Melanogaster coccolobae sp. nov. (Paxillaceae, Boletales),
a tropical hypogeous fungus from the urban areas of Quintana Roo, Mexico

Abstract:
Background and Aims: The genus Melanogaster is characterized by its hypogeous to semi hypogeous habit, brownish basidiomata, gel-filled gle- bo locules, and globose to ellipsoid basidiospores. The genus is distributed in temperate zones, but sequences from Coccoloba root tips and a few basidiome collections have revealed its presence in the tropics. The aim of this article is to describe a new species of Melanogaster based on ecological, molecular, and morphological data.

Methods: Specimens were collected in urban vegetation of Quintana Roo in the Yucatán Peninsula, Mexico. For morphological description, the classic protocols for sequestrate fungi were followed. The dried material was deposited in the mycological herbarium “José Castillo Tovar” of the Instituto Tecnológico de Ciudad Victoria (ITCV) and the herbarium of the Universidad Autónoma de Yucatán (UADY).

Key results: Melanogaster coccolobae is presented as a new species from the urban gardens of Quintana Roo based on ecological, molecular, and morphological evidence. This species is characterized by its hypogeous to semi hypogeous basidioma, greyish orange, brown to reddish brown peridium composed of two layers, sweet smell, subglobose, ellipsoid or periform basidiospores, and by its mycorrhizal association with Coccoloba spicata.

Conclusions: Melanogaster coccolobae is the first species described from the Mexican Caribbean from urban gardens with Coccoloba spicata. More studies about the tropical sequestrate fungi are recommended.

Key words: Agaricomycetes, Basidiomycota, Polygonaceae symbionts, sequestrate fungi, tropical fungi.

Resumen:
Antecedentes y Objetivos: El género Melanogaster se caracteriza por su hábito hipogeo a semi hipogeo, basidiomas parduscos, gleba con lóculos llenos de gel y basidiosporas globosas a elipsoides. El género se distribuye en zonas templadas, pero secuencias de ectomicorrizas de Coccoloba y pocas colecciones de basidiomas han revelado su presencia en los trópicos. El objetivo de este artículo es describir una nueva especie de Melanogaster a partir de datos ecológicos, moleculares y morfológicos.

Métodos: Los especímenes fueron recolectados en jardines urbanos de Quintana Roo en la Península de Yucatán, México. Para la descripción morfológica se siguieron los protocolos clásicos para hongos sequestrados. El material se depositó en el herbario micológico “José Castillo Tovar” del Instituto Tecnológico de Ciudad Victoria (ITCV) y en el herbario de la Universidad Autónoma de Yucatán (UADY).

Resultados clave: Melanogaster coccolobae se presenta como una nueva especie de los jardines urbanos de Quintana Roo con base en evidencia morfológica, ecológica y molecular. Esta especie se caracteriza por sus basidiomas hipogeos a semi hipogeos, peridio naranja grisáceo, marrón o marrón rojizo, compuesto por dos capas, olor dulce, basidiosporas subglobosas, elipsoides o periformes y por formar asociación micorrízica con Coccoloba spicata.

Conclusões: Melanogaster coccolobae es la primera especie descrita del Caribe mexicano en jardines urbanos con Coccoloba spicata. Se recomiendan más estudios sobre los hongos sequestrados tropicales.

Palabras clave: Agaricomycetes, Basidiomycota, Polygonaceae sibionts, sequestrate fungi, tropical fungi.
Introduction

The genus *Melanogaster* Corda (Paxillaceae, Boletales) comprises hypogeous to semi hypogeous species characterized by a brownish to yellowish peridium, sometimes with mycelial strands at the base or sides, sequestrate hymenophore composed of rounded to irregular locules filled with black basidiospores embedded in gel, separated by whitish to yellowish veins or walls; and dark brown, globose, ellipsoid, pyriform or citriform basidiospores. Most of the species have an odor ranging from sweet and pleasant to garlicky or nauseating (Castellano et al., 1986; Miller Jr. and Miller, 1988; Pegler et al., 1993; Montecchi and Sarasin, 2000; Cázares et al., 2008; Trappe et al., 2009). It differs from related sequestrate Paxillaceae such as *Alpova* C.W. Dodge, *Neoalpova* Vizzini, and *Paralpova* Cabero & P. Alvarado by the peridium structure (prostrated or interwoven hyphae in *Melanogaster* vs. pseudoparenchymatous with inflated hyphae in *Alpova, Neoalpova*, and *Paralpova*), and *Rhizopogon* Fr. (Rhizopogonaceae) by the locules without gel and basidiospore color (hyaline in *Rhizopogon*). The relationships between these have been widely discussed by Trappe (1975), Grubisha et al. (2001), Vizzini et al. (2010), Moreau et al. (2011; 2013), and Alvarado et al. (2021).

