








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

## *Melanogaster coccolobae* sp. nov. (Paxillaceae, Boletales), un hongo hipogeo tropical de las áreas urbanas de Quintana Roo, México

Javier Isaac de la Fuente<sup>1</sup> , César Ramiro Martínez-González<sup>2</sup> , Iván Oros-Ortega<sup>3</sup> , Gonzalo Guevara<sup>1</sup> , Víctor M. Bandala<sup>4</sup> , Iván Córdova-Lara<sup>5</sup> , Reyna Yazuly Vela-Hernández<sup>3</sup> , Caribell Yuridia López<sup>6</sup>  y Jesús García Jiménez<sup>1,7</sup> 

### Abstract:

**Background and Aims:** The genus *Melanogaster* is characterized by its hypogeous to semi hypogeous habit, brownish basidiomata, gel-filled gleba 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 piriform 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 secuestrados. 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 piriformes y por formar asociación micorrízica con *Coccoloba spicata*.

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

**Palabras clave:** Agaricomycetes, Basidiomycota, hongos secuestrados, hongos tropicales, simbiosis de Polygonaceae.

<sup>1</sup>Tecnológico Nacional de México, Instituto Tecnológico de Ciudad Victoria, Boulevard Emilio Portes Gil 1301, 87010 Cd. Victoria, Tamaulipas, Mexico.

<sup>2</sup>Universidad Nacional Autónoma de México, Departamento de Biología, Facultad de Ciencias, Ciudad Universitaria, 04510 CdMx., Mexico.

<sup>3</sup>Tecnológico Nacional de México, Instituto Tecnológico de la Zona Maya, Carretera Chetumal-Escárcega, km 21.5, 77965 Juan Sarabia, Quintana Roo, Mexico.

<sup>4</sup>Instituto de Ecología A.C., Red Biodiversidad y Sistemática, 91000 Xalapa, Veracruz, Mexico.

<sup>5</sup>Centro de Investigación Científica de Yucatán (CICY), Unidad de Biotecnología, calle 43, Col. Chuburná de Hidalgo, 97025 Mérida, Yucatán, Mexico.

<sup>6</sup>Tecnológico Nacional de México, Instituto Tecnológico de Chetumal, Av. Insurgentes 330, Col. 17 de Octubre, 77013 Chetumal, Quintana Roo, Mexico.

<sup>7</sup>Author for correspondence: jgarjim@yahoo.com.mx


Received: May 10, 2021.

Reviewed: June 8, 2021.

Accepted by Marie-Stéphanie Samain: September 22, 2021.

Published Online first: September 29, 2021.

Published: Acta Botanica Mexicana 128 (2021).

 This is an open access article under the Creative Commons 4.0 Attribution-Non commercial Licence (CC BY-NC 4.0 Internacional).

To cite as: de la Fuente, J. I., C. R. Martínez-González, I. Oros-Ortega, G. Guevara, V. M. Bandala, I. Córdova-Lara, R. Y. Vela-Hernández, C. Y. López and J. García Jiménez. 2021. *Melanogaster coccolobae* sp. nov. (Paxillaceae, Boletales), a tropical hypogeous fungus from the urban areas of Quintana Roo, Mexico. Acta Botanica Mexicana 128: e1896. DOI: <https://doi.org/10.21829/abm128.2021.1896>

## 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 Sarasini, 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 *Variiegati* P.-A. Moreau (including the species with basidiospores of 6-10 µm long on average, not associated with *Alnus* Mill.) and section *Rivulares* 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 umbrinigliebus* Trappe & Guzmán, *M. variegatus* (Vittad.)

