



Guapirioid ectomycorrhiza: a novel fungus-plant subtype is described associated to *Guapira opposita* (Nyctaginaceae) in the Brazilian restinga

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Abstract

Aim of study: Despite its recognized ecosystemic importance, knowledge about mycorrhizal associations in Brazil is still dearth, and the diversity of the native mycorrhizal fungi remains unknown. In this study, we characterized morpho-anatomically and molecularly the ectomycorrhizae found associated with *Guapira opposita* in the restinga, vegetation established on sandy soil.

Area of study: Coastal of the Atlantic Forest of southern Brazil, Florianópolis: Parque Natural Municipal das Dunas da Lagoa da Conceição, Monumento Natural Municipal da Lagoa do Peri and Parque Natural Municipal da Lagoa do Jacaré das Dunas do Santinho.

Materials and methods: The ectomycorrhizae found were morphotyped and described according to standard criteria. Plant and fungi were molecularly identified using sequences of the ITS region of rDNA.

Main results: Ten morphotypes were identified associated with *G. opposita*, including the native threatened species *Austroboletus festivus*. Members of the clade /tomentella-thelephora were the most representative in our study, with six taxa. Based on particular characteristics, such as short, and simple or long and thin branched ectomycorrhizal systems, close connections between the layered ectomycorrhizal mantle and the cortical root cells, absence of a Hartig net and other fungal elements in the cortex, we propose the name 'Guapirioid ectomycorrhiza' for this new morphology.

Research highlights: The results presented in this work confirm the presence of ectomycorrhizae in the restinga and suggest the existence of a diversity, above and below-ground, much greater than previously known. Furthermore, our data confirm the morphological and possibly ecological differences of tropical ectomycorrhizae from those known from temperate forests.

Additional key words: Atlantic Forest; Basidiomycota; Brazilian Funga; diversity; mycorrhizal symbiosis; nurse plant.

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Introduction

Mycorrhizal fungi establish symbioses with approximately 95% of the plants, stimulating plant growth, drawing nutrients from the soil, and making plants less susceptible to environmental stresses such as drought and salinity (Dighton, 2016). Based on morpho-anatomical structural features, several types of mycorrhizal symbioses are recognized (Martin et al., 2016), with four main fungal groups forming the two major associations: arbuscular mycorrhizae (AM) and ectomycorrhizae. The AM are formed mainly by the Glomeromycota and have emerged with early land plants (Schüßler et al., 2001), while fungi forming ectomycorrhizae have a more recent evolutionary history and are spread across multiple lineages of Mucoromycotina, Ascomycota, and Basidiomycota (Brundrett & Tedersoo, 2018). Although much information has been produced about taxa that form AM associations, there is less knowledge about ectomycorrhizae, especially in tropical and neotropical ecosystems (Corrales et al., 2018). In ectomycorrhiza, Hartig net and fungal mantle are the main structural characteristics, but these may be incompletely developed or patchy, as often seen in the ectomycorrhizae of herbs and shrubs (Smith & Read, 2008).

Investigations with ectomycorrhizal fungi in native ecosystems in Brazil began in the 1960s with Singer's research in the Amazon (Singer & Morello, 1960; Singer et al., 1983). After these, other studies continued to reveal the diversity of ectomycorrhizal fungi in Brazilian forests (Roy et al., 2016; Sulzbacher et al., 2020). Still, the relative scarcity of knowledge about ectomycorrhizae and many other belowground interactions stress the need for new research in the neotropics.

It is likely that in neotropical ecosystems, ectomycorrhizae optimize plant fitness and enhance their nutrient acquisition and allow the exchange of nutrients and signals between plants through common mycelium network (Huang et al., 2019). This is especially true where there are conditions limiting the decomposition process (Smith & Read, 2008), such as the restinga, an ecosystem that occupies 79% of the coastal Atlantic Forest. Restinga has sandy, acidic and nutrient-poor soils, often with high salinity and high irradiance (SOS Mata Atlântica, 2021). Several plant species manage to establish and survive in the restinga, and ectomycorrhizal fungi may be a key component that allows it (Weidlich et al., 2020).

Restinga and mangroves are essential as a buffer between the ocean and the forest, and to shelter many living beings, serving as habitat and refuge for several species in at least one part of their life cycles (Steiner et al., 2015). The restinga also stores rainwater, controls flooding and regulates the water cycle (Lessa et al., 2000). Sadly, anthropic activities are negatively affecting the diversity and functionality of the ectomycorrhizal community in forest soils due to soil erosion, changes in land use, inorganic toxins, fire, and invasion of introduced

non-native plants (Asmelash et al., 2016). Recently, the 'Parque Municipal Natural das Dunas da Lagoa da Conceição', one of the largest fragments of restinga in Florianópolis, Santa Catarina, Brazil, and one of the places where we carried out this study, suffered an environmental disaster. The disruption of a water and sewage treatment system released excess organic matter and a large amount of nitrogen and phosphorus in the area, leaving the ecosystem out of balance.

Guapira opposita (Vell.) Reitz (*Nyctaginaceae*) is a polymorphic species with a wide distribution in Brazil. In southern Brazil, the species grows in the rainforest and in the restinga within the Atlantic Forest domain (Magnago et al., 2012). Members of the species can reach 25 m tall but are often shorter when growing in shallow or rocky soils. In restinga, plants of *G. opposita* grow as short trees and have leaves that are slightly shorter and more coriaceous than the plants growing in the rainforest. The fruits are fleshy, dark red to purple, and attract birds that act as dispersers (Reitz, 1970). *Guapira opposita* acts as nurse plant (Castanho et al., 2012), promoting the growth of other plants around itself and therefore contributing to regeneration processes in restinga by increasing biodiversity and helping to maintain other plant species (Dalotto et al., 2018).

Since the restinga constitutes a fragmented ecosystem marked by an intense and accelerated process of deforestation and territorial decrease (SOS Mata Atlântica, 2021), studies that aim to understand the fungi of this ecosystem are important to help develop conservation and restoration projects. Indeed, a more extensive knowledge of ectomycorrhizal symbiosis in the restinga might be pivotal for its ecological restoration, as recently noted by Weidlich et al. (2020). In this work, we present the morpho-anatomical and genetic characterization of Guapirioid ectomycorrhizae formed between *G. opposita* and the fungi from restinga, including new records of symbiotic associations in the Atlantic Forest.

Material and methods

Collecting site and fungal sampling

Collections of *G. opposita* roots and fungi basidiomata were made between October 2017 and May 2019 in three restinga areas in Florianópolis, Brazil: 'Parque Natural Municipal das Dunas da Lagoa da Conceição' (-27.694028, -48.506587), 'Monumento Natural Municipal da Lagoa do Peri' (-27.728243, -48.510175) and 'Parque Natural Municipal da Lagoa do Jacaré das Dunas do Santinho' (-27.467783, -48.393395). The vegetation in these areas is associated with Quaternary coastal sand deposits and rocky coastal habitats (Magnago et al., 2012) and grows on sandy soil between the ocean and forest.

Besides *G. opposita*, members of the following potential ectomycorrhizal families grow in restinga: *Fabaceae*, *Moraceae*, *Myrtaceae*, *Nyctaginaceae*, *Polygonaceae*, and *Salicaceae* (Binfaré & Falkenberg, 2017). Soil samples (approx. 20 cm³) were collected below the basidiomata and left in water overnight. The isolated roots were further cleaned and carefully selected under a stereomicroscope. The roots that had an ectomycorrhizal mantle were morphotyped. From each root system with a mantle, several tips were transferred to 70% ethanol and stored at -20°C for subsequent DNA extraction. Also, part of the root system with the mantle was fixed in 4% glutaraldehyde for morphological analyses. Voucher material of the basidiomata, mycorrhizae and the plants are deposited in the FLOR herbarium and fungarium and the permanent collection of the mycology laboratory (Micolab) at the Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brazil.