*Melanogaster* species are common in the Northern hemisphere, but also occur in the Southern one due to introduction of *Quercus* L. species (Trappe et al., 2009). They can develop from the sea level to high forests and form ectomycorrhizal association with conifers and hardwoods (Castellano et al., 1986; Rinaldi et al., 2008; Tedersoo et al., 2010). According to Binder and Hibbett (2006), the genus belongs to the clade Paxillinae along with *Gyrodon* Opat., *Paxillus* Fr., and *Alpova*. Moreau et al. (2011) provisionally separated the European species in three sections: section *Melanogaster* P-A. Moreau (including the species with basidiospores with more than 10 μm long in average), section *Variegeti* P-A Moreau (including the species with basidiospores of 6-10 μm long on average, not associated with *Alnus* Mill.) and section *Rivulaires* P-A Moreau (including species with basidiospores of 5-6 μm long on average). Ten species are recognized from Europe (Moreau et al., 2011), 19 from North America (Zeller and Dodge, 1936), and only three are known from Mexico: *Melanogaster umbriniglebus* Trappe & Guzmán, *M. variegatus* (Vittad.) Tul. & C. Tul., and *M. minisporus* Cázares, G. Guevara, J. García & Trappe (Trappe and Guzmán, 1971; Cázares et al., 1992, 2008). All the species known from northern Mexico form mycorrhizal symbioses with *Quercus* species (Zeller and Dodge, 1936; Trappe and Guzmán, 1971; Cázares et al., 1992, 2008). Despite the fact that several species have been described from Asia, America and Europe, molecular information is only available for eight, mostly European ones (Vizzini et al., 2010; Moreau et al., 2011, 2013). Furthermore, several unnamed *Melanogaster* sequences available in public databases are waiting to be formally described or nested with previously known taxa (Alvarado et al., 2021).

The ectomycorrhizal fungi from the tropics are still poorly studied in comparison with those from the template zones. Although several studies have been carried out in the Antilles and Central America about ectomycorrhizal fungi associated to Polygonaceae species (Pegler and Fiard, 1979; Pegler, 1983; Singer et al., 1983; Miller Jr. et al., 2000; Hasselquist et al., 2011; Pölme et al., 2017; de la Fuente et al., 2018), the knowledge about the fungi associated to *Coccoloba spicata* Lundell is still scarce in Mexico. Furthermore, *C. spicata* is a common tree of the disturbed vegetation, backyards and urban gardens in Quintana Roo state (Yucatán Peninsula, Southern Mexico). In recent years, several hypogeous fungi have been collected growing under *C. spicata* from the urban gardens of some localities in Quintana Roo. The aim of this study is to describe *Melanogaster coccolobae* based on ecological, morphological, and molecular evidence.

Material and Methods

Sampling

The mycological explorations were carried out in the state of Quintana Roo, Mexico, in urban gardens from Chetumal and Felipe Carrillo Puerto (Fig. 1). The vegetation within the sampling zones is disturbed. Methods for collecting, sampling and describing sequestrate fungi were used following Castellano et al. (1986). Handcut sections were made from dried specimens mounted in KOH 5% and Meltzer reagent for microscopic description. Color terminology was according to the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). At least 30 microscopic structures (basidiospores, basidia and peridium hyphae) were measured.
with an optical microscope (Motic ba310, San Antonio, USA). The Q range, average length (L) and average width (W) were obtained for basidiospores. The scanning electron microscope (JEOL JSM-6010PLUS, LA, Tokyo, Japan) of El Colegio de la Frontera Sur (ECOSUR, Chetumal, Mexico) was utilized to observe basidiospores. All the specimens were curated and deposited in the mycological herbarium “José Castillo Tovar” of the Instituto Tecnológico de Ciudad Victoria (ITCV, Ciudad Victoria, Mexico) and Universidad Autónoma de Yucatán (UADY, Mérida, Mexico).