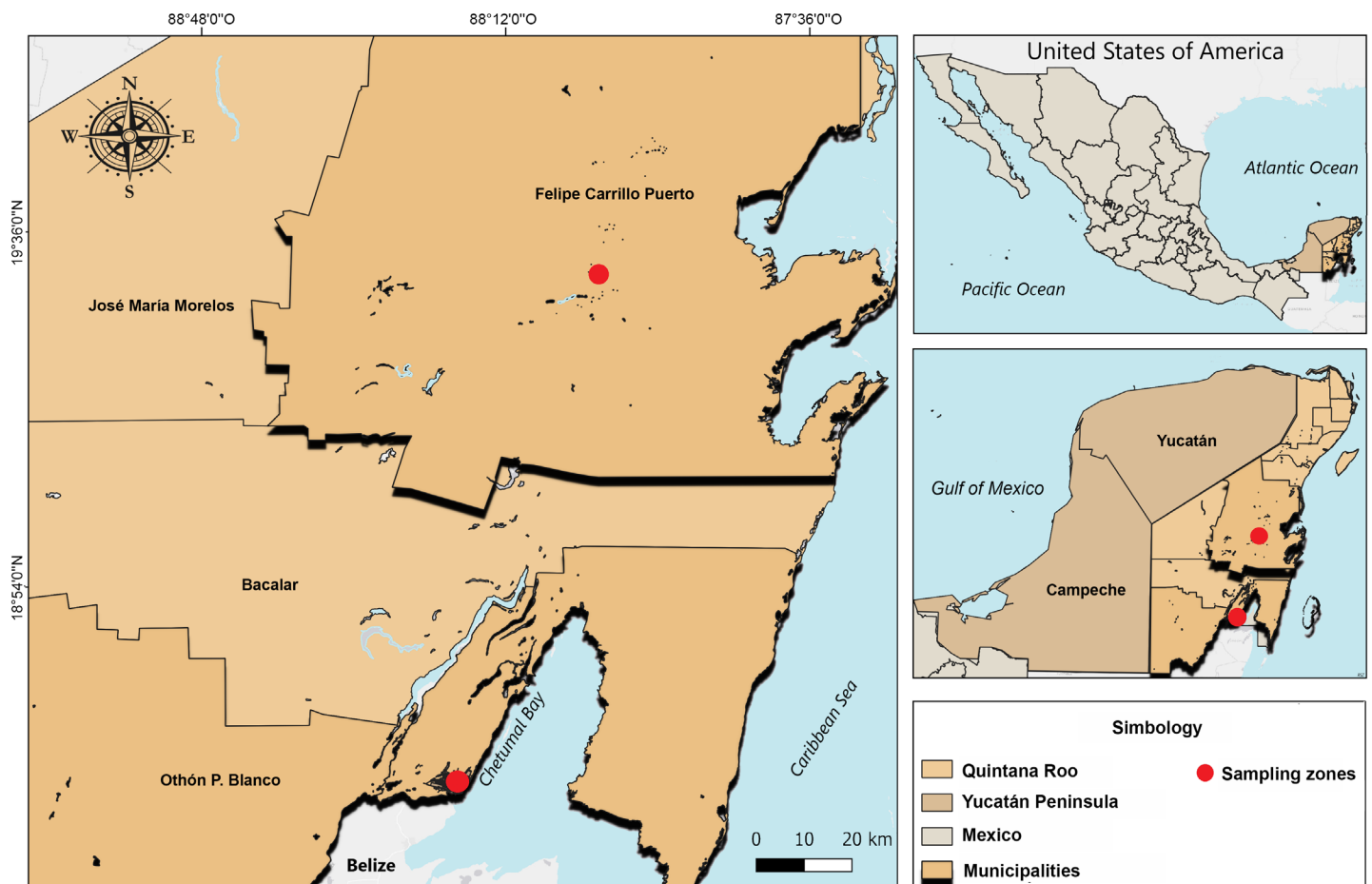
Tul. & C. Tul., and *M. minisporus* Cázares, G. Guevara, J. García & Trappe (Trappe and Guzmán, 1971; Cázares et al., 1992, 2008). All the species known from northern Mexico form mycorrhizal symbioses with *Quercus* species (Zeller and Dodge, 1936; Trappe and Guzmán, 1971; Cázares et al., 1992, 2008). 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 temperate 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