The basidiomata were identified by comparing them with the morphology described in the literature (Menolli Jr. et al., 2009; Magnago & Neves, 2014) and by using DNA barcoding of the ITS region. The specimens were collected next to *G. opposita* plants. After making morpho-anatomical analyses, the basidiomata were dried at 40 °C for further preservation. The identification of the plant was made by a botanist and confirmed by DNA barcoding (ITS region) (Cheng et al., 2015).

Morpho-anatomical analysis of mycorrhizae

Mantle preparations of ectomycorrhizae fixed in glutaraldehyde were made under a Leica MZ6 stereomicroscope and fixed on microscope slides for observation under a light optical microscope. Observations and descriptions were made with a Zeiss Axioplan 2 microscope, at 1000 × total magnification, at the University of Cagliari, Italy. Images were taken using a digital camera attached to the microscope. Illustrations of microscopic features from the mantle layers were based on observations under the light microscope. The morphotype descriptions followed the standard methodology and terminology used for studying ectomycorrhizae (Agerer, 1991; 1995). Munsell Soil Colour Charts (Munsell Color Co., Grand Rapids, MI, USA) were used as references to describe the colors of ectomycorrhizae. The ectomycorrhizae descriptions are based on 6-10 root tips of each morphotype recorded.

The anatomical analyses of the ectomycorrhizae were conducted at the University of L'Aquila (UnivAq), Italy. For these, mycorrhizal tips were embedded in LR White resin (Multilab Supplies, Surrey, UK), sectioned at 2.5 µm thick with a ultramicrotome (Ultracut R, Leica), stained with 1% toluidine blue in 1% sodium borate buffer for 10-15 s at 60°C, observed with a microscope, and photographed using a digital camera. Ultrathin sections (60-80 nm) were made with a diamond knife, mounted on

copper grids, and stained with UranylLess EM (Electron Microscopy Sciences, Hatfield, PA, USA). The sections were examined using a transmission electron microscope (CM100, Philips, Eindhoven, Netherlands) operating at 80 kV and photographed with a digital camera (PHURONA, Emsis, Münster, Germany).

Molecular and phylogenetic analysis

The molecular characterization of the ectomycorrhizae was done at Laboratório Multiusuário de Estudos de Biologia, Florianópolis, Brazil. We accessed fungal DNA (from the basidiomata and ectomycorrhizal mantle) using two approaches:

1) Genomic DNA was extracted from the basidiomata using a PowerPlant®Pro DNA Isolation Kit (MO BIO Laboratories, Inc.), following the manufacturer's protocol adapted for fungi. The internal transcribed spacer of ribosomal nuclear DNA (nrITS) region was amplified using the primers ITS1F and ITS4 (Gardes & Bruns, 1993) and the following cycling parameters: an initial denaturation at 94°C for 2 min; 40 cycles of 30 s at 94°C, 45 s at 55°C and 1 min at 72°C; and a final extension at 72°C for 7 min.

2) A direct PCR approach was applied to amplify the ITS region from the ectomycorrhizal tips isolated from soil samples (Iotti & Zambonelli, 2006). The ectomycorrhizal tips were selected and directly amplified using the same pair of primers (ITS1F/ITS4) (Gardes & Bruns, 1993). Two microliters of 20 mg/mL BSA (bovine serum albumine) solution was added to each reaction tube to prevent PCR inhibition. The parameters applied to the PCR cycles followed Leonardi et al. (2013).

To identify the plant host, plant DNA was extracted from the ectomycorrhizal root tips using an isolation kit (see above), and the barcoding region (ITS) for the plant was amplified using the ITS-u1 and ITS-u4 primer pair (Cheng et al., 2015) and the following cycle parameters: an initial denaturation at 94°C for 4 min; 34 cycles of 30 s at 94°C, 40 s at 55°C and 1 min at 72°C; and a final extension at 72°C for 10 min. The DNA extracted from *G. opposita* leaves was used as a positive control. PCR products were purified using PEG (polyethylene glycol, Sambrook et al., 1989). Sanger sequencing was performed with a BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) at the company Myleus Biotecnologia, in Minas Gerais, Brazil, following the manufacturer's instructions and using the same primers cited above for the correspondent symbionts. When needed, the generated sequences and their respective chromatograms were manually edited with Geneious R9 (Kearse et al., 2012).

The consensus sequences were used as operational taxonomic units (OTUs) and they were accessed into Genbank. The taxonomic identity of the newly generated sequences from *G. opposita* leaves, basidiomata and the

Table 1. Identification of ectomycorrhizal morphotypes by their phylogenetic affinity in GenBank.

Species	Accession No.	Voucher	E ^[1]	%Id ^[2]	Best BLAST match	Symbiont of best match (potential)	Location of best match
<i>Amanita viscidolutea</i>	MW000471	M70	0.0	99.37	<i>Amanita</i> sp. (MN123998)	-	Brazil
<i>Austroboletus festivus</i>	OP819290	M41	0.0	97.56	<i>Austroboletus festivus</i> (KY886203)	-	Brazil
<i>Inocybe</i> sp.	OP819291	M51	0.0	90.20	Uncultured <i>Inocybe</i> (JN663843)	<i>Dicymbe corimbosa</i>	Guyana
			2e-174	87.68	<i>Inocybe amazoniensis</i> (JN642232)	<i>Dicymbe</i> sp.	Guyana
<i>Thelephoraceae</i>	OP819292	M53	0.0	96.71	Uncultured <i>Thelephoraceae</i> (AY667418)	<i>Neea</i> sp.	Ecuador
<i>Tomentella</i> sp1	OP819288	M36	0.0	90.25	<i>Tomentella longisterigmata</i> (MG136837)	-	Finland
			0.0	91.38	<i>Tomentella</i> sp. (KX398760)	<i>Coccoloba uvifera</i>	Caribbean region
<i>Tomentella</i> sp2	OP819289	M36'	0.0	91.32	Uncultured fungus (LC622630)	<i>Pinus tabuliformis</i>	China
			0.0	90.52	<i>Tomentella</i> sp. (MW027950)	<i>Quercus pubescens</i>	-
<i>Tomentella</i> sp3	OP819293	M57	2e-157	91.79	Uncultured <i>Tomentella</i> cf. <i>stuposa</i> (FJ013054)	<i>Pinus pinaster</i>	Spain
			1e-154	91.67	Uncultured fungus (KT289957)	<i>Dicymbe corimbosa</i>	Guyana
<i>Tomentella</i> sp4	OP819286	M30	0.0	92.73	<i>Tomentella</i> sp. (JX548271)	<i>Pisonia taina</i>	Puerto Rico
<i>Tomentella</i> sp5	OP819287	M34	0.0	97.85	Uncultured ectomycorrhizal fungus - <i>Tomentella</i> (FN557576)	<i>Coccoloba</i> sp.	Ecuador
			0.0	92.75	<i>Tomentella</i> sp. (KF472145)	<i>Coccoloba uvifera</i>	France/ Guadeloupe
<i>Tomentella</i> sp6	OP819294	M68	0.0	92.99	<i>Fungal</i> sp. (MG761298)	<i>Pinus ponderosa</i>	USA
			0.0	93.40	Uncultured <i>Tomentella</i> sp. (EU625804)	<i>Corallorhiza odontorhiza</i>	USA

^[1] e-value: indicates the probability of similarity in the identification between the sequences generated in our study and the GenBank sequences.

