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Hysterangium atlanticum sp. nov., forms ectomycorrhizae with *Coccoloba* species (Polygonaceae) from the Atlantic rainforest of Northeastern Brazil

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Abstract
Hysterangium basidiomata were collected associated with *Coccoloba alnifolia* and *C. laevis* (Polygonaceae), in the Guaribas Biological Reserve in the Atlantic rainforest, of northeastern Brazil during the rainy seasons of 2012–2013. Based on its unique morphological and molecular traits, this new taxon is described as *Hysterangium atlanticum* sp. nov. The most prominent morphological characters that separate *H. atlanticum* from other close relatives are the large size of the basidiomata, the white peridium that rapidly turns greyish-orange to pale-red where bruised or exposed to air, and the ellipsoid to suboblong spores with a minutely verrucose surface. Molecular analyses of the LSU, SSU, *atp*6, and EF-1α markers were done. The analyses of the concatenated *atp*6–EF-1α matrix confirmed the placement of the new species in the /hysterangium lineage. Moreover, at the infra-generic level, *Hysterangium atlanticum* sp. nov. forms a sister clade with *Hysterangium* sp. from *Dicymbe* forests located in neighboring Guyana. Moreover, the ectomycorrhizae (EcM) formed by *H. atlanticum* and roots of *Coccoloba* species was confirmed, based on identical ITS nrDNA sequences obtained from basidiomata and EcM root tissues. The main conspicuous features of the EcM are: a well-developed plectenchimatous mantle, the ramarioid, abundant emanating hyphae with clamps and covered with crystals, the presence of oleoacanthocystidia, and the whitish rhizomorphs. This is the first report of a *Hysterangium* species forming EcM with native members of *Coccoloba* spp. in South America.

Keywords Ectomycorrhizae · Hypogeous fungi · Hysterangiales · Neotropics · Phylogeny

1 Introduction

Ectomycorrhizal (EcM) fungal associations have been for long time considered unusual in the tropics (Alexander 1989; Béreau et al. 1997). Historically, they were though to be restricted to temperate and boreal regions of the world. In contrast, the more recent literature have shown that various tropical and subtropical tree species form ECM associations with a wide range of plant families, including Dipterocarpaceae, Fabaceae (Caesalpinioideae and Faboideae), Fagaceae, Gnetaceae, Nyctaginaceae, Polygonaceae and Sapotaceae (Moyersoen et al. 1998;

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Henkel et al. 2012; Smith et al. 2013; Moyersoen and Weiß 2014; Roy et al. 2017; Corrales et al. 2018).

The genus *Hysterangium* Vittad. belongs to the Hysterangiaceae E. Fisch., in the order Hysterangiales, subclass Phallomycetidae (Hosaka et al. 2006). The genus harbors over 50 species, all forming hypogeous sporocarps, diagnosed by the enclosed basidiomata, an irregularly developed columella, a cartilaginous gleba, and narrowly ellipsoid or fusoid, smooth to rugose basidiospores, covered by a membranous utricle or perisporium (Kirk et al. 2008). *Hysterangium* is a globally distributed genus known to form EcM with Fagaceae, Myrtaceae, Nothofagaceae and Pinaceae (Beaton et al. 1985; Castellano 1999; Hosaka et al. 2008).

EcM symbiosis also influence plant productivity and plant diversity, and connect plants below ground via a hyphal network, allowing the movement of resources among coexisting plants (van der Heijden et al. 2015). Species of *Hysterangium* are hyphal-mat-forming fungi (Agerer 2001) and have the capacity to modify soil chemistry and microbial biomass (Griffiths et al. 1994; Entry et al. 1992; Trappe et al. 2012), playing an important role in the cycling of nutrients, water uptake and also in soil stabilization of forest ecosystems (Entry et al. 1991, 1992; Trappe et al. 2012).

This genus is widely distributed in both the Northern and Southern Hemispheres, with characteristic discrete host ranges (Castellano 1999). The most comprehensive revision of the genus *Hysterangium* diversity in South America was conducted by Castellano and Muchovej (1996). In that study, four new species associated with *Eucalyptus* and *Nothofagus* were described; furthermore, *Hallingea* Castellano, a new genus related to *Hysterangium* and exclusively found in South America, was proposed. Currently, based on DNA analysis and intensive sampling of unexplored areas, new species of *Hysterangium* from various world regions have been described (Xu and Liu 2003; Hosaka et al. 2007; Guerrero et al. 2008; Elliott et al. 2015).

Despite its global distribution, the genus is poorly known in the neotropics and subtropics. In Brazil, only a few records of *Hysterangium* species are available, primarily from introduced eucalypt and pine plantations. Among them, *H. australe* Speg. (Rick 1961), *H. gardneri* E. Fisch. (Giachini et al. 2000), *H. affine* Massee & Rodway and *H. inflatum* Rodway (Cortez et al. 2011). *Hysterangium thaxteri* Zeller & Dodge, also reported for Brazil by Zeller and Dodge (1929) is currently considered a member of the genus *Gelopellis* Zeller.

This study reports morphological and molecular characteristics of a novel species of *Hysterangium* associated to *Coccoloba* (Polygonaceae) in northeast Brazil. In addition, the morpho-anatomical description of the EcM and the ITS nrDNA sequences analyses of DNA extracted from basidiomata, and from root mantle confirmed its mycorrhizal status and its association with *Coccoloba*.

2 Material and methods

Specimens were collected during the rainy season, from July to September 2012 and in June 2013, at the Guaribas Biological Reserve, 6°44′14”S, 35°8′55”W. This area is located in the State of Paraíba, Brazil, covering 4029 ha of the Atlantic rainforest. The vegetation ranges from lowland semideciduous forest to savannas. The forests contain primarily members of the families Fabaceae, Melastomataceae, Myrtaceae, Nyctaginaceae, Rubiaceae, Polygonaceae Cyperaceae and Poaceae (Barbosa et al. 2011). Soils are Tertiary sediments of the “Barreiras” formation (Barbosa et al. 2011) the topsoil is sandy, composed mainly of marine quartz sand (Quartzarenic Neosoil).

