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Original article

Association of *Phlebopus sp.* with arborean species of the urban trees of Asunción, Paraguay

Asociación de *Phlebopus sp*. con especies forestales del arbolado urbano de Asunción, Paraguay

Associação de *Phlebopus sp*. com espécies florestais das árvores urbanas de Assunção, Paraguai

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ABSTRACT

Paraguayan ecosystems are home to a great diversity of organisms in urban trees that include fungus-plant interactions, commonly called mycorrhizae. These mycorrhizae present a number of subterranean hyphae that are difficult to visually appreciate and basidiocarps that grow together with certain forest species. Therefore, the objective of this research was to identify mycorrhizal associations between macrofungi and forest species that interact with each other in the urban trees of Asunción, Paraguay. For this, two squares (De las Américas and Infante Rivarola) and the Carlos Antonio López Park

were selected as reference points. A tour was carried out to identify the floristic composition of urban trees in the aforementioned green spaces, and the observation of the existence of microrhizal fungi. The fruiting bodies of the macrofungi found were collected and taken to the Biology Laboratory of the Faculty of Agrarian Sciences of the National University of Asunción, they were dried for microscopic observation and identification. The forest species were identified using taxonomic keys, taking into account the size of the tree, the stem, the wood, the bark and the leaves. Three specimens of macrofungi of the genus *Phlebopus were found associated with the trees of the* Bignoniaceae and Fabaceae families in Asuncion green spaces. Two native forest species *Handroanthus heptaphyllus* (Vell.) Mattos, *Jacaranda mimosifolia* D.Don and an exotic *Bauhinia variegate* L. with association of the fungus *Phlebopus sp* .

Keywords: Mycorrhizae; Native forest species; Exotic forest species; Macrofungi.

RESUMEN

Los ecosistemas paraguayos albergan una gran diversidad de organismos en el arbolado urbano que incluyen las interacciones hongo-planta, comúnmente llamadas micorrizas. Estas micorrizas presentan una cantidad de hifas subterráneas de difícil apreciación visual y basidiocarpos que crecen junto a ciertas especies forestales. Por ello, el objetivo de esta investigación fue identificar asociaciones micorrízicas entre macrohongos y especies forestales que interactúan entre de sí en el arbolado urbano de Asunción, Paraguay. Para ello, fueron seleccionados como puntos de referencia dos plazas (De las Américas e Infante Rivarola) y el Parque Carlos Antonio López. Se realizó un recorrido para la identificación de la composición florística del arbolado urbano en los mencionados espacios verdes, y la observación de la existencia de hongos microrrízicos. Los cuerpos fructíferos de los macrohongos encontrados fueron recolectados y llevados al Laboratorio de Biología de la Facultad de Ciencias Agrarias de la Universidad Nacional de Asunción, se procedió al secado de estos para su observación microscópica e identificación. Las especies forestales fueron identificadas mediante las claves taxonómicas teniendo en cuenta el porte del árbol, el fuste, la madera, la corteza y las hojas. Tres ejemplares de macrohongos del género Phlebopus fueron encontrados asociados a los árboles de las familias Bignoniaceae y Fabaceae en los espacios verdes asuncenos. Se identificaron dos especies forestales nativas *Handroanthus heptaphyllus* (Vell.) Mattos, Jacaranda mimosifolia D. Don y una exótica *Bauhinia variegata* L. con asociación del hongo *Phlebopus sp*.

Palabras clave: Micorrizas; Especies forestales nativas; Especies forestales exóticas; Macrohongos.

RSUMO

Os ecossistemas paraguaios abrigam uma grande diversidade de organismos em árvores urbanas que incluem interações fungo-planta, comumente chamadas de micorrizas. Estas micorrizas apresentam uma série de hifas subterrâneas de difícil apreciação visual e basidiocarpos que crescem junto com certas espécies florestais. Portanto, o objetivo desta pesquisa foi identificar associações micorrízicas entre macrofungos e espécies florestais que interagem entre si nas árvores urbanas de Assunção, Paraguai. Para isso,

