



Arbuscular mycorrhizal fungi (Glomeromycotina) in natural and agroecosystems in Biosphere Reserve Ciénaga de Zapata, Cuba

Hongos micorrizógenos arbusculares (Glomeromycotina) en ecosistemas naturales y agrícolas en la Reserva de la Biosfera Ciénaga de Zapata, Cuba

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Key words: arbuscular mycorrhizal, Glomeromycota, spore density, wetlands
Palabras clave: densidad de esporas, Glomeromycota, humedales, micorriza arbuscular

Recibido: 19/12/2017

Aceptado: 21/03/2018

ABSTRACT

Species composition and richness of arbuscular mycorrhizal fungi (AMF) in natural and agroecosystems in two localities of Reserve Biosphere Ciénaga de Zapata, Cuba were determined. In Pálpite were evaluated a swamp forest and three agricultural plots with different crops into one agroecosystem (normally used for local feeding) on charcoal as wadding substrate. In Playa Máquina were studied two natural ecosystems: one semideciduous forest (periodically inundated) and one coastal semideciduous forest. From compound soil samples from each ecosystem were estimated the spore density and identified the AMF species and morphotypes. Density of AMF spores was lowest in sweet potato plot with 480 spores 100 g⁻¹ of dry soil, while the highest values were observed in swamp forest with 7124 spores. In total 37 species and/or morphotypes of AMF were observed belonging to twelve different genera: *Archaeospora*, *Acaulospora*, *Diversispora*, *Funneliformis*, *Gigaspora*, *Glomus*, *Kuklospora*, *Pacispora*, *Racocetra*, *Rhizoglomus*, *Septoglomus* and *Viscospora*. The genus *Glomus* was the better represented with 16 species and morphotypes. The highest number of AMF species was observed at cassava crop, plantain crop and a semideciduous forest (periodically inundated) with 13, 11 and 9 AMF species and/or morphotypes, respectively.

RESUMEN

En el presente trabajo se determinó composición, diversidad de especies y morfoespecies de hongos micorrizógenos arbusculares en ecosistemas naturales y agroecosistemas en dos localidades de la Reserva de la Ciénaga de Zapata, Pálpite y Playa Máquina. En Pálpite fue evaluado un bosque de pantano y tres parcelas agrícolas con diferentes cultivos dentro de un agroecosistema (normalmente usado para autoconsumo) sobre restos de carbón como substrato. En Playa Máquina fueron estudiados dos ecosistemas naturales: un bosque semideciduo (con inundaciones periódicas) y un bosque semideciduo costero. A partir de muestras compuestas de cada ecosistema fue estimada la densidad de esporas en el suelo e identificadas las especies y morfoespecies de hongos micorrizógenos arbusculares. La menor densidad de esporas fue observada en la parcela agrícola sembrada con boniato con 480 esporas 100 g⁻¹ de suelo seco mientras los valores más altos fueron observados en el bosque de ciénaga con 7124 esporas. En total fueron observadas 37 especies y/o morfoespecies de HMA pertenecientes a once géneros diferentes: *Acaulospora*, *Archaeospora*, *Diversispora*, *Funneliformis*, *Gigaspora*, *Glomus*, *Kuklospora*, *Pacispora*, *Racocetra*, *Septoglomus* y *Viscospora*. El género *Glomus* fue el mejor representado con 16 especies y/o morfoespecies. El mayor número de especies de HMA fue observado en las dos parcelas agrícolas y el bosque semideciduo de pantano con 13, 11 y 9 especies y/o morfoespecies, respectivamente.

