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## **A survey of an ectotrophic sand dune forest in the northeast Brazil**

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### **Abstract**

Ectomycorrhizal (ECM) species are poorly known from tropical lowlands of South America. Recent systematic surveys in the reserve Parque Estadual das Dunas do Natal, in the state of Rio Grande do Norte, Brazil, using a purposive sampling approach revealed new and yet undocumented community of ectomycorrhizal fungi in the reserve. Collections made in areas with a ectotrophic tree genera present next to the established walking paths revealed six genera of putative ECM fungi. These fungi belong to the Basidiomycota family Amanitaceae, Boletaceae, Hymenochaetaceae, Russulaceae and Sclerodermataceae, all of which are poorly documented from Brazil. The study contributed to the fungal diversity in an ectotrophic sand dune forest in the northeast Brazil, necessary for better understanding of the role of these organisms and their impact in the ecosystem. Discoveries opened additional questionings about the distribution, ecology and taxonomy of the ectomycorrhizal genera in tropical moist broadleaf forests.

**Key words** – Basidiomycota – ectomycorrhizal fungi – sequestrate fungi – forest reserve – tropical rainforest

### **Introduction**

Plant families associated with ectomycorrhizal (ECM) fungi in tropical and subtropical moist broadleaf forest regions in Brazil belong primarily to the Nyctaginaceae (*Neea*, *Pisonia*, *Guapira*), Polygonaceae (*Coccoloba*), and Fabaceae (Caesalpinioideae and Faboideae) (Singer et al. 1983, Singer & Aguiar 1986, Haug et al. 2004). Additionally, members of the family Gnetaceae (*Gnetum*) and Sapotaceae (*Glyoxylom*) were also described as presenting ECM fungi in the Campinarana forests in the Amazon (Singer & Araújo 1979).

Until recently, tropical and subtropical moist broadleaf forests were rarely considered for studies on ECM fungi and the knowledge about ECM forests distribution and the related ECM communities is still limited. Worldwide, most surveys and ecological studies on ectomycorrhizal fungi have been done in temperate and boreal regions, especially in the Northern hemisphere (Brundrett 2009, Tedersoo et al. 2010). Exceptions are tropical montane and submontane regions where species of oaks are found (Nixon 2006). Endomycorrhiza, on the other hand, are predominant in tropical ecosystems as tree species common in the lowland forests are typically associated to arbuscular mycorrhizal (AM) fungi (Thomazini 1977, Hogberg 1982). Studies have also confirmed that AM are predominant in *Araucaria* forests and Atlantic rainforest of Southern Brazil (Andrade et al. 2000).

The presence of ectotrophic tropical forests has been supported by numerous collections of Agaricomycetes obtained from ECM genera in surveys carried out in many tropical regions of the world, both in the high altitudes and lowland forests (Henkel et al. 2002, 2012, Bâ et al. 2012).

Basidiomes of putative ectomycorrhizal fungi have been described to occur related to caesalpinoid species in Africa (Heinemann 1951, Onguene & Kuyper 2001, Bâ et al. 2012) and in the Guyanas (Henkel et al. 2002, McGuire 2007, Henkel et al. 2012). Above and below ground studies have shown that species of the Dipterocarpaceous forests in Asia are also associated with ECM fungi (Ducouso et al. 2003, Neves et al. 2012). In the Seychelles Islands members of both caesalpinoid and Dipterocarpaceae were found to be associated with ECM fungi (Tedersoo et al. 2007). A rich diversity of ECM fungi was related to the Patagonian temperate forests of *Nothofagus* species (Nouhra et al. 2012, 2013).

Recently, two new species of *Elaphomyces* were discovered in the Guyana forests (Castellano et al. 2012) and a new genus of sequestrate basidiomycete, namely *Guyanagaster*, was found and described from the Pakaraima Mountains of Guyana, using ecological, molecular and morphological data (Henkel et al. 2010). This new genus is a parasite/saprophyte, closely related to *Armillaria*. On the other hand the two new species of *Elaphomyces* have been reported for the first time associated with specimens of Fabaceae from the lowland tropics of South America (Castellano et al. 2012). These studies (Henkel et al. 2010, Castellano et al. 2012) show that tropical South American forests are very likely to present a highly unexplored hypogeous fungal diversity.

