Arbuscular mycorrhizal fungi: new records in Northeast of Brazil

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RESUMO: **Fungos Micorrízicos Arbusculares: novos registros no Nordeste do Brasil**. Integrantes da mais ampla associação entre plantas e fungos na natureza, e desempenhando papel fundamental na manutenção dos ecossistemas terrestres, os fungos micorrízicos arbusculares (FMA - Glomeromycota) ainda não recebem o devido destaque e só há pouco tempo vêm sendo considerados no campo da biologia da conservação. Nesse contexto, objetivou-se registrar a ocorrência de espécies de FMA, indicar os principais avanços no conhecimento sobre a diversidade desses fungos em áreas naturais do Nordeste do Brasil, gerados a partir do Programa Sisbiota-Brasil (Sistema Nacional de Pesquisa em Biodiversidade) e contribuir com uma discussão sobre as espécies registradas na região. O trabalho incluiu o levantamento bibliográfico das ocorrências citadas para áreas naturais e agrícolas e dados de coletas de solo rizosférico realizadas em seis dos nove Estados nordestinos, no período de 2010-2013. Registraram-se 28 gêneros e 125 espécies de FMA; dessas, 11 constituíram

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novas espécies, nove representam novas ocorrências para o Brasil e oito para o Nordeste. Houve aumento de 25% no conhecimento sobre a diversidade de FMA na região, e com a ampliação desses registros observa-se que 50% das espécies de Glomeromycota descritas no mundo estão representadas no Nordeste. Os resultados sobre a ocorrência e distribuição de FMA no Nordeste do Brasil reforçam a importância de estudos em áreas pouco ou ainda não exploradas para o conhecimento da biodiversidade, cujos dados podem auxiliar na definição de estratégias de manejo e conservação dessas áreas.
Palavras-chave: biodiversidade, Glomeromycota, micorriza, Sisbiota, taxonomia.

ABSTRACT: Arbuscular mycorrhizal fungi (AMF - Glomeromycota) comprise the largest association of plants and fungi in nature yet have only recently been considered within the context of conservation biology. The aim of this work was to document the occurrence of AMF species and highlight recent advances in our knowledge of their diversity in Northeast Brazil. This new information has been generated by the Sisbiota-Brazil Program (National System of Biodiversity Research) and provides the basis for a discussion on the AMF species found in the region. The work included a bibliographic review of the records from natural and agricultural area plus data generated by collections made in natural areas in six of the nine Northeastern states during the period 2010-2013. Overall we recorded 28 genera and 125 species of AMF: 11 were new species, nine represented new records for Brazil and eight to the Northeast. This represents a 25% increase in our knowledge of the diversity of AMF in the region. Thus the Northeast of Brazil now accounts for approximately 50% of all AMF species described worldwide. This finding reinforces the need for more studies in areas that are poorly studied in order to extend our understanding of biodiversity and to help define future strategies for management and conservation.
Key words - biodiversity, Glomeromycota, mycorrhiza, Sisbiota, taxonomy.

Introduction

Arbuscular mycorrhizal fungi (AMF) appear first in the fossil record in the Ordovician Period, approximately 460 million years ago (Redecker *et al*., 2000). Yet despite being ancient organisms taxonomic studies on AMF are relatively recent. The first taxonomic classification to include representatives of the group was proposed in 1974 by Gerdemann & Trappe. At this point AMF were included in the order Endogonales (Zygomycetes). After nearly three decades Morton & Benny (1990) retained the AMF within the same

class, but proposed a monophyletic Glomales order to encompass "all soil borne fungi which form arbuscules in obligate mutualistic associations with terrestrial plants." Later Cavalier-Smith (1998) moved the AMF into the new Glomeromycetes class which brought together all arbuscular mandatory symbionts. After a further three years the AMF were finally redefined as a phylum, Glomeromycota (Schüβler *et al*., 2001), today subdivided into three classes, 5 orders, 14 families, 32 genera and about 270 species (Oehl *et al*., 2011a; Goto *et al*., 2012b; Blaszkowski & Chwat, 2013).

The AMF are extremely abundant and may represent 10% or more of the soil microbial biomass (Fitter *et al*., 2011; Turrini & Giovannetti, 2012). They contribute significantly to plant nutrition by means of their mycelial network and are responsible for transferring phosphorus, nitrogen and other mineral nutrients from the soil to the plant which, in turn, provides carbohydrates for the symbiont fungi (Smith & Read, 2008).