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are considered among more largely distributed on the Earth (Dodd *et al.*, 1996) and a key functional component in soils (Rillig, 2004; Smith and Read, 2008). The symbiotic relation that these establish with plant roots finds in the majority of the environments and kind of plants. Evidences exist of plants fossils that indicate AMF colonized the roots of the first terrestrial plants (Taylor *et al.*, 1995) and therefore both components are coevolved probably during the past 460 million years (Redecker *et al.*, 2000a, b). It is well recognized that AMF play significant roles in terrestrial ecosystems and development of different crops, due to their impacts on nutrient cycling, improvement in soil quality, carbon transport and limiting erosion due to the mechanical aggregation of soil particles (Brundrett *et al.*, 1996; Andrade *et al.*, 1998; Bago, *et al.*, 2000; Johansson *et al.*, 2004)

In the literature is accepted that different soil and climate factors like soil water content, temperature, organic matter, light and pH affect the arbuscular mycorrhizal symbiosis (Gibson and Daniels Hetrick; 1988, Fitter *et al.*, 2000; Covacevich *et al.*, 2007, 2012). On the other hand, wetlands are unique habitats at the interface between terrestrial and aquatic ecosystems and because of that, their plant communities, edaphic factors and water level fluctuations vary greatly (Turner and Friese, 1998)

AMF are present at a great range of wetlands (Cornwell *et al.*, 2001; Ipsilantis and Sylvia 2007; Wang *et al.*, 2010). The association of these fungi with wetland plants may be of considerable ecological significance for their establishment and growth, because these fungi enhance plant nutrient uptake, increase plant tolerance to drought and salt stress, and protect against soil pathogens (Stevens *et al.*, 2002; Dunham *et al.*, 2003; Koske *et al.*, 2004). Besides these soils are saturated and in consequence the levels of available oxygen for the aerobic organisms of the soil, like AMF is low (Wolfe *et al.*, 2007).

It is known that flooding conditions can suppress arbuscular mycorrhizal symbiosis also (Khan and Belik, 1995; Miller, 2000) owed to AMF need well-aired soils to develop and therefore present few conditions to live in very humid environments. However, they have been found in many wetland ecosystems including cypress swamps (Kandalepas *et al.*, 2010) bottomland hardwood forests (Stevens *et al.*, 2010), tropical river flood plains (de Marins *et al.*, 2009), and tropical marshes (Radhika and Rodrigues, 2007).

Some other results evidence that the condition of flooding does not deem adverse effects on the colonization of

roots by AMF (Stevens and Peterson, 1996; Ypsilantis and Sylvia, 2006) while Turner and Friese (1998) have shown presence of arbuscular mycorrhizae in the plants roots growing at wetlands recently rehabilitated, respectively. Other results suggest that does not seem to exist relation between the percentage of roots colonized for AMF and hydrologic category of plants (Aziz *et al.*, 1995; Turner *et al.*, 2000).

In Cuba have been developed some studies on the diversity of AMF in natural and agriculture systems (Ferrer and Herrera, 1980; Furrazola *et al.*, 2011, Furrazola *et al.*, 2015), but in general way, few works have been published on this subject. Little has been published about presence of AMF in Cuban wetlands (Rodríguez-Rodríguez, *et al.*, 2014; Torres-Arias *et al.*, 2015). The aim of the present study was study the presence of AMF species and/or morphotypes present at plots with different biological functioning at Biosphere Reserve Ciénaga de Zapata, the biggest wetland in the Caribbean.

MATERIALS AND METHODS

Studied ecosystems

The study was developed in two areas at Biosphere Reserve Ciénaga de Zapata: Pálpite (81°10' W, 22°19'N) and Playa Máquina (81°11' W, 22°17' N). In Pálpite were evaluated one swamp forest and three plots with different crops into one agroecosystem (normally used for local feeding) over a charcoal. In Playa Máquina were studied two natural ecosystems: one semideciduous forest (periodically inundated) and one coastal semideciduous forest.

Pálpite

Swamp forest: It is characterized by the presence of forest with a canopy at 5 -15 m (sometimes up to 20 m high) and prevalence of perennifolium trees. Shrubby stratus presents, some herbs, lianas and abundant palms and epiphytic plants, generally. This kind of forest is developed on peaty soils, which can remain inundated temporally with fresh water during four to six months during the year. The swamp forest is one of the richest vegetal formations in this area. Characteristics species are: *Bucida buceras* L., *Bucida palustris* Borhidi *et* Muñiz, *Lonchocarpus domingensis* (Pers.) DC., *Tabebuia angustata* Britt., *Hibiscus elatus* Sw., *Salix caroliniana* Schuttl., *Sabal maritima* (Kunth) Burret, *Annona glabra* L., *Myrica cerifera* L., *Erythroxylum confusum* Britt.

