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First Records and Community Pattern of Arcellinida Inhabiting a Pristine and Remote Island from Southeastern Pacific, Chile

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Summary. We investigate for the first time the species composition and community structure of lobose thecamoebians (Arcellinida) inhabiting an unpopulated and pristine island from the southeastern Pacific. Results revealed low alpha diversity and a high proportion of cosmopolitan species. One genus, four species and two subspecies were identified for the first time for southwestern South America. Further, four morphotypes were not identified to species level, and one could not be identified to species or genera level. They are probably endemics of this poorly studied and remote zone. These results were consistent with the moderate endemicity hypothesis of microbial biogeography. We hypothesized that the low diversity of species recorded on the island is due to selective colonization-extinction dynamics, processes that determines the low species richness of insular macro-organisms. However, this hypothesis needs to be evaluated in the future. Statistical analysis showed that testate amoebae were distributed in two discrete communities in the island. The first consisted of organisms inhabiting habitats located within a forest and the second by organisms inhabiting habitats located outside the forest. The suggested primary factor differentiating these both communities was the availability of appropriate habitat for the different species of testate amoebae.

Key words: Chile, Guamblin Island National Park, lobose testate amoebae, North Patagonian rainforest, southwestern South America.

INTRODUCTION

In opposition to the idea that all the microorganisms have a cosmopolitan or ubiquitous distribution, many studies support the hypothesis that some free-living protists have biogeographic patterns (e.g. see Foissner and Hawksworth 2009 or Fontaneto 2011 for many examples). However, if we compare the number of investigations on protists made in the southern hemisphere with those made in the northern hemisphere, it is easy to conclude that the former has been historically less studied (Fenchel and Finlay 2003, Mitchell and Meisterfeld 2005). Consequently, the general approval for this hypothesis has been undermined mainly by the lack of data from unexplored regions of the planet. This lack

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of information on protists is especially evident in southwestern South America, Chile, a region where research on biota is strongly biased towards vertebrates (Simonetti *et al*. 1995). Particularly, remotes areas of the southern Chilean coastline, archipelago and fjordland (41–56°S) are amongst the most poorly studied areas of the planet (Schrödl and Grau 2003, Arriagada 2010); and except for a number of recent studies on foraminifera (e.g. Zapata 1999; Hromic 2002, 2009; Fernández 2010; Fernández and Zapata 2010a, b), free-living protists belonging to these remote areas remain still unknown. Moreover, most of the studies carried out on testate amoebae in Chile correspond to taxonomic reports (Fernández and Zapata 2011); as a result, little is known on the community pattern of these microorganisms in southwestern South America.

In order to reduce the gap in the global knowledge of these microorganisms, this study presents the first records and community patterns of testate amoebae (Arcellinida) inhabiting the southern Chilean Guamblin Island, one of the most remote and oceanic islands of Los Chonos Archipelago. The outcomes supplied by this study could provide valuable antecedents to the current debate on the biogeography of protists.

MATERIALS AND METHODS

Study site

The Chonos Archipelago is made up of over 150 islands separated by saltwater channels and fjords scattered along the western margin of Aysén Region, southern Chile, between latitudes 44°S and 46°S (Haberle and Bennett 2004). The region lies within a zone of high precipitation, produced by the coupled ocean–atmospheric influence of the Southern Polar Front that migrates seasonally between 50°S (austral summer) and 45°S (austral winter) (Haberle and Bennett 2004). The climate is strongly oceanic, with annual precipitation in the region in the order of 3,000 mm. Annual average temperatures at sea level range between 8 and 10°C, and decrease further to the east with increasing continentality and with increasing altitude (Szeicz *et al.* 2003). Guamblin Island is the most oceanic of the islands composing the Chonos Archipelago and lies at about 83 nautical miles from the mainland. This island has an area of 15,912 ha and a maximum attitude of 218 m. In 1967 a National Park was designated in order to protect both the biodiversity and the purity of its landscapes (CONAMA 1987). Guamblin Island is an excellent natural laboratory to test the dispersal capability of protist since it is an extremely inaccessible and pristine mass of land, without any anthropogenic intervention; nobody lives or have lived on the island in the past (it is completely uninhabited); has many habitats similar to those found in the nearby mainland (e.g. North Patagonian rainforest, creeks, swamps), which in turn, are potentially suitable for the establishment of the arcellinids; and naturally, is isolated. These precedents lead us to assume that the occurrence of all of its biotic components can be attributed to natural processes and to consider a minimal (if any) anthropogenic influence on the dispersal mechanism and subsequent establishment of protists on island.

Sampling of testate amoebae and laboratory treatment

Fifteen surface samples (1,000 cm^3) were collected in January 2008 (austral summer) along a ca. 5,000 m longitudinal transect, covering the northeastern side (closest area to the mainland) of the Guamblin Island (Fig. 1). We sampled only this area because the remainder of the island was, at that time, totally inaccessible by foot or by sailing (trekking through the forest was almost impossible, and there are many cliffs and rocky outcrops along the coastline that do not allow a safely disembarkation). All samples were collected in potential habitats for these microorganisms, but bryophytes (a classical habitat for testate amoebae) were absent along this transect. Details of the sampling sites are given in Table 1 and two representative examples are shown in Fig. 2. All samples were stored wet to maintain the original moisture conditions during transport and were studied within three weeks after collection. The samples were maintained at air temperature (the austral summer is 12°C on average at this latitude) and were protected from direct sunlight during the whole stay on the island and the return trip. On return to the laboratory, samples were washed through 500, 250 μm mesh sieves to retain the coarse organic material, and then back-sieving over a 30 μm mesh sieve to retain microorganisms. The resulting filtrate of each sample was deposited on a Petri plate and air-dried at room temperature. All samples were examined under a light microscope at high magnification and all individuals were isolated and counted. The determination of species was based on morphological characters of the shell (morphospecies), according to a wide range of literature. Important sources used in the determinations included those of Certes (1889), Wailes (1913), Jung (1942), Bonnet (1966), Meisterfeld (2002) and Zapata *et al.* (2007). For scanning electron microscopy, the organisms (shells) were isolated, mounted on coverslips and finally air-dried. The shells on coverslips were coated with gold and examined with a JEOL JSM-6380 operating at 20 kV.