Singer was the first to report the presence of ectotrophic forests in the lowlands of the Brazilian Amazon (Singer & Araújo 1979, Singer et al. 1983). Very few studies have been carried out in Brazil to determine the presence of ECM fungi in native forests. For the most, studies on ECM fungi from Brazil have been done in introduced *Pinus* and *Eucalyptus* forests (Giachini et al. 2000, Baseia & Milanez 2002, Giachini et al. 2004, Cortez et al. 2008, Sulzbacher et al. 2010, Cortez et al. 2011), especially in Southern Brazil. Only a few records exist on ECM fungi for Southern Brazil from native, non-planted forests, which were comprehensive reviewed in the work of Sulzbacher et al. (2013). Recent studies in the Northeast of Brazil have reported some putative ECM fungi collected from the Atlantic Rain Forest and the Semi-Arid Region. These include *Amanita crebresulcata* Bas (Wartchow & Maia 2007), *A. liloi* Singer (Wartchow et al. 2007), *A. lippiae* Wartchow & Tulloss (Wartchow et al. 2009), *Cantharellus aurantioconspicuus* Wartchow & Buyck (Wartchow et al. 2012), *C. protectus* Wartchow & F.G.B. Pinheiro (Pinheiro & Wartchow 2013), *Clavulina amazonensis* Corner (Wartchow 2012a), *C. incrustata* Wartchow (Wartchow 2012b), *Lactarius rupestris* Wartchow (Wartchow & Cavalcanti 2010), and *Lactifluus dunensis* Sá & Wartchow (Sá et al. 2013).

A common feature found in the Brazilian ECM fungi is the fragmented distribution. In this respect they do not always strictly follow the distribution of their plant symbionts (*sensu* Halling et al. 2007a, Tedersoo et al. 2007), which may result in high endemism at the species level due to the specific habitats that they occupy (Henkel 1999, Osmundson et al. 2007, Halling et al. 2007b, Fulgenzi et al. 2008, Henkel et al. 2011).

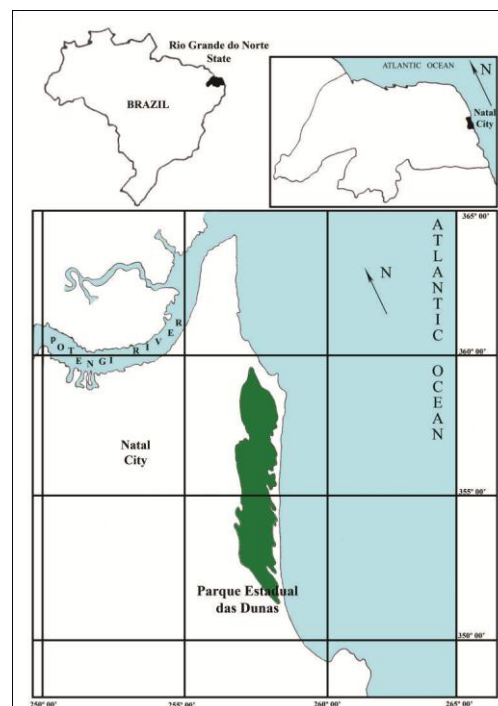
This article aims to report the first records of putative ECM taxa from a survey of epigeous and hypogeous ECM fungi in a newly discovered ectotrophic sand dune forest in the reserve Parque Estadual Dunas do Natal, located in the city of Natal, Rio Grande do Norte state, in the northeast of

Brazil, representing a part of the widely distributed ectotrophic sand dune forest along the Brazilian Atlantic coast.