^[2] % Id: percentage of similarity between the sequences generated in our study and the GenBank sequences.

ectomycorrhizal roots with the morphotypes was determined by phylogenetic affinity of the consensus sequences with homologous sequences from Genbank database (<https://www.ncbi.nlm.nih.gov/nucleotide/>) using a Blast search (Altschul et al., 1990). New sequences generated during

this work were included in GenBank (Sayers et al., 2009) and the accession numbers for all sequences used are shown in Table 1. Sequenced ectomycorrhizal vouchers are in bold in the section “Studied material” following the description of the equivalent Guapirioid ectomycorrhiza.

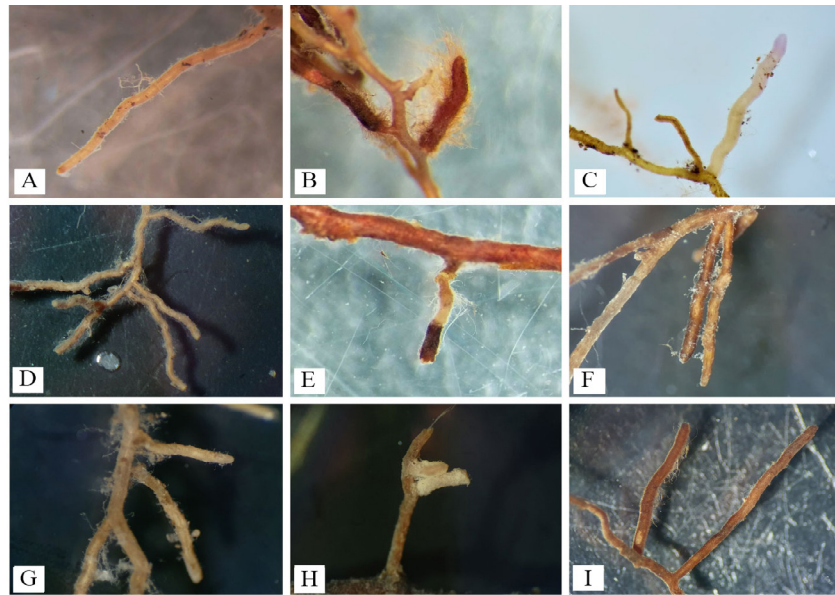


Figure 1. Ectomycorrhizal morphotypes associated with *Guapira opposita* roots in the restinga of Santa Catarina Island, Brazil. A, *Amanita viscidolutea* (morphotype M70). B, *Austroboletus festivus* (M41). C, *Inocybe* sp. (M51). D, *Thelephoraceae* (M53). E, *Tomentella* sp1 and *Tomentella* sp2 (M36 and M36', respectively). F, *Tomentella* sp3 (M57). G, *Tomentella* sp4 (M30). H, *Tomentella* sp5 (M34). I, *Tomentella* sp6 (M68).

Results

Ten ectomycorrhizal morphotypes were found associated with *G. opposita* roots corresponding to Basidiomycota taxa (Fig. 1), based on the barcoding (molecular studies). The best represented genus was *Tomentella* with six taxa (Table 1). Some interesting findings of this work include the collection of *Amanita viscidolutea* and *Austroboletus festivus* (Fig. 2) basidiomata, two native species from the restinga of the Atlantic Forest.

Description of ectomycorrhizae

— *Amanita viscidolutea* Menolli, Capelari & Baseia (Figs. 1A, 3A).

For a complete description of the ectomycorrhiza of *A. viscidolutea* on *G. opposita*, see Furtado et al. (2022).

— *Austroboletus festivus* (Singer) Wolfe (Fig. 1B)

Morphological characters: Mycorrhizal system simple, unramified, mostly dense and long, short-distance explora-



Figure 2. Basidiomata of *Austroboletus festivus* collected in restinga in Santa Catarina Island, Brazil.

tion system. *Main axis* 1.5-4.0(-7.0) mm long and 0.6-0.9 mm in diameter, sometimes tortuous, but mostly straight to bent. *Mantle surface* grainy to woolly, with some portions stringy, strong brown (HUE 7.5YR 4/6 and 5/8) with yellowish red parts (HUE 5YR 4/6). Older parts dusky red (HUE 10R 3/4). *Hyphal strands* not observed. *Emanating hyphae* abundant. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* plectenchymatous, a net of branching hyphae in a regular ring-like arrangement. Hyphae densely incrustated with small crystals, rough, frequently branched, hiding any deeper structure, only some hyphal tips or cystidia-like end-cells visible at the margins. Hyphae (3.5-)4.0-4.6(-6.5) μm thick, brownish yellow,

hyphal walls slightly thick, less than 1 μm thick; clampless. *Middle mantle layer* as a transitional type between plectenchymatous and pseudoparenchymatous, irregularly shaped hyphae from a coarse net, hyphae brownish yellow, 6.0-12 μm thick, thin-walled, cell-walls slightly rough, some crystals, anastomoses mostly with a septum. *Inner mantle layer* pseudoparenchymatous, hyphae rusty brown, shape of the hyphae variable, 4-5 cells in a square of 20 \times 20 μm , 7.0-15 μm long, 5.0-6.5 μm in diameter, slightly thick-walled, cell walls often agglutinated, lacking crystals, anastomoses open.

Anatomical characters of emanating elements: *Hyphal strands* lacking. *Emanating hyphae* abundant, rusty brown, long, 4.0-5.5 (6.0) μm in diameter, ramification

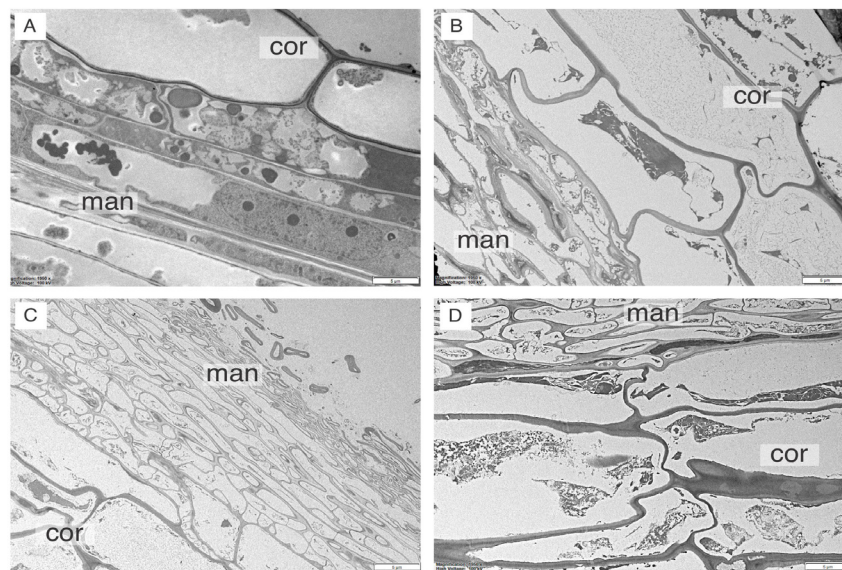


Figure 3. Longitudinal section of *Guapira opposita* ectomycorrhizae with *Amanita viscidolutea* (A), *Austroboletus festivus* (B), *Tomentella* sp1. (C) and *Tomentella* sp2. (D) showing the conspicuous ectomycorrhizal mantles, with the particularities of each species, covering the cortical cells of host plant root, without the establishment of the Hartig net. No intercellular or intracellular fungal penetration can be observed in the root cortex. Cor = cortical layer; Man = mantle. Bars: 5 μm .