Evaluation of sampling artifacts

In order to rule out possible artifacts on sampling effort we performed a species-accumulation curve method. Species-accumulation curve take account of the identity of the species and plot the rate of accumulation of the new species sampled as samples of identical size are pooled over the total area sampled (Ugland *et al*. 2003). Because all communities contain a finite number of species, if the surveyors continued to sample, the curves would eventually reach an asymptote at the actual community richness. Thus, the curves contain information about how well the communities have been sampled (i.e. what fraction of the species in the community have been detected) (Soberón and Llorente 1993). Here, we use the non-parametric abundance-based estimator Chao1 to estimate richness (Chao 1984, Colwell and Coddington 1994). Species accumulation curve was calculated with EstimateS v8 software (Colwell 2012) using 1,000 randomizations and sampling without replacement. To see if result was sensitive to choice of richness estimator, we also ran selected analyses using a number of other common non-para-

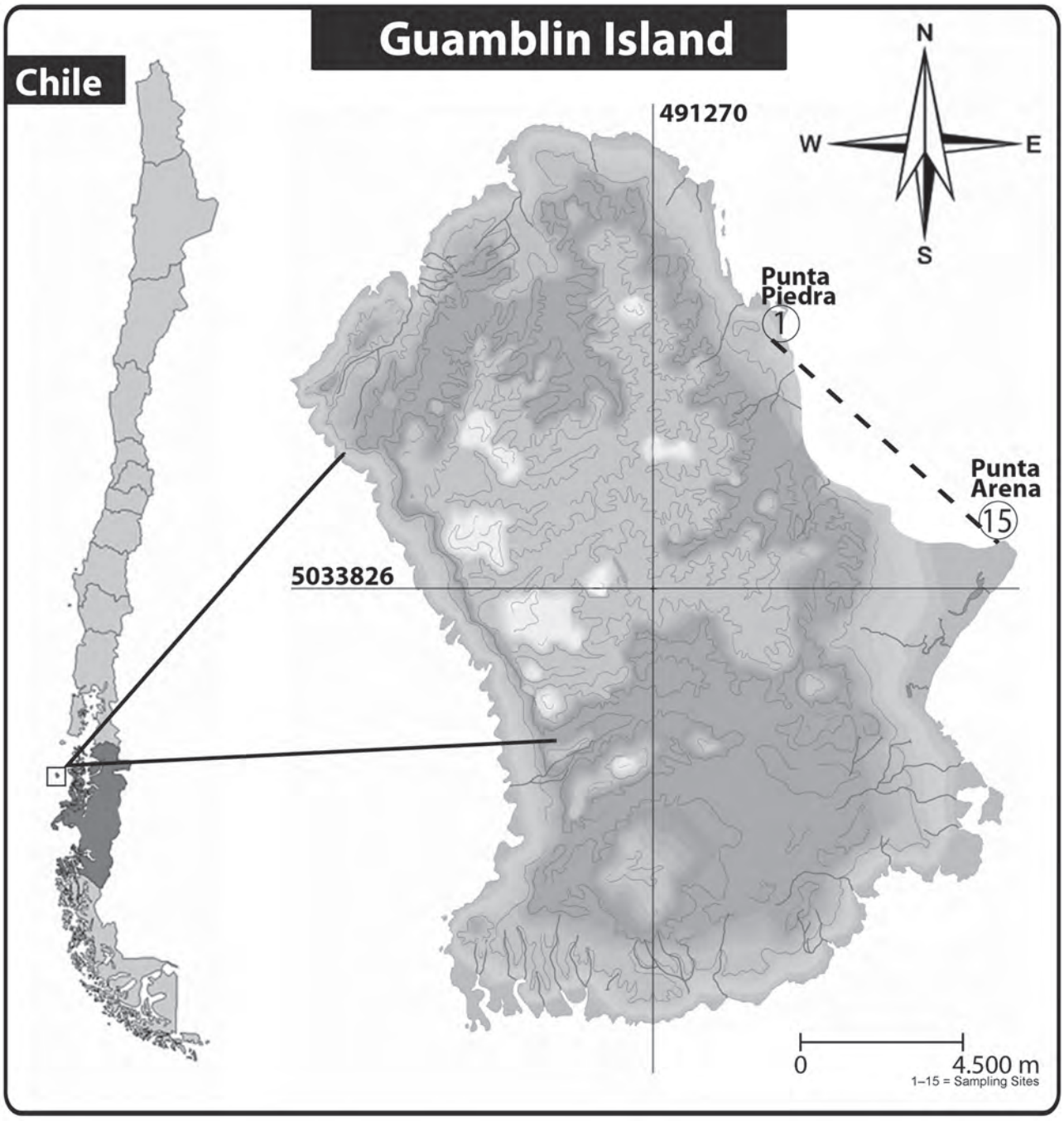

Fig. 1. Map of the Guamblin Island National Park. Samples were taken at equidistant distances, ranging from sector Punta Piedra (sampling site number 1) to sector Punta Arena (sampling site number 15).

metric estimators based in both incidence and abundance of species (i.e. Jack1, Jack2, Chao2, ICE and ACE).

Multivariate analyses

Individuals not identified to genera or species level were excluded from multivariate analyses. Although they may belong to new taxa, we must still confirm that they are not just aberrant individuals. In addition, we lumped together all subspecies (formerly considered as varieties) in their respective morphospecies, since many of them have not been confirmed for anyone else than the person who first described (Mitchell and Meisterfeld 2005), situation very common for this region of the globe. These procedures were performed to reduce skewed or distorted results. Then, we fourth root transformed data to down-weight the influence of