Fresh basidiomata were collected randomly by raking the litter and topsoil organic layer among the native vegetation, as described by Castellano et al. (2004). After analysis, basidiomata were dried in a forced air dryer at 40 °C for further preservation.

Firstly, coarse roots from *Coccoloba* were traced from the stem into the area of fruiting sporocarps and marked. Subsequently a soil core for EcM analyses was taken from a single plot in June of 2013 directly from under the basidiomata (Sulzbacher 455 – UFRN-fungos 1750). The soil sample including humus layer and mineral soil of 15 × 15 cm and 5 cm deep was collected, following Suz et al. (2008).

2.1 Morphological analyses

Collections were photographed in situ. Informative macro and micro characters were observed with the aid of a dissecting microscope (EZ4 Leica), and photographed using light microscopy at ×40–×100 (Eclipse Ni Nikon and digital camera DS-Ri1 Nikon). Spores were studied by scanning electron microscopy (XL30-ESEM Phillips). Line drawings of the microstructures were made with the aid of a drawing tube attached to the microscope (BX41 Olympus), with 100X magnifications. The basidiospore were measured as proposed by Tulloss et al. (1992) and based on 30 mature spores. Abbreviations include: *L(W)* = average basidiospore length (width), *Q* = the length to width ratio range as determined from all measured basidiospores, and Q_m = the *Q* value averaged from all measured basidiospores. Basidiomata coloration was registered from fresh material; color codes followed Kornerup and Wanscher (1978). Vouchers were deposited at the Universidade Federal do Rio Grande do Norte Herbarium, Natal, Rio Grande do Norte, Brazil (UFRN), with duplicate material deposited in Father Camille Torrend Herbarium, Recife, Pernambuco, Brazil (URM).

EcM root tips were carefully washed and separated from the soil sample with tap water. Morphological analyses of fresh EcM tips followed Agerer (1991), under a dissecting microscope (EZ4 Leica) and light microscopy at

magnifications of 40X–100X (Eclipse Ni Nikon) and photographed with a microscopy digital camera (DS-Ri1 Nikon). Line drawings of the microstructures were made using a drawing tube attached to the microscope (BX41 Olympus) at 100X magnification.

2.2 DNA extraction, amplification and sequencing

Total fungal DNA from gleba and EcM root tips tissue (5–15 tips per three samples) were extracted using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was re-suspended in pre-warmed, sterile milli-Q water to the approximate final concentration of 100 ng $\mu l^{-1}$and kept at −80 °C.

Four nuclear or mitochondrial markers were amplified for basidiomata: complete *nuc*-ITS-rDNA spacer (ITS), partial *nuc*-LSU-rDNA, partial *atp6*, and partial EF-1α, using primer pairs ITS1/ITS4 (White et al. 1990), NS1/NS4 (White et al. 1990), ATP6-2F/ATP6-3R (Kretzer and Bruns 1999) and rEF1-983F/rEF1-1953R (Rehner and Buckley 2005), respectively. For the fungal DNA isolated from EcM only the ITS marker was amplified. PCR reactions were performed as follows: 1.0 μl DNA; 2.5 μl PCR buffer 10×; 3.0 μl dNTPs (1.5 mM); 2.0 μl $MgCl_2$ (20 mM); 3.0 μl of each primer (25 pmol); 0.5 U *Taq* polymerase (5 U μl^{-1}); and 10.5 μl ultrapure water. PCR conditions followed previously published protocols for selected primers (ibid.) and modified for amplification of ITS (Sulzbacher et al. 2016). Amplifications were done in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Prior to sequencing, PCR products were purified from agarose gel using the Wizard SV Genomic DNA Purification System (Promega Corporation, Madison, WI, USA). Both strands were sequenced separately at Macrogen Korea (Seoul, Korea) with the same primers used in the amplification. Sequencher 5.1 (Gene Codes Corporations, Ann Arbor, MI, USA) was used to assemble the consensus sequence from the two strands of each isolate.

2.3 Molecular analyses

The new sequences generated were compared to those available at GenBank (Altschul et al. 1997); the accession numbers are indicated in Tables S1 and S2. Two datasets were aligned in Mafft v6.859b (Katoh et al. 2005) using a default alignment approach.

To assess the phylogenetic position of the new species, concatenated partial *atp*6 and EF-1α sequences from GenBank were included (Table S1), mainly from Hosaka et al. (2008); *Phallus hadriani* and *Ramaria flavobrunnescens* were used as outgroups (ibid.). Analyses were conducted using Maximum Parsimony (MP) and maximum likelihood (ML). The Maximum Parsimony (MP) phylogenetic reconstruction with Subtree-Pruning-Regrafting MP search method using all sites, and 1000 bootstrap repetitions (MPbs); while for Maximum Likelihood (ML), the General Time Reversible with a discrete Gamma distribution, and assuming invariable sites (+I) was selected after ModelTest in MEGA7.0.18 (Kumar et al. 2016), and 1000 bootstrap repetitions (MLbs), with a partial deletion of gaps/missing data (95% site coverage cutoff).

For the molecular identification of the EcM in the second dataset, ITS nrDNA sequences from basidiomes of the new species, and from the EcM of *Coccoloba* species were compared with partial homologous sequences belonging to genus *Hysterangium* retrieved from GenBank on January 15, 2018 (Table S2); *Gallacea* spp. were included as outgroup (Giachini et al. 2010). The MP analysis was done using the same parameters as mentioned above, and the ML analysis with the Tamura-2-parameter substitution model with a discrete Gamma distribution of sites, as selected by ModelTest; 1000 bootstrap repeats were run with complete selection of data to MP and ML analyses.