Y-shaped frequent, clampless; cell walls densely incrustated with small colorless crystals, cell walls up to 1.0 thick; awl-shaped, apical ends simple to bent, walls at the tips thick as remaining walls; diverticulate hyphae frequent. *Cystidia* like end-cells, clavate to cylindrical, abundant, (10-)12-19(-22) \times (4.0-)6.0-7.0(-7.8) μm , slightly thick-walled, colorless to light yellow; cell walls rough, mostly lacking crystals, but less dense and smaller crystal layer can also be found (Fig. 4A).

Anatomical characters, longitudinal section: Mantle (10-)15-18(-25) μm thick, at the very tip up to 20 μm thick, plectenchymatous throughout, very compact, no layers discernable; hyphal cells roundish, 5.0-6.0(-7.0) \times 2.0-3.5 μm , slightly thick-walled (up to 1.0 μm). Tannin cells

not observed. Cortical cells non-differentiated, in 2-3(-4) rows, elongated, (40-)50-75(-83) \times (13-)15-23(-25) μm , tangentially oriented. Hartig net not present (Fig. 3B).

Studied material: BRAZIL. Santa Catarina: Florianópolis, Monumento Natural Municipal da Lagoa do Peri, -27.728243, -48.510175, 03 Feb 2019, A.N.M. Furtado (M41(A)R208, M41(B)R209, **M41(C)R210**, M41(D)R211, M41(E)R212, M41(F)R213, M41(G)R214, M41(H)R217, M41(I)R218, M41(J)R220, M41(K)R277, M41(L)R287).

— *Inocybe* sp. (Fig. 1C)

Morphological characters: Mycorrhizal system simple, unramified, contact exploration system. *Main axis*

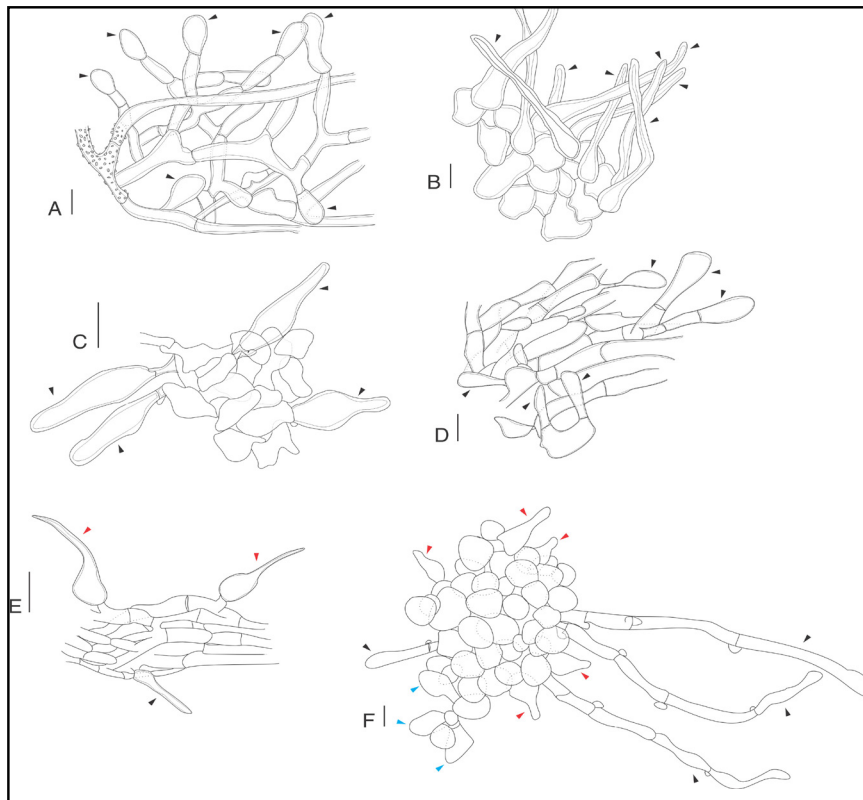


Figure 4. Cystidia diversity in the mantle of Guapirioid ectomycorrhizae from the Brazilian restinga. A. Clavate to cylindrical cystidia (black arrows) of *Austroboletus festivus*. B. Bottle-shaped with straight neck cystidia (black arrows) of *Toментella* sp1. C. Lageniform cystidia (black arrows) of *Toментella* sp2. D. Clavate cystidia (black arrows) of *Toментella* sp4. E. Bottle-shaped with straight neck (red arrows) and flask-shaped (black arrow) cystidia of *Toментella* sp5. F. Lageniform (light blue arrows) and bottle-shaped with straight neck (red arrows) cystidia; and cystidia like a normal clamped hypha but often twisted like a cork-screw (black arrows) of *Toментella* sp6. Bars: 10 μ m.

(0.5–)0.7–4.5(–5.5) mm long and 0.1–0.3 mm in diameter, straight, rarely bent. *Mantle surface* smooth, slightly silver, mostly dense, light-yellow brown (HUE 2.5Y 6/4) to light gray (HUE 5YR 7/1); purplish gray (HUE 10PB 8/4) at the very tip. Older mycorrhizae with a uniform pale-yellow color (HUE 2.5Y 7/4). *Hyphal strands* absent. *Emanating hyphae* abundant, not specially distributed. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* plectenchymatous, hyphae rather irregularly arranged, no special pattern discernible, with a gelatinous matrix between the hyphae. Hyphae hyaline, but sometimes membranaceous yellowish, cylindrical, constricted at the septa, abundant oleaginous contents, 4.0–5.0(–7.0) μ m thick, slightly inflated in middle portion, Y-connections frequent, thin-walled, smooth; clampless. *Middle mantle layer* plectenchymatous as well as the outer layer but hyphae often growing in longitudinal directions regarding root orientation. Hyphae cylindrical, hyaline, 3.5–4.5 μ m thick, thin-walled; clampless. *Inner*

mantle layer plectenchymatous, with a ring-like arrangement of hyphae bundles. Hyphae cylindrical, 3.0–4.0(–6.0) μ m thick, sometimes slightly inflated in middle portion, squarrosely branched, smooth, thin-walled; clampless. *Anatomical characters of emanating elements.* *Hyphal strands* lacking. *Emanating hyphae* awl-shaped but with bent tips, sometimes curved (sickled-shaped), (–87)94–112 μ m long, (–3.0)4.0–5.0 μ m thick, membranaceous yellowish brown, smooth, not striking, thin-walled, slightly constricted at the septa, clamped; oleaginous contents covering the surface. *Cystidia* absent.

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Monumento Natural Municipal da Lagoa do Peri, –27.728243, –48.510175, 04 Feb 2019, A.N.M. Furtado (M51(A)R230, M51(B)R232, M51(C)R234, M51(D)R237, M51(E)R240, M51(F)R243, M51(G)R257).

— *Thelephoraceae* sp1 (Fig. 1D)

Morphological characters: *Mycorrhizal system* strongly ramified, irregularly pinnate, 7.0–9.5 mm long, contact

exploration system. *Main axis* 0.15-0.3 mm in diameter. *Mantle surface* smooth, becoming stringy with age, dark reddish brown (HUE 2.5YR 2.5/4) to dark brown (HUE 7.5YR 3/4) in older parts with silvered patches all over the surface. Unramified ends tortuous, beaded in some parts, (2.3–)2.9-4.7 mm long and 0.21-0.27 mm in diameter. *Hyphal strands* absent. *Emanating hyphae* present, abundant, slightly cottony, all over the surface but concentrated in the middle portion of the branch. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* plectenchymatous, with a ring-like arrangement of hyphae bundles. Hyphae 5.0-6.5(–8.0) μm thick, membranaceous brownish yellow, smooth, thick-walled (up to 1.0 μm thick), anastomoses at contact-septum, very agglutinated; clampless. *Middle mantle layer* as a transition between plectenchymatous and pseudoparenchymatous layers. Hyphae irregularly shaped forming a coarse net, (16–)18-22 μm long, 5.0-7.0 μm in diameter, thin-walled; clampless. *Inner mantle layer* loosely plectenchymatous, hyphae arranged net-like. Hyphae membranaceous brownish yellow, smooth, thin-walled, (3.0–)4.0-5.5 μm thick, densely agglutinated; clampless.