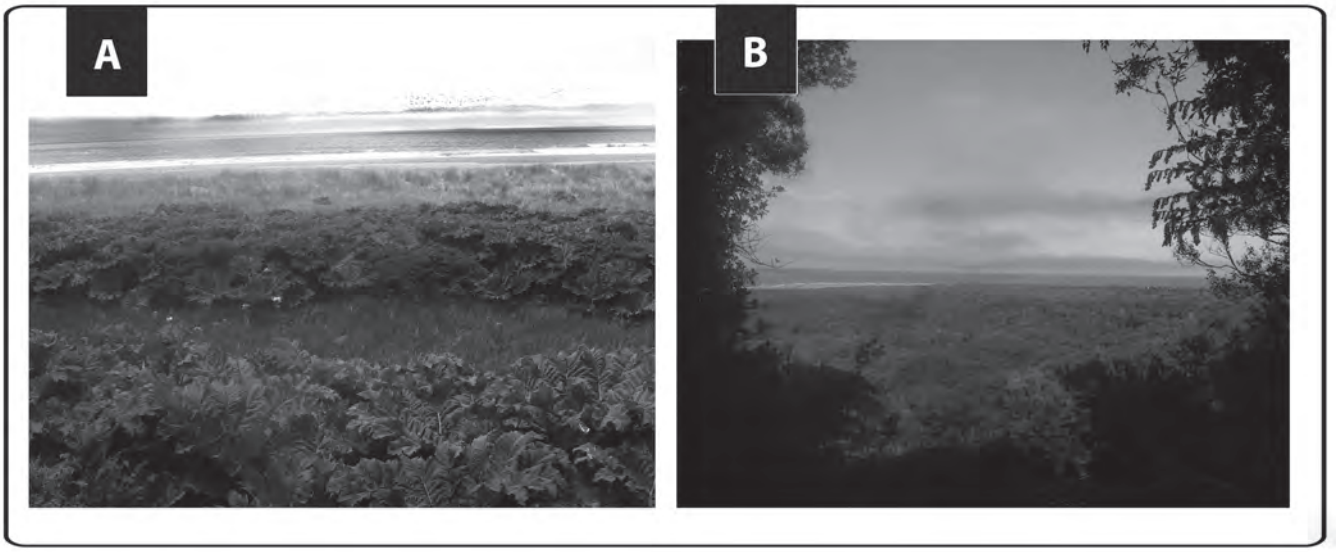

Fig. 2. Both images correspond to the northeastern side of the Guamblin Island ('closest' area to mainland). These images are useful to show the contrasting nature of the two types of sampled environments (treeless and forested environments). **A** – this photo corresponds to the sampled coastal swamp, which is located in sector Punta Piedra (see Fig. 1). This environment is dominated by *Gunnera* spp. and *Juncus* sp.; **B** – this photo corresponds to sector Punta Arena (see Fig. 1), and was taken from an elevation of 150 m a.s.l. In the foreground are some ferns (*Lomatia ferruginea*) and trees (*Nothofagus* spp., *Amomyrtus luma* and *Maytenus boaria*). In the background is the North Patagonian rain forest (which is mainly dominated by *Nothofagus* spp.) and towards the end the Southeastern Pacific. Photo credits by J. Pérez.

Table 1. Description of sampling sites surveyed on Guamblin Island. See some global examples in Fig. 2.

Sampling sites	Habitat	General remarks
S1–S2	Creeks	Aquatic soil from two small creeks located outside the forest, close to a swamp and surrounded by marsh vegetation
S3	Swamp	Coastal swamp, located very close to sea, dominated by *Gunnera* spp. and *Juncus* sp. (probably *J. procerus*)
S4	Swamp	Swamp located at forest margins, dominated by *Gunnera* spp., *Baccharis linearis*, *Blechnum chilense* and *Juncus* sp. (probably *J. procerus*)
S5	Cave	Mud from a little cave located at forest margins, without vegetation.
S6, S7, S9, S12–S15	Forest	Litter and soil from sites located within North Patagonian rain forest, dominated mainly by *Nothofagus dombeyi* and *N. nitida*
S8, S10, S11	Creek	Three small creeks located within North Patagonian rain forest and surrounded mainly by *Gunnera* spp., *B. chilense* and *Drimys winteri*

abundant taxa and account for rare taxa as well (Kreutzweiser *et al*. 2005). Cluster analysis (group average method on Bray–Curtis similarity index) was used to quantify community differences and similarities between environments sampled. To confirm that the groups (i.e. communities) created by the cluster analysis were natural, we subjected the similarity matrix to a 2-d non-metric multidimensional scaling (nMDS), which is a robust ordination method for ecological community data (Clarke and Gorley 2005). In order to identify significant groupings of testate amoebae according to species composition (i.e. deviated from null structure at $\alpha = 0.05$) we performed a similarity profile (SIMPROF) analysis (Clarke 1993). Here, each of the variables (species) was randomized across samples (sampling sites) and the rank order of similarities recalculated over 1,000 permutations. Similarity of percentage (SIMPER) analysis, with a cut off for low contributions of 50%, was used to determine the percentage contribution of individual species towards describing the communities of testate amoebae by species composition (Clarke 1993). Bubble-plots of the most conspicuous species were selected because they were identified as primary factor differentiating the major communities and were superimposed onto nMDS plots. All the analyses were performed in PRIMER v6 software (Clarke and Gorley 2005).

RESULTS

A total of 18,620 thecamoebians, distributed in 12 genera, 33 species and five subspecies were identified and counted from Guamblin Island. One genus, four species and two subspecies are mentioned for first time for southwestern South America (Table 2). On the other hand, four morphotypes were not identified to species level, and one could not be identified to species or genera level (Table 2). We do not make a formal description of these taxa, because the number of individuals per sample was too low to confirm that they are not just aberrant individuals. Except for these individuals, all of testate amoebae identified in the present study have been recorded previously in other regions of the world, including continental Chile (i.e. cosmopolitan or ubiquitous microorganisms) (Fig. 3).

The most diverse genera were *Centropyxis* and *Difflugia*, with five and seven species correspondingly (Table 2). In addition, the five most abundant species (taxa with > 5% of total individuals) made up 56.1% (10,460 individuals) of the total testate amoebae recorded in the Guamblin Island (Table 2). These taxa encompassed *Centropyxis aculeata* 32.5% (6,050 individuals, lumping all the subspecies as one taxon), *Apodera vas* 6.8% (1,260 individuals), *Centropyxis elongata* 5.7% (1,070 individuals), *Cyclopyxis arcelloides* 5.7% (1,060 individuals), and *Centropyxis aerophila* 5.5% (1,020 individuals) (Table 2). Moreover, our species-accumulation curve was close to saturation, showing that our sampling effort provides a reliable species richness for the sampling sites, since all the data points estimated with Chao1 fall within the 95% probability intervals (Fig. 4). Estimation of species richness was not changed when we explored the use of species richness estimators other than Chao1. Indeed, species-accumulation curve using Jack1, Jack2, Chao2, ICE and ACE produced estimations that also are within the 95% probability intervals given by Chao1 analysis (results are not shown).

