

## Diversity of zoosporic Fungi and Stramenopila at terrestrial habitats from the Reserva Natural Selva Marginal Punta Lara, Buenos Aires, Argentina

by

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With 4 figures and 3 tables

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**Abstract:** A study of soil samples was carried out at five contiguous habitats of different vegetation types from the Reserva Natural Selva Marginal Punta Lara (Buenos Aires, Argentina): coast, dry forest, floodplain marsh, grassland and marginal forest. Soil samples were processed with a baiting technique, thus producing an inventory of twenty four saprophytic organisms belonging to the Blastocladiomycota, Chytridiomycota and Oomycetes. The frequency analysis showed that all species were of rare occurrence on a macro scale although there were distributional differences between habitats. Cluster analysis showed three groups: (i) the marsh and the grassland, (ii) the dry and the marginal forests and (iii) the coast, which appeared to be the poorest habitat with only three isolations and two taxa recorded.

**Key words:** abundance, ecology, Fungi, soil, Stramenopila.

### Introduction

Zoosporic Fungi and Stramenopila are common inhabitants of soil, but due to the difficulties in culture and identification they are often omitted in ecological and biological studies of soil (Ellanskaya et al. 2000; Gusmao et al. 2001). These organisms play a crucial role in the decomposition of plant structural polymers, such as cellulose, hemicellulose and lignin, thus contributing to the maintenance of the global carbon cycle (Gadd 2004).

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Although several studies related to terrestrial zoospore organisms have been carried out worldwide (e.g. Letcher & Powell 2001, 2002; Letcher et al. 2004; Schoenlein-Crusius et al. 2006), in Argentina there is little information concerning their occurrence in soil (González & Steciow 2006; Marano et al. 2006 a,b,c; Steciow et al. 2006).

The Reserva Natural Punta Lara is located on the riverine side of the Río de la Plata on the northeast of Ensenada and Berazategui districts, Buenos Aires, Argentina (Cabrera 1960). This area includes 6000 ha (Martínez et al. 2006) that consists of basins and levees (50-500 m width) with alluvial ("fluvacuantes") to gley humic soils (Cappannini & Mauriño 1966), high organic-carbon content and low dissolved oxygen availability (Martínez et al. 2006).

The aim of this study was to compare the composition, abundance and diversity of soil zoospore organisms (Blastocladiomycota, Chytridiomycota and Oomycetes) at five contiguous habitats with different soil features and vegetation types.

### Material and methods

**SELECTION OF HABITATS:** Five terrestrial habitats at the Reserva Selva Marginal Natural Punta Lara were selected (Fig. 1): (i) Floodplain marsh: is characterized by gley flooded soils, thus allowing the growth of *Scirpus giganteus* (C.A.Mey.) Steud., *Zizaniopsis bonariensis* (Balansa et Poitr.) Speg., *Schoenoplectus californicus* (C.A.Mey.) Soják, *Typha latifolia* L. and *Erythrina crista-galli* L.; (ii) Grassland: it develops next to the floodplain marsh and it is characterized by gley-sandy soils and a mixture of grasses and herbaceous species; (iii) Dry forest: is placed on shelly ridges between floodplain marshes. Xerophytic species such as *Celtis tala* Gill. ex Planch., *Scutia buxifolia* Reiss., *Schinus longifolius* (Lindl.) Speg., *Jodina rombifolia* H. et A. and *Acacia caven* (Mol.) Mol. are typical at this habitat because of the low water levels in soil; (iv) Marginal forest: it develops onto the stream shoreline and it is characterized by the presence of dominant arboreal species such as *Allophylus edulis* (Camb.) Radlk., *Citharexylon montevidense* (Spreng.) Mold., *Lonchocarpus nitidus* (Vog.) Benth., *Ocotea acutifolia* (Nees) Mez, *Pouteria salicifolia*, (Spreng.) Radlk., *Ligustrum lucidum* Ait. and a great number of lianas and epiphytes (Cabrera & Dawson 1944; Dascanio et al. 1994); and (v) Coast: it lies on the riverine coast of the Río de la Plata. This habitat has few plant species, such as *Mimosa bomplandii* (Gill.) Benth., *Phyllanthus sellowianus* Muell. Arg., *Myrceugenia glaucescens* (Camb.) Legrand et Kausel and *Oxalis* sp.