duas praças (De las Américas e Infante Rivarola) e o Parque Carlos Antonio López foram selecionados como pontos de referência. Foi realizado um passeio para identificar a composição florística da arborização urbana nos referidos espaços verdes, e a observação da existência de fungos microrrizais. Os corpos de frutificação dos macrofungos encontrados foram coletados e levados ao Laboratório de Biologia da Faculdade de Ciências Agrárias da Universidade Nacional de Assunção, onde foram secos para observação microscópica e identificação. As espécies florestais foram identificadas por meio de chaves taxonômicas, levando em consideração o tamanho da árvore, o caule, a madeira, a casca e as folhas. Três espécimes de macrofungos do gênero Phlebopus foram encontrados associados às árvores das famílias Bignoniaceae e Fabaceae em espaços verdes de Assunção. Duas espécies florestais nativas *Handroanthus heptaphyllus* (Vell.) Mattos, *Jacaranda mimosifolia D*. Don e uma exótica Bauhinia variegata L. com associação do fungo Phlebopus sp.

Palavras-chave: Micorrizas; Espécies florestais nativas; Espécies florestais exóticas; Macrofungos.

INTRODUCCIÓN

The organisms of the Fungi Kingdom have been studied throughout universal history, showing that hundreds of them present a way of life that interacts with the hundreds of hosts of the Plantae Kingdom (Pham *et al.*, 2012).

Castro (2009) mentions that the mycorrhiza is a mutualistic symbiosis where its function is to increase the absorption surface of the roots through hyphal systems. extraradical, since the plant can absorb more water, essential nutrients such as (nitrogen and phosphorus) and less mobile ions such as (copper, zinc, ammonia and phosphoric acid), favoring water balance and nutrition (Barrera 2009).

Therefore, from a nutritional perspective, one of the main contributions of mycorrhizae is the absorption of phosphorus, since this nutrient is assimilated from the root pathways, redistributing throughout the plant as it is a fundamental element for its growth, that reflects a fundamental contribution in the increase of the dry mass, growth and foliar area of the plant (Barrera 2009).

Within the edaphic community, the microbiological activity may be due to the mutualistic associations of the microrhizal fungi, such is the case that these organisms may also interact with other agents, no matter if they are bacteria or fungi, thus contributing to perform synergistic actions for the control of phytopathogens, added all this for a better growth of the plant as mentioned (Cano 2011).

Research carried out by Álvarez-Manjarrez *et al*. (2018) refer that mycorrhiza have certain preferences for botanical families such as (Pinaceae, Fagaceae and Betulaceae) in the northern hemisphere, since they are families of great importance due to their diversity in species and in relation to the southern hemisphere, the colonized botanical families for ectomycorrhizae are the *Nothofagaceae, Myrtaceae, Dipterocarpaceae*, and the order Fabales, as they are dominant.

In relation to forest species, also from a positive point of view, they can be a source of improvement in obtaining size and quality in seed production through the use of

mycorrhizae through the incorporation of phosphorus and other nutrients Montaño *et al.* (2007).

Within urban trees, there are also fungi associated with roots in a negative way, such as *Ganoderma, Fomitiporia, and Hydnopolyporus, which* degrade the quality of the wood, affecting its sanitary measures (Moreira-Rivas and Díaz-Lezcano, 2021).

The objective of the research was to identify mycorrhizal associations between macrofungi and forest species that interact with each other in the urban trees of Asunción, Paraguay.

MATERIALS AND METHODS

Research Location

The fieldwork was carried out in the Plazas de las Américas, and Infante Rivarola and Carlos Antonio López Park, in the city of Asunción, Paraguay (Latitude: -25.2819, Longitude: -57.635 25° 162 553 South, 57° 382 63 West). At the end of January 2021, a tour was carried out to identify the floristic composition of urban trees, in the aforementioned green spaces, and the observation of the existence of microrhizal fungi of the genus Phlebopus. In the city of Asunción, a total rainfall of 300 mm was recorded during the month of January and an average temperature of 26ºC. This temperature is within the range considering, the average maximum temperature of 33 °C and 23 °C as minimum temperature, the warmest month of the year in Asunción is January according to Grassi (2020).

Firstly, the forest species present in the aforementioned plazas and park were identified according to the taxonomic keys of López *et al*.,(2002), Pérez de Molas (2016), consigning the botanical family, the scientific name and the common name. For this, the size of the tree, type of stem bark, type and shape of leaves, presence of lenticels, type of inflorescences and fruiting have been considered.