Agroecosystems: agricultural plots of land on charcoal, used for subsistence farming with *Manihot esculenta* Crantz. (cassava), *Ipomoea batatas* (L.) Lam. (sweet potato) and *Musa paradisiaca* L. (banana plantain) were also studied.

Playa Máquina

Semideciduous forest (periodically inundated): This kind of forest grows on soils flooded for a short time, and they localize themselves between the swamp forest and semideciduous forests. It shows two arboreal dense strata of 12-20 m, a shrub like well-represented stratum, some grasses, lianas and epiphytes. The species that characterize it are: *Copernicia curtissii* Becc., *Juniperus lucayana* Britt., *Bucida buceras* L., *Calophyllum calaba* L., *Cupania glabra* Sw. and *Hibiscus elatus* Sw.

Coastal semideciduous forest: With the predominance of *Gymnanthes lucida* Sw., *Harrisia eriophora* (Pfeiff.) Britt., *Bursera simaruba* (L.) Sargent, *Malpighia cubensis* H.B.K., *Cordia gerascanthus* L., *Selenicereus grandiflorus* (L.) Britt. et Rose, *Malpighia infestissima* (A.L.Juss.) L.C.Rich. ex Ndz., *Drypetes lateriflora* (Sw.) Krug et Urb. and *Ficus aurea* Nutt.

Collection of samples for analysis of diversity of HMA

In each studied ecosystems four plots (50 x 25 m) were selected and five soil sub-samples were taken at 10x10x15 cm and were homogenized and stored in a polyethylene bag as a compound sample per plot. At the laboratory, soil samples were air-dried for 72 hours, sieved (2 mm) and maintained at room temperature (24-27°C) in order to avoid that high soil moistness causes AMF spores germination. That prevent the attack of other soil microorganisms also, knowing that a common feature of inundated soils is a high organic material content, which attract a wide array of soil organisms enhancing the parasitic pressure on AMF spores.

Processing of soil for isolation spores of AMF and determined spore density

Once dry the soil at room temperature, were taken 100 g to make the process acquaintance like wet sieving and decanting method (Gerdemann and Nicolson, 1963) followed of centrifugation in sucrose 2M (Sieverding, 1991). Spore density was determined counting AMF spores in Doncaster plates with water and extrapolates these data to 100 g of dry soil.

Identification of HMA species and/or morphotypes

The AMF fungal spores were extracted of the sieves and placed in Doncaster's dish with water under a stereomicroscope (CARL ZEISS model AXIOSKOP 2 Plus). After that, healthy spores, with typical contents of lipids droplets and brilliant colors were placed in poly-vinyl alcohol lactoglycerine (PVLG) (Omar *et al.*, 1979) and PVLG Melzer (1:1, v v) to study all its attributes under optic microscope Carl Zeiss model Axioscop 2 Plus with magnify 200x-1000x. The photos were taken using a camera Axiocam and software Axiovision (software v. 3.1 and 1300 x 1030 dpi). For the classification of AMF

species, taxonomic available descriptions compiled by Schenck and Pérez (1990), INVAM's Web page (International Vesicular Arbuscular Mycorrhizal Collection), Schüßler and Walker (2010), Dr's. Blaszkowski Web page, and the issues of herbarium of CCHMA (Colección Cubana de Hongos Micorrizógenos Arbusculares) located in the IES-CITMA (Instituto de Ecología y Sistemática) were used.

Statistical analysis

In order to explore ecosystems grouping based on presence of different species and/or morphotypes of AMF, a dendrogram was developed using PAST program version 1.81. Tree is joined by Ward's method in which case, clusters are joined such that increase in within-group variance is minimized.