**SAMPLING AND LABORATORY ANALYSIS:** At each habitat three random collection sites were sampled in December 2007. Subsamples of 300-400 g of soil were collected and transported to the laboratory in ziploc® bags. The subsamples of each habitat were mixed. Soil was diluted (10 g soil/30 ml water), decanted (Pires-Zottarelli 1999) and the pH of the solution was measured (Jenco electronics Co. Ltd., 6091). Three Petri dishes per soil sample were prepared. Five grams of soil (Mettler Toledo PB 302, Max: 310 g, Min: 0.2 g) was flooded with sterile deionized water (30 ml) and different bait material was added, according to the baiting technique (Sparrow 1960; Stevens 1974). Baits were five units of leaves of *Zea mays* L. (corn leaves), *Liophis anomulus* (Günther) skin (snake skin), of 5 mm in diameter and *Sesamum indicum* L. seeds (sesame seeds), according to Marano & Steciow (2006). Each Petri dish was considered a sample unit. A total of 45 Petri dishes and 225 substrate units (45 substrate units for each bait) were analyzed. Cultures were kept at room temperature (20-25°C) for 60 d (Letcher & Powell 2002) and examined at 4, 7, 10, 15, 20, 30, 42, 60 d of the incubation period.

The species composition of zoospore organisms (Blastocladiomycota, Chytridiomycota and Oomycetes) was characterized. Taxonomic identifications were made according to Coker (1923), Sparrow (1960), Seymour (1970), Karlung (1977) and Johnson et al. (2002), with an Olympus BX 40 microscope (Olympus Optical Co. Ltd., Tokyo, Japan).

Presence-absence (occurrence) of the taxa in each Petri dish was recorded. A taxa was recorded as present for a sample if it was observed at any of the observational times (4-60 d). Frequency and

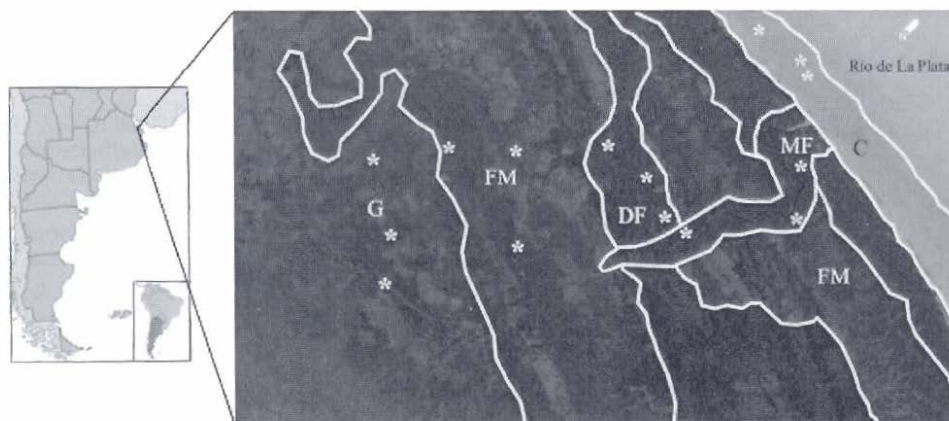


Fig. 1. Location of the terrestrial habitats at the Reserva Natural Punta Lara: C (coast), DF (dry forest), FM (floodplain marsh), G (grassland) and MF (marginal forest). (\*) indicates the collection sites.

abundance was calculated according to Marano & Steciow (2006): (i) Frequency of colonization (FC): (number of sample units colonized by a taxon/number of sample units examined)  $\times 100$  (Figuereda & Barata 2007), and (ii) Abundance (A): (number of isolations recorded for a taxon/number of substrate units employed)  $\times 100$ .

Frequency and abundance were determined for each habitat and type of bait (corn leaves, snake skin and sesame seeds). The taxa were assigned to five frequency groupings of the Braun-Blanquet scale: ubiquitous: 100-80.1%; common: 80-60.1%; often present: 60-40.1%; scarce: 40-20.1% and rare: 20-0.1% occurrence (Kershaw 1973; Letcher & Powell 2001; 2002).