Amplification and sequencing
Total DNA was extracted from dried herbarium specimens using a modification of the Murray and Thompson (1980) protocol. The PCR amplification, based on Mullis and Faloona (1987), included 35 cycles with an annealing temperature of 54 °C, and was carried out with the ITSS and ITS4 primers (White et al., 1990; Gardes and Bruns, 1993) for the ITS nrDNA region, the LR0R and LR5 primers (Vilgalys and Hester, 1990; Cubeta et al., 1991) for the 28S nrDNA region (LSU), and the primers bRPB2-6F2 (reverse of bRPB2-6R2), and bRPB2-7R2 for the second largest subunit of the RNA polymerase II gene (rpb2) (Matheny et al., 2007). The PCR products were verified by agarose gel electrophoresis. The gels were run for 1 h at 95 V cm⁻³ in 1.5% agarose and 1× TAE buffer (Tris Acetate-EDTA). The gel was stained with GelRed (Biotium, USA) and the bands were visualized in an Infinity 3000 transilluminator (Vilber Lourmat, Germany). The amplified products were purified with the ExoSAP Puriﬁcation kit (Affymetrix, USA), following the manufacturer’s instructions. They were quantified and prepared for the sequence reaction using a BigDye Terminator v. 3.1 (Applied Biosystems, USA). These products were sequenced in both directions with an Applied Biosystem model 3730XL (Applied BioSystems, USA), at the Instituto de Biología of the Universidad Nacional Autónoma de México (UNAM).
sequences obtained were compared with the original chromatograms to detect and correct possible reading errors.

Phylogenetic analyses

To explore the phylogenetic relationships of the new species, an alignment was made based on the taxonomic sampling employed by Halášz (2009), Moreau et al. (2011, 2013), Rochet et al. (2011), Vizzini et al. (2010), and Alvarado et al. (2021). The ITS dataset included sequences from 42 fungal specimens representing 35 taxa. The ITS region was aligned using the online version of MAFFT v. 7 (Katoh et al., 2002, 2017; Katoh and Standley, 2013). The alignment was revised in PhyDE v. 10.0 (Müller et al., 2005), followed by minor manual adjustments to ensure character homology between taxa. The matrix was composed of 42 individuals (700 characters) (Appendix). Phylogenetic inferences were estimated with maximum likelihood in RaxML v. 8.2.10 (Stamatakis, 2014) with a GTR + G model of nucleotide substitution. To assess branch support, 1000 rapid bootstrap replicates were run with the GTRCAT model. For Bayesian posterior probability, the best evolutionary model for alignment was sought using PartitionFinder v. 2 (Lanfear et al., 2014; 2017; Frandsen et al., 2015), the best-fit model selected for these three partitions of ITS sequences was GTR+G for ITS1, JC for 5.8s, and HKY+G for ITS2. Phylogenetic analyses were performed using MrBayes v. 3.2.6 x64 (Huelsenbeck and Ronquist, 2001). The information block for the matrix included two simultaneous runs, four Montecarlo chains, temperature set to 0.2 and sampling 10 million generations (standard deviation ≤0.1) with trees sampled every 1000 generations. The first 25% of samples were discarded as burn-in, and stationarity was checked in Tracer v. 1.6 (Rambaut et al., 2014). Trees were visualized and optimized in FigTree v. 1.4.4 (Rambaut, 2014).

Results

Molecular analyses

The two simultaneous Bayesian runs continued until the convergence parameters were met, and the standard deviation fell below 0.002 after 4 million generations. No significant changes in tree topology trace or cumulative split frequencies of selected nodes were observed after about 0.25 million generations, which were discarded as 25% burn-in. In the present study, a new species based on morphological characters and phylogenetic analysis of ITS sequences is described (Fig. 2). Due to the lack of multilocus sequences from other species of the genus Melanogaster and the family Paxillaceae, we cannot construct a multilocus phylogenetic tree at this time. We have sequenced the ITS, 28S and rpb2 genes, hoping they will be useful for future studies. The analysis of ITS produced a phylogenetic tree where Melanogaster is shown as a monophyletic clade (1 Bayesian Posterior Probability (PP) and 100% bootstrap proportion (BP) for Maximum Likelihood). The new species is phylogenetically distant from the European and North American species and forms a well-supported clade (0.99 Bayesian PP and 98% BP for ML) near two unidentified Melanogaster sequences from Guadeloupe, in the Caribbean (0.98 Bayesian PP and 97% BP for ML) (Séne et al., 2015) (Fig. 2).

Taxonomy

Melanogaster coccolobae de la Fuente, J. García & Guevara sp. nov. Fig. 3.

TYPE: MEXICO. Quintana Roo, municipality Othón Pompeyo Blanco, urban gardens of the Instituto Tecnológico de Chetumal under Coccoloba spicata, 18°31’12”N, 88°18’22”W, 10 m a.s.l., 16.XI.2017, de la Fuente 375 (holotype: ITCV; isotype: UADY). Mycobank: MB 839197.