RESULTS

Arbuscular mycorrhizal fungal spore density was higher at swamp forest with a total of 7124 spores. g soil⁻¹, followed by coastal semideciduous forest and semideciduous forest periodically inundated with 2932 and 1848 spores, respectively (Fig. 1), whereas the lowest values were observed at sweet potato crop plot with 480 spores. The spores isolated from field samples represented 22 species and 15 un-described morphotypes in 12 genera of the Glomeromycota (Table 1). The species were distributed as following: one *Archaeospora* species, eight *Acaulospora* species, one *Diversispora* species, two *Funneliformis* species, one *Gigaspora* species, 16 *Glomus* species, one *Kuklospora* species, two *Pacispora* species, one *Racocetra* species, one *Rhizoglomus* species, one *Septoglomus* species and one *Viscospora* species (Fig. 2).

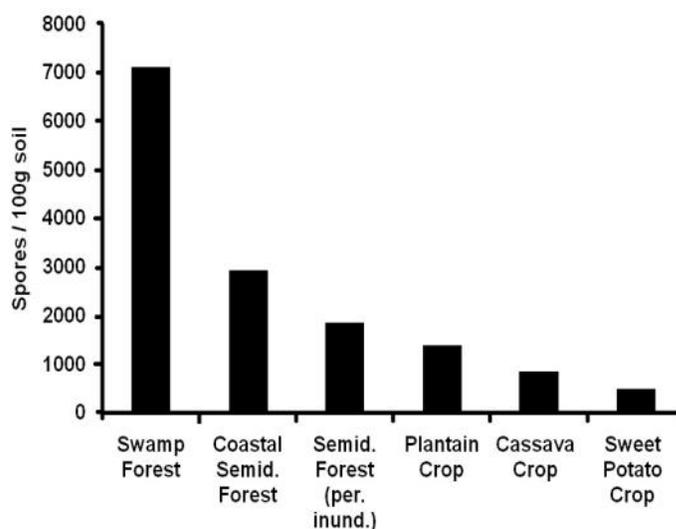


Figure 1. AMF spore density at different analyzed ecosystems.

Figura 1. Densidad de esporas de HMA en los diferentes ecosistemas analizados.

Table 1. Presence of AM fungal species and/or morphospecies observed at the studied ecosystems. CSF, Coastal semideciduous forest; SF, Semideciduous forest periodically inundated; SW F, Swamp forest; P crop, Plantain crop; C crop, Cassava crop; SP crop, Sweet potato crop.

Tabla 1. Presencia de especies y/o morfoespecies de hongos MA observados en los ecosistemas estudiados. CSF, bosque semideciduo costero; SF, bosque semideciduo periódicamente inundado; SW F, bosque de pantano, P crop, parcela de plátano; C crop, parcela de yuca, SP crop, parcela de boniato.