**DATA ANALYSIS:** Community structure was analyzed by: (i) species richness (S); (ii) Shannon's diversity index  $H' = -\sum_{i=1}^S p_i \cdot \log_2(p_i)$ , where  $p_i$  is the abundance of the species  $i$ , that contributes to total diversity; (iii) Evenness  $E = H'/H'_{max}$ , where  $H'_{max}$  is the maximum value of the diversity for the number of species that are present (Magurran 1988); (iv) Simpson's dominance index  $D = 1/\sum_{i=1}^S (p_i)^2$ , which was calculated for each habitat based on species abundance; and (v) Sorensen's similarity index  $SI = 2j/(a+b)$ , where  $j$  is the number of species common to both habitats,  $a$  is the number of species in habitat A and  $b$  is the number of species in habitat B (this index is equal to 1 in cases of complete similarity and 0 if have no species in common).

Diversity indexes were tested for significance with the H-t test using the software program Biodap<sup>®</sup> (Clay & Thomas 1996). Kruskal-Wallis test was used to explore differences in frequency and abundance and the results analyzed by multiple pairwise comparisons using the Dunn's procedure and performed with XLSTAT, version 2007 (Dunn 1961).

Relationships between habitats based on the assemblages of zoospore organisms were evaluated by means of Sorensen's index and UPGMA (Unweighted Pairgroup Method Average). Cluster analysis (Legendre & Legendre 1998) was performed using MVSP package (version 3.1).

## Results

**pH:** All habitats exhibited values within the acidic range: 5.12 (dry forest), 5.76 (floodplain marsh), 5.78 (grassland), 6.35 (marginal forest) and 6.40 (coast).

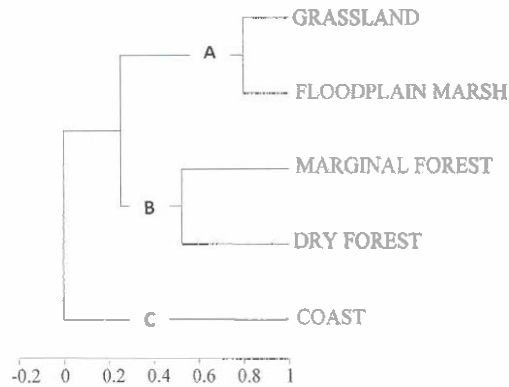


Fig. 2. Phenetic similarities between terrestrial habitats, using Sorensen's index as a similarity measure and UPGMA as a clustering algorithm.

**SPECIES COMPOSITION:** We recorded 23 taxa. Twelve out of them were Oomycetes, nine Chytridiomycota and only two Blastocladiomycota (Table 1). *Rhizophydium keratinophilum* Karling is a new record for Argentina.

Cluster analysis resulted in three defined groups (Fig. 2): Group A (SI: < 0.0001), Group B (0.52) and Group C (0.8).

**FREQUENCY, ABUNDANCE AND DIVERSITY:** We recorded 35 positive sample units and 198 isolations. The frequency of colonization and abundance of zoosporic organisms in soil samples were 90% and 88% respectively. The highest frequency was shared by the dry forest and floodplain marsh, followed by marginal forest and grassland with similar intermediate values, whereas the lowest was observed for the coast. The two habitats mentioned first differed in their abundance, whereas the marsh and dry forest had the same values. Differences of statistical significance were observed in the frequency and abundance ( $P < 0.05$ ; Fig. 3).

The marsh showed the greatest diversity ( $H'$ : 3.37;  $E'$ : 0.89) followed by grassland ( $H'$ : 3.18;  $E'$ : 0.89), marginal forest ( $H'$ : 3.05;  $E'$ : 0.92) and dry forest ( $H'$ : 2.62;  $E'$ : 0.83), whereas the coast had the lowest values ( $H'$ : 1.0;  $E'$ : 1.0). The same trends were observed for the species richness (Fig. 4). The H-t test showed that some habitats exhibited differences of statistical significance ( $P < 0.05$ ; Table 2). Floodplain marsh presented the lowest value of Simpson's index ( $D$ ) indicating a more equitable distribution of abundance (0.09). The grassland (0.11), marginal forest (0.11) and dry forest (0.19) showed intermediate values, whereas the coast exhibited the highest value of this index (0.33), thus the lowest species richness and evenness.