The Cluster and SIMPROF analyses identified two broad and statistically significant groups (i.e. two communities, $p = 0.001$, Fig. 5). The first of these groups (community A) consisted of samples taken exclusively outside the forest (creeks and swamps, S1–S4), which in turn, was subdivided into two statistically significant smaller clusters ($p < 0.01$, Fig. 5). In contrast, the second group (community B) was composed of samples taken both within and at forest margins (forest soil, creeks and cave, S5–S11), which was subdivided into two significant clusters: mud from a little cave and the rest ($p < 0.01$, Fig. 5). The nMDS ordination analysis also supported graphically the existence of these two broad and discrete communities (Kruskal stress = 0.13, Fig. 6).

The SIMPER analysis revealed that the average Bray–Curtis similarity between all pairs of sampling sites in the community A was 60.44%, made up mainly of contributions from four species (Table 3, columns 2 and 4). Among these were the species *C. aculeata*, *Cy. arcelloides*, *Difflugia oblonga curvicollis* and *D. globularis* with a cumulative contribution of about 50% of the total similarity of 60.44% (Table 3, column 5). Furthermore, the community B obtained a within-group average Bray–Curtis similarity of 63.91%, made up mainly of contributions from five species (Table 3, columns 2 and 4). *C. aculeata* was again prominent among these species, but the remaining four species (*Argynnia dentistoma*, *A. vas*, *Certesella martiali*, *C. aerophila*) were completely different from those listed as major contributors in the community A. These five species contributed with about 50% of the total similarity of 63.91% (Table 3, column 5). We bubble-plotted onto nMDS plots the two most conspicuous species making up the observed similarity within each community in Fig. 7 (excluding a priori *C. aculeata*, since was present in both communities).

Additionally, the SIMPER analysis also found that the average of the Bray–Curtis dissimilarities between both communities was 56.31%. Ten species accounted for more than 50% of average dissimilarity between both communities (Table 4). For instance, this dissimilarity was made up of 4.45% from *Cy. arcelloides*, 3.94% from *D. globularis* and so on (Table 4, column 3). The *Cy. arcelloides* contribution was 7.55% of the total of 56.31%, *D. globularis* gived 6.99% of this total (Table 4, column 5). These values percentages were accumulated in the column 6 (Table 4), until the cut-off of > 50% was reached. The ratio of the average contribution/standard deviation (column 4) was always high for each of these ten species. We bubble-plotted onto nMDS plots the two most conspicuous species making up the observed distinction between both communities (Fig. 8).

DISCUSSION

The so-called ‘ubiquitous dispersal hypothesis’ states that all microorganisms can be found anywhere on the planet as long as suitable conditions are met

Table 2. Occurrences of testate amoebae in each sampling site on Guamblin Island, Southern Chile (individual abundances were divided by the total for that sample).

	Samples														
	Community A								Community B						
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15
Apodera vas Certes	0.0	0.0	1.9	4.3	6.8	7.8	11.7	4.8	19.6	4.0	0.0	12.4	7.9	13.7	31.3
Argynnia dentistoma Penard	0.0	0.0	1.0	3.5	4.5	4.9	7.8	4.8	4.7	6.0	9.1	11.3	6.3	3.8	10.0
Argynnia vitrea Penard	0.0	0.0	0.5	4.3	6.8	3.9	0.0	0.0	0.0	5.0	4.5	0.0	7.1	7.6	6.3
Centropyxis aculeata aculeata (Ehrenberg)	15.6	15.3	36.3	47.8	36.4	42.2	16.9	54.2	13.1	45.0	47.8	18.5	35.4	30.5	15.0
Centropyxis aculeata oblonga (Ehrenberg)	5.5	8.3	0.0	0.0	1.1	2.9	3.9	2.4	2.8	2.0	0.0	0.0	2.4	3.8	0.0
Centropyxis aerophila Deflandre	10.1	8.7	0.0	4.3	0.0	11.8	7.8	2.4	4.7	8.0	3.4	3.1	4.7	6.9	0.0
Centropyxis constricta (Ehrenberg)	6.9	8.3	0.0	0.0	5.7	7.8	3.9	0.0	0.0	0.0	5.7	3.1	6.3	2.3	2.5
Centropyxis discoides Penard	9.6	7.9	1.0	0.0	0.0	0.0	0.0	0.0	4.7	8.0	6.8	3.1	0.0	0.0	10.0
Centropyxis elongata (Penard)	6.9	3.3	1.4	0.0	26.1	7.8	2.6	0.0	0.0	0.0	5.7	20.6	9.4	8.4	0.0
Certesella martiali (Certes)	0.0	0.0	1.0	0.0	1.1	2.0	10.4	3.6	24.3	2.0	0.0	12.4	4.7	3.8	10.0
Cyclopyxis arcelloides (Penard)	9.6	8.3	24.9	5.2	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0	1.6	0.0	0.0
*Cyclopyxis intermedia** Kufferath	2.3	0.8	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyclopyxis kahli Deflandre	3.7	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyclopyxis eurystoma Deflandre	2.8	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyclopyxis sp.	0.9	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Difflugia bryophila Penard	0.0	1.2	0.0	0.0	0.0	2.0	15.6	2.4	14.0	3.0	0.0	2.1	6.3	5.3	6.3
*Difflugia cylindrus** (Thomas)	7.3	12.8	11.0	0.0	0.0	2.0	5.2	2.4	4.7	3.0	0.0	0.0	0.0	0.0	0.0
Difflugia globularis Wallich	0.9	11.6	7.2	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Difflugia lanceolata** Penard	2.3	1.2	4.8	1.7	1.1	0.0	1.3	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
Difflugia mitriformis Wallich	2.3	0.8	3.8	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Difflugia oblonga curvicollis*** Ehrenberg c.f.	8.3	9.1	2.4	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0
*Difflugia lata** (Ehrenberg)	1.8	1.2	1.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Heleopera petricola Leidy	0.9	0.8	0.0	0.0	0.0	0.0	2.6	3.6	0.0	0.0	2.3	0.0	0.0	0.8	0.0
Heleopera sphagni (Leidy)	0.0	0.0	0.0	0.0	0.0	2.0	2.6	1.2	0.0	0.0	0.0	3.1	1.6	1.5	0.0
Lagenodifflugia sp.	0.0	0.0	1.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nebela barbata psilonata Leidy	0.9	0.4	0.0	0.9	2.3	2.0	2.6	6.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0
Nebela collaris (Ehrenberg)	0.0	0.0	0.0	1.7	0.0	0.0	2.6	6.0	2.8	2.0	5.7	3.1	0.8	1.5	6.3
Nebela penardiana Deflandre	0.0	0.0	0.0	1.7	0.0	1.0	2.6	0.0	4.7	3.0	0.0	2.1	3.1	2.3	2.5
Nebela sp.1	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0
Nebela sp.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0
Padaungiella (*Nebela*) *lageniformis* (Penard) c.f.†	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	2.0	0.0	5.2	0.0	4.6	0.0
*Plagiopyxis glyphostoma major*** Bonnet	1.4	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pontigulasia compressa Rhumbler	0.0	0.0	0.0	2.6	0.0	0.0	0.0	6.0	0.0	6.0	3.4	0.0	0.0	0.0	0.0
Undetermined individual	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Species mentioned for first time for southwestern South America; ** subspecies mentioned for first time for southwestern South America; †formerly *Nebela lageniformis*, now transferred to new genus *Padaungiella* (Kosakyan *et al*. 2012).