Because of their key role in maintaining terrestrial ecosystems, studies on the conservation of AMF are crucial. Several projects designed to increase our knowledge of the country's biodiversity have been recently supported by the Brazilian federal government. These include SISBIOTA (National System of Biodiversity Research), Protax (Taxonomy Program) and PPBio (Biodiversity Prospecting Program), together with other programs to improve the capacity and quality of national collections. For example, the National Institute of Science and Technology (INCT) Virtual Herbarium of Plants and Fungi of Brazil provide free and open access to information and data from most of the herbaria in the country (http://inct.florabrasil.net). Together these initiatives aim to expand our knowledge of plant and fungal diversity and to evaluate the effectiveness of monitoring and interpreting the occurrence of species over time. These new data will help to identify gaps in taxonomic and geographical knowledge, supporting conservation and sustainable use of Brazilian biodiversity. The main objective of this study was therefore to document the occurrence and indicate the major advances in knowledge about the diversity of AMF in ecosystems of the Northeast, generated from efforts supported by the SISBIOTA program.

Material and Methods

Bibliographical survey of the recorded species in northeastern Brazil. Descriptions of AMF species published before the start of the SISBIOTA project were used as a basis for this study. These included the review by de Souza *et al*. (2010), together with data provided by Cardozo Júnior *et al*. (2012), Ferreira (2010), França (2004), Maia *et al*. (2005, 2010),

Mello (2010), Mello *et al*. (2012), Mergulhão *et al*. (2009, 2010), Nobre *et al*. (2010), Pagano *et al*. (2013), da Silva *et al*. (2012), Sousa *et al*. (2012, 2013), and Souza *et al*. (2013).

Some of these reports were published after 2010, but since the corresponding data collections were made before that year we considered that they were still representative of the period preceding the field research project. We did not discriminate whether the data came from natural areas in the analysis of these past records. Indeed most of them relate to cultivated and/or degraded areas.

Collection and sampling. Collections of rhizospheric soil were carried out from October 2010 until June 2013 in various natural ecosystems - Caatinga, Savana, Atlantic forest, Restinga and "Brejo de Altitude" - located in six of the nine states of northeastern Brazil: Bahia, Pernambuco, Paraíba, Rio Grande do Norte, Ceará and Piauí (Table 1). The other three states (Alagoas, Maranhão and Sergipe) have not yet been awarded AMF studies under the project.

The areas visited encompass a National Forest (Araripe-EC), an Ecological Tourist Park (Extremoz-RN), National Parks (Catimbau-PE and Serra das Confusões-PI), and others areas not included as conservation units (Cabo de Santo Agostinho, Goiana, Ipojuca Itamaracá, Petrolina and Triunfo in Pernambuco, Mataraca in Paraíba, Parnamirim in Rio Grande do Norte and Santa Teresinha in Bahia). In each area 5-10 soil samples were collected, each composed of five sub-samples taken at a depth of 0-20 cm.

Species identification. Glomerospores were extracted from soil by wet sieving (Gerdemann & Nicolson, 1963) followed by water and sucrose centrifugation (Jenkins, 1964), separated and mounted on slides with PVLG and PVLG + Melzer's reagent (1:1 v / v). Species identification was done by micromorphological analysis, using the descriptions of AMF found in Schenck & Perez (1990) and Błaszkowski (2012), other available descriptions and the sites http://www.agro.ar.szczecin.pl/ jblaszkowski and http://invam.caf.wvu.edu. The classification used was based on Oehl *et al*. (2011a), Goto *et al*. (2012b) and Blaszkowski & Chwat (2013), while the nomenclature employed follows that used by the Mycobank (www.mycobank.org).

Results

Twenty eight genera, represented by 125 species of AMF were recorded. Of these, 11 were described as new, nine represent new records for Brazil and eight are new records for the Northeastern region (Table 2). There was an increase in the number of records of AMF species in all six states studied.

Table 1. Sampling locations used for the extraction and identification of mycorrhizal fungi in Northeastern Brazil.