*Achlya* sp.# 1, *Catenophlyctis variabilis*, *Nowakowskiella* sp., *Nowakowskiella elegans* and *Pythium* sp.# 2 showed the highest frequency (> 10%), whereas the rest of the taxa had values < 10%. Regarding the habitats, in the coast the two taxa recorded showed the same frequency, whereas in the other, the taxa are listed in groups of decreasing frequency: DRY FOREST: (a) *Pythium* sp.# 2, (b) *N. elegans*, (c) *Achlya*

Table 1. Species composition and their presence (\*) in the studied habitats. In gray, the taxa restricted to one habitat.

Taxa/Habitats	C	DF	FM	G	MF
<b>BLASTOCLADIOMYCOTA</b>					
<b>Blastocladales</b>					
<b>Blastocladiaceae</b>					
<i>Catenophlyctis variabilis</i> (Karling) Karling		*	*	*	
<b>Catenariaceae</b>					
<i>Catenaria</i> sp.# 1	*				
<b>CHYTRIDIOMYCOTA</b>					
<b>Chytridiales</b>					
<b>Chytridiaceae</b>					
<i>Cylindrochytridium johnstonii</i> Karling			*		
<b>Cladochytriaceae</b>					
<i>Cladochytrium</i> sp.# 1		*			
<i>Cladochytrium replicatum</i> Karling			*		
<i>Nowakowskiella</i> sp.			*	*	*
<i>Nowakowskiella elegans</i> (Nowak.) J.Schröt.		*			*
<i>Nowakowskiella profusa</i> Karling					*
<b>Endochytriaceae</b>					
<i>Catenochytridium</i> sp.					
<b>Rhizophydiales</b>					
<b>Rhizophydiaceae</b>					
<i>Rhizophydium keratinophilum</i> Karling					
<b>Spizellomycetales</b>					
<i>Rhizophlyctis rosea</i> (de Bary et Woronin) A.Fisch.					
<b>OOMYCETES</b>					
<b>Saprolegniales</b>					
<b>Saprolegniaceae</b>					
<i>Achlya</i> sp.# 1		*	*	*	
<i>Achlya caroliniana</i> Coker		*			*
<i>Aphanomyces</i> sp.# 1					*
<i>Aphanomyces</i> sp.# 2			*		
<i>Dictyuchus</i> sp. (sterile)			*	*	*
<i>Dictyuchus monosporus</i> Leitgeb					
<b>Leptomitales</b>					
<b>Leptolegnellaceae</b>					
<i>Aphanomycopsis saprophytica</i> Karling					
<b>Pythiales</b>					
<b>Pythiaceae</b>					
<i>Pythium</i> sp.# 1	*				
<i>Pythium</i> sp.# 2		*			*
<i>Pythium</i> sp.# 3				*	
<i>Pythium</i> sp.# 4				*	*
<b>Peronosporales</b>					
<i>Phytophthora</i> sp.				*	

*caroliniana*, *C. variabilis*, *Rhizophydium keratinophilum* and *Rhizophlyctis rosea*, (d) *Achlya* sp.# 1, *Cladochytrium* sp.# 1 and *Dictyuchus monosporus*; FLOODPLAIN MARSH: (a) *Achlya* sp.# 1 and *Cladochytrium replicatum*, (b) *Pythium* sp.# 2,