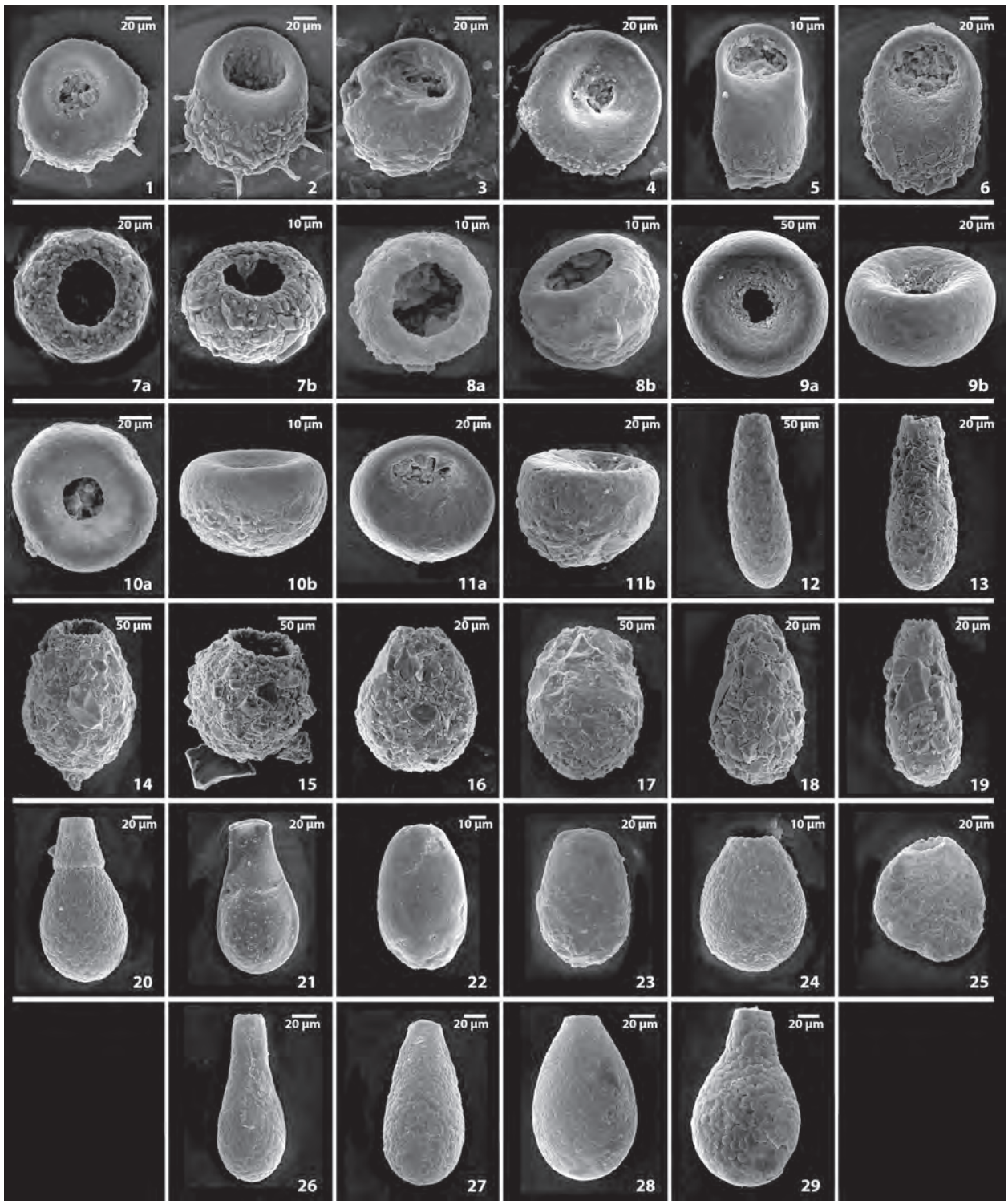

Fig. 3. Species observed on the Guamblin Island. **1** – *Centropyxis aculeata aculeata*; **2** – *C. aculeata oblonga*; **3** – *C. aerophila*; **4** – *C. discoides*; **5** – *C. elongata*; **6** – *C. constricta*; **7a, b** – *Cyclopyxis arcelloides*; **8a, b** – *C. eurystoma*; **9a, b** – *C. intermedia*; **10a, b** – *C. kahli*; **11a, b** – *Plagiopyxis glyphostoma major*; **12** – *Difflugia lanceolata*; **13** – *D. cylindrus*; **14** – *D. mitriformis*; **15** – *D. globularis*; **16** – *Pontigulasia compressa* c.f.; **17** – *D. lata*; **18** – *D. oblonga curvicollis* c.f.; **19** – *D. bryophila*; **20** – *Apodera vas*; **21** – *Certesella certesi*; **22** – *Heleopera sphagni*; **23** – *H. petricola*; **24** – *Argynnia dentistoma*; **25** – *A. vitrea*; **26** – *Nebela barbata psilonata*; **27** – *N. penardiana*; **28** – *N. collaris*; 29 – *Padaungiella* (*Nebela*) *lageniformis*. The background of SEM images were retouched in some cases to highlight the organisms, however, the microorganisms *per se* were not manipulated in any way.

