for each single alignment was estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 0.1.1 (Posada 2008). GTR+G and GTR+I+G models were chosen respectively for the ITS and LSU alignments. *Tubaria dispersa* (Berk. & Broome) Singer (EF051054) was chosen as outgroup taxon for the LSU alignment (members of the *Tubariaceae* Vizzini were used as outgroup per Matheny 2005 and Alvarado et al. 2010), while *Inocybe geophylla* (Sowerby) P. Kumm. (HQ604291) was chosen for the combined ITS and LSU dataset. Phylogenetic hypotheses were constructed under Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) criteria.

Bayesian inference of phylogeny using Markov chain Monte Carlo (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). For each analysis four incrementally heated simultaneous MCMC chains were run 10 000 000 generations under model assumption. Trees were sampled every 1000 generations resulting in an overall sampling of 10 001 trees. The burn-in value was evaluated with Tracer 1.4 (Rambaut and Drummond 2007). The first 15% trees were discarded as burn-in. For the remaining trees a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. Only BPP values greater than 0.50 are reported in the resulting trees. This Bayesian analysis was repeated three times, always using random starting trees and random starting values for model parameters to test the independence of the results from revisiting of prior topologies during chain growth (Huelsenbeck et al. 2002).

The maximum likelihood estimation was performed with RAxML 7.0.4 (Stamatakis 2006) with 1000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMAI and GTRGAMMA (LSU and ITS analyses respectively) algorithm to perform tree inference and topology reconstruction. Support values (MLB) from both bootstrapping runs were mapped on the globally best tree with the `-f a` option of RAxML and `-x 12345` as a random seed to invoke the novel rapid bootstrapping algorithm. Only MLB values greater than 50% are reported in the resulting trees.

Finally, parsimony reconstruction was performed with PAUP 4.0b10 (Swofford 2002). MP analysis was conducted with the heuristic search mode with 1000 random addition sequence replicates and tree bisection reconnection (TBR) branch swapping but keeping only 10 trees per replicate to discover possible islands of maximum parsimony. All character states were treated as unordered and equally weighted. Gaps were treated as missing data. Branch robustness was estimated by nonparametric bootstrapping (Felsenstein 1985) with 1000 replicates with 10 random addition replicates per bootstrap. Only bootstrap values (MPB) exceeding 50% are visualized in the resulting trees. Support values for major clades that are supported by either BI, ML and MP analyses are visualized in the resulting tree. Analysis of the pairwise percent identity values for the *Neopaxillus* sequences (see RESULTS) were calculated with MEGA 5 (Tamura et al. 2011).

## RESULTS

### TAXONOMY

*Neopaxillus dominicanus* Angelini et Vizzini, sp. nov.

FIGS. 1, 2

Mycobank MB561643

A N. echinospermo differt lamellis in juventute albis deinde saepe lilaceis, pileo haud umbilicato, cheilocystidia vere manifesta, spinulis sporalis conico-cylindraceis atque structura molecularis in spatiis internis transcriptis ITS.

*Typus:* Dominican Republic, Puerto Plata, Sosua, ad locum dicto "El Castillo", 10/1/2010, leg. C. Angelini (MCVE n. 25727, holotypus).

*Etymology:* The specific epithet refers to the country, Dominican Republic, where the type collection was made.

Habit chroogomphoid to paxillo-phylloporoid (FIG. 1). Pileus 50–80(–100) mm diam; at first convex, expanded to plano-convex to applanate, rarely with a slightly depressed center; margin for a long time involute, inflexed, then plane, crenulate, undulate, ribbed; surface mat dry, minutely velutinous-tomentose, somewhat reticulately wrinkled, coarsely venose at center in young pilei (FIGS. 1b, 2a, as in *Pluteus thomsonii* [Berk. & Broome] Dennis); at first ochraceous brown (5C7-8), then ochraceous yellow, fading to lemon yellow (4A8, B7-8) when fully expanded; white at the margin. Lamellae distant (L = 12–20; l = [1–]3–4[–5]), thick, strongly decurrent, extending halfway down stipe, up to 7 mm broad, often