**Figure 1:** Distribution of *Melanogaster coccolobae* de la Fuente, J. García & Guevara.

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 ITS5 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<sup>-3</sup> 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). The

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 Halász (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 Rax-ML 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  $\leq 0.1$ ) 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

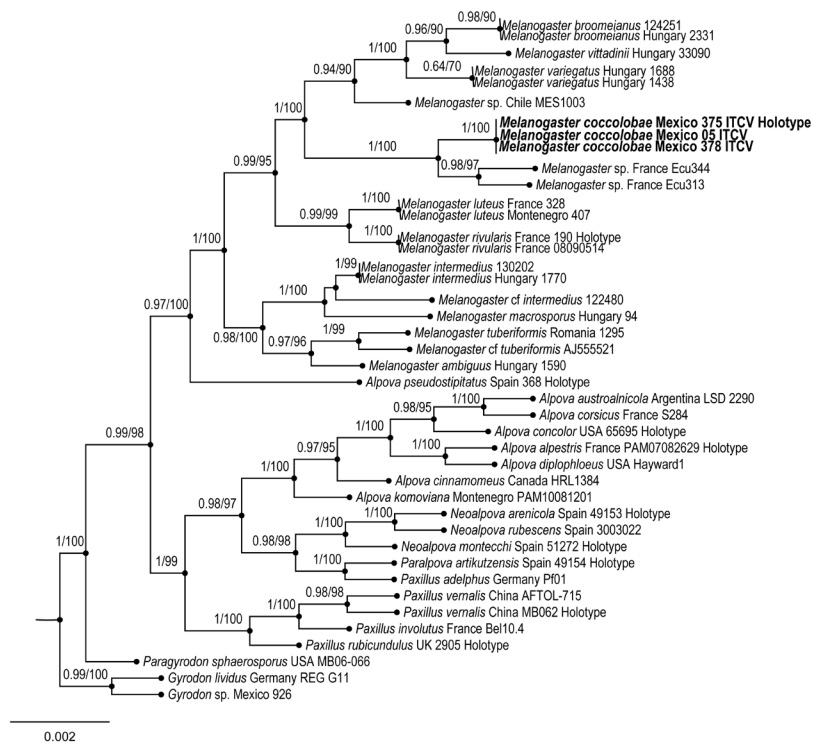
***Melanogaster coccolobae*** de la Fuente, J. García & Guevara sp. nov. Fig. 3.

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





**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  $\mu\text{m}$  thick, composed of two layers; epicutis 48-96  $\mu\text{m}$  thick, composed of interwoven hyphae with conspicuous erect hyphae, fusoid to cylindrical terminal cells, dark orange to slightly reddish brown, 2-5  $\mu\text{m}$  in diameter, thick-walled, sometimes with incrustated dark brown pigments; subcutis 116-140  $\mu\text{m}$  thick, composed of interwoven tubulose, globose to irregular hyphae, yellowish to hyaline near the hymenophore, 3.5-14  $\mu\text{m}$  diameter, thin-walled; clamp connections present, hymenophoral trama 37-75 diameter, hyaline, irregular composed of strongly gelatinized and interwoven slender hyphae, 4-10  $\mu\text{m}$  diameter, thin-walled; basidia 18.8-29.7  $\times$  8.6-10.4  $\mu\text{m}$ , 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  $\times$  5.2-