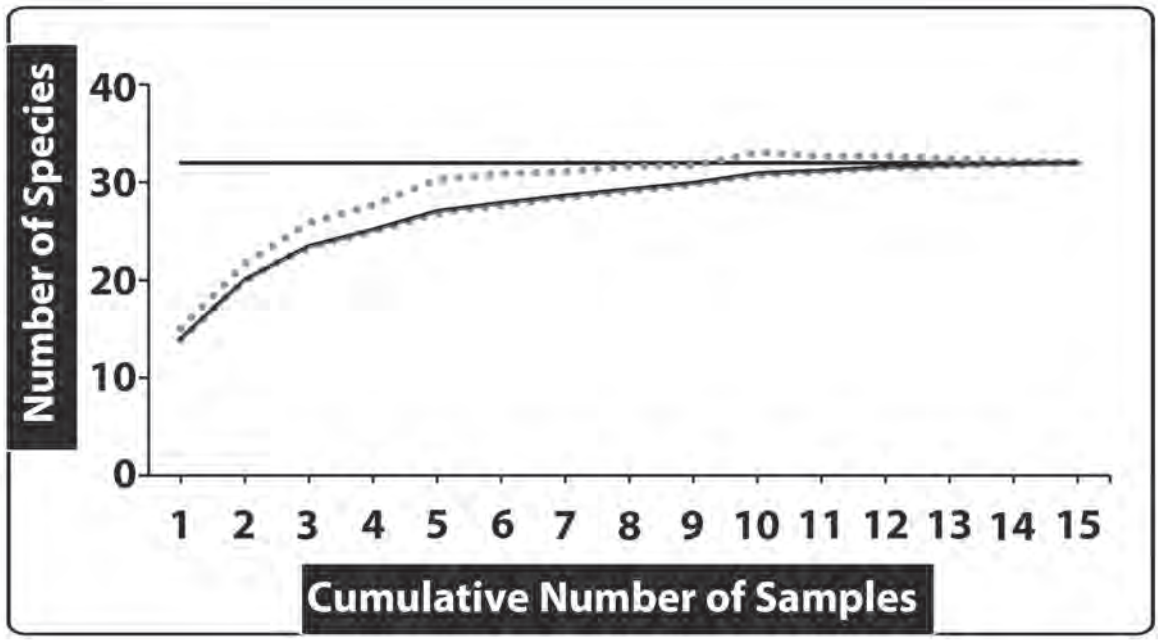

Fig. 4. Species-accumulation curve for testate amoebae collected in the Guamblin Island. Our observed number of species was 33 (black horizontal line). This value was consistent with the estimate species richness curve based on Chao1 (black irregular line). Upper and lower confidence intervals are shown as gray dotted lines (calculated using 1,000 permutations without replacement).

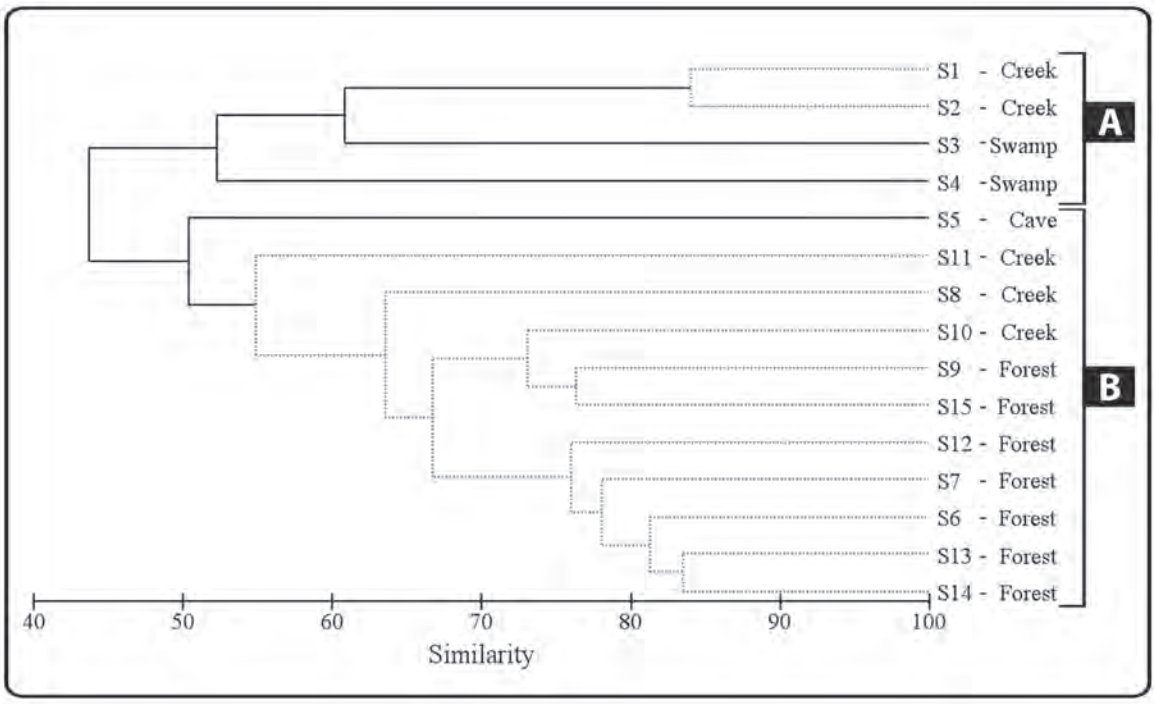

Fig. 5. Bracketed samples encompass groupings (i.e. communities) that are dissimilar from other sampling sites at 43.69% (p = 0.001). **A** – Community A, includes those sampling sites located outside the forest (treeless-group); and **B** – Community B, includes those sampling sites located within the forest and at the forest margins (forested-group). Groupings are according to the group average method on Bray–Curtis similarity index and fourth root transformed abundance-data. Black solid lines correspond to significant clusters and dotted lines correspond to clusters without significant internal structure (based on SIMPER analysis using 1,000 permutations).