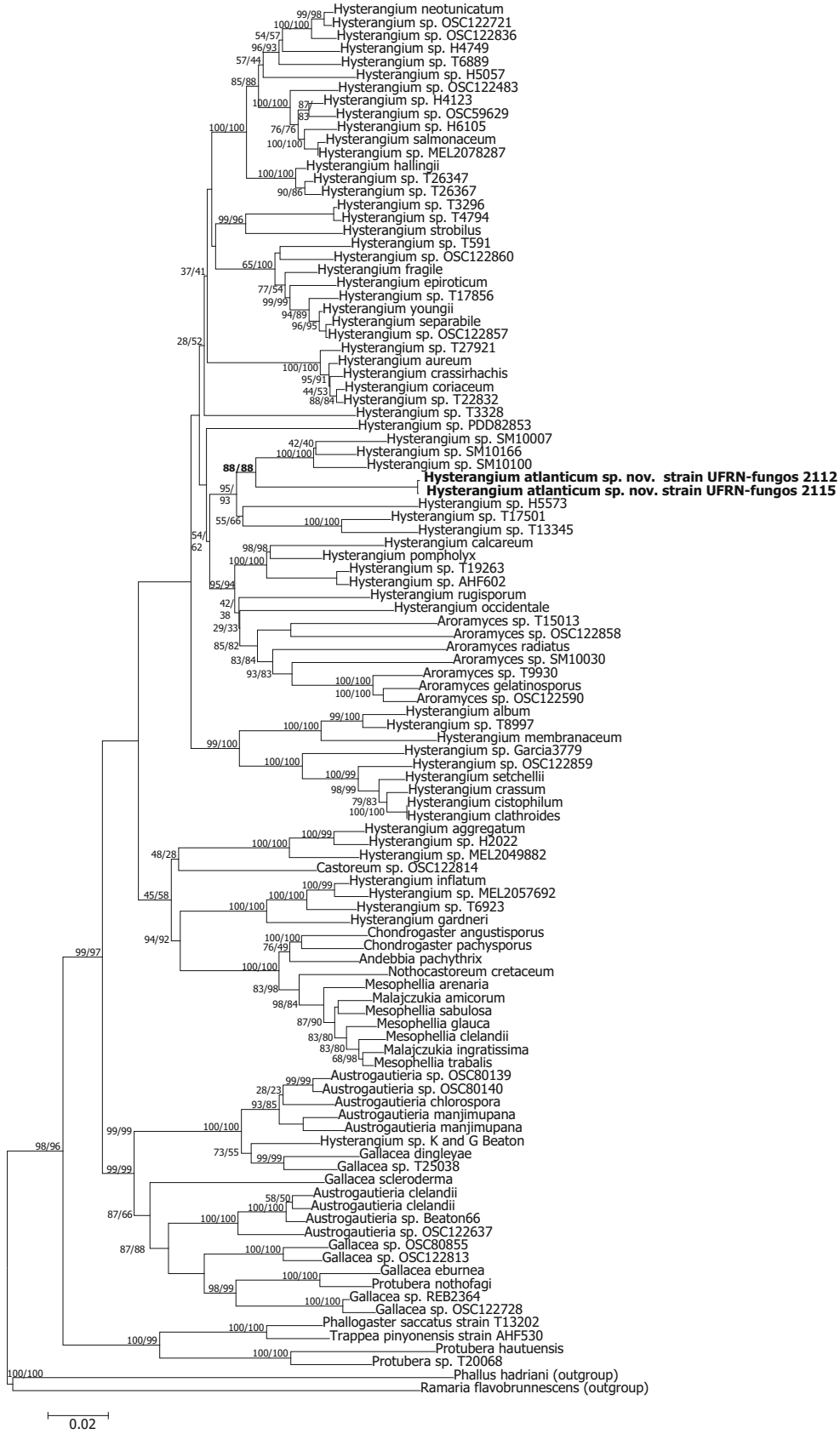
All phylogenetic analyses were run in MEGA7.0.18. Phylogenetic trees were drawn and annotated in the same software and subsequently edited in *Inkscape* 0.91.

3 Results

3.1 Molecular analyses

For reconstruction of the Hysterangiales phylogeny and taxonomic positioning of the new hypogeous species, LSU and complete ITS sequences were not included in the analysis because LSU sequences were poorly represented in nucleotide databases for a relevant analysis, and the complete ITS region was too heterogeneous within Hysterangiales to be aligned with confidence. As a result, a concatenated dataset (*atp6*/EF-1α) was prepared. The matrix contained 108 taxa (Table S1) and 1314 positions. The Maximum Parsimony (data not shown) and Maximum Likelihood analyses (Fig. 1) resulted in trees with similar topologies, where the new species forms a well-supported terminal clade (MPbs = 88; MLbs = 88), sister group to three sequences of *Hysterangium* sp., e.g.: SM10007 (DQ218869), SM10166 (DQ218871), and SM10100 (DQ218870). The latter sequences were collected in tropical forest of Guyana (Hosaka et al. 2008). All these sequences form the sister group of three *Hysterangium* sp.: *Hysterangium* sp. H5573 (DQ218863), *Hysterangium* sp. T17501 (DQ218841), and *Hysterangium* sp. T13345 (DQ218872), all from Asia (Hosaka et al. 2008).

In the second dataset, the ITS matrix contained 51 taxa and 609 positions. Sequences from the basidiomata of *H. atlanticum* sp. nov. (ITS sequence GenBank LT623204; LT623205; LT623206), and from EcM rootips of *Coccoloba* (ITS sequence GenBank LT623207; LT623208; LT623209;