Biome/Ecosystem	Municipality/State	Geographic coordinates	Climatic data	Conservation Unit	Reference
Caatinga	Buíque – PE	08º30'48.3"S 037º14'57.8"W	Hot and dry with seasonal rainfall. Average annual rainfall between 650 and 1100 mm	National Park of Catimbau	Marinho (2014)
	Caracol – PI	9°7'30"S 43°48'11"W	Hot and dry with seasonal rainfall	National Park of Serra das Confusões	Assis (2012)
	Petrolina – PE	09º09'S 40º22'W	Semi-arid tropical	No	Pontes (2013)
	Triunfo – PE	7º52'45.29"S 38º06'13.64"W	Hot and humid	No	Silva *et al.* (2014)
	Crato – CE	7º17'S 39º33'W	Tropical rainy	National Forest of Araripe	de Lira *et al.* (2014)
Cerradão	Crato – CE	07º14'S 39º28'W	Tropical rainy	National Forest of Araripe	de Lira *et al.* (2014)
Mata Atlântica	Goiana – PE	07º38'20''S 034º57'10''W	Rainy tropical with dry summer. Temperature and mean annual precipitation: 24 ° C and 2000 mm	No	Pereira et al. (2014)
Brejo de Altitude	Triunfo – PE	7º52'29.42"S 38º06'12.07"W	Hot and humid	No	Silva *et al.* (2014)
	Santa Teresinha – BA	2º51'S 39º28'W	Tropical wet to semi-humid. Temperature and mean annual precipitation: 21 ° C and 1200 mm, respectively	No	Assis (2012)

Table 1 (cont.)

Biome/Ecosystem	Municipality/State	Geographic coordinates	Climatic data	Conservation Unit	Reference
	Crato – CE	07º14'S 39º28'W	Tropical rainy climate	National Forest of Araripe	de Lira *et al.* (2014)
Restingas	Mataraca – PB	6º28'20"S 34º55'50"W	Tropical rainy climate. Temperature and mean annual precipitation: 25.5 º C and 1700 mm, respectively	Private Natural Heritage Reserve	Silva (2013)
	Cabo de Santo Agostinho – PE	8°17'15"S 35°02'00"W	Tropical rainy climate; average annual rainfall about 2000 mm	No	Vieira (2013)
	Ipojuca – PE	08°31'48"S 35°01'05"W	Tropical rainy climate; average annual rainfall about 2000 mm	No	Vieira (2013)
	Itamaracá – PE	07º45'00"S 34º49'30"W	Tropical rainy climate; average annual rainfall about 2000 mm	No	Vieira (2013)
	Extremoz – RN	05º40'40"S 35º12'56"W	Tropical rainy climate with dry summer and temperatures oscillating between 21 ° C (minimum) and 30 ° C (maximum), with a mean of 26.1 º C	Turistic Ecological Park Dunes de Genipabu	Jobim & Goto (data not published)
	Parnamirim – RN	05°55'30"S 35°09'47"W	Tropical rainy climate with dry summer; average annual temperature of 27.1 º C, annual rainfall of 1650 mm	Não	Jobim & Goto (data not published)

Table 2. Classification of AMF and comparison between the numbers of species described worldwide per genus and those recorded in Northeast Brazil.

Class	Order	Family	Genus	Number of AMF species	
				Described	Recorded in NE
Archaeosporomycetes	Archaeosporales	Archaeosporaceae	*Archaeospora*	1	1
			Intraspora	1	1
		Ambisporaceae	*Ambispora*	9	4
Paraglomeromycetes	Paraglomerales	Paraglomeraceae	*Paraglomus*	8	4
Glomeromycetes	Diversisporales	Acaulosporaceae	*Acaulospora*	46	27
			Kuklospora	1	1
		Diversisporaceae	*Corymbiglomus*	4	1
			Diversispora	15	3
			Otospora	1	0
			Redeckera	6	1
			Tricispora	1	0
		Pacisporaceae	*Pacispora*	7	1
		Sacculosporaceae	*Sacculospora*	1	1
	Gigasporales	Dentiscutataceae	*Dentiscutata*	9	4
			Fuscutata	4	4
			Quatunica	1	1
		Intraornatosporacee	*Intraornatospora*	1	1
			Paradentiscutata	2	2
		Gigasporaceae	*Gigaspora*	8	5
		Racocetraceae	*Cetraspora*	7	3
			Racocetra	12	9
		Scutellosporaceae	*Orbispora*	2	1
			Scutellospora	8	4
	Glomerales	Entrophosporaceae	*Albahypha*	2	0
			Entrophospora	1	1
			Claroideoglomus	5	3
			Viscospora	1	0
		Glomeraceae	*Funneliformis*	13	5
			Glomus	77	33
			Septoglomus	8	4
			Simiglomus	1	1
Total = 3	**5**	**14**	**32**	**263**	**125**

In the states of Piauí and Ceará there was a 95% increase in records of AMF species, while in Rio Grande do Norte all the species found are new records for the state - there were no studies previously to this project (Figure 1). The new species collected in these states will be described in a future publication. Families with more species records in the region are Glomeraceae (43) and Acaulosporaceae (28), followed by Racocetraceae (12), Dentiscutataceae (9), Diversisporaceae, Gigasporaceae and Scutellosporaceae (5), Ambisporaceae, Paraglomeraceae and Entrophosporaceae (4), Intraornatosporaceae (3) Archaeosporaceae (2) and Pacisporaceae and Sacculosporaceae (1) (Table 2).