crisped and connected by veins, white, gray-white (1A1,B1) (FIG. 1a, b), then whitish-lilac (7B3-4, C3-4) (FIG. 1c), belatedly violaceous brown due to spore maturation; edges concolorous or slightly paler, sinuous, sterile. Stipe 30–50 × 3–8 mm; equal, solid, terete, central to slightly eccentric, straight to somewhat curved, flexuous, white, basal mycelium white. Context white, unchanging, firm and fibrous, odorless. Spore-print dark brown with violet shades (7B3-4, C3-4, D4-5). Basidia bi- and tetrasporic, clavate, 30–32 × 7–10 μm (FIG. 2d); sterigmata 4.5–5.5 μm long. Cheilocystidia septate, catenulate, lageniform, fusiform or cylindrical, with 30–50 × 10–15 μm terminal elements (FIG. 2e). Pleurocystidia absent. Spores (n = 90) globose, (6.2–)6.5–7.6(–8.0) μm, on average 7.1 μm, Q = Qm = 1.0, nonamyloid, uniguttulate, aculeate-spinulose; echinules conico-cylindrical, 0.5–0.85(1.0) μm high, isolate or sometimes interconnected by a network of thin ridges (FIG. 2f). Pileipellis a trichodermial palisade made up of erect, cylindrical, yellow-brown, encrusted hyphae with usually clavate terminal element, 20–40 × 8–12 μm (FIG. 2c). Hymenophoral trama slightly bilateral, with hyphae 2–8 μm wide. Thromboplerous hyphae (= oleiferous hyphae sensu Cléménçon 2004) present (FIG. 2g). Clamp connections abundant.

*Habitat and distribution.* Terricolous, gregarious to subcaespitose in small clusters, on bare soil, under broadleaf trees (Fabaceae Lindl., Fagaceae Dumort., Rubiaceae Juss., Rutaceae Juss.). October-February. Known only from the Dominican Republic and Mexico.

*Specimens examined.* DOMINICAN REPUBLIC, Puerto Plata, Sosua, El Castillo, 100 m, 2 km from the sea, bare soil under broadleaf trees, 1 Jan 2004, leg. C. Angelini (Angelini pers herb); ibidem, 22 Dec 2007, leg. C. Angelini (Angelini pers herb); ibidem, 3 Feb 2009, leg. C. Angelini (Angelini pers herb); ibidem, 10 Jan 2010, leg. C. Angelini (HOLOTYPE MCVE n. 25727; ISOTYPE TO AVAC11); ibidem, 12 Jan 2011, leg. C. Angelini (MCVE 26928; duplo in TO AVAC12). MEXICO, Nuevo Leon, Mpio. De Santiago, El Cercado, 520 m, mixed forest with *Zanthoxylum* L., *Acacia* Mill. and *Randia* L., 8 Oct 1983, leg. Jesús García J. (F 1059091-Jesús García J. 3167, as *N. echinospermus*); ibidem, 450 m, mixed mesophytic forest, under *Quercus virginiana* P. Mill., 22 Oct 1988, leg. Gregory M. Mueller (F 1133966-Gregory M. Mueller 3831, as *N. echinospermus*).

*Additional specimens examined. Neopaxillus echinospermus*: BRAZIL, Paraná State, Sao José dos Pinhais, Roça Velha, 900 m, mixed ombrophilous forest, on ground, under native dicotyledonous trees, 23 Jan 2001, leg. A.A.R. de Meijer (MA-Fungi 49404- MPM 2886). *Neopaxillus plumbeus*: USA- PUERTO RICO, El Verde, Luquillo Mountains, Luquillo National Park, on clay soil in wet tropical montane forest, 5 Sep 1985, leg. Lodge & Prieto (HOLOTYPE F 1068564 - Lodge No. PR 38). *Neopaxillus reticulatus*: SRI LANKA, Nuwara Eliya Distr., Hakgala, on dead wood, May 1912, leg. T. Petch (HOLOTYPE K(M) 168990 - T. Petch 3536).

*DNA sequencing and phylogenetic analyses.*—Amplification and sequencing of the ITS and LSU rDNA regions were successful for all specimens selected for molecular study (TABLE I), with the exception of *N. reticulatus* (specimen too old and strongly infected by a hyphomycete). Comparing these sequences with those from GenBank revealed that *Neopaxillus* species do not belong to the Boletales, but they show affinities with species of the genus *Crepidotus* (Fr.) Staude of the Crepidotoid clade of the Agaricales as defined by Matheny et al. (2006), Matheny (2009) and Alvarado et al. (2010) (= Crepidotaceae s.l. according to Petersen et al. 2010). The results of our phylogenetic analyses are presented (FIGS. 3, 4).

The Crepidotoid LSU data matrix comprises a total of 49 sequences (including 43 from GenBank). This dataset includes 934 positions with 243 (26.01%) as variable sites and 170 (18.2%) parsimony informative. The BI, ML and MP trees are congruent, but only the Bayesian tree is shown (FIG. 3). All analyses show that *Neopaxillus* species are nested in the Crepidotoid clade (BPP 0.99, MLB 87%, MPB 79%) and sister to *Crepidotus* (BPP 1, MLB 81% and MPB 58%).

The combined ITS and LSU data matrix comprised 22 sequences (including 10 from GenBank). This dataset includes 1641 positions with 420 (25.6%) as variable sites and 197 (12.0%) parsimony informative. The topology and branches support values of all the analyses are consistent, but only the Bayesian tree is shown (FIG. 4). All *Neopaxillus* sequences are

closely related and form a well supported clade (BPP 1, MLB 100% and MPB 100%) sister to *Crepidotus* and *Simocybe* P. Karst.