No.	Fungal species	CSF	SF	SW F	P Crop	C crop	SP crop
1	<i>Archaeospora trappei</i> (R.N. Ames & Linderman) J.B. Morton & D. Redeckeremend. Spain						x
2	<i>Acaulospora excavata</i> Ingleby & C. Walker					x	
3	<i>Acaulospora rehmi</i> Sieverd. & S. Toro					x	
4	<i>Acaulospora scrobiculata</i> Trappe		x		x	x	x
5	<i>Acaulospora tuberculata</i> Janos & Trappe		x			x	x
6	<i>Acaulospora</i> sp 1	x					
7	<i>Acaulospora</i> sp 2				x		
8	<i>Acaulospora</i> sp 3				x		
9	<i>Acaulospora</i> sp 4				x		
10	<i>Diversispora spurca</i> (C.M. Peiff. et al.) C. Walker & A. Schüssler					x	
11	<i>Funneliformis geosporum</i> (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler					x	
12	<i>Funneliformis halonatus</i> (S.L. Rose & Trappe) Oehl, G.A. Silva & Sieverd.		x	x			
13	<i>Gigaspora decipiens</i> I.R. Hall & L.K. Abbott					x	
14	<i>Glomus ambisporum</i> G.S. Sm. & N.C. Schenck,		x	x	x		
15	<i>Glomus brohultii</i> Sieverd. & R.A. Herrera		x				
16	<i>Glomus crenatum</i> Furrazola, R.L. Ferrer, R.A. Herrera & B.T. Goto			x			
17	<i>Glomus diaphanum</i> J.B. Morton & C. Walker					x	
18	<i>Glomus microcarpum</i> Tul. & C. Tul.	x					
19	<i>Glomus rubiforme</i> (Gerd. & Trappe) R.T. Almeida & N.C. Schenck		x		x		
20	<i>Glomus sinuosum</i> (Gerd. & B.K. Bakshi) R.T. Almeida & N.C. Schenck				x	x	x
21	<i>Glomus</i> sp 1	x					
22	<i>Glomus</i> sp 2	x					
23	<i>Glomus</i> sp 3	x	x				
24	<i>Glomus</i> sp 4			x			
25	<i>Glomus</i> sp 5					x	x
26	<i>Glomus</i> sp 6	x					
27	<i>Glomus</i> sp 7					x	x
28	<i>Glomus</i> sp 8						x
29	<i>Glomus</i> sp 9		x				
30	<i>Kuklospora kentinensis</i>						x
31	<i>Pacispora</i> cf. sp 1	x					
32	<i>Pacispora</i> cf. sp. 2				x		
33	<i>Racocetra alborosea</i> (Ferrer & R.A. Herrera) Oehl, F.A. Souza & Sieverd.		x	x			
34	<i>Rhizoglosum aggregatum</i> (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl						
35	<i>Rhizoglosum intraradices</i> (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl				x		
36	<i>Septoglosum constrictum</i> (Trappe) Sieverd., G.A. Silva & Oehl			x	x	x	
37	<i>Viscospora viscosa</i> (T.H Nicolson) Sieverd., Oehl & G.A. Silva					x	
Total AMF species		7	9	6	11	13	8



Figure 2. Some species of AMF observed in the present study. 1 *Acaulospora scrobiculata* 2. *Glomus brohultii* 3. *Glomus sinuosum* 4. *Glomus rubiforme*

Figura 2. Algunas especies de HMA observadas en el presente estudio. 1 *Acaulospora scrobiculata* 2. *Glomus brohultii* 3. *Glomus sinuosum* 4. *Glomus rubiforme*

Different studied ecosystems were clustered in two principal groups (**Fig. 3**) where upper group was composed of natural ecosystems and lower group was formed by agricultural plots, which respond to the AM fungal species distribution of such plots.

DISCUSSION

The explanation of the high number of morphotypes observed at the agricultural plot sown with cassava can be due to several factors, if well intensive agricultural management may not always reduce the abundance or diversity of AMF (Wang *et al.* 2009; Borriello *et al.* 2012; Gosling *et al.* 2013). It is known that soils handling in agroecosystems implies soil removal, use of agricultural machinery, vegetable propagules, organic amendments, etc., which makes easy dispersion and re-introduction of mycorrhizal propagules in soils (spores, roots colonized by AMF, external mycelium, etc.) all that can cause the presence of a bigger number of species in the studied area. These soils also present a bigger diversity of vegetable species as Gramineae, and these agroecosystems other own agrophytes, which can act as potential host plants for these fungi that prove to be obliged symbiont, factor that can contribute to raise AMF's diversity (Hijri *et al.*, 2006). Finally, we know that the cassava is a plant highly micotrophic, as has suggested Sieverding (1991), which through its radical system favors the present multiplication of AMF species at the soils where the same grows itself.