Pernambuco, Paraíba and Bahia have been subject to a higher number of studies on the occurrence and diversity of AMF and this is reflected in the smallest increase of species records in these states (Figure 1).

Discussion

Before this project, 101 AMF species had been recorded for the Northeast (Cardozo Junior *et al*., 2012; Ferreira, 2010; França, 2004; Maia *et al*., 2005; Maia *et al*., 2010; Mello, 2010; Mello *et al*., 2012; Mergulhão *et al*.,

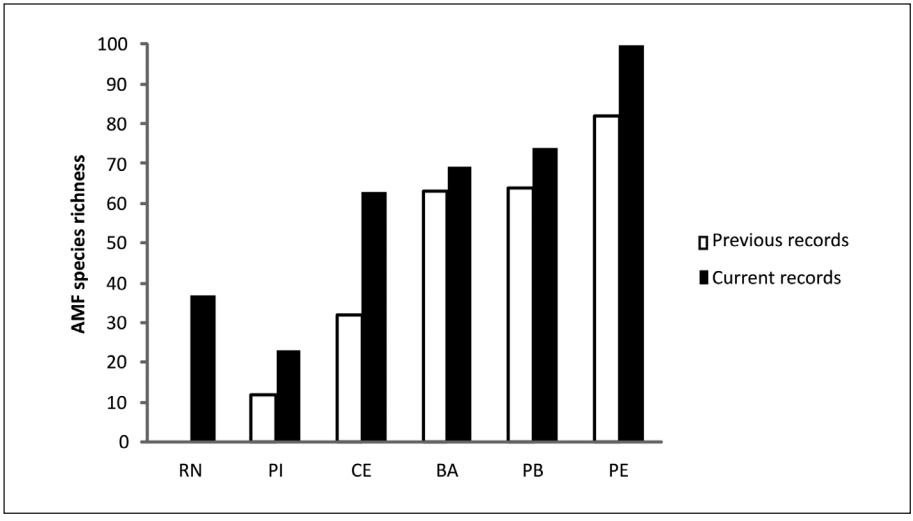

Figure 1. Number of species of arbuscular mycorrhizal fungi (AMF) occurring in the following states of Brazilian Northeast: Rio Grande do Norte (RN), Piauí (PI), Ceará (CE), Bahia (BA), Paraíba (PB) and Pernambuco (PE). Species richness recorded before (previous record) and after (current record) the beginning of implementation of the SISBIOTA project Rede Integrada em Taxonomia de Plantas e Fungos.

2009; Mergulhão *et al*., 2010; Nobre *et al*., 2010; Pagano *et al*., 2013; da Silva *et al.*, 2012; Sousa *et al.*, 2012; Sousa *et al.*, 2013; de Souza *et al*., 2010; Souza *et al*., 2013). Including the presented data, this number has increased to 125 species, representing a 25% increase in knowledge about the diversity of AMF in the region. Thus the Northeast of Brazil now accounts for approximately 50% of all AMF species described worldwide.

All classes, orders and families of Glomeromycota are registered in the region. Most common are the Acaulosporaceae and Glomeraceae whose species have been commonly reported in the Caatinga (Maia *et al*., 2010) and in other Brazilian biomes (de Souza *et al*., 2010). Besides having several genera with wide distribution, such as *Acaulospora* and *Glomus*, these families have the highest number of species within the Glomeromycota. *Glomus* predominates in Brazilian ecosystems, with the exception of maritime dunes areas, where species that produce larger spores, such as those of the order Gigasporales, notably *Gigaspora* and *Scutellospora* are commonly found (da Silva *et al*., 2012; Stürmer & Siqueira, 2008; Stürmer *et al*., 2013). Of the 77 species of *Glomus* and 46 of *Acaulospora* recorded worldwide, respectively 33 and 27 occur in Northeastern Brazil.