Hysterangium neotunicatum
Hysterangium sp. OSC122721
Hysterangium sp. OSC122836
Hysterangium sp. H4749
Hysterangium sp. T6889
Hysterangium sp. H5057
Hysterangium sp. OSC122483
Hysterangium sp. H4123
Hysterangium sp. OSC59629
Hysterangium sp. H6105
Hysterangium salmonaceum
Hysterangium sp. MEL2078287
Hysterangium hallingii
Hysterangium sp. T26347
Hysterangium sp. T26367
Hysterangium sp. T3296
Hysterangium sp. T4794
Hysterangium strobilus
Hysterangium sp. T591
Hysterangium sp. OSC122860
Hysterangium fragile
Hysterangium epiroticum
Hysterangium sp. T17856
Hysterangium youngii
Hysterangium separabile
Hysterangium sp. OSC122857
Hysterangium sp. T27921
Hysterangium aureum
Hysterangium crassirhachis
Hysterangium coriaceum
Hysterangium sp. T22832
Hysterangium sp. T3328
Hysterangium sp. PDD82853
Hysterangium sp. SM10007
Hysterangium sp. SM10166
Hysterangium sp. SM10100
Hysterangium atlanticum sp. nov. strain UFRN-fungos 2112
Hysterangium atlanticum sp. nov. strain UFRN-fungos 2115
Hysterangium sp. H5573
Hysterangium sp. T17501
Hysterangium sp. T13345
Hysterangium calcareum
Hysterangium pompholyx
Hysterangium sp. T19263
Hysterangium sp. AHF602
Hysterangium rugisporum
Hysterangium occidentale
Aroramyces sp. T15013
Aroramyces sp. OSC122858
Aroramyces radiatus
Aroramyces sp. SM10030
Aroramyces sp. T9930
Aroramyces gelatinosporus
Aroramyces sp. OSC122590
Hysterangium album
Hysterangium sp. T8997
Hysterangium membranaceum
Hysterangium sp. Garcia3779
Hysterangium sp. OSC122859
Hysterangium setchellii
Hysterangium crassum
Hysterangium cistophilum
Hysterangium clathroides
Hysterangium aggregatum
Hysterangium sp. H2022
Hysterangium sp. MEL2049882
Castoreum sp. OSC122814
Hysterangium inflatum
Hysterangium sp. MEL2057692
Hysterangium sp. T6923
Hysterangium gardneri
Chondrogaster angustisporus
Chondrogaster pachysporus
Andebbia pachythrix
Nothocastoreum cretaceum
Mesophellia arenaria
Malajczukia amicorum
Mesophellia sabulosa
Mesophellia glauca
Mesophellia clelandii
Malajczukia ingratissima
Mesophellia trabalis
Austrogautieria sp. OSC80139
Austrogautieria sp. OSC80140
Austrogautieria chlorospora
Austrogautieria manjimupana
Austrogautieria manjimupana
Hysterangium sp. K and G Beaton
Gallacea dingleyae
Gallacea sp. T25038
Gallacea scleroderma
Austrogautieria clelandii
Austrogautieria clelandii
Austrogautieria sp. Beaton66
Austrogautieria sp. OSC122637
Gallacea sp. OSC80855
Gallacea sp. OSC122813
Gallacea eburnea
Protubera nothofagi
Gallacea sp. REB2364
Gallacea sp. OSC122728
Phallogaster saccatus strain T13202
Trappea pinyonensis strain AHF530
Protubera hautuensis
Protubera sp. T20068
Phallus hadriani (outgroup)
Ramaria flavobrunnescens (outgroup)
0.02

◀ **Fig. 1** Phylogram based on Maximum Likelyhood analysis of concatenated *atp6* and EF-1α genes of sequences included in Table S1. The two new sequences generated from *Hysterangium atlanticum* sp. nov. basidiomata are marked in bold. *Phallus hadriani* and *Ramaria flavobrunnescens* were included as outgroup taxa. Maximum Parsimony and Maximum Likelyhood bootstrap percentages are indicated on the branches (MP/ML)

LT623210) were identical (Fig. 2); thus, the identity of *H. atlanticum* sp. nov. as the fungal symbiont of the EcM rootips collected was confirmed.

3.2 Taxonomy

Hysterangium atlanticum Sulzbacher, Grebenc, Baseia et Nouhra, sp. nov.

MycoBank MB 817856.

Diagnosis – The combination of basidiomes up to 25 mm in diam., the white peridium that rapidly turns greyish orange to pale red where bruised or exposed to air, basidiospores 11–15 × 5–7 μm, ellipsoid, utricle present, are the main features that characterize this species growing under *Coccoloba* spp.