The four *N. dominicanus* sequences (two from the Dominican Republic, two from Mexico) are almost identical (99.9% of pairwise percent identity for both ITS and LSU regions). These are sister to the *N. echinospermus* sequence with 93.3% and 98.5% of pairwise identity of ITS and LSU regions respectively. A third *Neopaxillus* species, basal to the others, is *N. plumbeus*. According to these ITS pairwise percent identity values and accepting an intraspecific variability lower than 3% (Nilsson et al. 2008), *Neopaxillus dominicanus*, *N. echinospermus* and *N. plumbeus* should be considered distinct species.

#### DISCUSSION

*Neopaxillus species delimitation.*—*Neopaxillus dominicanus* is easily recognized in the field by the combination of its whitish, strongly decurrent lamellae with pink-lilac tinges and the applanate, rarely depressed pileus. Microscopically the species is remarkable due to the combination of well developed catenulate cheilocystidia and strongly ornamented spores. These collections represent the first record of a *Neopaxillus* from the Dominican Republic. Furthermore the photographs provided here are the first so far published of a *Neopaxillus* species.

The closely allied taxon of our new species, *N. echinospermus*, originally was described from Brazil (Singer 1948, 1951); it differs in having a clearly depressed pileus with yellow-brown lamellae, inconspicuous noncatenulate cystidia, lower truncate spore spinules (Singer 1949, 1964; Singer et al. 1990, Watling and Meijer 1997) as well as different ITS and LSU sequences. Singer (1964, PLATE 1/FIGS. 1–4) and Watling and Meijer (1997) describe the lamellae of *N. echinospermus* as pale buff, mustard yellow, later brownish, and clay brown to dark brown when fully mature respectively, always without lilac tinges. The same authors describe the pileus of this species as soon irregularly applanate and centrally depressed to



deeply or shallowly infundibuliform. Regarding the presence/absence of cheilocystidia in *N. echinospermus*, in the protolog Singer (1948) as well as Watling and Meijer (1997) the species is reported as lacking cheilocystidia. Singer (1964) and Singer et al. (1990) found sterile elements not well differentiated from basidia in old basidiomes. Horak (1968), who examined the type collection, reported inconspicuous, noncatenulate cheilocystidia and pleurocystidia. Our observations on a Brazilian collection (MA-Fungi 49404, see *Additional specimens examined*) are in agreement with Horak's description.

The geographical distribution of *N. echinospermus* also is limited, so far confined to tropical and subtropical South America. In addition to Brazil it has been reported from Bolivia, Paraguay and Argentina (Singer and Digilio 1952, Singer 1964, Singer et al. 1990, Pegler 1997, Watling and Meijer 1997, Neves and Capelari 2007). Mexican collections determined as *N. echinospermus* show hymenial cystidia and lilac-violet tinges in the lamellae (Guzmán 1983, García and López 1993, Guzmán and Guzmán-Dávalos 1984, Singer et al. 1990). As stated by Singer et al. (1990, p 20) with regard to these collections: “If this is indeed a *Neopaxillus*, it is a third species, perhaps intermediate between *N. echinospermus* and *N. plumbeus*.” Based on our morphological and molecular analyses of two Mexican collections (F 1059091, F 1133966), these are clearly referable to *N. dominicanus* (FIGS. 3, 4).

*Neopaxillus plumbeus* Singer & Lodge from Puerto Rico is macro- and microscopically characterized by small basidiomes (pileus 4–9 mm broad), a gray to purple-blue pileus, gray pigmentation in lamellae, stipe and context, and versiform, nonseptate cheilocystidia (Singer and Lodge 1988).

*Neopaxillus reticulatus* (Petch) Pegler from Sri Lanka has a lignicolous habit (on dead logs), an eccentric stipe, an infundibuliform pileus, a fertile gill edge, a pileipellis made up of a cutis, and pale spores with smaller and shorter echinulae (Pegler 1986 and our observations on type collection). According to Pegler (1986), its spores look like those of *Crepidotus* spp.

This is the only *Neopaxillus* species described outside Meso- and South America. Due to its aberrant habit and spores and its non-neotropical distribution, the placement of this taxon in *Neopaxillus* is doubtful. According to our analysis of the type collection (K[M] 168990), it could be a *Crepidotus*.

