

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 tissues. The main conspicuous features of the EcM are a well-developed plectenchimatus 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.

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1 Title:

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

4
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35

36 **Abstract** *Hysterangium* basidiomata were collected associated with *Coccoloba alnifolia*
37 and *C. laevis* (Polygonaceae), in the Guaribas Biological Reserve in the Atlantic
38 rainforest, of northeastern Brazil during the rainy seasons of 2012-2013. Based on its
39 unique morphological and molecular traits, this new taxon is described as *Hysterangium*
40 *atlanticum* sp. nov. The most prominent morphological characters that separate *H.*
41 *atlanticum* from other close relatives are the large size of the basidiomata, the white
42 peridium that rapidly turns greyish orange to pale red where bruised or exposed to air,
43 and the ellipsoid to suboblong spores with a minutely verrucose surface. Molecular
44 analyses of the LSU, SSU, *atp6*, and EF-1 α markers were done. The analyses of the
45 concatenated *atp6*/EF-1 α matrix confirmed the placement of the new species in the
46 */hysterangium* lineage. Moreover, at the infra-generic level, *Hysterangium atlanticum*
47 sp. nov. forms a sister clade with *Hysterangium* sp. from *Dicymbe* forests located in
48 neighboring Guyana. Moreover, the ectomycorrhizae (EcM) formed by *H. atlanticum*
49 and roots of *Coccoloba* species was confirmed, based on identical ITS nrDNA
50 sequences obtained from basidiomata and EcM tissues. The main conspicuous features
51 of the EcM are a well-developed plectenchimatous mantle; the ramarioid, abundant
52 emanating hyphae with clamps and covered with crystals; the presence of
53 oleoacanthocystidia and the whitish rhizomorphs. This is the first report of a
54 *Hysterangium* species forming EcM with native members of *Coccoloba* spp. in South
55 America.

56

57 **Key words** Ectomycorrhizae . Hypogeous fungi . Hysterangiales . Neotropics . Phylogeny

58

59

60 **Introduction**

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

69 Species of *Hysterangium* are hyphal-mat-forming fungi (Agerer 2001) and have the
70 capacity to modify soil chemistry and microbial biomass (Griffiths et al. 1994; Entry et al. 1992;

71 Trappe et al. 2012), playing an important role in the cycling of nutrients, water uptake and also
72 soil stabilization in forest ecosystems (Entry et al. 1991, 1992; Trappe et al. 2012).

73 This genus is widely distributed in both the Northern and Southern Hemispheres, with
74 characteristic discrete host ranges (Castellano 1999). The most comprehensive revision of the
75 genus *Hysterangium* diversity in South America was prepared by Castellano and Muchovej
76 (1996). In that study, four new species associated with *Eucalyptus* and *Nothofagus* were
77 described; furthermore, *Hallingea* Castellano, a new genus related to *Hysterangium* and
78 exclusively found in South America, was proposed. Currently, based on DNA analysis and
79 intensive sampling of unexplored areas, new species from various world regions have been
80 described (Xu and Liu 2003; Hosaka et al. 2007; Guerrero et al. 2008; Elliott et al. 2015).

81 Despite its global distribution, the genus is poorly known in the neotropics and subtropics.
82 In Brazil, only a few records of *Hysterangium* species are available, primarily from introduced
83 eucalypt and pine plantations. Among them, *H. australe* Speg. (Rick 1961), *H. gardneri* E.
84 Fisch. (Giachini et al. 2000), *H. affine* Masee & Rodway and *H. inflatum* Rodway (Cortez et al.
85 2011). *Hysterangium thaxteri* Zeller & Dodge, also reported for Brazil by Zeller and Dodge
86 (1929) is currently considered a member of the genus *Gelopellis* Zeller.