Anatomical characters of emanating elements. *Hyphal strands* absent. *Emanating hyphae* hyaline, ornamented with irregularly shaped crystals (except at the apical end), thin-walled, 80-126 μm long, (1.5–)2-2.5 μm in diameter, not ramified; clamped, anastomosis with a clamp, bent at the apical end, strongly glutinous. *Cystidia* lacking.

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Monumento Natural Municipal da Lagoa do Peri, -27.728243, -48.510175, 04 Feb 2019, *A.N.M. Furtado* (M53(A)R235, M53(B)R236, M53(C)R239, M53(D)R243).

— *Tomentella* sp1 (Fig. 1E)

Morphological characters: *Mycorrhizal system* simple, unramified, sometimes with short monopodial pinnate portions, mostly dense, frequently starting from a distinct yellowish-brown mycorrhiza, short-distance exploration system. *Main axis* 3.2-6.0 mm long and 0.2-0.4 mm in diameter, sometimes tortuous, but mostly bent. *Mantle surface* mostly reddish black (HUE 10R 2.5/1) to very dusky red (HUE 10R 2.5/2) and dark reddish brown (HUE 5YR 2.5/2) with some darker grains that give the mantle's spongy texture. *Unramified ends* straight to bent, 0.2-0.3(–1.0) mm long and 0.2-0.3 mm in diameter. *Hyphal strands* not observed. *Emanating hyphae* frequent, sparse over the mantle. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* pseudoparenchymatous, composed by angular to epidermoid cells, of various shapes and dimensions. Hyphae reddish brown to yellowish, 5-6 cells in a square of $20 \times 20 \mu\text{m}$, (9.0–)12-18(–22) μm long, (7.0–)10-15 μm in diameter, slightly thick-walled, smooth, cell walls often agglutinated, aggregating soil particles; clampless. *Middle*

mantle layer very similar to the outer mantle layer but here hyphae are much more branched than in the outer part. *Inner mantle layer* pseudoparenchymatous, more compact and organized than the outer layer, formed by variable shaped cells, some containing droplets, 4-5 cells in a square of $20 \times 20 \mu\text{m}$, 7.0-18(–22) μm long, 5.0-10(–13) μm in diameter, brownish, slightly thick-walled.

Anatomical characters of emanating elements. *Hyphal strands* lacking. *Emanating hyphae* similar to elongated cystidia, 5.0-6.0 μm in diameter, not ramified, clamped; cell walls smooth, thick-walled, yellowish brown, sometimes filled with homogeneous yellow contents; straight, apical ends simple or inflated, sometimes flattened, walls at tips as thick as remaining walls. *Cystidia* abundant, bottle-shaped with straight to bent neck, 59-67(–82) μm long, at basis 7.0-10(–12) μm in diameter and 2.0-3.0 μm in diameter apically, smooth, thick-walled, slightly yellow, clampless (Fig. 4B).

Anatomical characters, longitudinal section: Mantle 35-40(–50) μm thick, hyphae thick-walled, often with two discernible layers: outer layer 22-28 μm thick, formed by densely arranged hyphae, hyphal cells roundish to different shapes, 2.5-6.5 \times 3.5-6.0 μm ; inner layer 7.5-12.5(–15) μm thick, just as compact as the outer layer, hyphae narrow and longitudinally elongated, 12-15 \times 3.7-6.0 μm . Tannin cells not observed. Cortical cells non-differentiated, in 3-4 rows, longitudinally rectangular, (50–)55-70(–82) \times (15–)22-32(–37) μm , tangentially oriented. Hartig net not present (Fig. 3C).

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Monumento Natural Municipal da Lagoa do Peri, -27.728243, -48.510175, 03 Feb 2019, *A.N.M. Furtado* (M36(A)R200, M36(B)R202, M36(C)R203, M36(D)R204, M36(E)R210, M36(F)R212, M36(G)R215, M36(H)R216, M36(I)R220).

— *Tomentella* sp2 (Fig. 1E)

Morphological characters: *Mycorrhizal system* simple, unramified, often making the connection between a distinct dark brown ectomycorrhiza and the root without the mantle, medium-distance exploration system, smooth subtype. *Main axis* 14-25 mm long, 0.2-0.5 mm in diameter, straight to slightly curved. *Mantle surface* frequently stringy, sometimes cottony, olive yellow (HUE 2.5Y 6/6) to olive brown (HUE 2.5 4/3 to 4/4). *Hyphal strands* present, relatively abundant, sparse, branched, connected in a restricted point to the mantle; pale yellow (HUE 2.5Y 8/3). *Emanating hyphae* abundant, like hyphal fans. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* pseudoparenchymatous with angular cells bearing a delicate hyphal net of irregularly shaped angular-triangular or barrel-like cells on surface. Hyphae slightly yellow, 3-4 cells in a square of $20 \times 20 \mu\text{m}$, 4.5-5.5(–7.0) μm long, 12-15 μm in diameter, thin-walled, smooth; clampless. *Middle mantle layer* not clearly ob-

servable. *Inner mantle layer* as a transition type between plectenchymatous and pseudoparenchymatous, compacted, formed by polygonal, elongated or irregular hyphal segments, 4.0-6.0 μm thick, slightly yellow, thick-walled (up to 1.0 μm thick); clampless.

Anatomical characters of emanating elements. Hyphal strands (35–)50-70(–85) \times 7.0-8.5(–10) μm , with smooth margins; ramification with one or two side branches at nodia. Hyphae slightly differentiated, densely intertwined and aggregated: central hyphae clamped, smooth, 5.0-5.5 μm in diameter, thick-walled (2 μm thick), membranaceous yellow; peripheral hyphae clamped, smooth, 3.0-4.5 μm in diameter, hyphae wall up to 1.0 μm thick, slightly tortuous, loosely woven, repeatedly branched, hyaline. *Emanating hyphae* similar to elongated cystidia, 3.0-5.0 μm in diameter, secondarily septate, clamped; ramification Y-shaped below the clamps; cell walls smooth, slightly thick-walled (up to 0.5 μm thick), hyaline, filled with oil yellow contents; bent, apical ends simple or inflated, sometimes undifferentiated. *Cystidia* on hyphal strands, rare, lageniform, clamped, 12-17 μm long, at basis 3.0-4.5 μm in diameter and 2.0-2.5 μm apically, smooth, hyphae wall up to 1.0 μm thick, hyaline (Fig. 4C).

Anatomical characters, longitudinal section: Mantle (25–)32-42(–45) μm thick, hyphae slightly thick-walled, two hardly discernible layers: outer layer (in some places weakly developed) 17.5-22.5 μm thick, hyphal cells often elongated, (10–)12-18(–20) \times 2.0-3.0 (–4.5) μm ; inner layer (10–)12.5-15 μm thick, more compact than the outer layer, hyphae of varied shapes, 3.0-4.0(–5.5) μm thick. Tannin cells not observed. Cortical cells non-differentiated, in 3-4 rows, longitudinally rectangular, (45–)57-125 \times 10-12.5(–23) μm , tangentially oriented. Hartig net not present (Fig. 3D).