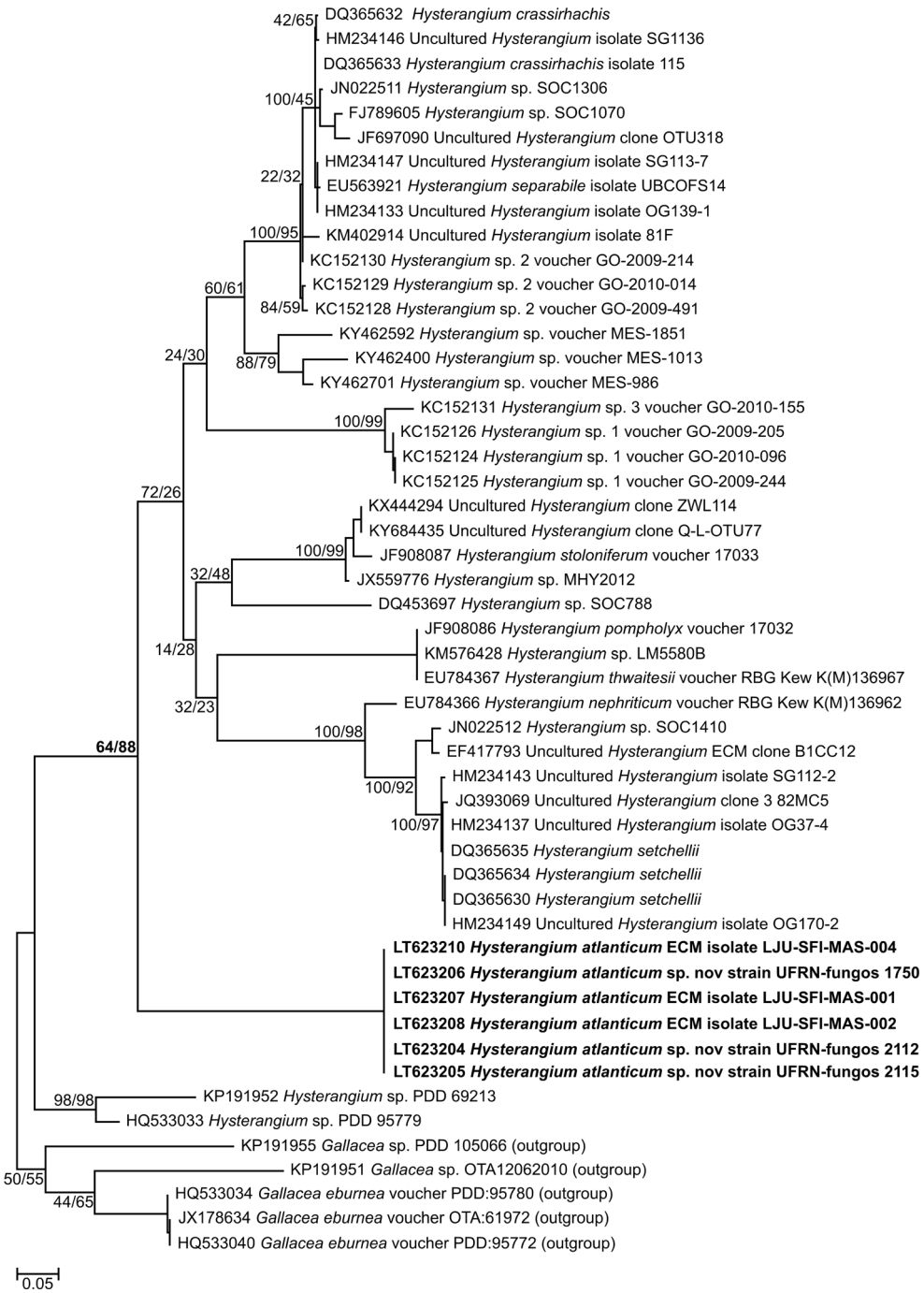


Fig. 2 Phylogram based on Maximum Likelyhood analysis of ITS marker. *Hysterangium atlanticum* sp. nov. basidiomata and EcM are indicated in bold. *Gallacea* spp. were used as an outgroup. Maximum Parsimony and Maximum Likelyhood bootstrap percentages are indicated on the branches (MP/ML)

Holotype – BRAZIL. Paraíba. Mamanguape, Guaribas Biological Reserve, under *Coccoloba*, 27 Jul 2012, leg. *Sulzbacher 412* (UFRN-Fungos 2115 holotype! URM 88220 isotype!; ITS sequence GenBank LT623204; LT623205; LT623206, *atp6* sequence Number LT635647; LT635648, EF-1α sequence LT635645; LT635646).

Etymology – The epithet refers to the Atlantic rainforest type of habitat.

Description – *Basidiomata* (4–7) 11–25 mm diam., (3–6) 8–19 mm high, globose to somewhat depressed, reniform, with a distinct rhizomorphic base (Fig. 3b). Peridium <1 mm thick, white (1A1) to yellowish white (1A2), yellowish grey (2B2), rapidly turns greyish orange (6B3) to reddish grey (7B2) or pale red (9A3) where bruised or exposed to air. Peridium surface tomentose under hand lens at immature stages, smooth and glabrous at maturity, covered by scattered to numerous rhizomorphs, roots or debris are frequently attached to the peridium (Fig. 3a, b). Gleba finely loculate, gelatinized, compacted, olive (3F3, 3F8) to olive brown (4F4), with rounded to irregular locules (<1 mm diam.) radially arranged. Columella dendroid and irregular in shape, 1–3 mm wide, 3–7 mm high, distinctly gelatinous, translucent, yellowish grey (3D2), to greyish beige (4C2), arising from a sterile base (Fig. 3c). Rhizomorphs 0.1–1.5 mm diam., white (1A1), yellowish grey (3B2) to greyish yellow (4B3), short and numerous going into the ground, at the base of the basidiomata. – *Microscopic characters*: Rhizomorphs 2–4 μm diam., constituted by hyaline, thin-walled hyphae, ramified and frequently encrusted by irregular shaped crystals 2–4.5 μm diam., which dissolve in 5% KOH (Fig. 5a–c), clamps frequent at the septa, with ampullated or inflated hyphal portions (4–8 μm diam.). The hyphae at the core of the rhizomorph are smooth, thick walled (up to 1.5 μm diam.), clamped, with brown contents, 2–3.5 μm diam. (Fig. 5c). Peridium (Fig. 3f, 4a) easily separable from gleba, 2-layered; external layer plectenchymatous (25–50 μm thick) formed by cylindrical yellowish interwoven hyphae 1–5 μm diam., slightly thickened walls, encrusted with crystalline particles,

Fig. 3 *Hysterangium atlanticum* sp. nov. (holotype). **a-b** Basidiomata. **c** Longitudinal section of basidiomata showing the gelatinized gleba. **d** Basidiospores (all mounted in 5% KOH with Congo Red). **e** Gleba structure. **f** Peridium. **g-h** Basidiospores under scanning electron microscopy (SEM) showing the verrucose ornamentation and heavily wrinkled utricle. *Scale bars* represent 10 mm (**a-c**), 10 μm (**d**), 100 μm (**e**), 20 μm (**f**), 2 μm (**g**), and 5 μm (**h**). Photos: M.A. Sulzbacher