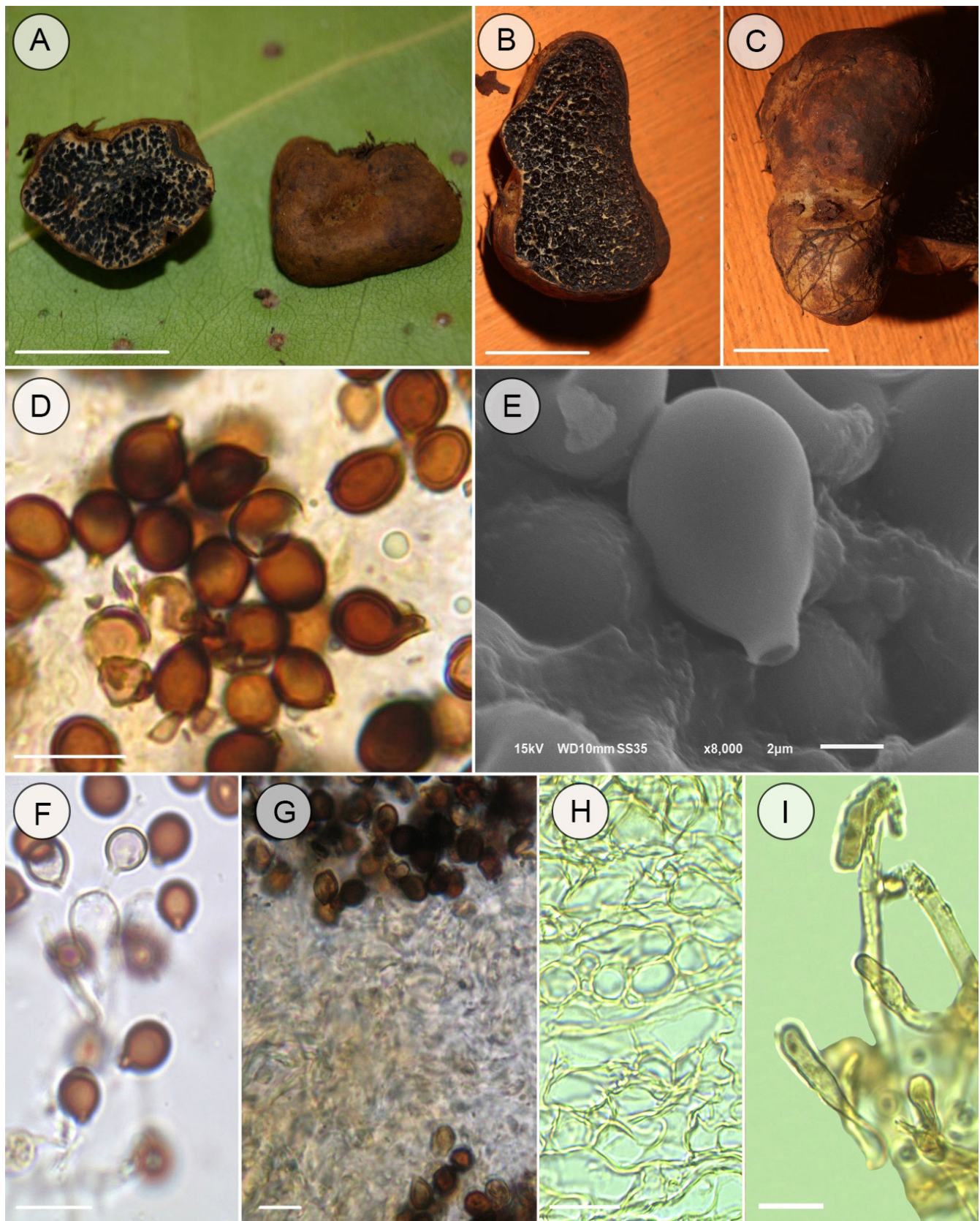
10  $\mu\text{m}$  (Q=1.1-1.7, L= 9.9, W=7.5, N=30), hyaline when immature, becoming dark brown, smooth, with conspicuous hilar appendage, 0.5-1.5  $\times$  1.4-2  $\mu\text{m}$ , cylindrical and hollow, sometimes cracked, giving to the hilar appendage a spiny appearance in optical microscopy, thick-walled.

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  $\mu$ m (E); 10  $\mu$ m (D,F,G,H,I).





**Figure 4:** Type locality of *Melanogaster coccolobae* de la Fuente, J. García & Guevara, with *Coccoloba spicata* Lundell.