Melanogaster coccolobae can be distinguished from all other Melanogaster species by the following combination of characters: greyish orange, brown to reddish brown peridium, hypogeous to semi hypogeous, basidiomata 19-59 × 10-17 mm, odor sweet, subglobose, basidiospores ellipsoid or piriform, 6.2-12 × 5.2-10 µm, and the association with Coccoloba spicata.

Basidiomata 19-59 × 10-17 mm, scattered, hypogeous or semi hypogeous, globose to subglobose, lobate, greyish orange (5B6), brown (7E7) to reddish brown (8E8), staining dark brown (7F8) when touched, dry, smooth or slightly velvety, sometimes exuding a bright yellow liquid, with mycelial strands attached in some parts or around the

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Figure 2: Bayesian inference phylogram of ITS sequences data. Posterior probability (left of slash) from Bayesian analysis and Bootstrap support (right of slash). The new species Melanogaster coccolobae de la Fuente, J. García & Guevara is shown in bold. Boldface names represent samples sequenced for this study.

basidiomata, dark brown (7F8); hymenophore composed of locules covered by a thin layer of black basidiospores when young, filled with hyaline gel, blackened due to the basidiospores when mature, without color changes when injured, with pale orange intermixed veins (5A3); columella absent; odor pleasant, sweet to alcoholic; peridium 170-230 µm thick, composed of two layers; epicutis 48-96 µm thick, composed of interwoven hyphae with conspicuous erect hyphae, fusoid to cylindrical terminal cells, dark or orange to slightly reddish brown, 2-5 µm in diameter, thick-walled, sometimes with incrusted dark brown pigments; subcutis 116-140 µm thick, composed of interwoven tubulose, globose to irregular hyphae, yellowish to hyaline near the hymenophore, 3.5-14 µm diameter, thin-walled; clamp connections present, hymenophoral trama 37-75 diameter, hyaline, irregular composed of strongly gelatinized and interwoven slender hyphae, 4-10 µm diameter, thin-walled; basidia 18.8-29.7 × 8.6-10.4 µm, clavate with a long and sinuous stalk, basidiospores 2-4, hyaline, poorly reviving in KOH 5%, thin-walled, occurring randomly in the locules; basidiospores subglobose, ellipsoid or pyriform, 6.2-12 × 5.2-10 µm (Q=1.1-1.7, L = 9.9, W = 7.5, N=30), hyaline when immature, becoming dark brown, smooth, with conspicuous hilar appendage, 0.5-1.5 × 1.4-2 µm, cylindrical and hollow, sometimes cracked, giving to the hilar appendage a spiny appearance in optical microscopy, thick-walled.

Habit and habitat: hypogeous to semi hypogeous under Coccoloba spicata in urban gardens (Fig. 4).

Distribution: only known from Quintana Roo, Mexico.

Etymology: named coccolobae due to the putative micorrhizal association with Coccoloba spicata.

Additional material examined: MEXICO. Quintana Roo, Municipality Othón Pompeyo Blanco, gardens of the Instituto Tecnológico de Chetumal (ITCh), 05.II.2014, J. de la Fuente 05 (ITCV); loc. cit., 14.X.2018, J. de la Fuente et al. 436 (ITCV). Municipality Felipe Carrillo Puerto, Felipe Carrillo Puerto, in urban gardens, 17.XI.2017, A. Hernández-Cach and J. de la Fuente 378 (ITCV).
Figure 3: Melanogaster coccolobae de la Fuente, J. García & Guevara. A. basidiomata, de la Fuente 375 (ITCV-Holotype); B. details of the hymenophore, de la Fuente 05 (ITCV); C. details of the pileus, showing the dark mycelial strands, de la Fuente 05 (ITCV); D. basidiospores; E. basidiospore under scanning electron microscope (SEM); F. basidia; G. hymenophoral trama; H. subcutis; I. epicutis. Scale bars: 10 mm (A, B, C); 2 µm (E); 10 µm (D, F, G, H, I).
Notes: the most remarkable characteristic of this new species is its putative micorrhizal association with *Coccoloba spicata*. Furthermore, *M. coccolobae* has an interesting peridium structure, which is composed of two layers with different hyphal arrangement. Due to the size and shape of spore and basidiome size, it resembles *M. tuberiformis* Corda. Nevertheless, that species has a thinner peridium composed only by interwoven hyphae and is associated with *Pinus* L., *Quercus* and *Erica* L.

