

Sclerotium-forming fungi from soils of the Atlantic rainforest of Northeastern Brazil

Marcelo A. Sulzbacher^{1,*}, Tine Grebenc², Admir J. Giachini³ & Iuri G. Baseia⁴

¹Departamento de Micologia/CCB, Universidade Federal de Pernambuco, Av. Prof. Nelson Chaves, s/n, CEP 50670-901, Recife, Pernambuco, Brazil

²Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia

³Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Santa Catarina, CEP 88040-970, Florianópolis, Santa Catarina, Brazil

⁴Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte, Campus Universitário, CEP 59072-970, Natal, Rio Grande do Norte, Brazil

*Author for correspondence: marcelo_sulzbacher@yahoo.com.br

Background and aims – Many South American ecosystems remain unexplored and neglected as to the occurrence and distribution of fungi. Data about occurrence of sporocarp forming hypogeous fungi in Atlantic rainforests and Caatinga biome are even less frequently published. For this reason, intensive studies on this group are needed. During studies of hypogeous sequestrate fungi in Northeastern Brazil several sclerotium-like structures were encountered in soil and here determined based on phylogenetic affiliation.

Methods – The fungal structures in soils were sampled by raking the litter and top soil organic layer at four different forest sites, all located in the Atlantic rainforest or in the ‘brejo de altitude’ (upland wet forest enclaves) of the Atlantic rainforest remnants within the Caatinga biome. Samples were studied by optical microscopy and molecular analyses. Internal Transcribed Spacer of nuclear ribosomal DNA (ITS nrDNA) sequences (barcoding of fungi) were used to identify some selected specimens.

Key results – Among the nine sclerotium samples, four were related to the */trechisporales* lineage, three related to */entolomataceae*, and two to the */amylocorticiales* lineages.

Conclusions – This study provides new information about sclerotium-forming fungi occurring naturally in soils and litter layers from native Atlantic rainforests in Brazil. It is a starting point for more detailed and systematic studies planned for epigeous and hypogeous sequestrate fungi. Such studies are needed to understand the distribution, evolutionary affiliations and trophic modes of those fungi.

Key words – Fungal diversity, ITS, tropical forests, restinga forests, sandy soils.

INTRODUCTION

The fungal phylum Basidiomycota comprises approximately 31 500 described species (Kirk et al. 2008), with agaricoid, clavarioid, corticioid, cyphelloid, gasteroid and stereoid basidiomes including those with either epigeous or hypogeous habits. These fungi are widely dispersed among terrestrial ecosystems as symbionts (mycorrhizal), pathogens, or mainly saprotrophs.

The tropical South American ecosystems remain unexplored and neglected as to the occurrence and distribution of fungi (Mueller et al. 2007) and lag behind in collection efforts when compared to North America, Europe, and Australasia. Records on the fungal partners and their distribution

can contribute to a more concise and accurate phylogenetic placement within the kingdom, as concluded by Tedersoo et al. (2010). Although diverse, data about hypogeous sequestrate fungi occurring in these regions are even more rarely found in the literature (Mueller et al. 2007, Henkel et al. 2010, Sulzbacher et al. 2013, 2017, Bâ et al. 2014).

In addition to sexual fruiting bodies, many genera of Basidiomycota produce sclerotia, specialized hyphal bodies involved in dormancy survival (Deacon 2006). In challenging conditions such as desiccation, long-term absence of a host, forest fire, microbial attack or freezing, fungi can survive by producing sclerotia. In addition, several sclerotium-forming fungi have complex life cycles or even uncertain trophic modes (Smith et al. 2015). Sclerotia are known for 41 genera

of Basidiomycota. They are most frequent in Agaricales, Boletales and Polyporales, with a wide trophic diversity (Smith et al. 2015).

A recent study has shown an unexpected and variable morphological diversity of sclerotium-forming fungal species in soils, litter layer and decayed wood in forests of North and South America (Smith et al. 2015). Smith et al. (2015) showed that these fungal structures are widely dispersed across Basidiomycota, and some of the lineages were not linked with any known fungal species. The authors inferred that these structures have been overlooked in most mycological studies, and therefore deserve more attention.

During recent taxonomic studies on the coastal sand dunes of the Atlantic rainforest and in the 'brejo de altitude' (upland wet forest enclaves) of Northeast Brazil, numerous sclerotia were discovered in soil samples and collected for analysis. To determine their identities and evolutionary affiliations, morphological and molecular (Internal Transcribed Spacer of nuclear ribosomal DNA) analyses were performed.