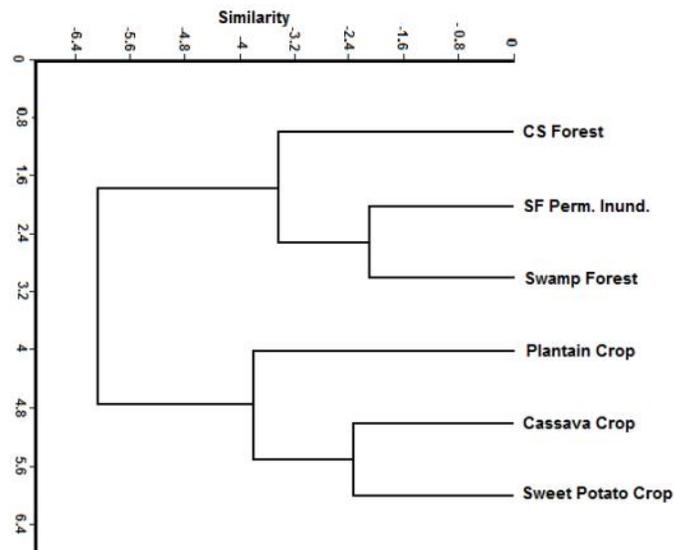


Figure 3. Relationship between the studied ecosystems based on absence/presence AMF species. Cophenetic correlations = 0.76.

Figura 3. Relación entre los ecosistemas estudiados basado en la ausencia/presencia de las especies de HMA. Correlación cofenética = 0.76.

In fact, the high numbers of AMF species and spores were observed in those soils and the frequent occurrence of spores of those species found in this study in the field of other natural and agricultural sites of Cuba and the world indicate that soils of Ciénaga de Zapata swamps seem to favor sporulation of AMF. The number of AMF species found in this study exceeds in seven species the number observed by Torres-Arias *et al.* (2015), although the species composition of both studies varies, besides other changes in absence/presence of species. They showed 14 unidentified *Glomus* spp. while in the present study only nine were observed.

The *Glomus* genus, with over 70 species described now was the genus with the biggest number of species in the present study, with 22 species and/or morphotypes. The high predominance and diversity of members of the genus *Glomus* in swamp soils supports earlier reports of a good adaptation of these fungi to a wide range of physical and chemical soil conditions (Grey, 1991).

The representatives of this genus predominate in numerous diversity studies made in other parts of the world, like in the studies accomplished by D'Souza and Rodrigues (2013) who found a total of 11 AM fungal species from two mangrove ecosystems in Goa, India, where predominant plant species were *Acanthus ilicifolius* L. (Acanthaceae), *Excoecaria agallocha* L. (Euphorbiaceae), and *Rhizophora mucronata* Poir. (Rhizophoraceae). Here *Glomus* was also the dominant

genus followed by *Acaulospora*, *Rhizophagus*, *Funneliformis*, and *Racocetra*. On the other hand, Seerangan and Thangavelu (2014) assessed the incidence of AM and DSE symbiosis in eight hydrophytes and 50 wetland plants from four sites in south India. Spore morphotypes belonging to only five AM fungal species, such as *Glomus macrocarpum* Tul. & Tul., *Acaulospora laevis* Gerd., *Glomus ambisporum* G.S. Sm. & N.C. Shenck, *Glomus invermaium* I. R. Hall., and an *Acaulospora* sp. were isolated from the rhizospheres of that wetland plants. These authors attributed that low AM fungal species number richness to the collection of the soils sediments during the wrong season as sporulation has been shown to be seasonal for many AM fungi. Beena *et al.* (2000) analyzing diversity of arbuscular mycorrhizal fungi in 10 geographical locations consisting of moderately disturbed and severely disturbed dunes during wet and dry seasons on the west coast of India found until 31 species, with the prevalence of *Glomus* genus (17 species and/or morphotypes for a 55% of the total observed species).

Sengupta and Chaudhuri (2002) also studied AMF species associated to thirty-one species of mangrove and mangrove associates and 23 species of transported flora, belonging to 25 families at four physiographic stages of succession at the terminal part of the Ganges river estuary in India. Seven species of AM fungi in common with those plants were isolated from soils of the mangroves, three of which predominated in root association. Six of these were identified as belonging to *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, *G. fasciculatum* (Thaxter) Gerdemann & Trappe *emend.* Walker & Koske, *G. macrocarpum* Tul. & Tul., *G. multicaulis* Gerdemann & Bakshi, *Gigaspora margarita* Baker & Hall and *Acaulospora mellea* Spain & Schenck. One species of *Entrophospora* remained unidentified. Among these, *G. mosseae*, *G. fasciculatum* and *Gigaspora margarita* appeared to be the dominant species as these were present in all successional stages and in rhizospheres of all mycorrhizal plants.