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Monumento Natural Municipal da Lagoa do Peri, -27.728243, -48.510175, 03 Feb 2019, *A.N.M. Furtado* (M36'(A)R200, **M36'(B)R202**, M36'(C)R203, M36'(D)R204, M36'(E)R210, M36'(F)R212, M36'(G)R215, M36'(H)R216, M36'(I)R220).

— *Tomentella* sp3 (Fig. 1F)

Morphological characters: *Mycorrhizal system* irregularly pinnate, dichotomous like, sometimes simples (unramified), 11.5-13 mm long, contact exploration system. *Main axis* 0.1-0.2 mm in diameter. *Mantle surface* visible, decaying when older, smooth, loosely grainy becoming stringy with age, yellowish red (HUE 5YR 4/6) becoming dark reddish brown (HUE 2.5YR 2.5/4) aging. Unramified ends straight, (0.6–)1.3-3.0(–5.0) mm long and 0.1-0.2 mm in diameter. *Hyphal strands* and *emanating hyphae* absent. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* pseudoparenchymatous, composed of angular cells. Hyphae compact, membranaceous light brown, smooth, thin-walled (up to 0.5 μm thick), 2-4 cells in a

square of 20 \times 20 μm , 11-15(–17) μm long, 10-12 μm in diameter; clampless. *Middle mantle layer* as a transitional type between plectenchymatous and pseudoparenchymatous, irregularly shaped hyphae forming a coarse net, looser than the outer layer. Hyphae smooth, thin-walled, plasmatol light brown, 11-12.5(–17) μm long, (6.0–)9.0-11 μm in diameter; clampless. *Inner mantle layer* plectenchymatous, hyphae rather irregularly arranged, but sometimes arranged net-like. Hyphae hyaline, smooth, thin-walled, 4.0-6.0 μm thick; anastomosing with a clamp.

Anatomical characters of emanating elements. Hyphal strands absent. *Emanating hyphae* absent, although when old the outer mantle becomes loose from the mycorrhiza surface imitating these structures. *Cystidia* lacking.

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Parque Natural Municipal das Dunas da Lagoa da Conceição, -27.694028, -48.506587, 04 Feb 2019, *A.N.M. Furtado* (M57(A)R247, **M57(B)R248**, M57(C)R249, M57(D)R250, M57(E)R252, M57(F)R253, M57(G)R254, M57(H)R255, M57(I)R256, M57(J)R257, M57(K)R258, M57(L)R259, M57(M)R261).

— *Tomentella* sp4 (Fig. 1G)

Morphological characters: *Mycorrhizal system* simple, sometimes irregularly pinnate, 2.5-4.0 mm long, medium distance exploration system, smooth subtype. *Main axis* 0.1-0.25 mm in diameter. *Mantle surface* visible, smooth, dense becoming spongy with age, shiny, yellowish brown (HUE 10YR 5/4) to dark yellowish brown (HUE 10YR 4/4) in older parts. Unramified ends mostly straight, (0.4–)1.3-3.7(–4.5) mm long and 0.15-0.25 mm in diameter. *Hyphal strands* present but infrequent, growing in flat angles, mostly in the proximal and median portions. *Emanating hyphae* scarce, like subtle hyphal fans, more concentrated in the proximal portion of the mycorrhizal system. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* plectenchymatous, hyphae arranged net-like, sometimes with a weak ring-like pattern and prominent cystidia departing from it. Hyphae 5.0-7.0(–10) μm thick, yellowish, smooth, thick-walled (1.0 μm thick); clampless. *Middle mantle layer* as a thin layer, representing a transitional type between plectenchymatous and pseudoparenchymatous systems. Hyphae yellowish brown, smooth, 12-16 \times 4.5-7.0 μm , thin-walled. *Inner mantle layer* denser than the outer layer, pseudoparenchymatous, irregularly shaped hyphae form a coarse net, puzzle-like. Hyphae brownish yellow, thick-walled (1.0 μm thick), 7-9 cells in a square of 20 \times 20 μm , 9.6-11(–13) μm long, 4.0-6.0 μm in diameter; clampless.

Anatomical characters of emanating elements. Hyphal strands present, albeit rare, sparsely distributed, never at the very tip, 20-24 μm thick, becoming tapered towards the apex; reversely oriented ramification, several hyphae grow out of the margin. Very agglutinate sticking soil particles on the surface. Hyphae undifferentiated rather loose-

ly woven of uniform diameter, secondarily septate, thick-walled (1.0 µm thick), membranaceous brownish yellow, irregularly shaped crystals on the surface, 3.0-5.0 µm in diameter; clamped, slightly enlarged near the septum. *Emanating hyphae* rare, awl-shaped (bristle-like) with a slightly bent apex, brownish yellow, 119-197(-310) µm long, 3.0-5.0 µm in diameter, abundant secondary septation, Y-ramifications present, thin-walled (<0.5 µm thick), smooth but agglutinated, holding soils particles on the surface; clamped irregularly, but anastomoses with the septum. *Cystidia* abundant, slightly clavate, 37-42(-72) µm long, at basis 4.0-6.0(-7.0) µm in diameter and 4.0-5.0 µm apically, smooth, thick-walled (1.0 µm thick), brownish yellow; clampless (Fig. 4D).

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Monumento Natural Municipal da Lagoa do Peri, -27.728243, -48.510175, 20 Mar 2018, *A.N.M. Furtado* (M1(R2)R172, **M1(S2)R173**, **M30(A)R173**).

— *Tomentella* sp5 (Fig. 1H)

Morphological characters: *Mycorrhizal system* simple, unramified, contact exploration system. *Main axis* 0.6-1.5 mm long and 0.15-0.2 mm in diameter, bent ends. *Mantle surface* visible, felty, slightly floury, very pale brown (HUE 10YR 8/4) to brownish yellow (HUE 10YR 6/6) aging. *Hyphal strands* and *emanating hyphae* absent. *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable, but hyphae often growing in longitudinal directions. Hyphae glutinous, 3.0-4.0 µm thick, slightly yellow, smooth, thin-walled; clampless. *Middle mantle layer* as a transition between plectenchymatous and pseudoparenchymatous layers, hyphae irregularly shaped forming a coarse net. Hyphae hyaline, thin-walled; clampless. *Inner mantle layer* plectenchymatous, hyphae arranged hash-like. Hyphae hyaline, strongly glutinous, smooth, thin-walled, 3.5-5.0 µm thick; clampless.

Anatomical characters of emanating elements. *Hyphal strands* and *emanating hyphae* absent. *Cystidia* present, but rare, parting from the inner layer, smooth, strong brown, slightly thick-walled (0.9 µm thick), bottle shaped with a straight neck [(11.5-)13-15 µm long, at basis 4.5-5.5 µm in diameter and 2.5-3.0(-4.5) µm apically] or flask-shaped [19-23 µm long, 3.0-4.0 µm in a diameter]; clampless (Fig. 4E).

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Parque Natural Municipal da Lagoa do Jacaré das Dunas do Santinho -27.467783, -48.393395, 08 Nov 2018, *A.N.M. Furtado* (M34(A)R180, M34(B)R183, M34(C)R185, M34(D)R187, **M34(E)R198**).

— *Tomentella* sp6 (Fig. 1I)

Morphological characters: *Mycorrhizal system* monopodial-pinnate, 12.9-14 mm long, contact exploration

system. *Main axis* 0.18-0.27 mm in diameter. *Mantle surface* visible, densely grainy (warty) to short spiny, shiny and coppery, dark reddish brown (HUE 5YR 2.5/2) to red (HUE 2.5YR 4/8) in older parts. Unramified ends straight with some becoming bent, (3.6-)5.0-12.8(-17) mm long and 0.26-0.31 mm in diameter. *Hyphal strands* absent. *Emanating hyphae* frequent, branched, yellowish red (HUE YR 5/8). *Sclerotia* not observed.