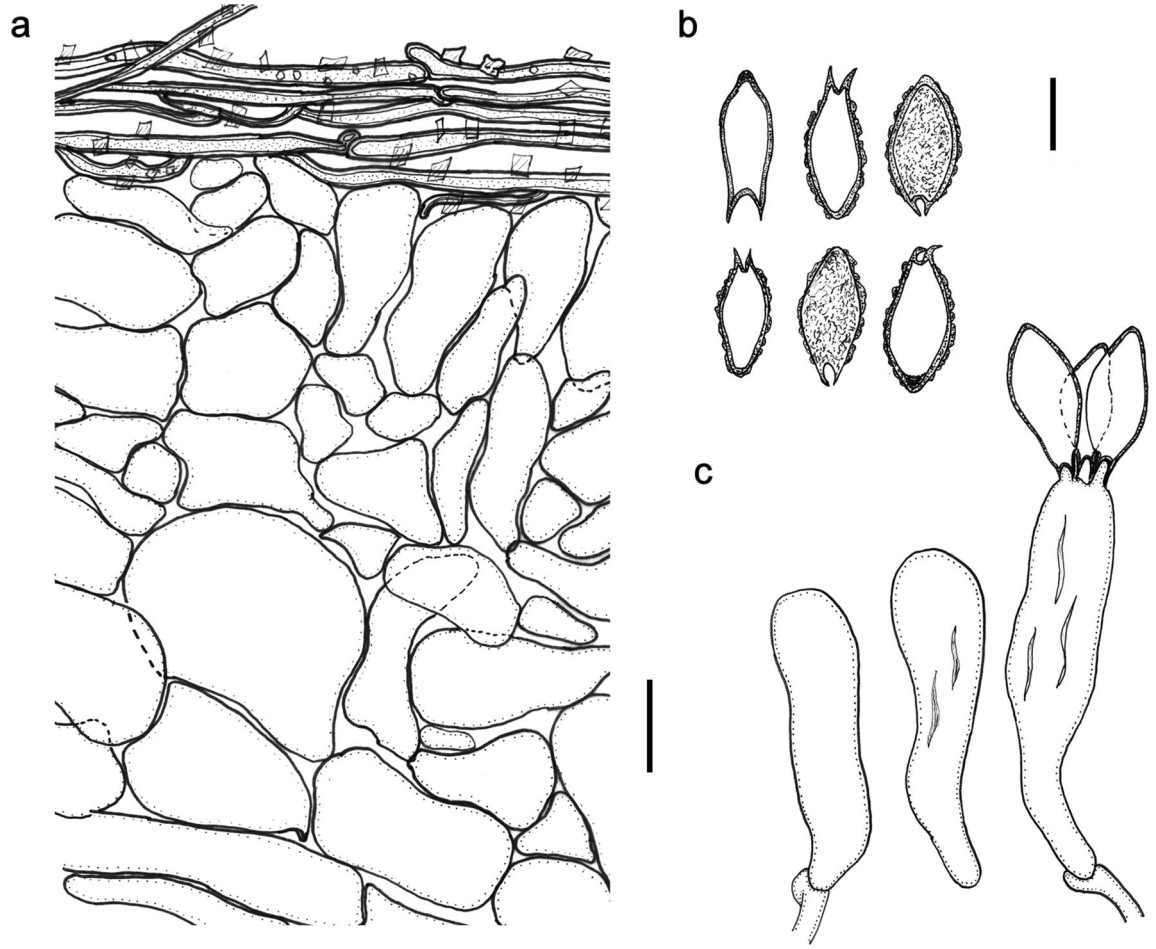


Fig. 4 *Hysterangium atlanticum* sp. nov. (holotype). **a** Peridium showing external and internal layers. **b** Basidiospores. **c** Basidia. *Scale bars* represent 10 μm (**a-c**)

clamp connections present; internal layer (230–307 μm thick) pseudoparenchymatous, composed by subglobose or angular in shape, more or less elongated hyaline hyphae, smooth and thin-walled, 7–20 μm diam., clamp connections present. Tramal plates of 38–140 μm thick, constituted by hyaline, mostly collapsed, subparallel to interwoven hyphae, smooth and thin-walled from 1 to 8 μm diam. (Fig. 3e). Basidioles 21–38 × 3–9 μm, clavate, hyaline. Basidia 28–45 × 6–9 μm, cylindrical to clavate, 1–4 spored, hyaline (Fig. 4c). Basidiospores (10–) 11–15 × 5–7 μm (ornamentation and sterigmal attachment base excluded), $L = 13$ μm, $W = 6$ μm, $Q = 1.8–2.6$, $Q_m = 2.2$; or 13–17 × 5–7 μm (attachment base included), $L = 15.2$ μm, $W = 6.3$ μm, $Q = (1.8–)1.9–3.0$, $Q_m = 2.4$, ellipsoid to suboblong, smooth, apex and base tapered, hyaline in KOH, slightly thickened wall 0.2–1.5(−2) μm thick, with a sterigmal attachment base (up to 3 μm high), utricle present and heavily wrinkled under SEM (Fig. 3g, h).

Ectomycorrhiza description: mycorrhizal root tips simple, monopodial-pinnate to irregularly pinnate, terminal tips of various lengths, the whole EcM system up to 20 mm long, white, the older parts yellowish white; ectomycorrhizae shiny with wooly surface, abundant and with a closed distribution in substrate. – *Rhizomorphs* abundant, especially in well-developed mycorrhizal systems, shiny, white to whitish, when handled turning ochre, frequently ramified, rhizomorphs connection to mantle oblique and in places not clearly visible (Fig. 3a). – *Margin* cottony. – *Exploration type* long distance. – *Sclerotia* absent. – *Morphology of the unramified ends* curved to bent, not inflated, tips very straight, white, shiny; older parts ochre to yellowish ochre. – *Anatomical characters of mantle in plan views*. *Mantle* not transparent, no latex, no dots, not carbonizing, with a lot of emanating hyphae over all of the surface. – *Anatomical characters of the outer mantle layer* plectenchymatous (Fig. 6b), inner mantel layers densely plectenchymatous, hyphae of the same diameter (3–5 μm diam.), septate, walls thin to slightly thickened (0.5–1 μm diam.) hyphae from which emanating hyphae and rhizomorphs originate, colorless, crystals on the surface and septa with clamp connections frequent. *Matrix* absent. *Hartig net* present. *Emanating hyphae* present, abundant, all over the mantle, white. – *Anatomical characters of emanating elements*: *Rhizomorphs* abundant, not differentiated, thin-walled, clamp connection frequent, no central hyphae observed, very similar to those of basidiomata, ramarioid (Fig. 6a, b), ampullated