Wilde *et al.* (2009) based on morphological criteria identified 22 arbuscular mycorrhizal fungi species in two saline habitats in Northern Germany, 19 of them belonging to *Glomus* genus. According with these authors generally, more than 20 species are found at non-polluted stands in most studies and illustrated this information with the 21 phylotypes detected by sequencing of PCR products obtained from reed (*Phragmites australis*) at similarly wet but non-saline sites obtained by Wirsal (2004). Guadarrama and Alvarez Sánchez (1999) in Mexico yielded 16 HMA's morphotypes for a rainy tropical forest of lowlands in the Tuxtlas, Veracruz State, of them eight belonging to *Glomus*.

The number HMA species found in these ecosystems of Zapata's wetland is considered itself high if its compares with other studies made in ecosystems of the American continent. In Argentina, Becerra *et al.* (2011) for the case of the Yungas forest, 22 morphotypes of these fungi observed a humid subtropical ecosystem. However, other studies have yielded a so tall abundance of species like the observed in the present study. Bever *et al.* (2001) observed 37 morphotypes in only 1ha of an agricultural abandoned soil which bordering pasturelands campus of Duke's University, North Carolina, EE UU. Furrazola *et al.* (unpublished data) studied eight sequentially different plots located at the evergreen forests of the hillock El Salón, in the reserve of the Biosphere Sierra del Rosario. With an improvement of the methods of extraction of spores from soil (using chemical and physical disaggregation) observed 51 species and or morphotypes of AMF at only half a hectare of forest in these ecosystems.

It was observed a low presence of the representatives' members of Gigasporaceae family at the agricultural studied plots. It coincides with the results obtained for de la Providencia *et al.* (2005, 2007) related to that exist differences between the families Glomeraceae and Gigasporaceae as to the strategies that present both developmental of fungal colony and the way that they can recover before the several injuries within the hyphae developed in soil. According to these authors, in a general way, the number of anastomosis is bigger in the family's representatives Glomeraceae than in those belonging to the family Gigasporaceae, which contributes to *Glomus* species a better adaptation to the injuries of the soil.

Colonization of wetland plants by mycorrhizal fungi is sometimes greater in less fertile (low phosphorus) wetlands (Wetzel and Van der Valk 1996; White and Charvat, 1999), nutrient levels are probably not a major influence (Adamus *et al.*, 2001). These results on the other hand are not surprising, if it is known that AMF's high diversity for some agricultural grounds has been reported (Oehl *et al.*, 2003) depending on that the plowed soils do not have a high level of chemical fertilizers and support himself an adequate crop rotation.

As noted, it should be mentioned that *Acaulospora* cf. sp 4 whose wall outside is ornate with bifid spines, *Glomus* sp. 2 with the external wall ornamented with "foveas" and *Glomus* sp. 7 with cerebriform ornamentations equally in its external wall constitute possible new species. He proves to be of concern equally the existence of HMA species considered like "generalists" for Oehl *et al.* (2003) in order to find in variety of agricultural soils of different latitudes like *Septoglomus constrictum*, *Glomus diaphanum*, *Funneliformis geosporus* and *Glomus*

intraradices. It is known the existence of a functional complementary between the different AMF species proposed by Read (1998) and Koide (2000) and that it can exist even within a same AMF species. Munkvold *et al.* (2004) work proves to be of a great interest future works in the ecosystems studied in the present that they enable isolation and posterior essay of varied of the *Glomus* strains here referred.

ACKNOWLEDGMENTS

We thank Osbel Gómez Ricardo, Carlos Massia Méndez and Esher Collazo for their technical assistance and L. Hernández, conservation's worker, for its valuable help in field expeditions. The work was financially supported by the National Project "*Diversidad y conservación de hongos en tres humedales cubanos*".

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