*Phylogenetic position of Neopaxillus.*—Contrasting with the morphologically based position of *Neopaxillus* in the Boletales (Machol and Singer 1971, Singer 1986, Singer and Lodge 1988, Binder and Hibbett 2006), our phylogenetic analyses (FIGS. 3, 4) clearly indicate an affiliation of *Neopaxillus* with the Agaricales within the Crepidotoid clade, as delimited by Matheny et al. (2006), Matheny (2009) and Alvarado et al. (2010) (= Crepidotaceae sensu Petersen et al. 2010). In particular *Neopaxillus* occupied a sister relationship to *Crepidotus* in the LSU analyses (FIG. 3) and to the clade consisting of *Crepidotus* and *Simocybe* species in the combined ITS-LSU analyses (FIG. 4). Micromorphological characters, the spore shape and ornamentation and cheilocystidia of *Neopaxillus*, resemble those of *Crepidotus* (Hesler and Smith 1965, Singer 1986, Consiglio and Setti 2009). *Neopaxillus* differs morphologically from *Crepidotus* mainly in the chroogomphoid to paxilloid habit, bilateral hymenophoral trama and terricolous habitat.

*Neopaxillus* species are present mostly in Central and South America, including the Caribbean Islands (Neotropics). Species of *Neopaxillus* were suspected by Singer (1986) and Watling (2002) to be ectomycorrhizal, due to their presumed affiliation with the ectomycorrhizal Paxillaceae. According to our phylogenetic analyses, we predict all species of *Neopaxillus* could be saprotrophic, like most of the Crepidotaceae (*Crepidotus* and *Simocybe*; Senn-Irlet 1995).

In our LSU analyses (FIG. 3) the position of *Simocybe* in the Crepidotoid clade is unresolved; our results disagree with the analyses of Aime (2001), Aime et al. (2002, 2005), Matheny et al. (2006), Alvarado et al. (2010) and Petersen et al. (2010), where *Simocybe* is

sister to the *Crepidotus* species studied. *Simocybe* shares with *Crepidotus* a physiological character unique among the Agaricales (with the exception of *Pluteus*, Banerjee and Sundberg 1993): a long period of spore dormancy before germination (Aime 1999, Aime and Miller 2002). In future work it could be interesting to check for this character in *Neopaxillus* species.

#### ACKNOWLEDGMENTS

P. Brandon Matheny (University of Tennessee, Knoxville, USA) and two anonymous reviewers are acknowledged for valuable comments and suggestions. The authors thank the curators of K (B. Spooner) and F (R. Lücking) for allowing the study and the loan of herbarium specimens. Our most sincere thanks also to E. Grilli (Popoli, Italy) for improving the English text.

#### LITERATURE CITED

Aime MC. 1999. Generic concepts in the Crepidotaceae as inferred from nuclear large subunit, ribosomal DNA sequences, morphology, and basidiospore dormancy patterns (master's thesis]. Blacksburg: Virginia Polytechnic Institute Press. 127 p.

———. 2001. Biosystematic Studies in *Crepidotus* and the Crepidotaceae (Basidiomycetes, Agaricales) [doctoral thesis]. Blacksburg: Virginia Polytechnic Institute Press. 194 p.

———, Miller OK. 2002. Delayed germination of basidiospores in temperate species of *Crepidotus* (Fr.) Staude. *Can J Bot* 80:280–287.

———, Baroni TJ, Miller OK Jr. 2002. *Crepidotus thermophilus* comb. nov., a reassessment of *Melanomphalia thermophila*, a rarely collected tropical agaric. *Mycologia* 94:1059–1065.

———, Vilgalys R, Miller OK Jr. 2005. The Crepidotaceae (Basidiomycota, Agaricales): phylogeny and taxonomy of the family based on molecular evidence. *Am J Bot* 92:74–82.

Alvarado P, Manjón JL, Matheny PB, Esteve-Raventós F. 2010. *Tubariomyces*, a new genus of *Inocybaceae* from the Mediterranean region. *Mycologia* 102.

Banerjee P, Sundberg WJ. 1993. Preliminary observations on germination of *Pluteus* basidiospores. *Mycologia* 85:811–813.

Binder M, Hibbett DS. 2006. Molecular systematic and biological diversification of Boletales. *Mycologia* 98:971–981.

Cléménçon H. 2004. Cytology and plectology of the Hymenomycetes. *Bibl Mycol* 199:1–488.

Consiglio G, Setti L. 2009. Il Genere *Crepidotus* in Europa. Vicenza: A.M.B. Fondazione Centro Studi Micologici. 344 p.

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A. 2009. Geneious v5.1.6 (available from <http://www.geneious.com>).

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.

García J, López A. 1993. *Neopaxillus echinospermus* (Speg.) Sing. Notas Técnicas 2. Veracruz: Centro de Genética Forestal de la Universidad Veracruzana de México.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118.

Guzmán G. 1983. Los hongos de la Península de Yucatán II. Nuevas exploraciones y adiciones micológicas. *Biótica* 8:71–100.

———, Guzmán-Dávalos L. 1984. Nuevos registros de hongos en el estado de Veracruz. *Bol Soc Mex Micol* 19:221–244.