(Fenchel and Finlay 2003, Fenchel 2005). Guamblin Island has many habitats similar to those found in the nearby mainland, which in turn, are potentially suitable for the establishment of the arcellinids. Nonetheless, our outcomes did not support the ubiquity hypothesis; because we recorded only a small fraction of the total species richness observed in the nearby mainland (33 species on the island versus more than 100 species of Arcellinida recorded on the near Chilean continent, Fernández unpublished data). Such situations are usually attributed to a low sampling effort (Fenchel and Finlay 2003), but our species-accumulation curve, based on Chao1 species richness estimator, showed that estimated curve reach an asymptote at the actual community species richness (Fig. 3), suggesting that only few more species of arcellinids are to be expected in future surveys. These results were concordant with the so-called 'moderate endemicity hypothesis,' which proposes that at least some microorganisms have restricted geographical distribution (Foissner and Hawksworth 2009, Fontaneto 2011).

One of the leading causes discussed to explain why some protists have a limited distribution is the 'shell size' of these organisms (Wilkinson 2001, Wilkinson and Smith 2006, Yang *et al*. 2010, Lara *et al*. 2011, Wilkinson *et al.* 2012). This predicts that, for thecamoebians, limited geographical range becomes commoner above shell sizes of 100–150 μm, because it would be more difficult for larger microorganisms to become airborne and travel long distances than for the smaller ones (Wilkinson 2001). However, our results did not support this hypothesis, since we recorded many large species on the island (Table 2), such as *A. vas* and *C. discoides*, arcellinids with a mean size of 174 μm (± 25.1) and 160 (± 46.17) μm, respectively (Zapata and Fernández 2008, Lahr *et al*. 2008). In a series of recent papers Foissner (2006, 2007, 2008) argues that a minute size is not a good reason to be a widely distributed organism. He cites several cases of macroorganisms with limited geographical distribution (e.g. macrofungi, mosses, ferns, and flowering plants), that possess microscopic or very small dispersal stages. Based on these examples he sug-

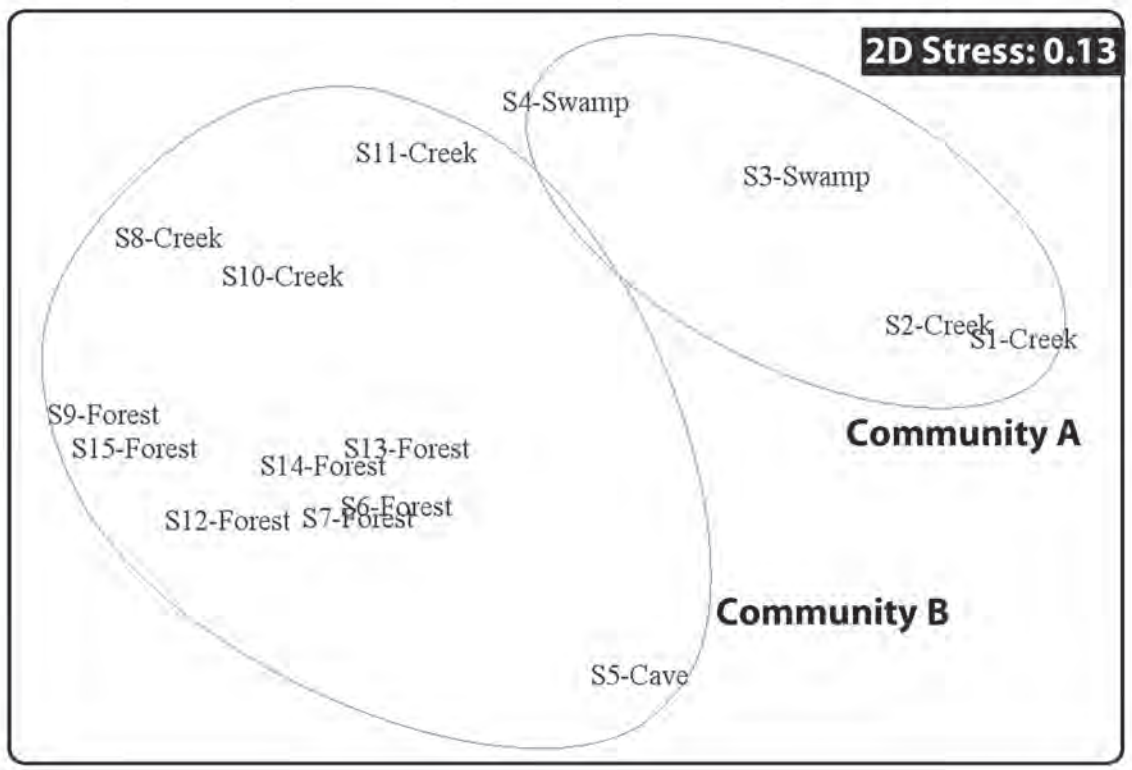

Fig. 6. Non-metric multidimensional scaling plot (nMDS) and Kruskal stress value for the nMDS configuration based on the abundance data of testate amoebae species found along the surveyed sampling sites on the Guamblin Island. Outlined circles represent groupings (i.e. communities) that are dissimilar from other sampling sites at 43.69% (based on SIMPER analysis using 1,000 permutations, $p = 0.001$). Groupings are according to the group average method on Bray–Curtis similarity index and fourth root transformed abundance-data.

Table 3. Species responsible for similarity within each community. The overall averages of the Bray–Curtis similarity (BC-s) between all sampling sites were 60.44% in the treeless-group (community A) and 63.91% in the forested-group (community B), respectively. Column 1 shows the average abundance for each species in each of the two communities. Columns 2 and 4 show the average and the percentage contribution respectively of each species to the BC-s. The column 3 corresponds to the ratio of the average contribution divided by the standard deviation. Column 5 comprises the cumulative percentage contribution to the BC-s, until the cut-off > 50% was reached.