MATERIAL AND METHODS

Material

Hypogeous sporocarps and fungal structures (basidiomata in early developmental stages or proper sclerotia) were collected at four localities. (1) "Parque Estadual das Dunas de Natal", municipality of Natal (05°48'S–05°43'S 35°09'W–35°12'W) in the State of Rio Grande do Norte that represents a fragment of Atlantic rainforest – 'restinga arbórea' (Oliveira-Filho 1993, Oliveira-Filho & Carvalho 1993); (2) "Reserva Particular do Patrimônio Natural (RPPN) Mata Estrela", Baía Formosa city in the State of Rio Grande do Norte (06°22'40"S 35°01'22"W) in the largest fragment of Atlantic rainforest of this state, with 2039 ha (1888 ha of forest, 81 ha of dunes, and 69 ha of lakes) composed of trees predominant on the 'restinga arbórea' forest (Lourenço & Barbosa 2012); (3) "Reserva Biológica de Guaribas", an Atlantic rainforest of 4029 ha in the State of Paraíba (06°44.545'S 35°08.533'W), where vegetation is a mixture of Atlantic rainforest and species of the Caatinga and Coastal tableland (Barbosa et al. 2011); (4) "Reserva Ecológica Estadual Mata do Pau Ferro" (06°58'12"S 35°42'15"W), a natural forest in the state of Paraíba covering about 600 ha, with altitude ranging from 400 to 600 m representing a 'brejo de altitude' (upland wet forest enclaves), humid 'islands' of Atlantic rainforest remnants isolated within the Caatinga biome (Barbosa et al. 2004, Tabarelli & Santos 2004), composed of podzolic soils (Jacomine et al. 1973). For the most (except 'brejo de altitude'), soils are sandy and predominantly poor (Pontes & Barbosa 2009). Surveys were performed during the rainy season in 2011, 2012 and 2013. All specimens studied are listed in the electronic appendix.

Sclerotium-like structures were sampled along with hypogeous fungi following the protocol described by Sulzbacher et al. (2013). A randomized-plot sporocarp survey was used (Castellano et al. 2004): the surface layer of litter and top soil organic layer was raked to a depth of 10 cm. Two or three portions of selected structures were placed separately in Eppendorf tubes with silica gel for rapid drying and in CTAB

extraction buffer for molecular identification. Vouchers are deposited at the Herbarium of the Federal University of Rio Grande do Norte (UFRN Herbarium).

Morphology

Fresh sclerotium-like structures were analyzed macro and microscopically with the aid of a light microscope at magnifications of 40–1000× (Eclipse Ni Nikon) and photographed with a digital camera (DS-Ri1 Nikon).

DNA extraction and ITS nrDNA amplification

DNA was extracted from fresh specimens with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The ITS nrDNA was amplified with the primer pair ITS1/ITS4 (White et al. 1990). PCR conditions followed previously published protocols for the selected primers (Grebenc et al. 2009). Prior to sequencing, products were cleaned from the gel with The Wizard SV Genomic DNA Purification System kit (Promega Corporation, Madison, WI, USA). Both strands were sequenced separately at Macrogen Korea (Seoul, Korea) with the same primer set mentioned above. Sequencher 5.1 (Gene Codes Corporations, Ann Arbor, MI, USA) was used to assemble the consensus sequences from the two strands of the ITS nrDNA of each isolate. Sequences obtained from the isolates were compared to those available at GenBank (Altschul et al. 1997) database with the BLASTN algorithm to identify the highest similarities.

Identification

The sequences were assigned to lineage level according to Matheny et al. (2006), Tedersoo et al. (2010), and Smith et al. (2015) using a preliminary BLAST search followed by search for the best lineage using partial ITS sequences. The sclerotia were identified at the lineage level by the ITS sequence similarities, INSD databases, and available literature (Matheny et al. 2006, Tedersoo et al. 2010, Smith et al. 2015). When query sequence displayed high-sequences similarity with existing data (> 80 %), the given names for these sequences / taxa were considered as the query lineage.

RESULTS

Several putative sclerotia structures were collected from all four sites (see electronic appendix). Morphological remarks to each of them are included in this appendix. The ITS nrDNA region was successfully amplified only for nine of the collected vouchers (table 1) enabling us to conclude on the lineage of the particular collections. Sclerotia structures commonly showed clamp connections in the sterile tissue, proving their belonging in Basidiomycota, and exhibited diverse morphological features and colors (fig. 1). Some appeared in the field to be angiosperm structures such as rhizomes or bulbs (e.g. *Sulzbacher* 413), but when examined under the microscope, true fungus structures were observed including hyphae, pseudoparenchyma and rhizomorphs.