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  $\mu\text{m}$ ) 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  $\mu\text{m}$  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 basidiomatas 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-

servation 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* Pegler, *Tremelloscypha gelatinosa* (Murrill) Oberw. & K. Wells, *Scleroderma bermudense* Coker, *Inocybe xerophytica* Pegler, *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.

## Funding

This project was supported by the Tecnológico Nacional de México-Instituto Tecnológico de Ciudad Victoria.

## Acknowledgements

The first author thanks Pablo Alvarado, León Ibarra, Anahí Hernández, Emilio Pérez, and Beni Arana for molecular and technical support. Martínez-González thanks Laura Márquez and Nelly López, Laboratorio Nacional de Biodiversidad (LaNaBio) of the Instituto de Biología, Universidad Na-

cional Autónoma de México for sequencing. To the Consejo Nacional de Ciencia y Tecnología (CONACYT) and El Colegio de la Frontera Sur (ECOSUR-Chetumal) for providing access to the scanning electron microscope.

## Literature cited

- Alvarado, P., J. Cabero, D. Moreno-Mateos, A. Vizzini, J. Alonso, R. Lebeuf, J. L. Siquer and J. M. Vidal. 2021. Phylogenetic relationships among false truffle genera of Paxillaceae—*Alpova*, *Melanogaster*, *Neopalova*, and *Paralpova*, gen. nov. *Mycologia* 113(4): 828-841. DOI: <https://doi.org/10.1080/00275514.2021.1911552>
- Binder, M. and D. S. Hibbett. 2006. Molecular systematics and biological diversification of Boletales. *Mycologia* 98(6): 971-981. DOI: <https://doi.org/10.3852/mycologia.98.6.971>
- Castellano, M. A., J. M. Trappe, Z. Maser and S. Maser. 1986. Key to Spores of the Genera of Hypogeous Fungi of North America, with Reference to Animal Mycophagy. Mad River Press. Eureka, USA. 185 pp.
- Cázares, E., J. García, J. Castillo and J. M. Trappe. 1992. Hypogeous Fungi from Northern Mexico. *Mycologia* 84(3): 341-359. DOI: <https://doi.org/10.2307/3760186>
- Cázares, E., G. Guevara, J. García and J. M. Trappe. 2008. *Melanogaster minisporus* sp. nov. A new sequestrate member of the Boletales from Mexico. *Revista Mexicana de Micología* 28: 67-69.
- Cubeta, M. A., E. Ehandi, T. Abernethy and R. Vilgalys. 1991. Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. *Phytopathology* 81(11): 1395-1400. DOI: <https://doi.org/10.1094/Phyto-81-1395>
- de la Fuente, J. I., O. Ayala-Vasquez, F. Garza-Ocañas, C. Y. López and J. García-Jiménez. 2018. Some interesting Boletales (Basidiomycota) from Quintana Roo, Mexico. *Scientia Fungorum* 48: 77-86. DOI: <https://doi.org/10.33885/sf.2018.48.1201>
- Frandsen, P. B., B. Calcott, C. Mayer and R. Lanfear. 2015. Automatic selection of partitioning schemes for phylogenetic analyses using iterative *k*-means clustering of site rates. *BMC Evolutionary Biology* 15(1): 1-17. DOI: <https://doi.org/10.1186/s12862-015-0283-7>
- García-Jiménez, J. and F. Garza-Ocañas. 2001. Conocimiento de los hongos de la familia Boletaceae de México. *Ciencia UANL* 4(3): 336-343.