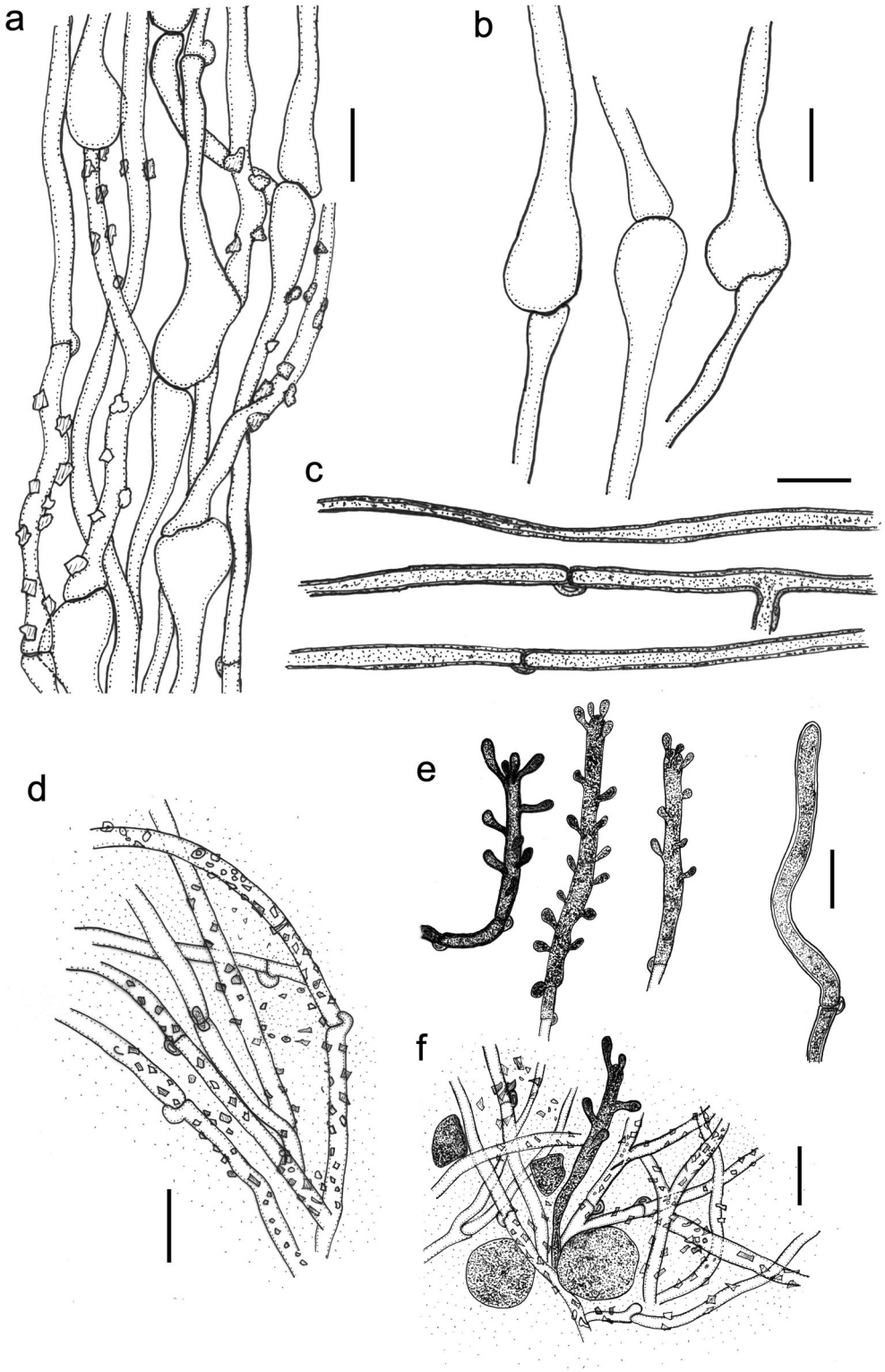


Fig. 5 *Hysterangium atlanticum* sp. nov. EcM (a-c: holotype; d-f: UFRN-fungos 1750). **a** Surface of rhizomophs with encrusted crystals and ampullate inflations at the septa. **b** Details of the ampullate inflations at the septa. **c** Thicker hyphae with simple septa, clamps and brown content. **d** Emanating hyphae. **e** Oleoacanthocystidia. **f** Emanating hyphae with roundish cells and cystidia, filled with contents. *Scale bars* represent 10 μm (a-f)

hyphae frequent (4–8 μm diam.), with open anastomoses; *Emanating hyphae* present, frequent, smooth, covered by numerous angular, irregular shaped crystals 1.5–5 μm diam., hyaline, cell walls thin, not filled, 3–7 μm diam., septate, septa clamped (Fig. 5d); *Cystidia* present (oleoacanthocystidia '*Hysterangium*-type' sensu Agerer 2006), frequently with short lateral outgrowths, roundish cells filled with yellowish or opaque contents, thick walled (0.4–1 μm diam.), (Fig. 5e, f, 6a).

Additional basidiomata examined: BRAZIL. Paraíba. Mamanguape, Guaribas Biological Reserve, SEMA II,

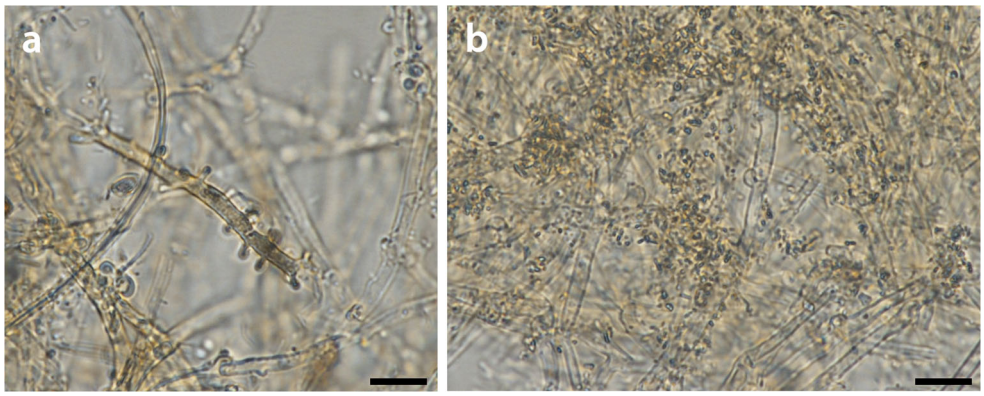


Fig. 6 *Hysterangium atlanticum* sp. nov. EcM (UFRN-fungos 1750). **a** Oleoacanthocystidia from emanating hyphae. **b** Plectenchymatous mantel with encrusted crystals. *Scale bars* represent 10 μm (**a**-**b**)

06°44.389′ S, 35°08.386′ W, under *Coccoloba alnifolia* 27 Jul 2012, leg. *Sulzbacher 408* (UFRN-Fungos 2112, URM 88222; paratypes); *idem,* under *Coccoloba* sp., 14 Jul 2012, leg. *Sulzbacher 396* (UFRN-Fungos 2207; paratype); *idem,* under *Coccoloba laevis*, 12 Sep 2012, leg. *Sulzbacher 438* (UFRN-Fungos 2205; paratype); *idem,* under *Coccoloba laevis* 30 Jul 2013, leg. *Sulzbacher 455* (UFRN-Fungos 1750; paratype).