Anatomical characters of mantle in surface views: *Outer mantle layer* pseudoparenchymatous, composed by roundish cells from which emerge cystidia and long emanating hyphae. Hyphae light brown, smooth, thick-walled (1.0 µm thick), 4-6 cells in a square of 20 × 20 µm, 10-14(-16) µm long, 9.0-11(-14) µm in diameter; clampless. *Middle mantle layer* pseudoparenchymatous, composed of angular hyphae, with large dimensions. Hyphae brownish yellow, smooth, thick-walled (up to 2.0 µm thick), 3-4 cells in a square of 20 × 20 µm, (19-)23-25 µm long, (15-)17-20 µm in diameter; clampless. *Inner mantle layer* plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable, but hyphae often growing in longitudinal directions regarding root orientation. Hyphae 3.0-6.0 µm thick, hyaline, smooth, thin-walled, with granular content and a gelatinous matrix between the hyphae; clamped, with Y-ramifications frequent.

Anatomical characters of emanating elements. *Hyphal strands* absent. *Emanating hyphae* 4.0-6.0 µm thick, bent in the apex, thick-walled (1.0 µm thick), brownish yellow, secondarily septate; clamped in all septa. Hyphae distributed all over the mantle surface but concentrated in middle and distal portions of the root. *Cystidia* abundant, of variable shapes, thin-walled (0.5 µm thick); lageniform [17-23(-26) µm long, at basis (7.0-)9.0-13 µm in diameter and 3.0-4.0 µm apically], bottle-shaped with a straight neck [25-36 µm long, at basis 9.0-11 µm in diameter and 3.0-5.0 µm apically] or like a normal hypha but often twisted like a cork-screw [27-33(-40) × 4.0-6.0] and in this case, clamped (Fig. 4F).

Studied materials: BRAZIL. Santa Catarina: Florianópolis, Parque Natural Municipal das Dunas da Lagoa da Conceição, -27.694028, -48.506587, 25 Mar 2019, *A.N.M. Furtado* (**M68(A)R291**, M68(B)R292).

The results presented here endorse the existence of morpho-anatomical particularities of ectomycorrhizae associated with *G. opposita*. Despite these genera being widely known to be ectomycorrhizal and usually having typical anatomical structures (mantle, Hartig net and extraradicular hyphae), the ectomycorrhizae of *A. viscidolutea* (Furtado et al., 2022), *A. festivus*, *Tomentella* sp1 and *Tomentella* sp2 exhibit particular characteristics (Fig. 3). It is possible that the lack of the Hartig net is common among other taxa that grow in restinga. The anatomy of the ectomycorrhizae studied revealed close connections between the layered ectomycorrhizal mantle and the cortical root cells. However, no intercellular or intracellular fungal pen-

etration was observed in the root cortex. Additionally, the epidermal cells of the root of *G. opposita*, where the mantle is established, are absent. Although we understand that this is not a taxonomic character for the symbiosis, we are working to understand if the absence of the epidermis may be an evolutionary step that helped the establishment of the association or maybe a physiological response of *G. opposita* to the fungal colonization.

Discussion

We have described the occurrence of ectomycorrhizae in the roots of *G. opposita* and propose the name Guapirioid ectomycorrhiza based on the particularities of the morphology. The short and simple or long and thin branched ectomycorrhizal systems, close connections between the layered mantle and the cortical cells, absence of a Hartig net and other fungal elements in the cortex are diagnostic characteristics that distinguish the Guapirioid from other ectomycorrhizae.

All the Basidiomycota fungi sequenced from *G. opposita* in this work belong to putatively ectomycorrhizal lineages (Table 1) and form visible ectomycorrhizal morphotypes with several layers of hyphae composing a distinguishable mantle. Some of the Guapirioid ectomycorrhizae are discussed below.

Sequences of the morphotype M70 had 99.37% (Table 1) similarity with sequences of *Amanita viscidolutea* basidiomata. This species was originally described for the northeast coast of Brazil, in Rio Grande do Norte, and it has been collected in Paraíba and Santa Catarina, probably having a distribution along the restinga. Records based on field observations made by other researchers suggested *Coccoloba* (Polygonaceae) (Menolli Jr. et al., 2009) or an unnamed *Nyctaginaceae* species (Wartchow et al., 2012) as possible symbionts. These suggestions have not been confirmed but are possibly not wrong. However, we proved that *A. viscidolutea* forms an association with *G. opposita*, and the morpho-anatomical features of this symbiosis have been recently described (Furtado et al., 2022). This was the first description of an *Amanita* ectomycorrhiza on *Guapira*.

An interesting species recorded in our site collection was *A. festivus*. Sequences from the ectomycorrhizal root tips (morphotype M41) had 97.56% (Table 1) of similarity with Brazilian sequences of *A. festivus* basidiomata. *Austroboletus festivus* was originally described from Pernambuco, northeastern Brazil (Singer, 1970). The species was also recorded in Paraná and Santa Catarina (Magnago & Neves, 2014) and probably has a wide distribution on the restinga. This is the first characterization of a native *Austroboletus* ectomycorrhiza with *G. opposita* in the restinga. Currently, and as contribution from our work about the ectomycorrhizae from the restinga, both *A. festivus* and *Amanita viscidolutea* were considered in The IUCN Red List of

Threatened Species and included in the vulnerable category according to IUCN criteria (Neves & Furtado, 2020; Neves et al., 2020; see also <http://iucn.ekoo.se/en/iucn/welcome>).

One Guapirioid ectomycorrhiza (morphotype 51 – *Inocybe* sp.) had 90.20% of similarity with an unknown *Inocybe* ectomycorrhiza associated with *Dicymbe corimbosa* in Guyana. Ectomycorrhizae of an unknown species of *Inocybe* were collected during our research. However, there are almost no voucher sequences with similarity greater than 90% in Genbank. *Inocybe* has ca. 750 species described (Matheny et al., 2020), but the taxon is undersampled in Brazil. There are only seven species known for Brazilian forests: *I. austrolilacina* Wartchow & RM Silveira, *I. cavalcantiae* Wartchow, *I. curvipes* P. Karst., *I. hyperhytra* Rick, *I. lepidospira* Wartchow, *I. martinica* Pegler and *I. violaceolamellata* Rick (Wartchow, 2018). The mycorrhizal potencial of *Inocybe* was not cited, but the authors suggest members of *Nyctaginaceae*, *Euphorbiaceae* and *Fabaceae* subfam. *Caesalpinoideae* as potential partners. The color and anatomical features of the *Inocybe* ectomycorrhizae is useful to distinguish morphotypes (Seress et al., 2015). The *Inocybe* ectomycorrhiza described here was compared to the seventeen descriptions previously published for the genus (Seress et al., 2015; DEEMY website <http://www.deemy.de/>). The *Inocybe* Guapirioid ectomycorrhiza can be differentiated mainly by two characters: (i) presence of a contact exploration system, as opposed to a short-distance system frequent in the other species of the genus; and (ii) the purple tip, in contrast to the white tip found in all other *Inocybe* ectomycorrhizae.