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

92

93 **Material and Methods**

94

95 Specimens were collected in the rainy season, from July to September 2012 and in June 2013, at
96 the Guaribas Biological Reserve, between 06°39'47''–06°42'57''S, and 35°06'46''–
97 35°08'00''W (Barbosa et al. 2011). This area is located in the State of Paraíba, Brazil, covering
98 4029 ha of the Atlantic rainforest protected under the Guaribas Biological Reserve. The
99 vegetation ranges from lowland semi-deciduous forest to savannas. The forests contain primarily
100 members of the families Fabaceae, Melastomataceae, Myrtaceae, Nyctaginaceae, Rubiaceae,
101 Polygonaceae Cyperaceae and Poaceae (Barbosa et al. 2011). Soils are Tertiary sediments of the
102 “Barreiras” formation (Barbosa et al. 2011) the topsoil is sandy, composed mainly of marine
103 quartz sand (Quartzarenic Neosoil).

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

107 A soil core for EcM analyses was taken from a single plot in June of 2013 directly from
108 under the basidiomata (Sulzbacher 455 – UFRN-fungos 1750). The soil sample including humus
109 layer and mineral soil of 15 × 15 cm and 5 cm deep was collected, following Suz et al. (2008).

110

111 **Morphological analyses**

112 Collections were photographed *in situ*. Informative macro and micro characters were observed
113 with the aid of a dissecting microscope (EZ4 Leica), and photographed using light microscopy at
114 40× and 100× (Eclipse Ni Nikon and digital camera DS-Ri1 Nikon). Spores were studied by
115 scanning electron microscopy (XL30-ESEM Phillips). Line drawings of the microstructures were
116 made with the aid of a drawing tube attached to the microscope (BX41 Olympus), with 100×
117 magnifications. Basidiospore measurements as proposed by Tulloss et al. (1992) and based on 30
118 mature spores. Abbreviations include $L(W)$ =average basidiospore length (width), Q =the length to
119 width ratio range as determined from all measured basidiospores, and Q_m =the Q value averaged
120 from all measured basidiospores. Basidiomata coloration was registered from fresh material;
121 color codes followed Kornerup & Wanscher (1978). Vouchers were deposited at the
122 Universidade Federal do Rio Grande do Norte Herbarium, Natal, Rio Grande do Norte, Brazil
123 (UFRN), with duplicate material deposited in Father Camille Torrend Herbarium, Recife,
124 Pernambuco, Brazil (URM).

125 EcM root tips were carefully washed and separated from the soil sample with tap water.
126 Morphological analyses of fresh EcM tips followed Agerer (1991), under a dissecting
127 microscope (EZ4 Leica) and light microscopy at magnifications of 40× and 100× (Eclipse Ni
128 Nikon) and photographed with a microscopy digital camera (DS-Ri1 Nikon). Line drawings of
129 the microstructures were made using a drawing tube attached to the microscope (BX41
130 Olympus) at 100X magnification.

131 **DNA extraction, amplification and sequencing**

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

136 Four nuclear or mitochondrial markers were amplified for basidiomata: complete *nuc*-ITS-
137 rDNA spacer (ITS), partial *nuc*-LSU-rDNA, partial *atp6*, and partial EF-1 α , using primer pairs

138 ITS1/ITS4 (White et al. 1990), NS1/NS4 (White et al. 1990), ATP6-2F/ATP6-3R (Kretzer and
139 Bruns 1999) and rEF1-983F/rEF1-1953R (Rehner and Buckley 2005), respectively. For the
140 fungal DNA isolated from EcM only the ITS marker was amplified. PCR reactions were
141 performed as follows: 1.0 µl DNA; 2.5 µl PCR buffer 10×; 3.0 µl dNTPs (1.5 mM); 2.0 µl MgCl₂
142 (20 mM); 3.0 µl of each primer (25 pmol); 0.5 U *Taq* polymerase (5 U µl⁻¹); and 10.5 µl
143 ultrapure water. PCR conditions followed previously published protocols for selected primers
144 (*ibid.*) and modified for amplification of ITS (Sulzbacher et al. 2016). Amplifications were done
145 in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).
146 Prior to sequencing, PCR products were purified from agarose gel using The Wizard SV
147 Genomic DNA Purification System (Promega Corporation, Madison, WI, USA). Both strands
148 were sequenced separately at Macrogen Korea (Seoul, Korea) with the same primers used in the
149 amplification. Sequencher 5.1 (Gene Codes Corporations, Ann Arbor, MI, USA) was used to
150 assemble the consensus sequence from the two strands of each isolate.