Among the nine successfully sequenced samples, the */trechisporales* lineage was most frequently observed with

Table 1 – Taxonomic affiliations of sclerotium-forming fungi based on INSD (<http://ncbi.nlm.nih.gov/>) fungal ITS sequence database.

Voucher number/GenBank	Best Hit_ INSD	ITS similarity percentage	Related name INSD	Sclerotium-forming lineage
Sulzbacher 340; UFRN-Fungos 2057 (LT594979)	EF421110	349/413 (85 %)	<i>Rhodocybe truncata</i>	/entolomataceae
Sulzbacher 345; UFRN-Fungos 2066 (LT594980)	JX392819	518/622 (83 %)	<i>Trechispora</i> sp.	/trechisporales
Sulzbacher 346; UFRN-Fungos 2061 (LT594981)	DQ411529	226/252 (90 %)	<i>Trechispora alnicola</i>	/trechisporales
Sulzbacher 406; UFRN-Fungos 2064 (LT594982)	KJ720886	658/667 (99 %)	<i>Amyloathelia</i> sp.	/amylocorticiales
Sulzbacher 410; UFRN-Fungos 1752 (LT594983)	KJ720886	633/655 (97 %)	<i>Amyloathelia</i> sp.	/amylocorticiales
Sulzbacher 413; UFRN-Fungos 2062 (LT594984)	KP012803	368/442 (83 %)	<i>Rhodocybe</i> sp.	/entolomataceae
Sulzbacher 463; UFRN-Fungos 2070 (LT594985)	JX392819	494/597 (83 %)	<i>Trechispora</i> sp.	/trechisporales
UFRN-Fungos 2068 (LT594986)	JX392819	476/578 (82 %)	<i>Trechispora</i> sp.	/trechisporales
Sulzbacher 433 (LT594987)	AB973812	476/552 (86 %)	<i>Entoloma</i> aff. <i>conferendum</i>	/entoloma