## Materials & Methods

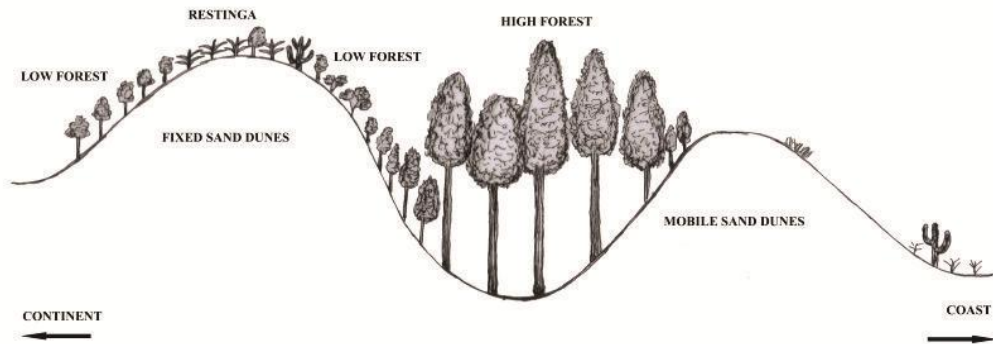
### Study site

The reserve Parque Estadual das Dunas do Natal is located at  $05^{\circ} 48' \text{ to } 05^{\circ} 53' \text{ S}$  and  $35^{\circ} 09' \text{ to } 35^{\circ} 12' \text{ W}$  in the city of Natal, Rio Grande do Norte state, in the northeast of Brazil. The park has approximately 1172 ha (Fig. 1). The climate is described as tropical humid, with mean annual temperatures close to  $25^{\circ} \text{ C}$ . Average annual precipitation ranges from 800 mm to 1,500 mm, with a rainy period from March to July. The vegetation has species representative from the Atlantic Forest, as well as species of the Caatinga and the Coastal Tableland forest types. The topsoil is sandy, composed mainly of marine quartz sand, dystrophic, low in fertility, and the landscape is plain with small slopes (Freire 1990).



**Fig. 1** – Map showing the state of Rio Grande do Norte and the relative location of the Parque Estadual Dunas do Natal (adapted from Freire 1990).

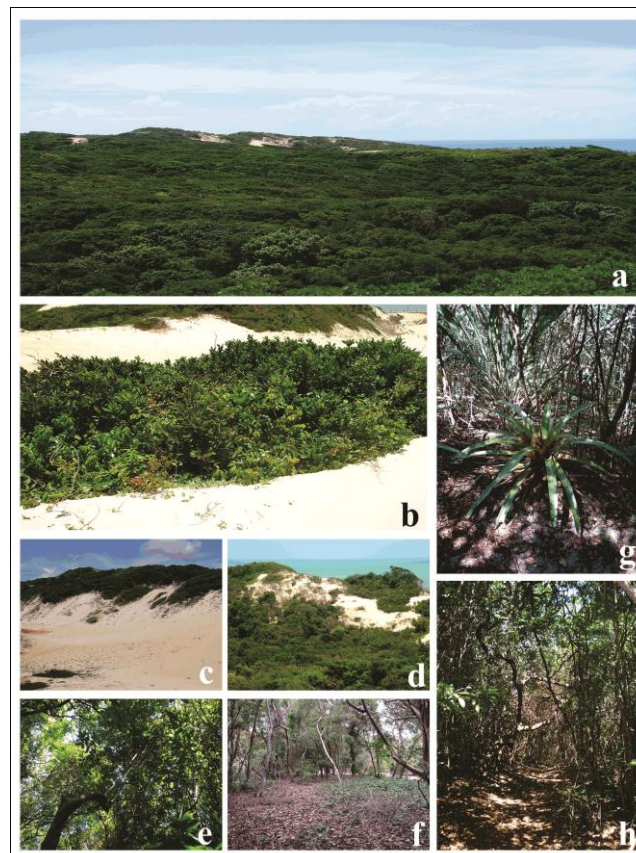
The dense vegetation helps to stabilize the sandy soil of the dunes present in the park. According to the features of the vegetation, as well as edaphic and climatic factors, three vegetation types are recognized in the Park (Freire 1990, Veloso et al. 1991): Coastal Forest or Forest of Littoral Dunes (High forests), beaches and dune foothills (Low forests), and “Restinga” (Fig. 2). The High forests have deep soil and are located in valleys (up to 30 meters in depth) and in between dunes, with trees up to two meters tall. These encompass members such as *Bowdichia virgilioides* Kunth, *Caesalpinia echinata* Lam. and *Manilkara salzmanii* (DC.) Lam. The low forests are formed on fixed dunes, covered by vegetation varying from three to seven meters tall. The low forests are predominantly covered by bushes and small trees such as *Eugenia ligustrina* (Sw.) Willd., *Maytenus erythroxyla* Reiss, and *Myrcia guianensis* DC. “Restinga” is an exposed area at the top of the dunes presenting sandy soil with a high incidence of light and heat. It is also more affected by sea winds. The vegetation is typically composed of *Byrsonima gardneriana* Juss., *Cryobalanus icaco* L., *Krameria tomentosa* A. St. -Hil., and some Cactaceae such as *Melocactus* spp. and *Pilosocereus* spp.