151

152 **Molecular analyses**

153 The new sequences generated were compared to those available at GenBank (Altschul et al.
154 1997); the accession numbers are indicated in Tables S1 and S2. Two datasets were aligned in
155 Mafft v6.859b (Katoh et al. 2005) using a default alignment approach.

156 To assess the phylogenetic position of the new species, concatenated partial *atp6* and EF-
157 1α sequences from GenBank were included (Table S1), mainly from Hosaka et al. (2008);
158 *Phallus hadriani* and *Ramaria flavobrunnescens* were used as outgroup (*ibid.*). Analyses were
159 conducted using Maximum Parsimony (MP) and maximum likelihood (ML). The Maximum
160 Parsimony (MP) phylogenetic reconstruction with Subtree-Pruning-Regrafting MP search
161 method using all sites, and 1000 bootstrap repetitions (MPbs); while for Maximum Likelihood
162 (ML), the General Time Reversible with a discrete Gamma distribution, and assuming invariable
163 sites (+I) was selected after ModelTest in MEGA7.0.18 (Kumar et al. 2016), and 1000 bootstrap
164 repetitions (MLbs), with a partial deletion of gaps/missing data (95 % site coverage cutoff).

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

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

175

176 **Results**

177

178 **Molecular analyses**

179 For reconstruction of Hysterangiales phylogeny and taxonomic positioning of the new
180 hypogeous species, LSU and complete ITS sequences were not included in the analysis because
181 LSU sequences were poorly represented in nucleotide databases for a relevant analysis, and the
182 complete ITS region was too heterogeneous within Hysterangiales to be aligned with confidence.
183 As a result, a concatenated dataset (*atp6/EF-1 α*) was prepared. The matrix contained 108 taxa
184 (Table S1) and 1314 positions. The Maximum Parsimony (data not shown) and Maximum
185 Likelihood analyses (Fig. 1) resulted in trees with similar topologies, where the new species
186 forms a well-supported terminal clade (MPbs = 88; MLbs = 88), sister group to three sequences
187 of *Hysterangium* sp., e.g.: SM10007 (DQ218869), SM10166 (DQ218871), and SM10100
188 (DQ218870). The latter sequences were collected in Guyana, in tropical forests (Hosaka et al.
189 2008). All these sequences form the sister group of three *Hysterangium* sp.: *Hysterangium* sp.
190 H5573 (DQ218863), *Hysterangium* sp. T17501 (DQ218841), and *Hysterangium* sp. T13345
191 (DQ218872), all from Asia (Hosaka et al. 2008).

192 In the second dataset, the ITS matrix contained 51 taxa and 609 positions. Sequences
193 from the basidiomata of *H. atlanticum* sp. nov. (ITS sequence GenBank LT623204; LT623205;
194 LT623206), and from EcM roottips of *Coccoloba* (ITS sequence GenBank LT623207;
195 LT623208; LT623209; LT623210) were identical (Fig. 2); thus, the identity of *H. atlanticum* sp.
196 nov. as the fungal symbiont of the EcM roottips collected was confirmed.

197

198 **Taxonomy**

199

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

201 Figs. 3–6

202 MycoBank MB 817856

203 *Diagnosis* – The combination of basidiomes up to 25 mm in diam., the white peridium that
204 rapidly turns greyish orange to pale red where bruised or exposed to air, basidiospores 11–15 \times
205 5–7 μ m, ellipsoid, utricle present, are the main features that characterize this species growing
206 under *Coccoloba* spp.