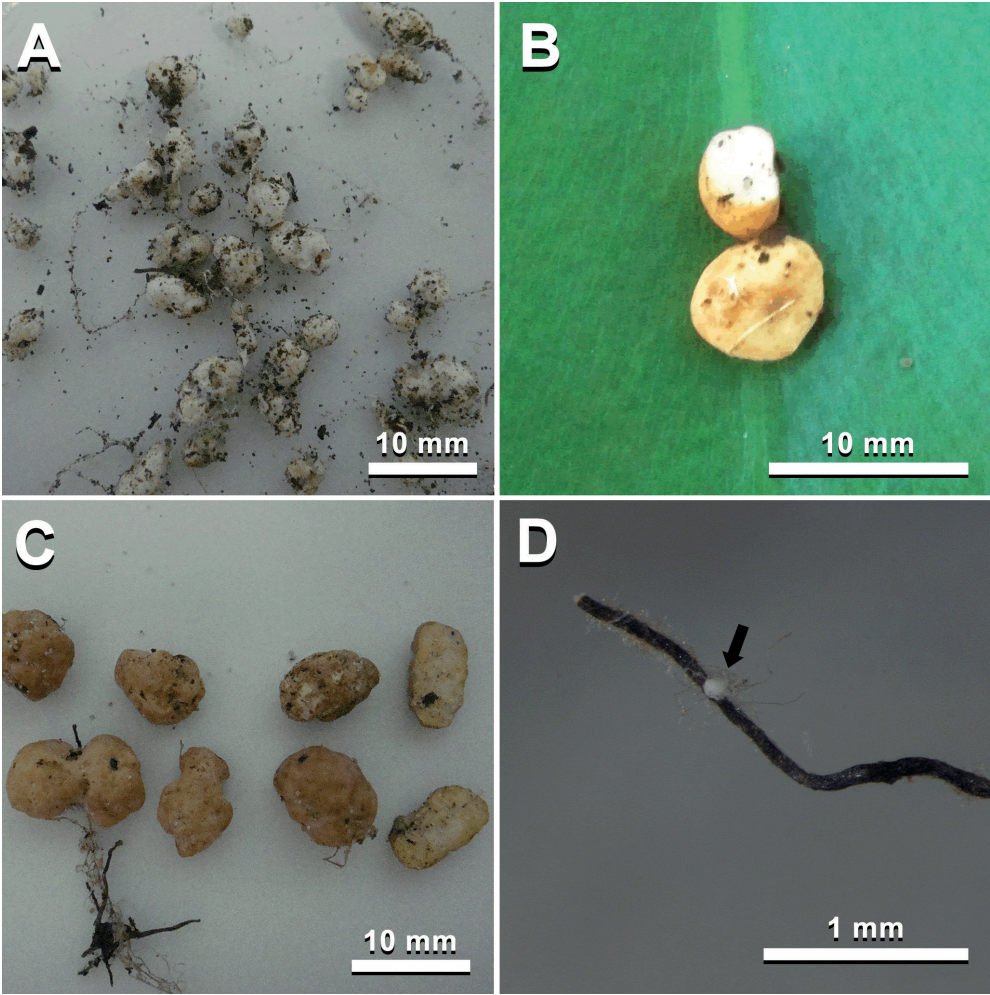


Figure 1 – Sclerotia collected in soils of native forests: A, /trechisporales lineage (Sulzbacher 346, UFRN-Fungos 2061); B, /amylocorticiales lineage (Sulzbacher 406, UFRN-Fungos 2064); C, unknown lineage (Sulzbacher 343, UFRN-Fungos 1755); D, /entoloma lineage (Sulzbacher 433).

four records (UFRN-Fungos 2066, 2061, 2070 and 2068). Two collections were related to the */amylocorticiales* lineage (UFRN-Fungos 1752 and 2064). Another three corresponded to the */entolomataceae* lineage: the Blast search related two sequences to two *Rhodocybe* species, with low ITS similarity; and a third specimen (Sulzbacher 433) to an *Entoloma* species. This last specimen was observed forming microsclerotia < 1 mm diam. (fig. 1D), frequently attached to fine roots of *Coccoloba laevis* Casar.

All specimens recorded were frequently observed near or attached to fine roots of native plants, potentially ectomycorrhizal partners. Sclerotia formed by the */trechisporales* lineage commonly occurred in the rhizosphere of the ‘restinga’ forests at “Reserva Particular do Patrimônio Natural (RPPN) Mata Estrela” and the “Parque Estadual das Dunas de Natal”, both in the State of Rio Grande do Norte. These lineages were not found in the state of Paraíba (see electronic appendix).

DISCUSSION

This study has confirmed that cryptic fungal communities can be detected by alternative sampling approaches, especially focusing on the hypogeous habit (Castellano et al. 2004). Soils from the Atlantic rainforest in Northeastern Brazil present diverse sclerotia types that need to be considered in future diversity studies. According to our results, a combination of morphological and molecular methods was successfully used to study and characterize the sclerotium-forming fungal diversity of a specific habitat.

In a recent review, Smith et al. (2015) observed that sclerotium-forming entities are frequently found in most habitats, belonging primarily to the Ascomycota and Basidiomycota phyla. They showed that sclerotia occur in 85 genera within twenty orders in Dikarya. They added three genera to the list of known sclerotium formers: *Cheilymenia*, a saprobic Ascomycota; *Ceriporia*, a wood decaying member of the Basidiomycota; and *Entoloma*, a putative ectomycorrhizal genus (Smith et al. 2015). All collections from Atlantic rainforest biome fit to pre-existing phylogenetic lineages. Furthermore, their study also produced data about sclerotia in tropical forest ecosystems. Séné et al. (2015) found 49 sclerotia of *Cenococcum geophilum* Fr. in two areas with *Coccoloba uvifera* L. in neotropical coastal forests. Most sclerotium reports before 2015 are from temperate Europe and North America (Agerer et al. 1993, Miller et al. 1994, Kovács et al. 2007, Münzenberger et al. 2009).

Our data add information about the diversity and community composition of sclerotium-forming fungi in the tropics, primarily in ecosystems of northeastern Brazil. Among the identified lineages, three sequences were related to *Entolomataceae*, with one forming microsclerotium. This is the first record of it occurring attached to fine roots of *Coccoloba* species. It is likely an ectomycorrhizal species, supporting previously described data from tropical South American forests (Tedersoo & Smith 2013, Smith et al. 2015). The corticioid */trechisporales* lineage appears to have a broad distribution within the restinga forests in northeastern Brazil (electronic appendix), occurring in high numbers, probably as a resource-storage or survival form (Smith et al. 2015). Based on molecular affiliation, the */amylocorticiales* lineage

(UFRN-Fungos 1752 and UFRN-Fungos 2064) was identified as close to *Amyloathelia* (INSD accession KJ720886). Smith et al. (2015) observed the same phylogenetic similarity between a native sclerotium-forming lineage from Guyana (MCA3930, FLAS-F-58921) and a member from INSD. Future collections in permanent plots should be established to monitor the fungal diversity in those sites, primarily focusing on sclerotium-forming fungi in natural or modified environments. In addition, more robust phylogenetic analyses are necessary to determine the phylogenetic position of these taxa.

SUPPLEMENTARY DATA

Supplementary data related to this article are available in pdf at *Plant Ecology and Evolution*, Supplementary Data site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consist of a table of fungal general collection and collecting information.

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