**Fig. 2** – Physiognomic regions of Parque Estadual Dunas do Natal (drawing by Tereza Cristina Galvão).

**Sampling**

Epigeous basidiomes of putative ECM fungi were collected in designated areas of the Reserve (Fig. 3) using a purposive sampling approach (Schreuder et al. 2004) always close to a potentially ectotrophic tree species. Walking paths were used to determine the survey locations. Surveys were performed during the rainy season in 2007, 2008, 2011 and 2012. In an approach to establish a below ground diversity assessment for the area, hypogeous fungi were collected in the same period using the methodology described by Castellano et al. (2004). All collections were initially assigned to family and further identified to the genus or species level based on the available literature (Dennis 1970, Guzmán 1970, Pegler 1977, 1983, 1986, Singer 1986, Pegler 1997).



**Fig. 3** – Sampling sites at Parque Estadual Dunas do Natal, Rio Grande do Norte, Brazil. a, e, f, g, h show the more arboreal physiognomy of the Atlantic coast restinga vegetation. b, c, d show low-lying shrub restinga vegetation that grows usually on sand dunes, and, thus, receiving direct sea influence.



The putative ECM status was determined and/or assumed either on the available description of ECM (Agerer 1987-2008, Agerer & Rambold 2004-2012) or on the taxonomic position (Miller 1983, Singer 1986, Rinaldi et al. 2008, Tedersoo et al. 2010). Specimens were dried according to traditional mycological methodologies (Largent 1986) and deposited as voucher at UFRN herbaria.

## Results

Thirty samples of epigeous/hypogeous basidiomes of putative ectomycorrhizal taxa were collected in all three vegetation types of the park and identified to the genus or species level as described in Table 1. All listed collections were observed growing near native plant species recognized as potential ectomycorrhizal partners. Species occurring in the plots belong to genera such as *Amanita*, *Coltricia*, *Lactifluus*, *Russula*, *Scleroderma* and *Tylopilus* (Fig. 4).



**Fig. 4** – Epigeous basidiomes frequently sampled in the Parque Estadual Dunas do Natal. a *Amanita viscidolutea*, b–c *Amanita* spp., d–f Boletaceae, g *Tylopilus* sp., h *Scleroderma nitidum*, i–j *Lactifluus* spp.