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

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

212 *Description* – *Basidiomata* (4–7) 11–25 mm diam., (3–6) 8–19 mm high, globose to somewhat
213 depressed, reniform, with a distinct rhizomorphic base (Fig. 3b). Peridium <1 mm thick, white
214 (1A1) to yellowish white (1A2), yellowish grey (2B2), rapidly turning greyish orange (6B3) to
215 reddish grey (7B2) or pale red (9A3) where bruised or exposed to air. Peridium surface
216 tomentose under hand lens at immature stages, smooth and glabrous at maturity, covered by
217 scattered to numerous rhizomorphs, roots or debris are frequently attached to the peridium (Fig.
218 3a, b). Gleba finely loculate, gelatinized, compacted, olive (3F3, 3F8) to olive brown (4F4), with
219 rounded to irregular locules (<1 mm diam.) radially arranged. Columella dendroid and irregular
220 in shape, 1–3 mm wide, 3–7 mm high, distinctly gelatinous, translucent, yellowish grey (3D2), to
221 greyish beige (4C2), arising from a sterile base (Fig. 3c). Rhizomorphs 0.1–1.5 mm diam., white
222 (1A1), yellowish grey (3B2) to greyish yellow (4B3), short and numerous going into the ground,
223 at the base of the basidiomata. – *Microscopic characters*: Rhizomorphs 2–4 μ m diam.,
224 constituted by hyaline, thin-walled hyphae, ramified and frequently encrusted by irregular
225 shaped crystals 2–4.5 μ m diam., which dissolve in 5% KOH (Fig. 5a, b, c), clamps frequent at
226 the septa, with ampullated or inflated hyphal portions (4–8 μ m diam.). The hyphae at the core of
227 the rhizomorph are smooth, thick walled (up to 1.5 μ m diam.), clamped, with brown contents, 2–
228 3.5 μ m diam. (Fig. 5c). Peridium (Fig. 3f, 4a) easily separable from gleba, 2-layered; external
229 layer plectenchymatous (25–50 μ m thick) formed by cylindrical yellowish interwoven hyphae 1–
230 5 μ m diam., slightly thickened walls, encrusted with crystalline particles, clamp connections
231 present; internal layer (230–307 μ m thick) pseudoparenchymatous, composed by subglobose or
232 angular in shape, more or less elongated hyaline hyphae, smooth and thin-walled, 7–20 μ m
233 diam., clamp connections present. Tramal plates of 38–140 μ m thick, constituted by hyaline,
234 mostly collapsed, subparallel to interwoven hyphae, smooth and thin-walled from 1–8 μ m diam.
235 (Fig. 3e). Basidioles 21–38 \times 3–9 μ m, clavate, hyaline. Basidia 28–45 \times 6–9 μ m, cylindrical to
236 clavate, 1–4 spored, hyaline (Fig. 4c). Basidiospores (10–) 11–15 \times 5–7 μ m (ornamentation and
237 sterigmal attachment base excluded), $L=13\mu$ m, $W=6\mu$ m, $Q=1.8-2.6$, $Q_m=2.2$; or 13–17 \times 5–7 μ m
238 (attachment base included), $L=15.2\mu$ m, $W=6.3\mu$ m, $Q=(1.8-1.9-3.0)$, $Q_m=2.4$, ellipsoid to
239 suboblong, smooth, apex and base tapered, hyaline in KOH, slightly thickened wall 0.2–1.5(–2)
240 μ m thick, with a sterigmal attachment base (up to 3 μ m high), utricle present and heavily
241 wrinkled under SEM (Fig. 3g, h).