Hesler LR, Smith AH. 1965. North American species of *Crepidotus*. New York: Hafner Publishing Co. 168 p.

- Horak E. 1968. Synopsis generum Agaricalium. Beitr Kryptogamenfl Schweiz 13:1–742.
- . 1980. Fungi, Basidiomycetes. Agaricales y Gasteromycetes secotioides. Flora Criptogámica Tierra Fuego 11:1–524.
- . 1988. On some extraordinary species of *Galerina* Earle from New Zealand, Australia and Indonesia, with annotations to related South American taxa. Sydowia 40:65–80.
- Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.
- , Larget B, Miller RE, Ronquist F. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst Biol 5:673–688.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066.
- Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Eyre Methuen & Co.
- Machol RE, Singer R. 1971. Bayesian analysis of generic relations in Agaricales. Nova Hedwigia 21:753–787.
- Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). Mol Phylogenet Evol 35:1–20.
- . 2009. A phylogenetic classification of the Inocybaceae. McIlvainea 18:11–21.
- , Curtis JM, Hofstetter V, Aime MC, Moncalvo J-M, Ge Z-W, Yang Z-L, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2006. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98:982–995.

———, Vellinga EC, Bougher NL, Ceska O, Moreau P-A, Neves MA, Ammirati JF. 2007. Taxonomy of displaced species of *Tubaria*. *Mycologia* 99:569–585.

Neves MA, Capelari M. 2007. A preliminary checklist of the Boletales from Brazil and notes on Boletales specimens at the Instituto de Botânica (SP) Herbarium, São Paulo, SP, Brazil. *Sitientibus. Rev Univ Estadual Feira Santana* 7:163–169.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H. 2008. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evol Bioinf* 4:193–201.

Pegler DN. 1986. Agaric flora of Sri Lanka. *Kew Bull, add ser* 12:1–519.

———. 1997. The Agaricales of São Paulo, Brazil. Kew, UK: Royal Botanic Gardens. 68 p.

Petersen G, Knudsen H, Seberg O. 2010. Alignment, clade robustness and fungal phylogenetics—Crepidotaceae and sister families revisited. *Cladistics* 26:62–71.

Posada D. 2008. jModeltest: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256.

Rambaut A, Drummond AJ. 2007. Tracer v1.4 (available from <http://beast.bio.ed.ac.uk/Tracer>).

Savolainen V, Cuénoud P, Spichiger R, Martinez MDP, Crèvecoeur M, Manen JF. 1995. The use of herbarium specimens in DNA phylogenetics: evaluation and improvement. *Plant Syst Evol* 197:87–98.

Senn-Irlet B. 1995. The genus *Crepidotus* in Europe. *Persoonia* 16:1–80.

Singer R. 1948. New Genera of Fungi IV. *Mycologia* 40:262–264.

- . 1949 (1951). The Agaricales in modern taxonomy. *Lilloa* 22:1–832.
- . 1964. Boletes and related groups in South America. *Nova Hedwigia* 7:93–132.
- . 1986. The Agaricales in modern taxonomy. 4th ed. Koenigstein, Germany: Koeltz Scientific Books. 981 p.
- , Digilio APL. 1952 (1951). Pródromo a la flora agaricina Argentina. *Lilloa* 25:5–462.
- , Lodge DJ. 1988. New tropical species in the Paxillaceae. *Mycol Helv* 3:207–213.
- , Garcia J, Gómez LD. 1990. The Boletineae of Mexico and Central America I & II. *Beih Nova Hedwigia* 98:1–70.
- Spazzolini C. 1889. Fungi Puiggariani. *Pugillus* 1. *Bol Acad Nac Ciencias, Córdoba* 11:381–622.
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
- Swofford DL. 2002. PAUP\*: phylogenetic analyses using parsimony (\*and other methods), Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* (In press). doi:10.1093/molbev/msr121
- Thiers B. 2011. [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium (<http://sweetgum.nybg.org/ih/>).

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246.

Watling R, Meijer A. 1997. Macromycetes from the state of Paraná, Brazil. Edinburgh J Bot 54:231–251.

———. 2002. Comparison of the macromycete biotas in selected tropical areas of Africa and Australia. In: Watling R, ed. Tropical mycology. Vol. 1. Macromycetes. Wallingford, UK: CABI Publishing. p 171–189.

White TJ, Bruns TD, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols. London: Academic Press. p 315–322.

## LEGENDS

FIG. 1. *Neopaxillus dominicanus* (from holotype). a, b, c. Basidiomes. Bars = 50 mm.

FIG. 2. *Neopaxillus dominicanus* (from holotype). a. Pileus surface. b. Stipe and lamellae. c. Pileipellis. d. Basidia. e. Cheilocystidia. f. Spores. g. Thromboplerous hypha. Bars: a, b = 40 mm; c, d, e, g = 30  $\mu\text{m}$ ; f = 20  $\mu\text{m}$ .