**Discussion**

*Melanogaster coccolobae* can be identified mainly by the subglobose, ellipsoid or piriform basidiospores, the two-layered peridium and the mycorrhizal association with *Coccoloba spicata*. It differs from other Mexican *Melanogaster* species, such as *M. umbriniglebus*, by the reddish reaction of the gleba when cut, and the larger (reaching up to 14 µm) and the light colored basidiospores (Trappe and Guzmán, 1971). *Melanogaster variegatus*, a common species in North America, has citriform to ellipsoid basidiospores shape and larger spores (reaching up to 17 µm long) and is associated with *Quercus* species (Zeller and Dodge, 1936; Trappe and Guzmán, 1971). *Melanogaster minisporus* differs by the yellowish pileus, the smaller spores and the ecological association with *Quercus* species (Cázares et al., 2008).

Close DNA sequences have been obtained from the Caribbean (KF472137 and KF472152), and also some basidiomata have been collected and have been identified as *Melanogaster* sp. (Séne et al., 2015). According to our phylogenetic analyses, these sequences are nested near *M. coccolobae*. These sequences have been collected under *Coccoloba uvifera* (L.) L. in coastal vegetation (Séne et al., 2015). Although the authors have exhaustively studied the ectomycorrhizal fungi associated to *Coccoloba P. Browne* trees in different vegetations with different con-
reservation degree in the Yucatán Peninsula, basidiomata of *M. coccolobae* have never been found elsewhere. This peculiar distribution has been observed before in the tropics by Singer and Morello (1960), who found greater diversity of ectomycorrhizal fungi in disturbed vegetation in comparison with well-conserved forest. This new species is common in urban gardens, sharing habitat with several ectomycorrhizal species such as *Xerocomus coccolobae* Peck, *Tremelloscypha gelatinosa* (Murrill) Oberw. & K. Wells, *Scleroderma bermudense* Coker, *Inocybe xerophytica* Peck, *Amanita arenicola* O.K. Mill. & Lodge, and *Cantharellus coccolobae* Buyck, P.-A. Moreau & Courtec. This fungal diversity has been partially studied by García-Jiménez and Garza-Ocañas (2001) and de la Fuente et al. (2018).

**Conclusions**

In this study, a new species of *Melanogaster* of Mexico based on morphological characters, ecological distribution and ITS phylogeny is described. *Melanogaster coccolobae* is the first species of the genus known from tropical vegetation in Mexico. The new species has a preference for disturbed vegetation or urban gardens. The discovery of this fungus from this unusual habitat shows that disturbed areas can harbor an interesting fungal diversity with several noteworthy species. More studies about the ectomycorrhizal fungi occurring in such areas are recommended.

**Author contributions**

JIF, RYVH and CYL collected the material. JIF, JGJ and GG described the species. CRMG, IOO, ICL and VMB made the molecular analysis. JIF, GGG, and JGJ wrote the manuscript. All authors contributed to the manuscript.

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Appendix: GenBank accession numbers corresponding to the sequences used in the phylogenetic analyses. In bold the accessions of the new species.