**Table 1** Putative ectomycorrhizal fungal taxa occurring on the Parque Estadual Dunas do Natal, northeast Brazil

Family	Genera	Voucher
Amanitaceae	<i>Amanita</i> sp. 1	UFRN-fungos 627
	<i>Amanita</i> sp. 2	UFRN-fungos 1870 (Sulzbacher 325)
	<i>Amanita</i> sp. 3	UFRN-fungos 1892 (Sulzbacher 349)
	<i>Amanita</i> sp. 4	UFRN-fungos 1876 (Sulzbacher 306)
	<i>Amanita</i> sp. 5	UFRN-fungos 1880 (Sulzbacher 426)
	<i>Amanita</i> sp. 6	UFRN-fungos 1877 (Sulzbacher 427)
	<i>Amanita</i> sp. 7	UFRN-fungos 1879
	<i>Amanita</i> sp. 8	UFRN-fungos 1878
	<i>Amanita</i> sp. 9	UFRN-fungos 1875
	<i>Amanita</i> sp. 10	UFRN-fungos 942
	<i>Amanita viscidolutea</i> Menolli, Capelari & Baseia	UFRN-fungos 958; UFRN-fungos 1872 (Sulzbacher 307)
	<i>Amanita nauseosa</i> (Wakef.) D.A. Reid	UFRN-fungos 1874 (Sulzbacher 309)
	Boletaceae	<i>Tylophilus</i> sp.1
<i>Tylophilus</i> sp. 2		UFRN-fungos 1885
Hymenochaetaceae	<i>Coltricia focicola</i> (Berk. & M.A. Curtis) Murrill	UFRN-fungos 1893 (Sulzbacher 331)
Russulaceae	<i>Lactifluus</i> sp. 1	UFRN-fungos 532
	<i>Lactifluus</i> sp. 2	UFRN-fungos 1873 (Sulzbacher 323)
	<i>Lactifluus</i> sp. 3	UFRN-fungos 1871 (Sulzbacher 326)
	<i>Russula</i> sp. 1	UFRN-fungos 530
	<i>Russula</i> sp. 2	UFRN-fungos 531
Sclerodermataceae	<i>Scleroderma nitidum</i> Berk.	UFRN-fungos 762; UFRN-fungos 1421; UFRN-fungos 1422
	Unidentified hypogeous sp. 1	UFRN-fungos 1891 (Sulzbacher 334)
	Unidentified hypogeous sp. 2	UFRN-fungos 1890 (Sulzbacher 335)
	Unidentified hypogeous sp. 3	UFRN-fungos 1888 (Sulzbacher 338)
	Unidentified hypogeous sp. 4	UFRN-fungos 1887 (Sulzbacher 350)
	Unidentified hypogeous sp. 5	UFRN-fungos 1886 (Sulzbacher 386)
	Unidentified hypogeous sp. 6	UFRN-fungos 1889 (Bezerra 07)

## Discussion

The forests of the reserve Parque Estadual das Dunas do Natal is represented predominantly by trees and shrubs bearing arbuscular mycorrhizal symbionts, since AM is common in tropical and subtropical moist broadleaf forests (Read 1991, Kraigher et al. 2013). On the other hand, Nyctaginaceae, Polygonaceae and Fabaceae from the region have been recognized as potential ectomycorrhizal hosts (Singer et al. 1983, Haug et al. 2004). Field observations showed that, unlike temperate regions, where ECM tree hosts are widely distributed and often dominate the forests, in the reserve the ECM associations were restricted to sharply demarcated stands where Fabaceae/Caesalpinioideae, Myrtaceae, Polygonaceae and Sapotaceae were present. Additionally, a high endemic level of native forests species to this particular ecosystem has also been previously recognized, suggesting it may have evolved co-associated with specific ECM fungal species (Sulzbacher et al. 2013).

The collected fungi from this region belong mainly to *Amanita*, *Lactifluus*, *Russula* and *Scleroderma*, genera with the broadest host ranges known (Smith & Read 2008) and frequently reported as ECM partners of Myrtaceae and Polygonaceae (Kreisel 1971, Pegler 1983, Miller et al. 2000, Guzmán et al. 2004, Bandou 2005, Bandou et al. 2006). The ECM fungal community

registered at the dunes is composed by the same genera found in temperate forests. Although the diversity seems to be considerable high, most collections are still unidentified at the species level. The task of identifying these species is generally arduous due to the lack of well defined voucher collections and appropriate literature to compare them to.