FIG. 3. Crepidotoid clade. Bayesian phylogram obtained from the general LSU sequence alignment. *Tubaria dispersa* was used as outgroup. Values for clades that are supported in either the Bayesian (posterior probabilities, BPP), maximum likelihood (ML bootstrap percentage, MLB) and maximum parsimony (MP bootstrap percentage, MPB) analyses are indicated. BPP above 0.50 and MLB/MPB above 50% are given above branches. Numbers (1–4) refer to the *N. dominicanus* collections reported (TABLE I).

FIG. 4. Bayesian phylogram obtained from the combined ITS-LSU sequence alignment. *Inocybe geophylla* was used as outgroup. Values for clades that are supported in either the Bayesian (posterior probabilities, BPP), maximum likelihood (ML bootstrap percentage, MLB) and maximum parsimony (MP bootstrap percentage, MPB) analyses are indicated. BPP above 0.50 and MLB/MPB above 50% are given above branches. Numbers (1–4) refer to the *N. dominicanus* collections reported (TABLE I).

## FOOTNOTES

Submitted 2 Nov 2010; accepted for publication 19 Jul 2011.

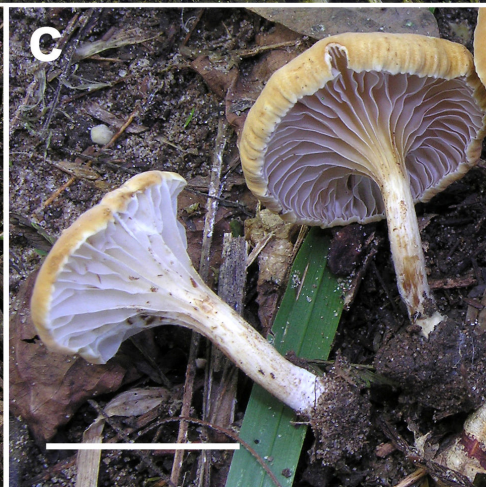


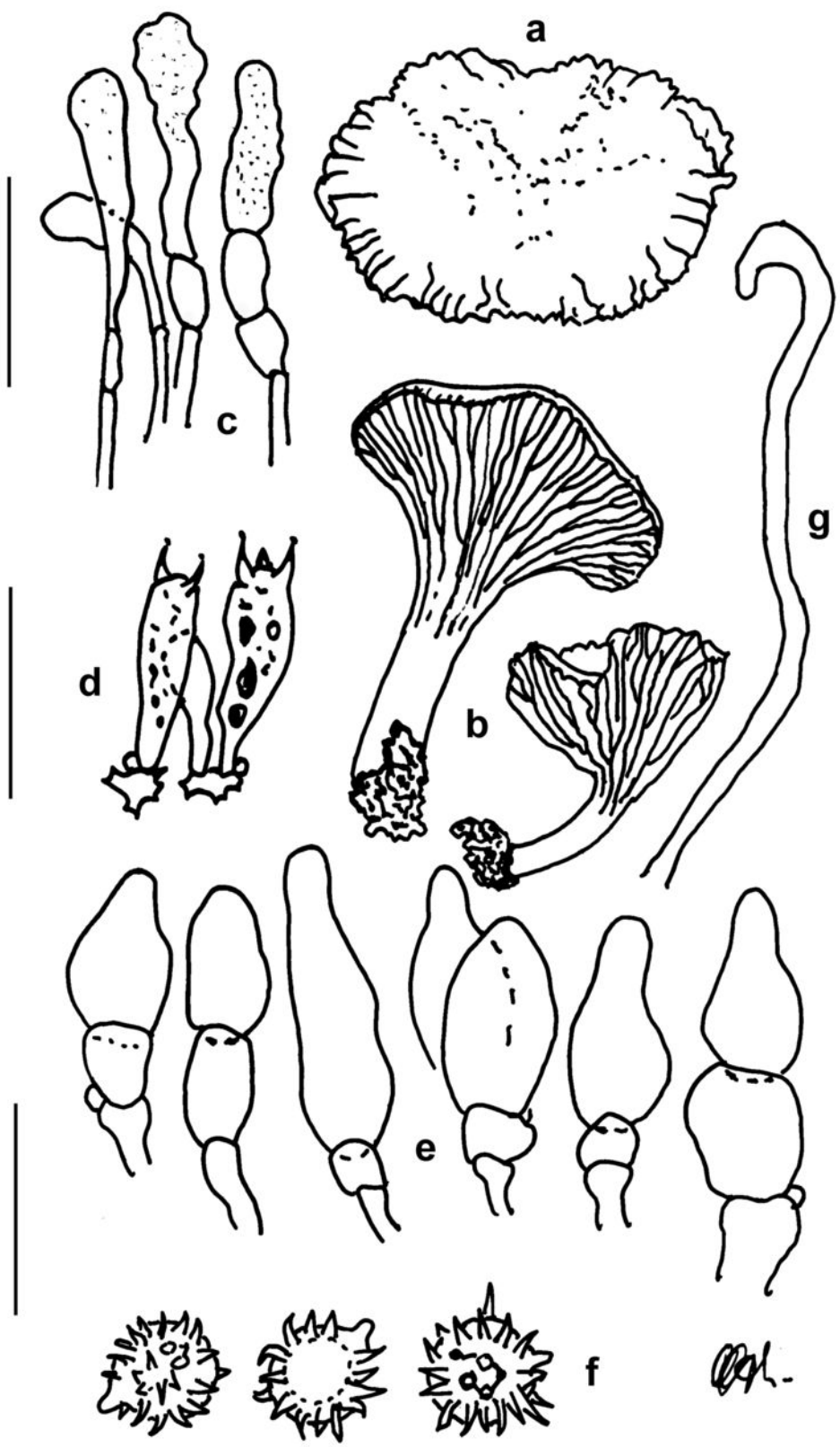
<sup>1</sup>Corresponding author. E-mail: [alfredo.vizzini@unito.it](mailto:alfredo.vizzini@unito.it)