Representatives of /tomentella-thelephora clade were the most frequently encountered taxa in our study (M53 – Thelephoraceae; *Tomentella* sp1 – M36; *Tomentella* sp2 – M36'; *Tomentella* sp3 – M57; *Tomentella* sp4 – M30; *Tomentella* sp5 – M34; and *Tomentella* sp6 – M68 morphotypes), as expected and reported in previous studies (Tedersoo et al., 2014). Indeed, studies based on root and soil analyses often find that the /tomentella–thelephora clade is diverse and dominant in neotropical habitats, but usually they are undersampled due to their inconspicuous basidiomata (Tedersoo et al., 2014), making it difficult to identify the sampled taxon. Unlike what has been reported in other works made in Latin America (Haug et al., 2005; Alvarez-Majarrez et al., 2017), the *Tomentella* ectomycorrhizae found in restinga often form morphotypes with a contact exploration system, or even with a medium distance exploration system with a smooth subtype.

Among the various *Tomentella* species found during this survey and linked to *G. opposita*, *Tomentella* sp1 (morphotype M36) and *Tomentella* sp2 (morphotype M36') were the most interesting. These taxa were frequent in our study and grew on the same root segment. Considering that *G. opposita* has secondary roots, with frequently smaller diameters that branch off from its main, larger-diameter root, we observe: *Tomentella* sp2 (morphotype M36') growing in the most proximal portion of the secondary root seg-

ment, and *Tomentella* sp1 (morphotype M36) growing more distal to the main root on the same root segment (Fig. 1E). Even though we do not have information about the biology of these species, there is possibly a functional co-dependency between them that could be explained if their growth and development could be monitored. Sequences of *Tomentella* sp1 had 91.38% similarity with a *Tomentella* mycorrhiza isolated from the root of *Coccoloba uvifera* from the Caribbean region and had 90.25% similarity with basidiomata sequences of *Tomentella longisterigmata* from coniferous forests from Finland (Table 1). *Tomentella* sp2 had 91.32% similarity with an ectomycorrhiza associated with *Pinus tabuliformis* from China (Lu et al., 2018) and 90.52% similarity with a *Tomentella* associated with *Quercus pubescens* (Mrak et al., 2021) (Table 1). *Tomentella* is a widespread genus, distributed throughout the temperate and tropical regions worldwide, forming abundant ectomycorrhizae in coniferous and deciduous forests. At least 100 species are currently known, and several have been described in recent years, thanks to molecular tools that allow to ascertain the existence of numerous cryptic species in a genus where basidiomata are inconspicuous and rarely found (Lu & Yuan, 2021). In extreme ecosystems like the restinga the constant disturbances, anthropogenic or biotic, can also affect the production of basidiomata.

Two morphotypes (M34 – *Tomentella* sp5 and M53 – *Thelephoraceae*) of the *tomentella-thelephora* ectomycorrhizae had high similarity with samples from Ecuador. The morphotype M34 presented 96.71% similarity with the sequence of an ectomycorrhiza isolated from the roots of *Neea* sp. (Haug et al., 2005). The morphotype M53 had 97.85% similarity with an ectomycorrhiza isolated from the roots of *Coccoloba* sp. (*Polygonaceae*) from Ecuador (Tedersoo et al., 2010).

There are records of *Guapira* and *Neea* species forming mycorrhiza with a single fungal taxon member of *Thelephoraceae*, where a fine root system made of only long roots was observed with an incomplete hyphal mantle development and with no suppression of root hairs formation (Moyersoen, 1993; Haug et al., 2005). However, we found in *G. opposita* that there were at least four different fungal species on the root system, including taxa from phylogenetically distant orders, e.g. *Austroboletus festivus* (M41) sharing the same root system with *Tomentella* sp1 (M36) and *Tomentella* sp2 (M36'). Also, even though *G. opposita* has a fine root system composed by long roots, there was a complete formation of the ectomycorrhizal mantle, as well as the suppression of the root hairs formation.

The morphotype M30 (*Tomentella* sp4) had a 92.73% similarity with a *Tomentella* sp. ectomycorrhiza isolated from the roots of *Pisonia taina* from Puerto Rico (Hayward & Horton, 2014), but there are no basidiomata sequences with similarity greater than 90% in Genbank. The morphotype M57 (*Tomentella* sp3) had a 91.79% similarity to *Tomentella stuposa* associated to *Pinus pinaster* roots, from Spain, but when the morpho-anatomical characteristics were compared it was clear that they were not

the same (Jakucs et al., 2005). Both have a similar general organization of the mantle layers; however, the arrangement of the hyphae differs (it is a star-like structure in *T. stuposa* morphotype and a net-like arrangement in M57 morphotype). Furthermore, the morphotype of *T. stuposa* has emanating elements and hyphal strands, features that are absent in the M57 morphotype. These are some of the characteristics that allow us to consider them as ectomycorrhizae from distinct species. An interesting particularity of the morphotype M57 is that even though it often occupies the entire root system, it is possible to find other morphotypes starting from it, such as M51 (*Inocybe* sp.) and M53 (*Thelephoraceae* sp.) morphotypes. The highest percentage of similarity for the *Tomentella* sp6 (morphotype M68 - 93.40%) was with an ectomycorrhiza of *Tomentella* sp. isolated from the rhizome of the orchid *Corallorhiza odontorhiza* (McCormick et al., 2009). Although orchids are often found forming endomycorrhizal associations with the genus *Rhizoctonia*, the ectomycorrhizal association with taxa of *Thelephoraceae* has been previously reported (Bidartondo & Read, 2008; Roy et al., 2013).

The Guapirioid ectomycorrhizae have potential to be used in *G. opposita* seedlings in restoration projects in restinga (Weidlich et al., 2020). The increase in the plant survival rate in the field would be directly related to the fact that both symbionts would be adapted to local conditions, thus without the risk of introducing alien plant hosts and/or fungal species (Dalotto et al., 2018). To use the Guapirioid ectomycorrhizal fungi in restoration projects the biology, physiology and ecology of the fungal community need to be better understood. Also, the fungi need to be isolated to be used as inocula, which has proven to be a great challenge, since many ectomycorrhizal species do not grow easily on culture media (Weidlich et al., 2020).

Conclusion

Our study showed that *G. opposita* forms symbiosis with at least ten taxa of ectomycorrhizal fungi in the restinga. We are just starting to explore the biological and functional complexity of the Guapirioid ectomycorrhiza and in future experiments we aim to better understanding how *G. opposita* acts as a nurse plant. We want to understand how the plant is affected by its fungal partners and how the mycelial network shared with neighboring host plants works, considering its importance in restinga. The apparent generalist status of *G. opposita* as a symbiont is probably due to its role as a nurse plant, but more studies on the mycorrhizal status and identification of the mycobionts are needed to draw conclusions about the evolution and potential host specificity in this and other species of *Nyctaginaceae*.

The collection of basidiomata of *Tomentella* and other *Thelephoraceae* continues to be a big challenge for those who study the species in *Thelephoraceae*. During this

study we did not find *Tomentella* basidiomata to check their correspondence with the ectomycorrhizae found in *G. opposita*, but we continue to search.

It is currently understood that ecosystem recovery is favored by the greater accessibility of disposable macronutrients in mycorrhizae-rich environments. The results obtained from this work make it possible to discuss the impact that these associations have on the formation and maintenance of the ecosystem and on the establishment of seedlings in sandy soils (Weidlich et al., 2020).

This work is the first to characterize the ectomycorrhizae native to restinga and to propose Guapirioid ectomycorrhiza as the first type described for the Atlantic Forest in Brazil. Unfortunately, the environmental disaster that happened in the study area and cited in the Introduction may have caused an irreversible disruption of the ecological communities in that point of the restinga. It is important to continue to study the fungal diversity above and below-ground in the restinga so better conservation and restoration projects can be proposed.

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