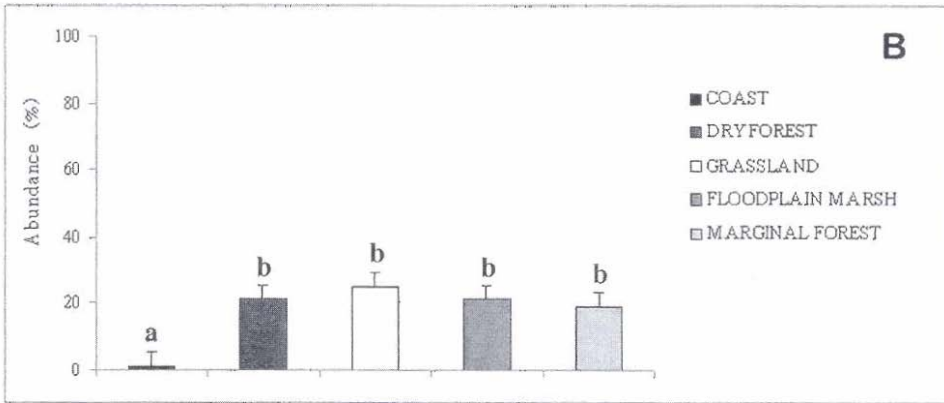
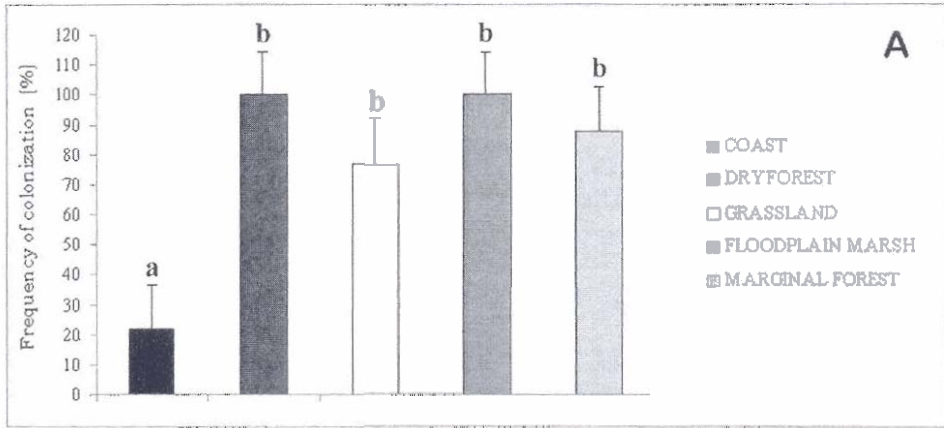


Fig. 3. A. Frequency of colonization (FC %) and B. Abundance (A %) of zoosporic organisms in the sampled habitats. Error bars: SE. The same letters above bars indicates that the values do not differ significantly by means of the Dunn's procedure ( $P < 0.05$ ).

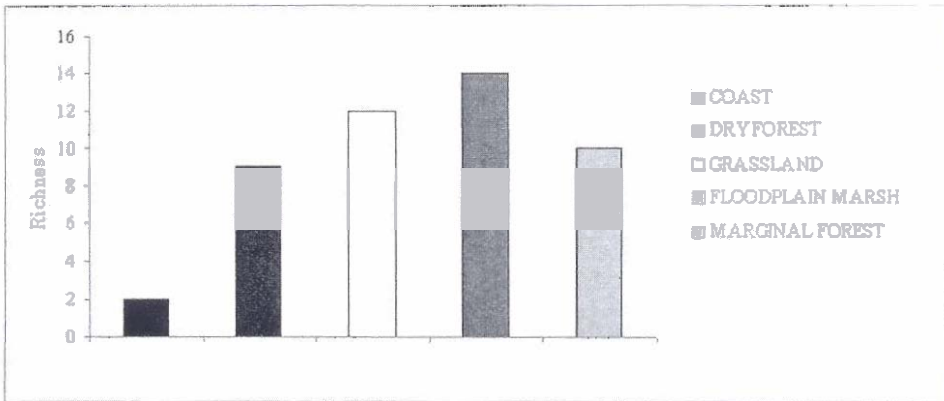


Fig. 4. Species richness (S) for the studied habitats.

Table 2. P-values for the habitats determined by the H-t test. In gray, values that differ significantly (P > 0.05).

Habitats	C	DF	G	FM	MF
C					
DF	8.56				
G	13.15	2.82			
FM	13.52	3.67	1.12		
MF	12.92	2.25	0.77	1.89	

(c) *Aphanomyces* sp.# 1, *Aphanomycopsis saprophytica*, *C. variabilis*, *Cylindrochytridium johnstonii*, *Dictyuchus* sp., *N. elegans*, *Pythium* sp.# 3 and *R. keratinophilum*; GRASSLAND: (a) *Nowakowskiella* sp. and *Pythium* sp.# 4, (b) *Achlya* sp.# 1, *Catenochytridium* sp. and *C. variabilis*, (c) *Aphanomyces* sp.# 2 and *A. saprophytica*, (d) *C. replicatum*, *C. johnstonii*, *Dictyuchus* sp. and *Pythium* sp.# 3; MARGINAL FOREST: (a) *Pythium* sp.# 3, (b) *Dictyuchus* sp., *Dictyuchus monosporus*, *Nowakowskiella* sp., *N. elegans* and *Achlya caroliniana*, (c) *Aphanomyces* sp.# 1, *Nowakowskiella profusa*, *Phytophthora* sp. and *R. rosea*.