From all 32 AMF genera known, only four were not recorded in the survey: *Otospora, Tricispora, Albahypha* and *Viscospora*. Three genera (*Intraornatospora, Orbispora* and *Paradentiscutata*) and 11 species were originally described from isolates recently collected in the Northeast: *Acaulospora endographis* B. T. Goto (Goto *et al*., 2013b), *Fuscutata aurea* Oehl *et al*. (de Mello *et al*., 2012), *Glomus trufemii* B. T. Goto *et al*. (Goto *et al*., 2012a), *Orbispora pernambucana* Oehl *et al*. (Oehl *et al*., 2011b), *Paradentiscutata bahiana* Oehl *et al*. e *P. maritima* B.T. Goto *et al*. (Goto *et al*., 2012b), *Paraglomus pernambucanum* Oehl *et al*. (Mello *et al*., 2013), *Racocetra tropicana* Oehl *et al*. (Goto *et al*., 2011), *Septoglomus furcatum* Blaszk. *et al*. (Blaszkowski *et al*., 2013), *S. titan* B.T. Goto & G.A. Silva (Goto *et al*., 2013a) e *Scutellospora alterata* Oehl *et al*. (Pontes *et al*., 2013). *Septoglomus furcatum* was not recorded in the present sampling, but the species was included in order to determine the current number of AMF species recorded in Northeastern Brazil. *Intraspora, Pacispora* and *Redeckera* were added as new records for the Northeast, while up to now *Intraornatospora, Paradentiscutata, Quatunica* and *Sacculospora* had been found only in this region.

With these inventories it was possible to record, in addition to the 11 new species, nine new records of AMF in Brazil: *Acaulospora gedanensis* Blaszk., *A. minuta* Oehl et al., *A. splendida* Sieverd. et al., *Cetraspora spinossisima* (C. Walker & Cuenca) Oehl et al., *Glomus aureum* Oehl & Sieverd., G. *irregulare* Blaszk. et al., G. *pachycaule* (C.G. Wu & Z.C. Chen) Sieverd. & Oehl, *Racocetra*

undulata T.C. Lin & C.H. Yen, *Sacculospora baltica* (Blaszk. et al.) Oehl et al. Beyond these, another eight species were new records for the Northeastern region: *Acaulospora colossica* P.A. Schultz et al., *Ambispora fecundispora* (N.C. Schenck & G.S. Sm.) C. Walker, *Claroideoglomus luteum* (L.J. Kenn et al.) C. Walker & A. Schüssler, *Corymbiglomus globiferum* (Koske & C. Walker) Blaszk. & Chwat, *Diversispora versiformis* (P. Karst.) Oehl et al., *Funneliformis vesiculiferum* (Thaxt.) C. Walker & A. Schüßler, *Fuscutata rubra* (Stürmer & J.B. Morton) Oehl et al. e *Redeckera fulva* (Berk. & Broome) C. Walker & A. Schüssler (Assis, 2012; de Lira et al., 2014; Jobim & Goto, data not published; Marinho, 2014; Pereira et al., 2014, Pontes, 2013; Silva, 2013; Silva et al., 2014; Vieira, 2013).

Only *Acaulospora scrobiculata* Trappe was recorded throughout the Northeast and there are records that it occurs from "the north to the south" of Brazil (Sturmer & Siqueira, 2008). Five other species occurred in eight of the nine states: *Acaulospora morrowiae Spain & N.C. Schenck, Ambispora appendicula (Spain et al.) C. Walker, Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßler, Fuscutata heterogama Oehl et al. and Gigaspora decipiens I.R. Hall & L.K. Abbott.* With the exception of *G. decipiens*, these species are known to have wide distribution in Brazil, as observed by de Souza *et al*. (2010).

Three states (Alagoas, Sergipe and Maranhão) have few studies on AMF diversity (França, 2004; Nobre *et al*., 2010). Of these states, only Sergipe is currently the subject of studies on AMF (Inned, personnel com.).

We highlight the importance of partnerships established with researchers from other institutions in the country and abroad, allowing the description of new taxa in Brazil, primarily in the Northeast. Thus, in the last seven years 15 new species of AMF have been described, of which 11 during the three years of development of this Sisbiota project.

The data presented here were the result of surveys carried out only in natural environments, whereas most previous studies focused on cultivated areas (Maia *et al*., 2006, 2010). Recently, Pereira *et al*. (2014) observed that the conversion of natural areas to plantations did not diminish the diversity of AMF, but changed the composition of species. For example, some rare species unique to the natural area were no longer recorded in the plantations. This highlights the importance of maintaining natural areas in order to conserve the unique local diversity of AMF. In a restinga area, the diversity of AMF was retained in replanted areas after extraction of minerals with the surrounding natural areas serving as a source of propagules (da Silva et al. 2012).

These results on the occurrence and distribution of AMF in Northeastern Brazil reinforce the importance of inventories and studies in areas little or not

yet explored allowing the expansion of knowledge concerning this important group of fungi. The data can serve as a resource for conservation policies and/or preservation of natural, impacted or degraded areas, and help to define management strategies in protected areas by taking into consideration the key role of AMF in the maintenance of terrestrial ecosystems.

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