Direct observations of the phytosanitary status of the trees were made and possible fruiting bodies of mycorrhizal fungi were sought, which were collected, labeled in bags and taken to the Biology Laboratory of the Faculty of Agricultural Sciences of the National University of Asunción (UNA).). Once the samples were collected, following the guidelines proposed by Miller and Miller (1988), they were dried and preserved for later microscopic observation and identification, using the Boeco Germany microscope with assemblies in Lactophenol Blue and 5 % Potassium Hydroxide. The photos were taken with a CANON PowerShot camera. Sx 410. The identification of macroscopic fungal species was carried out using the available taxonomic key (Baroni *et al*., 2015).

RESULTS AND DISCUSSION

Table 1 lists 27 botanical families and 69 tree species identified in the Plazas de las Américas and Infante Rivarola and the Carlos Antonio López Park in Asunción, capital of the Republic of Paraguay.

Table 1. - Tree floristic composition identified in Plazas de las Américas and Infante Rivarola and Carlos Antonio López Park in Asunción, Paraguay

Family	***scientific name***	**common name**
Altingiaceae	*Liquidambar styraciflua*	liquidámbar
Anacardiaceae	*Mangifera indica* L.	mango
Annonaceae	*Rollinia emarginata*	aratiku
Annonaceae	*Annona muricata*	corazon de india
Apocynaceae	*Tabernaemontana catharinensis.*	sapirangy
Apocynaceae	*Plumeria rubra*	jazmín magno
Arecaceae	*Roystonea regia*	palmera real
Arecaceae	*Arecaceae* sp.	palmera
Arecaceae	*Acrocomia aculeata*	mbokaja
Arecaceae	*Copernicia alba*	karanda
Bignoniaceae	*Handroanthus albus*	lapacho amarillo
Bignoniaceae	*Handroanthus heptaphyllus*	lapacho negro
Bignoniaceae	*Jacaranda mimosifolia*	jacaranda
Bignoniaceae	*Tecoma stans*	tecoma
Bignoniaceae	*Spathodea campanulata*	tulipán de la India
Boraginaceae	*Cordia americana*	guajayvi
Boraginaceae	*Cordia ecalyculata*	colita
Cecropiaceae	*Cecropia pachystachya*	amba
Clusiaceae	*Rheedia brasiliensi*	pakuri
Combretaceae	*Terminalia catappa*	sombrilla de playa
Euphorbiaceae	*Sapium haematospermum*	kurupika'y
Euphorbiaceae	*Actinostemon concolor*	yvyra hu
Fabaceae	*Delonix regia*	chivato
Fabaceae	*Bauhinia variegata*	lluvia de orquídea
Fabaceae	*Caesalpinia ferrea*	granadillo brasileño

Fabaceae	*Peltophorum dubium*	yvyra pyta
Fabaceae	*Parapiptadenia rigida*	kurupa'y
Fabaceae	*Anadenanthera colubrina*	kurupa'y kuru
Fabaceae	*Triplaris gardneriana*	villetana
Fabaceae	*Geoffroea spinosa*	manduvira
Fabaceae	*Enterolobium contortisiliquum*	timbo
Fabaceae	*Tipuana tipu*	tipa
Fabaceae	*Inga marginata*	inga
Fabaceae	*Albizia niopoides*	yvyraju
Fabaceae	*Leucaena leucocephala*	leucaena
Fabaceae	*Cassia fistula*	lluvia de oro
Fabaceae	*Acacia mangium*	acacia
Fabaceae	*Pterogyne nitens*	yvyra ro
Fabaceae	*Bauhinia forficata*	lluvia de orquídeas
Fabaceae	*Albizia niopoides*	yvyraju
Lamiaceae	*Vitex megapotamica*	taruma
Lauraceae	*Ocotea sp.*	laurel
Malpighiaceae	*Malpighia emarginata*	acerola
Malvaceae	*Ceiba chodatii*	samu'u
Malvaceae	*Guasuma ulmifolia*	kamba aka
Meliaceae	*Melia azedarach*	paraiso
Meliaceae	*Cedrela fissilis*	cedro
Moraceae	*Ficus luschnathiana*	ybapoy
Moraceae	*Ficus benjamina*	ficus
Moraceae	*Morus alba*	mora
Moraceae	*Ficus enormis*	guapo y
Moringaceae	*Moringa oleifera*	moringa
Myrtaceae	*Eucalyptus grandis*	eucalipto
Myrtaceae	*Eugenia uniflora*	ñangapiry
Myrtaceae	*Myrtus communis*	mirto