- Gardes, M. and T. D. Bruns. 1993. ITS primers with enhanced specificity for Basidiomycetes -application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2(2): 113-118. DOI: <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- Grubisha, L. C., J. M. Trappe, R. Molina and J. W. Spatafora. 2001. Biology of the ectomycorrhizal genus *Rhizopogon*. V. Phylogenetic relationships in the Boletales inferred from LSU rDNA sequences. *Mycologia* 93(1): 82-89. DOI: <https://doi.org/10.1080/00275514.2001.12061281>
- Halász, K. 2009. Különböző stressztűrő-képességű nagyombanemzetségek Kárpát-edencei leletanyagának molekuláris azonosítása és rendszerezése. *Mikológiai közlemények, Clusiana* 48(2): 245-250.
- Hasselquist, N. J., G. W. Douhan and M. F. Allen. 2011. First report of the ectomycorrhizal status of boletes on the Northern Yucatan Peninsula, Mexico determined using isotopic methods. *Mycorrhiza* 21(6): 456-471. DOI: <https://doi.org/10.1007/s00572-010-0355-x>
- Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8): 754-755. DOI: <https://doi.org/10.1093/bioinformatics/17.8.754>
- Katoh, K. and D. M. Standley. 2013. MAFFT Multiple Sequence Alignment Software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30(4): 772-780. DOI: <https://doi.org/10.1093/molbev/mst010>
- Katoh, K., J. Rozewicki and K. D. Yamada. 2017. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20(4): 1160-1166. DOI: <https://doi.org/10.1093/bib/bbx108>
- Katoh, K., K. Misawa, K. Kuma and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14): 3059-3066. DOI: <https://doi.org/10.1093/nar/gkf436>
- Kornerup, A. and H. Wanscher. 1978. *Methuen Handbook of Colour*, 7th Edition. Eyre Methuen. London, UK. 227 pp.
- Lanfear, R., B. Calcott, D. Kainer, C. Mayer and A. Stamatakis. 2014. Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology* 14: 1-14(1). DOI: <https://doi.org/10.1186/1471-2148-14-82>
- Lanfear, R., P. B. Frandsen, A. M. Wright, T. Senfeld and B. Calcott. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34(3): 772-773. DOI: <https://doi.org/10.1093/molbev/msw260>
- Matheny, P. B., Z. Wang, M. Binder, J. M. Curtis, Y. W. Lim, R. H. Nilsson, K. W. Hughes, V. Hofstetter, J. F. Ammirati, C. L. Schoch, E. Langer, G. Langer, D. J. McLaughlin, A. W. Wilson, T. Frøslev, Z. W. Ge, R. W. Kerrigan, J. C. Slot, Z. L. Yang, T. J. Baroni, M. Fischer, K. Hosaka, K. Matsuura, M. T. Seidl, J. Vauras and D. S. Hibbett. 2007. Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* 43(2): 430-451. DOI: <https://doi.org/10.1016/j.ympev.2006.08.024>
- Miller Jr. O. K. and H. H. Miller. 1988. *Gasteromycetes morphological and development features with keys to orders, families, and genera*. Mad River Press. California, USA. 157 pp.
- Miller Jr. O. K., D. J. Lodge and T. J. Baroni. 2000. New and interesting ectomycorrhizal fungi from Puerto Rico, Mona, and Guana Islands. *Mycologia* 92(3): 558-570.
- Montecchi, A. and M. Sarasini. 2000. *Funghi Ipogei d'Europa*. Associazione Micologica Bresadola, Fondazione Centro Studi Micologici. Vicenza, Italy. 714 pp.
- Moreau, P. A., S. Welti, B. Perić, P. Jargeat, S. Manzi and A. Vizzini. 2013. *Alpova komoviana* (Boletales, Paxillaceae), a new sequestrate fungus from Montenegro, with a revised phylogeny of the genus in Europe. *Mycological Progress* 12(1): 109-119. DOI: <https://doi.org/10.1007/s11557-012-0818-x>
- Moreau, P. A., J. Rochet, F. Richard, F. Chassagne, S. Manzi and M. Gardes. 2011. Taxonomy of *Alnus*-Associated Hypogeous Species of *Alpova* and *Melanogaster* (Basidiomycota, Paxillaceae) in Europe. *Cryptogamie Mycologie* 32(1): 33-62. DOI: <https://doi.org/10.7872/crym.v32.iss1.2012.033>
- Müller, K., D. Quandt, J. Müller and C. Neinhuis. 2005. PhyDE®-Phylogenetic data editor. Program distributed by the authors, version 10.0. <https://www.phyde.de> (accessed 2 March, 2021).
- Mullis, K. B. and F. A. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155: 335-350. DOI: [https://doi.org/10.1016/0076-6879\(87\)55023-6](https://doi.org/10.1016/0076-6879(87)55023-6)