TABLE I. Collections of *Neopaxillus* used in this study for the molecular analyses

Species	GenBank accession numbers		Source, country, date and collector
	ITS	LSU	
<i>Neopaxillus dominicanus</i> 1	HQ452479	HQ452478	MCVE 25727(holotype), DOMINICAN REPUBLIC, 10/01/2010, leg. C. Angelini
<i>Neopaxillus dominicanus</i> 2	JN033216	JN033217	MCVE 26928, DOMINICAN REPUBLIC, 12/01/2011, leg. C. Angelini
<i>Neopaxillus dominicanus</i> 3	JN033218	JN033219	F 1059091 (Jesús García J. 3167), MEXICO, 08/10/1983, leg. Jesús García J.
<i>Neopaxillus dominicanus</i> 4	JN033220	JN033221	F 1133966 (Gregory M. Mueller 3831), MEXICO, 22/10/1988, leg. Gregory M. Mueller
<i>Neopaxillus echinospermus</i>	AJ419194 <sup>a</sup>	JN033222	MA-Fungi 49404 (MPM 2886), BRAZIL, 23/01/2001, leg. A.A.R. de Meijer
<i>Neopaxillus plumbeus</i>	JN033223	JN033224	F 1068564 (Lodge No. PR 38) (holotype), USA, PUERTO RICO, 05/09/1985, leg. Lodge & Prieto
<i>Neopaxillus reticulatus</i>	—	—	K(M) 168990 (T. Petch 3536) (holotype), SRI LANKA, May 1912, leg. T. Petch