Myrtaceae	*Psidium guajava*	guayaba
Proteaceae	*Grevillea robusta*	grevillea
Rhamnaceae	*Hovenia dulcis*	hovenia
Rutaceae	*Citrus limonia*	limon
Rutaceae	*Citrus aurantiun*	apepu
Sapindaceae	*Sapinduous saponaria*	casita
Sapindaceae	*Melicoccus lepidopetalus*	yvapovo
Sapotaceae	*Chrysophyllum gonocarpum*	agua'i
Ulmaceae	*Phyllostylon rhamnoides*	juasy'y
Urticaceae	*Cecropia pachystachya*	amba'y

In all the sites of the urban trees, where the samplings and observations were carried out, the genus Phlebopus was identified (Figure 1). It is a light brown fungus, it has a fleshy basidiocarp, solitary to gregarious in habit, its crown is brown with the central stipe tapering upwards with a yellow apex. glabrous surface. It has an irregular margin, in which it splits when they mature.

At the microscopic level, the hyphal webs are gelatinous, the basidiospores have thin walls, and the basidia are clavate.

On the other hand, the genus *Phlebopus* is a fungus that lives in tropical areas characterized by basidiomata with typically robust structures with the presence of a thick central stipe, without holes, short and smooth with abundant olive -brown basidiospores, brown cystidia and an immeasurable number of intertwined hyphae. the basidioma with a wide distribution in the southern hemisphere as mentioned by Watling (2008).

Figure 1. - A) Observation of the pores. B) Entire basidiocarps with a gregarious growth habit. C) Basidiocarp color when old. D) Cross section of *Phlebopus sp.* associated with *Bauhinia variegate*

Forest associations with *Phlebopus sp.*

The specimens of the macrofungus *Phlebopus* sp. were found associated with three forest species mentioning the description and location of the host forest species of *Phlebopus* sp. as detailed in (Table 2). Moreira-Rivas and Díaz-Lezcano (2021), mention that in the urban trees of Asunción, species of the Fabaceae family predominate, followed by those of the Bignoniaceae family, being frequent to find in squares, parks and avenues of the city the presence of *H. heptaphyllus, J. mimosifolia* and *B. variegata*. Likewise, Díaz Lezcano *et al*. (2021) report that the floristic composition of forest species for ornamental use in squares and parks in Paraguay is very varied, with the species of the *Fabaceae, Bignoniaceae* and *Arecaceae* families being the most representative.

Table 2. - Specimens of the macrofungus Phlebopus sp. were found associated with three forest species, mentioning the description and location of the host forest species of Phlebopus sp.

Botanical family	Scientific name	Source	Location
Bignoniaceae	*Handroanthus heptaphyllus*	native	Americas Square (-25.2938788, -57.581541.16z)
Bignoniaceae	*Jacaranda mimosifolia*	native	Infante Rivarola Square (-25.294579, -57.5775188.15z)
Fabaceae	*Bauhinia variegate*	exotic	Carlos Antonio López Park (-25.2869366,-57.6510244.15z)

The fungus-plant association occurs through a series of signals that they present to each other, such is the case of the mycelium that the fungi of the genus *Phlebopus* present sp., with a worldwide distribution, which is associated with forest species such as eucalyptus (*Eucalyptus spp*.) and certain legumes (Ducousso *et al*., 2012).

However, there is evidence that *Phlebopus* sp. is an edible macrofungus, reports by Cristaldo *et al*. (2021), which confirm that this fungus has phenolic compounds (26.30 ± 5.06 mg GAE.$g^{-1)}$ and antioxidants (25.69 ± 3, 00 mg EAA.g^{-1}), the latter with an activity of 11 %, which is between the edible and non-toxic parameters for human consumption.

Baroni *et al*. (2015) comment that this genus has been reported in many American countries such as Bolivia, Brazil, Argentina, Colombia, Ecuador, Costa Rica, Puerto Rico, Venezuela and Mexico.