- Murray, M. G. and W. F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8(19): 4321-4326. DOI: <https://doi.org/10.1093/nar/8.19.4321>
- Pegler, D. N. 1983. *Agaric flora of the Lesser Antilles*. Her Majesty's Stationery Office. London, UK. 668 pp.
- Pegler, D. N. and J. P. Fiard. 1979. Taxonomy and Ecology of *Lactarius* (Agaricales) in the Lesser Antilles. *Kew Bulletin* 33(4): 601-628. DOI: <https://doi.org/10.2307/4109804>
- Pegler, D. N., B. M. Spooner and T. W. K. Young. 1993. *British Truffles: Revision of British Hypogeous Fungi*. Royal Botanical Gardens, Kew. London, UK. 215 pp.
- Pöhlme, S., M. Bahram, U. Köljalg and L. Tedersoo. 2017. Biogeography and Specificity of Ectomycorrhizal Fungi of *Coccoloba uvifera*. In: Tedersoo, L. (ed.). *Biogeography of Mycorrhizal Symbiosis. Ecological studies (Analysis and Synthesis)*. Vol. 230, Springer. Cham, Switzerland. 345-359 Pp. DOI: [https://doi.org/10.1007/978-3-319-56363-3\\_16](https://doi.org/10.1007/978-3-319-56363-3_16)
- Rambaut, A. 2014. FigTree version 1.4.2. Available at: <http://tree.bio.ed.ac.uk/software/figtree/> (consulted March, 2021).
- Rambaut, A., M. A. Suchard, D. Xie and A. J. Drummond. 2014. Tracer version 1.6. <http://beast.bio.ed.ac.uk/Tracer> (consulted March, 2021).
- Rinaldi, A. C., O. Comandini and T. W. Kuyper. 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. *Fungal Diversity* 33: 1-45.
- Rochet, J., P. A. Moreau, S. Manzi and M. Gardes. 2011. Comparative phylogenies and host specialization in the alder ectomycorrhizal fungi *Alnicola*, *Alpova* and *Lactarius* (Basidiomycota) in Europe. *BMC Evolutionary Biology* 11: 1-14. DOI: <https://doi.org/10.1186/1471-2148-11-40>
- Séne, S., R. Avril, C. Chaintreuil, A. Geoffroy, C. Ndiaye, A. G. Diédhiou, O. Sadio, R. Courtecuisse, S. N. Sylla, M. A. Selosse and A. Bâ. 2015. Ectomycorrhizal fungal communities of *Coccoloba uvifera* (L.) L. mature trees and seedlings in the neotropical coastal forests of Guadeloupe (Lesser Antilles). *Mycorrhiza* 25(7): 547-559. DOI: <https://doi.org/10.1007/s00572-015-0633-8>
- Singer, R., I. Araujo and M. H. Ivory. 1983. Ectotrophically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia. *BeihefteZur Nova Hedwigia* 77(2): 7-335.
- Singer, R. and J. H. Morello. 1960. Ectotrophic forest tree mycorrhizae and forest communities. *Ecology* 41(3): 549-551. DOI: <https://doi.org/10.2307/1933331>
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313. DOI: <https://doi.org/10.1093/bioinformatics/btu033>
- Tedersoo, L., T. W. May and M. E. Smith. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* 20: 217-263. DOI: <https://doi.org/10.1007/s00572-009-0274-x>
- Trappe, J. M. 1975. Revision of the genus *Alpova* with notes on *Rhizopogon* and the *Melanogastraceae*. *Nova Hedwigia Beiheft* 51: 279-310.
- Trappe, J. M. and G. Guzmán. 1971. Notes on some hypogeous fungi from Mexico. *Mycologia* 63(2): 317-332. DOI: <https://doi.org/10.2307/3757764>
- Trappe, J. M., R. Molina, D. L. Luoma, E. Cázares, D. Pilz, J. E. Smith, M. A. Castellano, S. L. Miller and M. J. Trappe. 2009. *Diversity, Ecology, and Conservation of Truffle Fungi in Forests of the Pacific Northwest*. General Technical Reports PNW-GTR-772-Department of Agriculture, Forest Service, Pacific Northwest Research Station. Portland, USA. 194 pp.
- Vilgalys, R. and M. Hester. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 72(8): 4238-4246. DOI: <https://doi.org/10.1128/jb.172.8.4238-4246.1990>
- Vizzini, A., M. Zotti, S. Ryman and S. Ghignone. 2010. Typification of *Octaviania rubescens* (Paxillineae, Boletales) and phylogenetic hypotheses for genus *Alpova*. *Mycologia* 102(4): 967-975. DOI: <https://doi.org/10.3852/09-256>
- White, T. J., T. D. Bruns, S. Lee and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., D. H. Gelfand, J. Sninsky and T. J. White (eds.). *PCR protocols: a guide to methods and applications*. Academic Press. San Diego, USA. 315-322 Pp.
- Zeller, S. M. and C. W. Dodge. 1936. *Melanogaster*. *Annals of the Missouri Botanical Garden* 23(1): 639-655.