242 *Ectomycorrhiza* description: mycorrhizal root tips simple, monopodial-pinnate to
243 irregularly pinnate, terminal tips of various lengths, the whole EcM system up to 20 mm long,
244 white, the older parts yellowish white; ectomycorrhizae shiny with wooly surface, abundant and
245 with a nested distribution in substrate. – *Rhizomorphs* abundant, especially in well-developed
246 mycorrhizal systems, shiny, white to whitish, when handled turning ochre, frequently ramified,
247 rhizomorphs connection to mantle oblique and in places not clearly visible (Fig. 3a). – *Margin*
248 cottony. – *Exploration type* long distance. – *Sclerotia* absent. – *Morphology of the unramified*
249 *ends* curved to bent, not inflated, tips very straight, white, shiny; older parts ochre to yellowish
250 ochre. – *Anatomical characters of mantle in plan views*. Mantle not transparent, no latex, no
251 dots, not carbonizing, with a lot of emanating hyphae over all of the surface. – *Anatomical*
252 *characters of the outer mantle layer* plectenchymatous (Fig. 6b), inner mantle layers densely
253 plectenchymatous, hyphae of the same diameter (3–5 µm diam.), septate, walls thin to slightly
254 thickened (0.5–1 µm diam.) hyphae from which emanating hyphae and rhizomorphs originate,
255 colorless, crystals and septa with clamp connections frequent. *Matrix* absent. *Hartig net* present.
256 *Emanating hyphae* present, abundant, all over the mantle, white. – *Anatomical characters of*
257 *emanating elements*: *Rhizomorphs* abundant, not differentiated, thin-walled, clamp connection
258 frequent, no central hyphae observed, very similar to those of basidiomata, ramarioid (Fig. 6a,
259 b), ampullated hyphae frequent (4–8 µm diam.), with open anastomoses; *Emanating hyphae*
260 present, frequent, smooth, covered by numerous angular, irregular shaped crystals 1.5–5µm
261 diam., hyaline, cell walls thin, not filled, 3–7µm diam., septate, septa clamped (Fig. 5d); *Cystidia*
262 present (oleoacanthocystidia ‘*Hysterangium*-type’ sensu Agerer 2006), frequently with short
263 lateral outgrowths, roundish cells filled with yellowish or opaque contents, thick walled (0.4–1
264 µm diam.), (Fig. 5e, f, 6a).

265 *Additional basidiomata examined*: BRAZIL. Paraíba. Mamanguape, Guaribas Biological
266 Reserve, SEMA II, 06°44.389' S, 35°08.386' W, under *Coccoloba alnifolia* 27 Jul 2012, leg.
267 *Sulzbacher 408* (UFRN-Fungos 2112, URM 88222; paratypes); *idem*, under *Coccoloba* sp., 14
268 Jul 2012, leg. *Sulzbacher 396* (UFRN-Fungos 2207; paratype); *idem*, under *Coccoloba laevis*, 12
269 Sep 2012, leg. *Sulzbacher 438* (UFRN-Fungos 2205; paratype); *idem*, under *Coccoloba laevis* 30
270 Jul 2013, leg. *Sulzbacher 455* (UFRN-Fungos 1750; paratype).

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

274 *Habitat and distribution*: Hypogeous, under organic soil and forest debris, occurring either
275 in large groups (±25 basidiomata was observed per single nest) or in small groups, and/or

276 isolated in sandy soil (Quartzarenic Neosoil), fixed to roots; associated with *Coccoloba alnifolia*
277 Casar. and *C. laevis* Casar.; known only from the type locality.

278

279 **Discussion**

280 *Hysterangium atlanticum* is a newly discovered hypogeous species from the Neotropics in
281 northeastern Brazil. Its habitat is quite unique, since it occurs in coastal sand habitats colonized
282 by ectomycorrhizal *Coccoloba alnifolia* and *C. laevis*, among other tropical plant species.
283 Macroscopically, *H. atlanticum* resembles the description of Montecchi and Sarasini (2000) of
284 the European species *H. stoloniferum* Tul. & C. Tul., specifically by the size of basidiomata (10
285 – 20 mm diam.), its smooth, whitish to reddish peridium and the presence of numerous ramified
286 whitish rhizomorphs connecting other basidiomes. However, *H. stoloniferum* has larger hyaline
287 spores (19–26 × 6–7 µm), shortly pedicellate at the base, and the peridiopellis is
288 pseudoparenchymatous, composed of hyaline cells, with an external layer formed by prostrate
289 and brownish hyphae, growing under deciduous trees (*Quercus* spp.), as indicated by Tulasne &
290 Tulasne (1843).