In the reports mentioned by Vasco *et al.* (2018), support that mycorrhiza are mostly linked by the environmental conditions of the place, in addition to parameters such as (pH, soil types, availability of phosporous, nitrogen, etc.). However, in environmental

conditions such as high relative humidity and intense rains that occurred in Asunción during the month of January 2021, the soil conditions were favorable for the growth of these mycorrhizae, and it can be observed that these soils have abundant organic matter that promotes saprotrophic growth of *Phlebopus sp.* (Figure 2).

Figure 2. - Complete basidiocarp with stereoscopic view and observation of the macrofungus associated with *Jacaranda mimosifolia*

The forest species *J. mimosifolia* is one of the dominant species in the urban trees of Asunción and, also, recommended in the Urban Tree Planting Guide for the Metropolitan Area of Asunción (MADES/PNUD/FMAM 2019), so its representativeness could be emphasizing the possibility of association with the fungus *Phlebopus* sp.

It was also possible to corroborate the presence of *Phlebopus sp*. in association with *H. heptaphyllus* (Figure 3). This reaffirms the evidence that mycorrhizal fungi arbusculares are the most prolific and ubiquitous root symbionts, colonizing 72 % of terrestrial plants (Brundrett and Tedersoo, 2018). Although the benefits of mycorrhizal symbiosis are well documented, little is known about the effects of urbanization on arbuscular mycorrhizal associations (Buil *et al*., 2021).

According to Kumla *et al.* (2016), *Phlebopus sp*. synthetically forms anatomically complete ectomycorrhizae with *Pinus* sp. and *Acacia sp.;* however, Palacio *et al.* (2015) maintain that species of the genus *Phlebopus* are facultative fungi. On the other hand, Singer *et al.* (1983) and Sánchez and Mata (2012) state that they are saprobes, which calls into question the ectomycorrhizal nature of the species. In addition, Flamini *et al.* 2018) and Sánchez and Mata (2012) maintain that species of the genus *Phlebopus* are food fungi.

Deschamps (2002) highlighted that, in San Luis and Córdoba, Argentina, the relationship between *Phlebopus bruchii and the Fagara coco* tree (Carrillo 2014), belonging to the Rutaceae family, has its origin in native flora, as well as in the case reported in the

present investigation where the association between this genus of fungus and two forest species is recorded. natives, *J. mimosifolia* and *H. heptaphyllus*.

Figure 3. - Macroscopic observations associated with *Handroanthus heptaphyllus* characterized by taxonomic keys

On the other hand, the species *Bauhinia variegata* is a legume that also presented symbiotic associations with the genus *Phlebopus sp*., as seen in Figure 1.

In the reports made by (Pérez-Moreno and Read 2004), it is mentioned that the genera *Acacia, Aldinia and Inga* present certain associations with the genera of the Boletales order, to which *Phlebopus* belongs *sp*.

Alexander and Hogberg (1986) have also reported genera of edible fungi associated with the subfamily Caesalpinoideae, these genera are: *Afzelia Brachystegia*, *Isoberlinia* and *Julbernardia*, this being the first indication of *Phlebopus sp.*. associated with *B. variegata* as mycorrhiza.

According to the FAO, (2005) there are many economic benefits produced by fungi from the forest point of view, since these are reflected in rural communities, especially in developing countries.

CONCLUSIONS

Specimens of the macrofungus were identified *Phlebopus* spp. associated with three forest species of the families Bignoniacea: Handroanthus heptaphyllus and Jacaranda mimosifolia and Fabaceae: Bauhinia variegata, very abundant species in the urban trees of Asunción.

The study suggests the execution of silvicultural management related to the health status of trees in the metropolitan area of Asunción and, above all, a record of fungus-tree interaction that allows further identification of Phlebopus species.

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Authors contribution

Esteban Isrrael Moreira-Rivas: Conception of the idea, literature search and review, instrument making, instrument application, compilation of information resulting from the instruments applied, statistic analysis, preparation of tables, graphs, and images, database preparation, general advice on the topic addressed, drafting of the original (first version), review and final version of the article, article correction, authorship coordinator, translation of terms or information obtained, review of the application of the applied bibliographic standard.

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