| Species name                          | Isolate/Voucher/strain | Locality          | GenBank Accessions |
|---------------------------------------|------------------------|-------------------|--------------------|
|                                       |                        |                   | ITS    | nrLSU   | rpb2  |
| *Alpova alpestris* P.-A. Moreau & F.  | PAM07082629            | France            | HQ714696 | ---     | ---   |
| Rich.                                 | Holotype               |                   |        |         |       |
| *Alpova austroalnicola* L.S. Domínguez | LSD 2290               | Argentina         | HQ714793 | AY377574 | ---   |
| *Alpova cinnamomeus* C.W. Dodge       | HRL1384, 29            | Canada            | MN594282 | MN594298 | MN594770 |
| *Alpova concolor* J. Hayward          | 65696 (OSC)            | USA               | KF835994 | ---     | ---   |
| *Alpova corsicus* P.-A. Moreau & F.   | S284                   | France            | HQ714766 | ---     | HQ714890 |
| Rich.                                 | Holotype               |                   |        |         |       |
| *Alpova diplophloeus* (Zeller & C.W. | Hayward 1              | USA               | KF835992 | ---     | ---   |
| Dodge) Trappe & A.H. Sm.              |                        |                   |        |         |       |
| *Alpova komovianus* B. Perić & P.-A. | PAM10081201            | Montenegro        | JQ436850 | ---     | JQ436862 |
| Moreau                                |                        |                   |        |         |       |
| *Alpova pseudostipitatus* Calonge &   | 36826 Holotype         | Spain             | MN594285 | ---     | ---   |
| Siquier                               |                        |                   |        |         |       |
| *Gyrodon lividus* (Bull.) Sacc.       | REG G11                | Germany           | DQ534568 | AF098378 | GU187701 |
| *Gyrodon sp.*                         | 926                    | Mexico            | HQ271432 | ---     | ---   |
| *Melanogaster ambiguus* (Vittad.)     | 1590                   | Hungary           | AJ555512 | ---     | ---   |
| Tul. & C. Tul.                        |                        |                   |        |         |       |
| *Melanogaster broomeianus* Berk.      | 124251                 | ---               | AJ555530 | ---     | ---   |
| *Melanogaster broomeianus* Berk.      | 2331                   | Hungary           | AJ555531 | ---     | ---   |
| *Melanogaster broomeianus* Berk.      | 2348                   | Hungary           | EU784370 | ---     | ---   |
| *Melanogaster coccobae de la Fuente, J. García & Guevara* | 375 ITCV Holotype | Mexico | MZ098622 | MZ098618 | MZ089614 |
| *Melanogaster coccobae de la Fuente, J. García & Guevara* | 378 ITCV | Mexico | MZ098623 | MZ098619 | MZ089615 |
| *Melanogaster coccobae de la Fuente, J. García & Guevara* | 05 ITCV | Mexico | MZ098624 | MZ098620 | MZ089616 |
| *Melanogaster intermedius* (Berk.)    | 130202                 | ---               | AJ555515 | ---     | ---   |
| Zeller & C.W. Dodge                   |                        |                   |        |         |       |
| *Melanogaster intermedius* (Berk.)    | 1770                   | Hungary           | EU784372 | ---     | ---   |
| Zeller & C.W. Dodge                   |                        |                   |        |         |       |
| *Melanogaster cf intermedius* (Berk.) | 122480                 | ---               | EU784371 | ---     | ---   |
| Zeller & C.W. Dodge                   |                        |                   |        |         |       |
| *Melanogaster luteus* Zeller          | 328                    | France            | HQ714780 | ---     | ---   |
| *Melanogaster luteus* Zeller          | 407                    | Montenegro        | HQ714794 | ---     | ---   |
| *Melanogaster macrosporus* Velen.     | 94                     | Hungary           | AJ555526 | ---     | ---   |
| *Melanogaster rivularis* P.-A. Moreau | 190 Holotype           | France            | HQ714731 | ---     | HQ714891 |
## Appendix: Continuation.

| Species name                        | Isolate/Voucher/strain | Locality | GenBank Accessions |
|-------------------------------------|------------------------|----------|--------------------|
| *Melanogaster rivularis* P.-A. Moreau & F. Rich. | 08090514            | France   | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Melanogaster* sp                  | Ecu344                | France   | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Melanogaster* sp                  | Ecu313                | France   | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Melanogaster tuberiformis* Corda  | 1295                  | Romania  | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Melanogaster cf* tuberiformis Corda | 48368                 | Hungary  | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Melanogaster variegatus* (Vittad.) Tul. & C. Tul. | 1688                  | Hungary  | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Melanogaster variegatus* (Vittad.) Tul. & C. Tul. | 1438                  | Hungary  | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Melanogaster vittadinii* Soehner & Knapp | 33090                 | Hungary  | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Neoalpova arenicola* Cabero & P. Alvarado | 49153 Holotype        | Spain    | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Neoalpova montecchii* Cabero, P. Alvarado & Vizzini | 51272 Holotype        | Spain    | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Neoalpova rubescens* (Vittad.) Vizzini | 3003022               | Spain    | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Paragyrodon sphaerosporus* (Peck) Singer | MB06-066              | USA      | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Paxillus vernalis* Watling        | AFTOL-715             | China    | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Paraalkova artikutzensis* Cabero, D. Moreno-Mateos & P. Alvarado | 49154 Holotype        | Spain    | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Paxillus adelphus* J.-P. Chaumeton, Gryta, Jargeat & P.-A. Moreau | Pf01                  | Germany  | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Paxillus involutus* (Batsch) Fr. | Bel10.4               | France   | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Paxillus rubicundulus* P.D. Orton | 2905                  | U.K.     | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |
| *Paxillus vernalis* Watling        | MB-062 Holotype       | China    | **ITS** 353427 | **nrLSU** 353427 | **rpb2** 353427 |