Additional EcM examined: deposited at the Mycotheca and herbarium GIS at the Slovenian Forestry Institute under accession numbers: LJU-SFI-MAS001; LJU-SFI-MAS002; LJU-SFI-MAS003; LJU-SFI-MAS004.

Habitat and distribution: Hypogeous, under organic soil and forest debris, occurring either in large groups (±25 basidiomata was observed per single nest) or in small groups, and/or isolated in sandy soil (Quartzarenic Neosoil), fixed to roots; associated with *Coccoloba alnifolia* Casar. and *C. laevis* Casar.; known only from the type locality.

4 Discussion

Hysterangium atlanticum is a newly discovered hypogeous species from the Neotropics in northeastern Brazil. Its habitat is quite unique, since it occurs in coastal sand habitats colonized by ectomycorrhizal *Coccoloba alnifolia* and *C. laevis*, among other tropical plant species. Macroscopically, *H. atlanticum* resembles the description of Montecchi and Sarasini (2000) of the European species *H. stoloniferum* Tul. & C. Tul., specifically by the size of basidiomata (10–20 mm diam.), its smooth, whitish to reddish peridium and the presence of numerous ramified whitish rhizomorphs connecting other basidiomes. However, *H. stoloniferum* has larger hyaline spores (19–26 × 6–7 μm), shortly pedicellate at the base, and the peridiopellis is pseudoparenchymatous, composed of hyaline cells, with an external layer formed by prostrate and brownish hyphae, growing under deciduous trees (*Quercus* spp.), as indicated by Tulasne and Tulasne (1843).

Multi-locus molecular data support the separation of the new species, indicating its close relation to several unnamed *Hysterangium* species including one from Guyana (Fig. 2).

Based on the morphology, *Hysterangium hallingi* Castellano & J.J. Muchovej and *H. spegazzinii* Castellano & J.J. Muchovej, both from southern South America (Argentina, Chile and Uruguay), are similar to *H. atlanticum.* However, *H. hallingi* has spore wall thickness of ±1 μm thick, narrower basidiospores (4.5–5.5 μm diam.), and a three-layered peridium (Castellano and Muchovej 1996), and *H. spegazzinii* presents spores minutely verrucose with walls thinner than 0.5 μm. The only available sequence for *H. hallingi* out of the two species is significantly different from *H. atlanticum* (Fig. 1). Moreover, *H. hallingi* putative EcM host plants are *Nothofagus betuloides* and *N. pumilio*, and for *H. spegazzinii, Eucalyptus* sp. and *Nothofagus dombeyi*; however, we confirmed that *H. atlanticum* forms EcM with *Coccoloba* species in the native Atlantic rainforest. In agreement with Castellano (1988), and considering the EcM host specificity displayed by *Hysterangium*, the natural geographic distribution of fungi and their hosts is a reliable character for species differentiation and identification in this genus.

Recently, some new *Hysterangium* species have been found in the Neotropics, associated with native tropical taxa. For example two undescribed *Hysterangium* species growing on a *Dicymbe*-dominated forest in the Guyana Shield region (Henkel et al. 2012), as part of an ectomycorrhizal community, previously unknown to occur in those latitudes (Hosaka et al. 2008; Henkel et al. 2010; Castellano et al. 2012). Similarly, our studies showed an undocumented community of EcM fungi, including some hypogeous taxa, co-occurring in native fragments of the Atlantic rainforest in northeast Brazil (Sulzbacher et al. 2013; Sulzbacher et al. 2016; Sulzbacher et al. 2017). It is possible that this ectotrophic sand dune forest along the Brazilian Atlantic coast is home for a unique community of EcM taxa (Menolli et al. 2009; Gurgel et al. 2008; Pinheiro and Wartchow 2013; Sá et al. 2013; Wartchow et al. 2015). However, tropical forest types in northeast Brazil do not have EcM tree hosts such as *Aldina* (Benth.) Endl. and *Dicymbe* Spruce ex Benth. (Freire 1990; Oliveira-Filho and Carvalho

1993; Barbosa et al. 2011); instead, as shown in this work, the putative EcM partners are represented by trees species in the Polygonaceae (e.g. *Coccoloba* spp.), and likely also in the Fabaceae (Caesalpinioideae), Nyctaginaceae (*Guapira* spp.), which are confirmed EcM genera (Smith and Read 2008; Tedersoo et al. 2010; Põlme et al. 2017; Séne et al. 2018).

The EcM status for the Hysterangiales members has not been investigated for all taxa (Hosaka et al. 2006); however, in *Hysterangium*, the symbiosis was described for *Hysterangium crassirhachis* Zeller & C. W. Dodge and *H. stoloniferum*, based on morpho-anatomical studies (Agerer and Iosifidou 2004; Agerer and Rambold 2004–2017; Agerer 2006). The most prominent difference of *H. atlanticum* compared to other *Hysterangium* ectomcorrhizae is the unique presence of oleoacanthocystidia and rhizomorphs which are cottony and not differentiated, compared to slightly differentiated rhizomorphs with central hypha present in *H. stoloniferum* (Raidl and Agerer 1998), and a combination of slightly differentiated and undifferentiated rhizomorphs in *H. crassirhachis* (Müller and Agerer 1996).

The description of *Hysterangium atlanticum* sp. nov. and its ectomycorrhizae is a new contribution unveiling a fungal community of the Atlantic rainforest biodiversity hotspot area. The Atlantic rainforest spans a considerable area in Brazil and the ectomycorrhizal fungal diversity is just starting to be discovered.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interests.

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