Since the highest abundance were observed for *Achlya* sp.# 1 and *Pythium* sp.# 2 (10%), almost all taxa presented A < 10% and five taxa < 1%. In the dry forest the dominant taxa was *Pythium* sp.# 2; in the marsh, *Cladochytrium replicatum* and *Achlya* sp.; in the grassland *Nowakowskiella* sp. and *Pythium* sp.# 4 and finally in the marginal forest *Pythium* sp.# 3 (Table 3).

**SUBSTRATE SPECIFICITY:** Our results suggest that some taxa might preferably developed on certain types of substrates: *Aphanomyces* sp.# 2, *Catenophlyctis variabilis*, *Rhizophyidium keratinophilum* and *Catenaria* sp. were restricted to snake skin; *Aphanomycopsis saprophytica*, *Catenochytridium* sp., *Cladochytrium replicatum*, *Cylindrochytridium johnstonii*, *Nowakowskiella* sp., *Nowakowskiella elegans*, *Nowakowskiella profusa* and *Rhizophlyctis rosea* to corn leaves, whereas *Achlya caroliniana* and *Achlya* sp.# 1 to sesame seeds. *Pythium* sp.# 2 did not showed any substrate preference, appearing in all kinds of substrate employed, whereas *Pythium* sp.# 4 was developed on both sesame seeds and snake skin, *Pythium* sp.# 3 was restricted to corn leaves and *Pythium* sp.# 1 to sesame seeds (Table 3).

## Discussion

Terrestrial zoosporic organisms of the Reserva Natural Punta Lara are ubiquitous and exhibit a great abundance. Letcher & Powell (2001) and Letcher et al. (2004) observed that in most habitats a few chytrid species are relatively frequent and abundant (i.e. *Rhizophlyctis rosea* in agricultural soils), whereas most species are infrequent and scarce to rare. In agreement with their observations, in our research all of the taxa recorded were rare according to the Braun-Blanquet scale.

Table 3. Frequency of colonization (FC %) and abundance (AR %) of the taxa recorded in each habitat: C (coast), DF (dry forest), FM (floodplain marsh), G (grassland) and MF (marginal forest).

Habitats	C		DF		FM		G		MF		Total FC %	Total A %
	FC %	A %	FC %	A %	FC %	A %	FC %	A %	FC %	A %		
<i>Achlya</i> sp.# 1	0	0	11	4	33	22	33	24	0	0	15.6	10
<i>Achlya caroliniana</i>	0	0	22	22	0	0	0	0	22	6	8.9	5
<i>Aphanomyces</i> sp.# 1	0	0	0	0	11	2	0	8	11	0	4.4	1
<i>Aphanomyces</i> sp.# 2	0	0	0	0	0	0	22	0	0	2	4.4	0.4
<i>Aphanomycopsis saprophytica</i>	0	0	0	0	11	8	22	8	0	0	6.7	3
<i>Catenaria</i> sp.# 1	11	4	0	0	0	0	0	0	0	0	2.2	0.9
<i>Catenochytridium</i> sp.	0	0	0	0	0	0	33	15	0	0	6.7	3
<i>Catenophlyctis variabilis</i>	0	0	22	8	11	8	33	15	0	0	13.3	6
<i>Cladochytrium</i> sp.# 1	0	0	11	2	0	0	0	0	0	0	2.2	0.4
<i>Cladochytrium replicatum</i>	0	0	0	0	33	11	11	4	0	0	8.9	3
<i>Cylindrochytridium johnstonii</i>	0	0	0	0	11	2	11	2	0	0	4.4	0.9
<i>Dictyuchus</i> sp.	0	0	0	0	11	4	11	2	22	8	8.9	3
<i>Dictyuchus monosporus</i>	0	0	11	2	0	0	0	0	22	15	6.7	3
<i>Nowakowskiella</i> sp.	0	0	0	0	0	0	44	8	22	13	13.3	4
<i>Nowakowskiella elegans</i>	0	0	33	11	11	2	0	0	22	6	13.3	4
<i>Nowakowskiella profusa</i>	0	0	0	0	0	0	0	0	11	8	2.2	2
<i>Phytophthora</i> sp.	0	0	0	0	0	0	0	0	11	4	8.9	1
<i>Pythium</i> sp.# 1	11	2	0	0	0	0	0	0	0	0	2.2	0.4
<i>Pythium</i> sp.# 2	0	0	55	37	22	18	0	0	0	0	15.6	10
<i>Pythium</i> sp.# 3	0	0	0	0	11	7	11	4	33	22	8.9	1
<i>Pythium</i> sp.# 4	0	0	0	0	0	0	44	24	0	0	8.9	3
<i>Rhizophydium keratinophilum</i>	0	0	22	11	11	6	0	0	0	0	6.7	4
<i>Rhizophlyctis rosea</i>	0	0	22	6	0	0	0	0	11	6	6.7	3