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

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

305 Recently, some new *Hysterangium* species have been found in the Neotropics, associated
306 with native tropical taxa. For example two undescribed *Hysterangium* species growing on a
307 *Dicymbe*-dominated forest in the Guyana Shield region (Henkel et al. 2012), as part of an
308 interesting ectomycorrhizal community, previously unknown to occur in those latitudes (Hosaka
309 et al. 2008; Henkel et al. 2010; Castellano et al. 2012). Similarly, our studies showed an
310 undocumented community of EcM fungi, including some hypogeous taxa, co-occurring in native

311 fragments of the Atlantic rainforest in northeast Brazil (Sulzbacher et al. 2013; Sulzbacher et al.
312 2016; Sulzbacher et al. 2017). It is possible that this ectotrophic sand dune forest along the
313 Brazilian Atlantic coast is home for a unique and interesting community of EcM taxa (Menolli et
314 al. 2009; Gurgel et al. 2008; Pinheiro et al. 2013; Sá et al. 2013; Wartchow et al. 2015).
315 However, tropical forest types in northeast Brazil do not have EcM tree hosts such as *Aldina*
316 (Benth.) Endl. and *Dicymbe* Spruce ex Benth. (Freire 1990; Oliveira-Filho and Carvalho 1993;
317 Barbosa et al. 2011); instead, as shown in this work, the putative EcM partners are represented
318 by trees species in the Polygonaceae (e.g. *Coccoloba* spp.), and likely also in the Fabaceae
319 (Caesalpinioideae), Nyctaginaceae (*Guapira* spp.), which are confirmed EcM genera (Smith and
320 Read 2008; Tedersoo et al. 2010).

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

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

334

335 **Compliance with ethical standards**

336 **Conflict of interest** The authors declare no conflicts of interests.

337

338 **References**

339

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462
 463
 464 **Fig 1.** Phylogram based on Maximum [Likelihood](#) analysis of concatenated *atp6* and EF-1 α genes of sequences
 465 included in Table S1. The two new sequences generated from *Hysterangium atlanticum* sp. nov. basidiomata are
 466 marked in bold. *Phallus hadriani* and *Ramaria flavobrunnescens* were included as outgroup taxa. Maximum
 467 Parsimony and Maximum [Likelihood](#) bootstrap percentages are indicated on the branches (MP/ML).
 468

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

472
473 **Fig 3** *Hysterangium atlanticum* sp. nov. (holotype). **a-b** Basidiomata. **c** Longitudinal section of basidiomata showing
474 the gelatinized gleba. **d** Basidiospores (all mounted in 5% KOH with Congo Red). **e** Gleba structure. **f** Peridium. **g-h**
475 Basidiospores under scanning electron microscopy (SEM) showing the verrucose ornamentation and heavily
476 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**).
477 Photos: M.A. Sulzbacher.

478
479 **Fig. 4** *Hysterangium atlanticum* sp. nov. (holotype). **a** Peridium showing external and internal layers. **b**
480 Basidiospores. **c** Basidia. *Scale bars* represent 10 µm (**a-c**).
481

482 **Fig. 5** *Hysterangium atlanticum* sp. nov. EcM (a-c: holotype; d-f: UFRN-fungos 1750). **a** Surface of rhizomorphs
483 with encrusted crystals and ampullate inflations at the septa. **b** Details of the ampullate inflations at the septa. **c**
484 Thicker hyphae with simple septa, clamps and brown content. **d** Emanating hyphae. **e** Oleoacanthocystidia. **f**
485 Emanating hyphae with roundish cells and cystidia, filled with contents. *Scale bars* represent 10 µm (a-f).
486

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

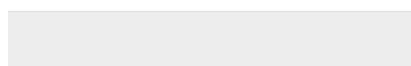
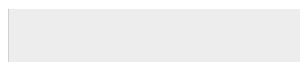
489
490
491 **Table S1:** Specimens/sequences used in the concatenated analyses of *atp6* and *EF-1α* genes. *Hysterangium*
492 *atlanticum* sp. nov., and the new sequences generated in this study are indicated in bold.