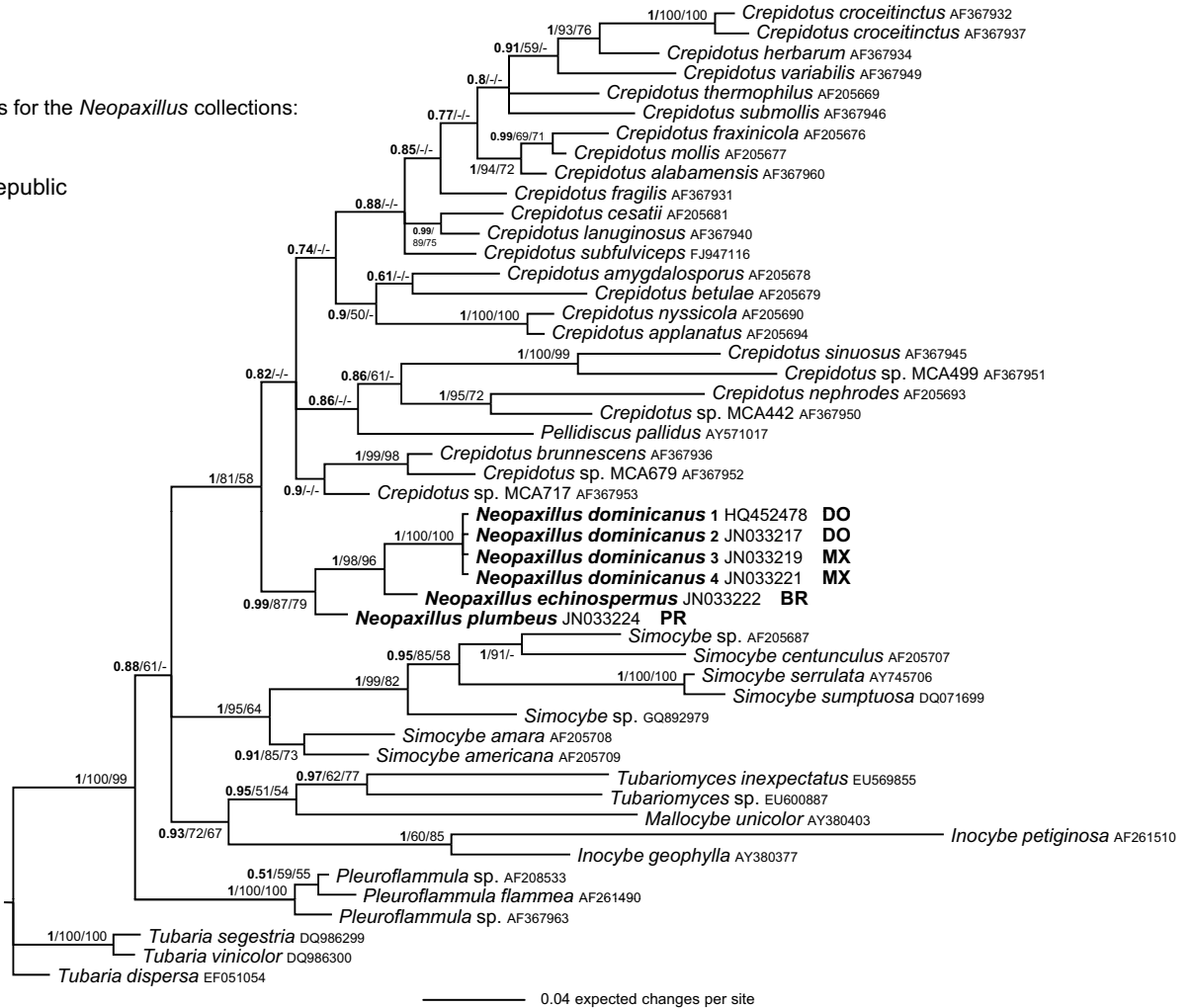
<sup>a</sup> Sequence retrieved from GenBank.





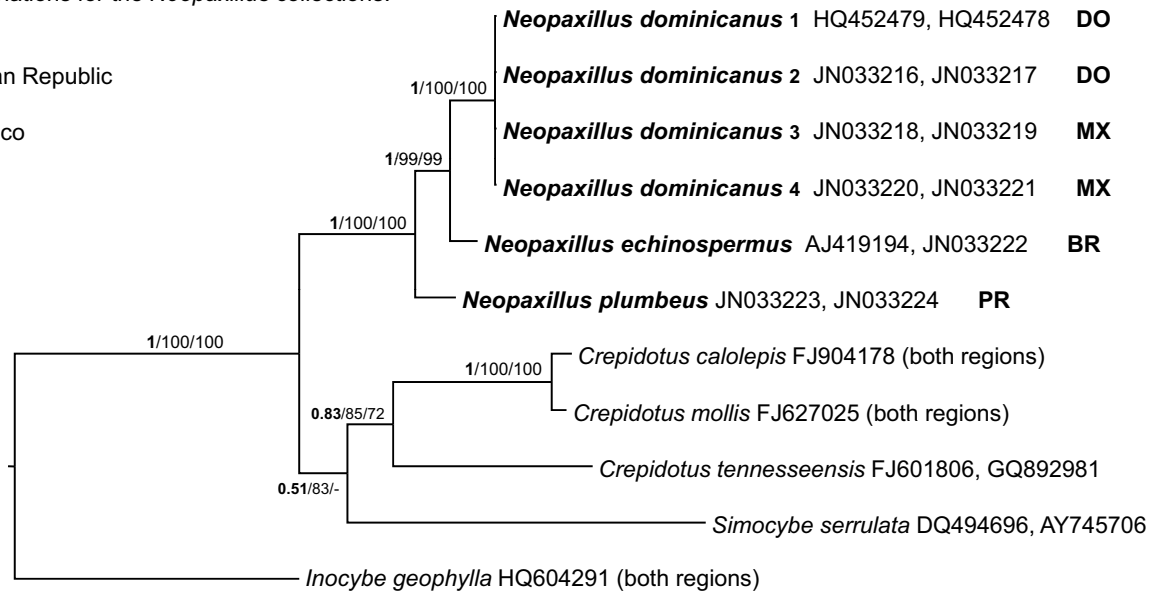
Country abbreviations for the *Neopaxillus* collections:

- BR - Brazil
- DO - Dominican Republic
- MX - Mexico
- PR - Puerto Rico



Country abbreviations for the *Neopaxillus* collections:

BR - Brazil  
 DO - Dominican Republic  
 MX - Mexico  
 PR - Puerto Rico



0.04 expected changes per site