The results indicate that there is a significant putative ectomycorrhizal fungal community in the Parque Estadual Dunas do Natal. This high diversity found in this preliminary study indicates that additional potential endemic species may be discovered when more sampling efforts are undertaken. Examples of this potentially high levels of endemic species associated to this ecosystem are available. Among them are a recently described species of *Amanita viscidolutea* (Menolli et al. 2009), and the occurrence of *Coltricia focicola* (Berk. & M.A. Curtis) Murrill, a typical ECM species found growing among dead woody debris and litter (Baltazar et al. 2010) in the park. Additionally, *Scleroderma nitidum* Berk. has been found growing near *Coccoloba* sp. (Gurgel et al. 2008) and *Lactifluus dunensis* Sá & Wartchow was recently described growing on the sandy soil at the dune areas (Sá et al. 2013). Furthermore, it is important to mention that at least one undescribed genus of hypogeous fungi, preliminarily clustered in the Phallales (Basidiomycota), and two new species of putative ECM epigeous agarics and boletes obtained from the park, very likely belonging to *Amanita* and *Tylopilus*, are currently being described and will be published elsewhere.

In respect to the hypogeous fungal sporocarps found in the reserve, this is one of the first references to a native hypogeous fungus discovered in Brazil. As pointed out by some authors (Bougher & Lebel 2001, Claridge 2002), a high number of hypogeous fungi have an obligate ectomycorrhizal association and depend on animals to disperse their spores. It is likely that a wide variety of fungal species with this habit is associated to forests around the world.

The Atlantic Forest is considered one of the 25 hotspots for biodiversity conservation around the globe (Myers et al. 2000), hosting more than 13,700 species of angiosperms (Stehmann et al. 2009), many of them endemic to the region. Consequently, the high diversity of potential ectomycorrhizae tree species suggests a high, unknown diversity of ectomycorrhizal partners. Additional studies focusing on the ECM communities, using basidiomes and ectomycorrhizae, are in need to expand the knowledge about the ECM community and its relationship to the host trees in these tropical broadleaf forest ecosystems.

The role of these putative ectomycorrhizal fungi in the sandy oligotrophic environment of the reserve Parque Estadual das Dunas do Natal is not only to promote the growth of the hosts by improving the capacity to gather nutrients in the poor soil (Read 1998), but also to increase the tolerance to high levels of minerals common in these seawater and coastal influenced zones (Bois et al. 2006). Additionally, it may also protect the plants during dry periods of the year (di Pietro et al. 2007). It has been proved that *Scleroderma bermudense* can alleviate salt stress in *Coccoloba*, enhancing considerably the tolerance of the plant species to this mineral (Bandou et al. 2006). Therefore, we predict that some of the ECM fungi occurring in the dunes in Natal may have the same role in the coastal tropic forest ecosystems. These findings agree with Singer & Araujo (1979) who showed that tropical ECM forests occur in highly oligotrophic white sandy soil ecosystems. This is not always the case since Henkel et al. (2002) found ectotrophic genera that apparently *Aldina* spp. and *Dicymbe* spp. do not have clear edaphic requirements. These particular types of plants can occur on different soils ranging from coarse, leached sands to tropical red clays with a wide variety of chemical and textural composition (Henkel et al. 2002).

This study represents a pioneer approach to reveal the potentially high fungal diversity hidden in sandy soils of the “restinga” in the northeast of Brazil. Future studies may uncover the patterns of occurrence and biogeographic distribution of ECM fungi and plants alike in this region. These results represent yet another piece of the puzzle trying to estimate the fungal diversity of tropical regions of Brazil. Surveys on ECM fungi in Brazil and other under-studied sites are necessary to better understand the role of this symbiosis and its impact in the ecosystems. Such discoveries bring about more questions to the discussion on the distribution of the ectomycorrhizal genera of fungi

and will give hints on the evolutionary placement and overall diversity of this ecologically important group of organisms.

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