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

## Appendix: Continuation.

Species name	Isolate/Voucher/strain	Locality	GenBank Accessions		
			ITS	nrLSU	<i>rpb2</i>
<i>Melanogaster rivularis</i> P.-A. Moreau & F. Rich.	08090514	France	NR132848	---	---
<i>Melanogaster</i> sp	Ecu344	France	KF472137	---	---
<i>Melanogaster</i> sp	Ecu313	France	KF472152	---	---
<i>Melanogaster tuberiformis</i> Corda	1295	Romania	AJ555527	---	---
<i>Melanogaster cf tuberiformis</i> Corda	48368	Hungary	AJ555521	---	---
<i>Melanogaster variegatus</i> (Vittad.) Tul. & C. Tul.	1688	Hungary	AJ555523	---	---
<i>Melanogaster variegatus</i> (Vittad.) Tul. & C. Tul.	1438	Hungary	AJ555524	---	---
<i>Melanogaster vittadinii</i> Soehner & Knapp	33090	Hungary	AJ555525	---	---
<i>Nealpova arenicola</i> Cabero & P. Alvarado	49153 Holotype	Spain	MN594292	MN594304	MN594775
<i>Nealpova montecchii</i> Cabero, P. Alvarado & Vizzini	51272 Holotype	Spain	MN594296	MN594306	MN594777
<i>Nealpova rubescens</i> (Vittad.) Vizzini	3003022	Spain	MT259304	---	---
<i>Paragyrodon sphaerosporus</i> (Peck) Singer	MB06-066	USA	GU187540	GU187593	GU187803
<i>Paxillus vernalis</i> Watling	AFTOL-715	China	DQ647827	AY645059	---
<i>Paralpova artikutzensis</i> Cabero, D. Moreno-Mateos & P. Alvarado	49154 Holotype	Spain	MN594297	---	MN594778
<i>Paxillus adelphus</i> J.-P. Chaumeton, Gryta, Jargeat & P.-A. Moreau	Pf01	Germany	EU084664	AF167680	---
<i>Paxillus involutus</i> (Batsch) Fr.	Bel10.4	France	KF261366	---	JQ436854
<i>Paxillus rubicundulus</i> P.D. Orton	2905	U.K.	KU163502	FM993114	---
<i>Paxillus vernalis</i> Watling	MB-062 Holotype	China	DQ647827	AY645059	---