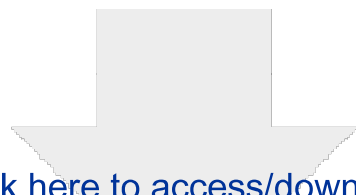
493
494 **Table S2:** Specimens/sequences used in the ITS analyses. Sequences from basidiomata, and EcM of *Hysterangium*
495 *atlanticum* sp. nov. are indicated in bold.
496



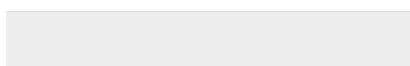
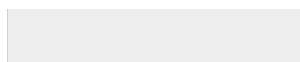
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Electronic Supplementary Material
Table S1_atp6_EF.docx





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Table S2_ITS.docx



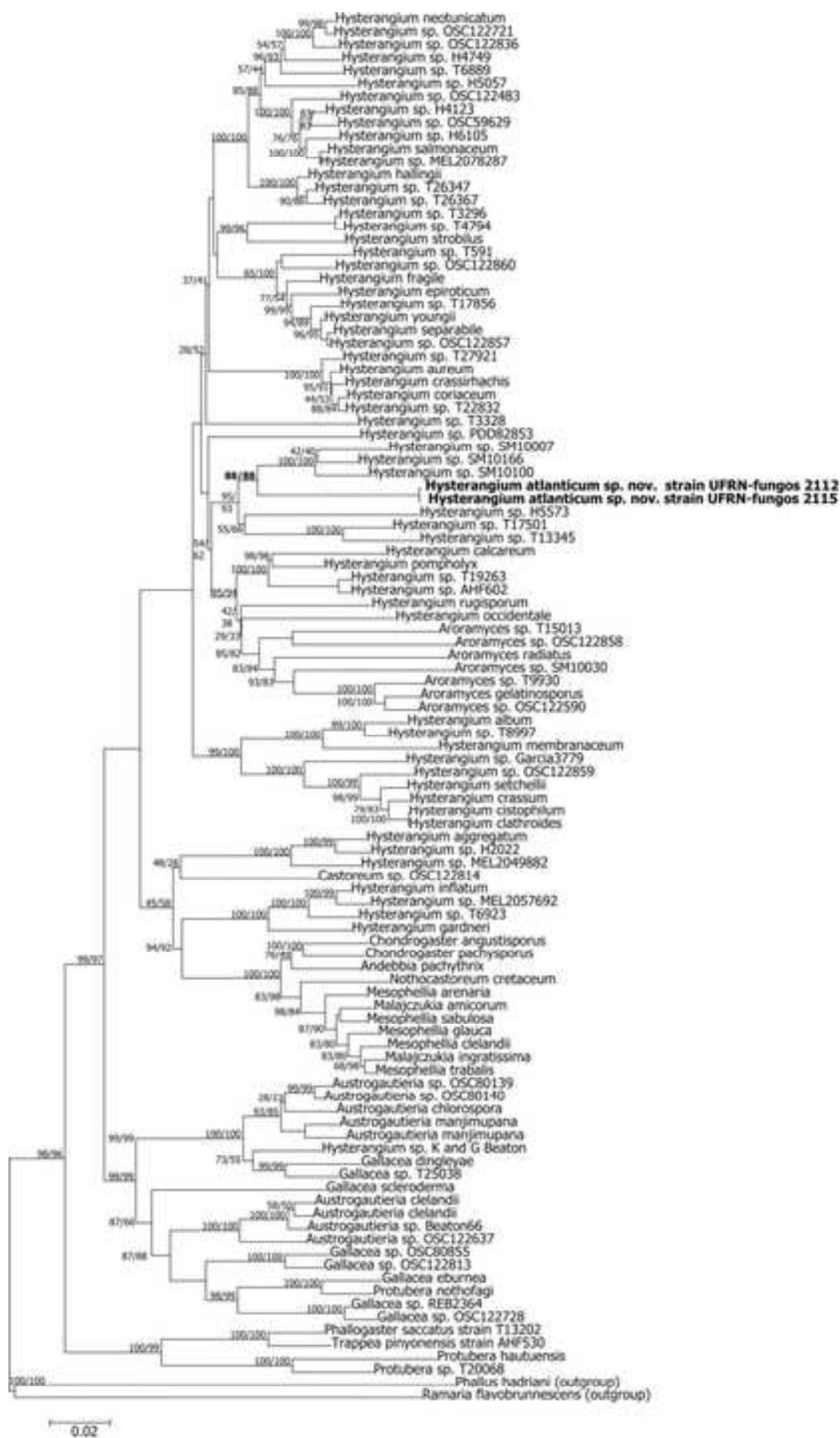
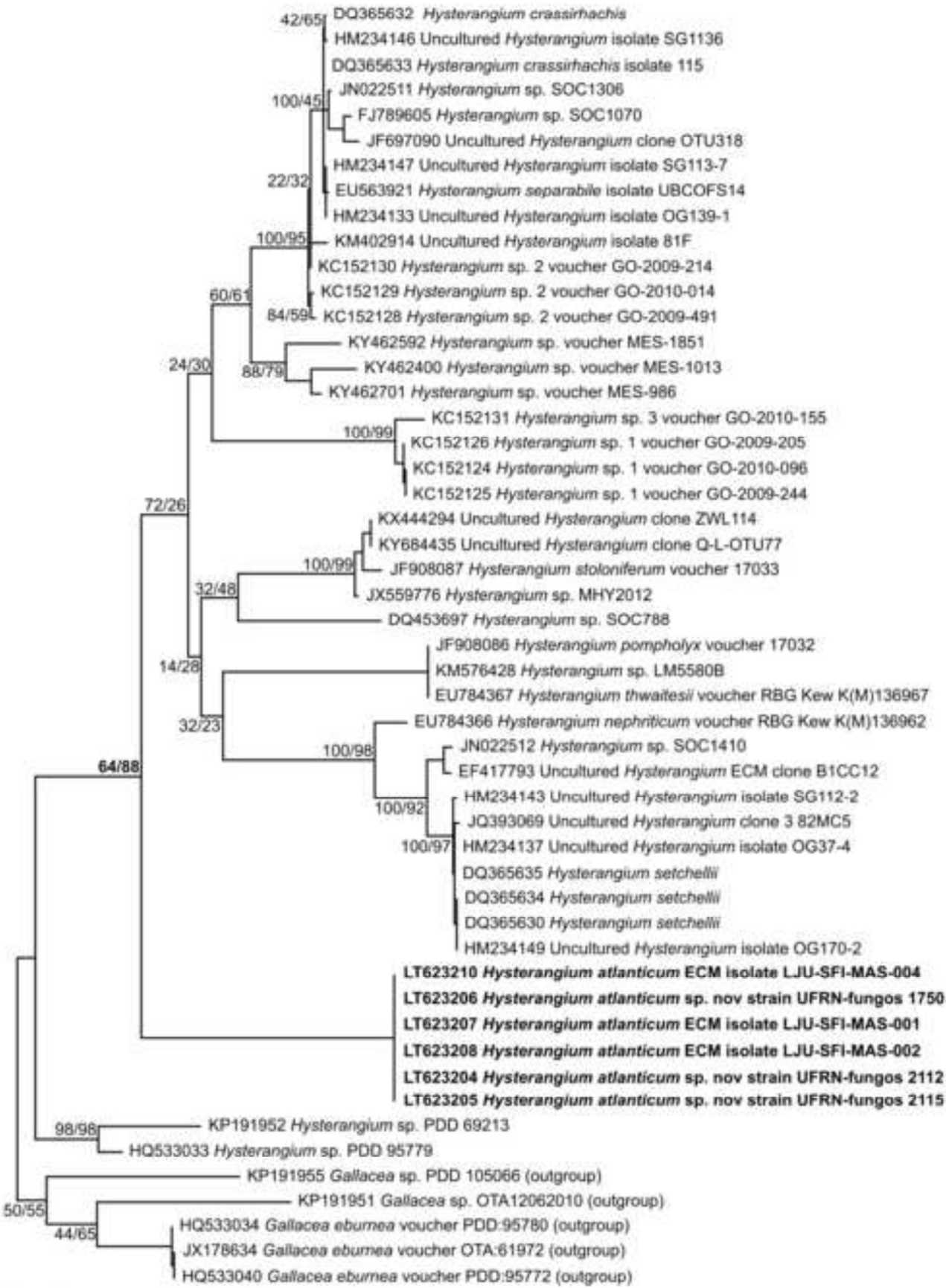


Figure 2

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