Dry forest-marginal forest and marsh-grassland are clustered into two groups probably due to their closeness, thus the zoospores are able to disperse across habitats by water flow or flooding. The coast presented the greatest dominance index and the lowest species richness that might be related to the little amount of organic matter and the great drainage of the soil. Letcher et al. (2004) suggested that chytrids inhabiting extreme environments must have morphological and physiological characteristics that enable their survival under harsh conditions. Factors such as the shortage of vegetation, the limited phosphorus and water availability (Gleason et al. 2004; Letcher et al. 2004), might explain the lowest diversity obtained for the coast. By contrast, Letcher et al. (2004) found a high diversity of chytrids in a harshest habitat (i.e. open heat). So according to them, the diversity of vegetation appears to be a less important factor in determining the diversity of chytrids. Grassland and marsh presented opposed values to the coast

probably due to the high availability of cellulose-rich materials and the great water retention which is necessary for zoospore dispersion through the soil pores. Willoughby (1961) concluded that the moisture content of soil samples represents the main factor in determining the occurrence of zoosporic organisms. Some chytrids are unable to survive drying in the laboratory and possibly in the soil. Members of the Blastocladales (e.g. *Catenaria* sp. and *Catenophlyctis variabilis*) and Spizellomycetales (e.g. *Rhizophlyctis rosea*) would be more likely to be found in dry soils than the Chytridiales which cannot survive drying (Gleason et al. 2005). Other edaphic factors such as soil pH and nutrients are correlated with distributional differences (Willoughby 1962, 1964). In our study, the pH values obtained are purely descriptive, so that more detailed studies are needed in order to establish a relationship between pH and the distribution of zoosporic organisms.

There were some taxa such as *Achlya* sp.# 1, *Catenophlyctis variabilis*, *Dictyuchus* sp. and *Nowakowskiella* sp. recorded in almost all habitats, which on a macro scale characterized the terrestrial zoosporic organisms community of the Reserva Natural Punta Lara. Four of the 24 taxa were common to both dry and marginal forests, other four to grassland and marsh and eight restricted to one habitat (three taxa restricted to the marsh, two to the coast and only one to the grassland, dry and marginal forests).

*Catenophlyctis variabilis* and *Rhizophyidium keratinophilum* are known as keratine decomposers (Karling 1946; 1947), whereas *Cladochytrium replicatum*, *Nowakowskiella* spp. and *Rhizophlyctis rosea* play an important role in the degradation of cellulose debris in soil (Willoughby 1998; Schoenlein-Crusius et al. 2006). Species adapted to restricted and different nutrient sources can co-exist, thus cellulophilic and keratinophilic species can be part of the same community. Therefore, the use of different kinds of baits allowed us to characterize this community as a whole.

This preliminary study establish some distributional trends between different terrestrial habitats, showing three defined groups based on their species composition. The community of the coast appears to differ significantly from the other four habitats. In this way, analysis at the microhabitat scale should be required to disclose differences in the distribution and frequency of zoosporic organisms in soil.

Our sample intensity was limited hence a greater sample will certainly reveal additional diversity beyond what we detected in the current research. Further studies are needed to clarify whether the distribution of zoosporic organisms are due to the different plant community types present in terrestrial habitats.

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Our sample intensity was limited hence a greater sample will certainly reveal additional diversity beyond what we detected in the current research. Further studies are needed to clarify whether the distribution of zoosporic organisms are due to the different plant community types present in terrestrial habitats.

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