Species	Average abundance*	Average similitude	Sim/SD	Percentage contribution	Cumulative percentage contribution
			Community A		
Centropyxis aculeata	2.76	10.58	9.92	17.50	17.50
Cyclopyxis arcelloides	2.13	7.26	6.97	12.02	29.52
Difflugia oblonga curvicollis	1.94	6.90	13.82	11.41	40.93
Difflugia globularis	1.74	5.67	3.78	9.38	50.31
Other taxa	14.12	30.03	–	49.68	100.00
Total	22.69	60.44	–	100.00	–
			Community B		
Centropyxis aculeata	2.36	11.38	7.78	17.80	17.8
Argynnia dentistoma	1.57	7.81	11.19	12.21	30.01
Apodera vas	1.63	6.80	1.99	10.64	40.65
Certesella martiali	1.39	5.49	1.91	8.59	49.25
Centropyxis aerophila	1.25	4.61	1.35	7.21	56.46
Other taxa	10.80	27.82	–	43.54	100.00
Total	19.00	63.91	–	100.00	–

* Values were fourth root transformed (see Materials and methods).

Table 4. Dissimilarity comparison between treeless- and forested-groups (communities A and B respectively). The overall average of the Bray–Curtis dissimilarities between all pairs of sampling sites (BC-d) was 56.31%. Columns 1 and 2 show the average abundance for each species in each of the two communities. Columns 3 and 5 show the average and the percentage contribution respectively, of each species to the BC-d. The column 4 corresponds to the ratio of the average contribution (column 3) divided by the standard deviation. The column 6 comprises the cumulative percentage contribution to the BC-d, until the cut-off > 50% was reached.

Species	Average abundance* Community A	Average abundance* Community B	Average dissimilitude	Diss/SD	Percentage contribution	Cumulative percentage contribution
Cyclopyxis arcelloides	2.13	0.24	4.25	2.65	7.55	7.55
Difflugia globularis	1.74	0.00	3.94	3.74	6.99	14.54
Difflugia oblonga curvicollis	1.94	0.22	3.86	3.34	6.86	21.40
Difflugia mitriformis	1.34	0.00	3.02	4.47	5.37	26.76
Difflugia cylindrus	1.64	0.60	2.98	1.65	5.30	32.06
Difflugia lanceolata	1.44	0.27	2.66	2.21	4.72	36.78
Certesella martiali	0.30	1.39	2.55	1.73	4.53	41.32
Centropyxis discoides	1.35	0.70	2.38	1.43	4.23	45.55
Difflugia bryophila	0.33	1.23	2.34	1.48	4.15	49.70
Centropyxis constricta	1.02	1.04	2.30	1.50	4.09	53.79
Other taxa	12.16	13.61	26.02	–	46.21	100.00
Total	25.39	19.30	56.30	–	100.00	–

* Values were fourth root transformed (see Materials and methods).

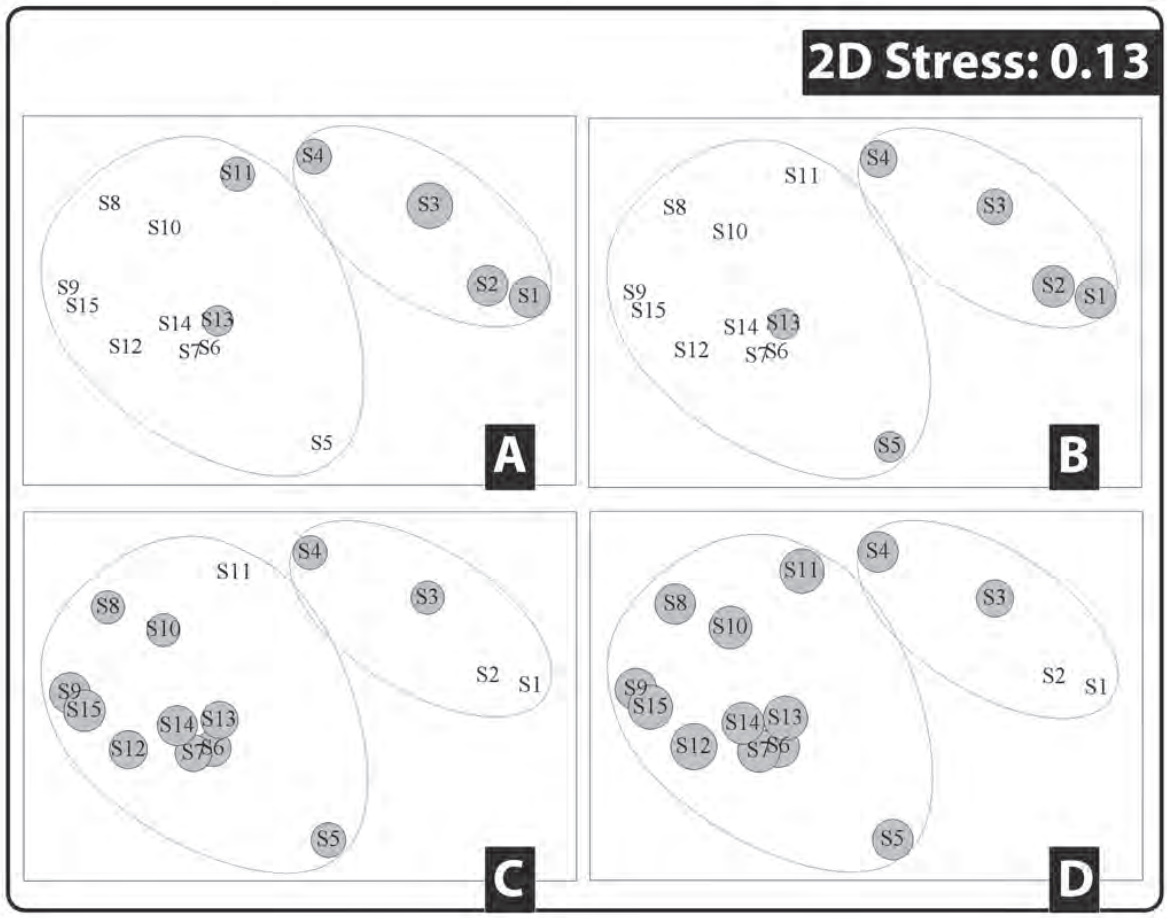

Fig. 7. Bubble plots of the two most conspicuous species making up the observed similarity within each community. For community A: **A** – *Cyclopyxis arcelloides*; **B** – *Difflugia oblonga curvicollis*; and for community B: **C** – *Argynnia dentistoma*; **D** – *Apodera vas*. Bubble plots are superimposed from the nMDS showed in Fig. 5. Bubble size approximates relative proportion of a given species in each sampling sites (gray circles with numbers) and each community type (outlined circles).

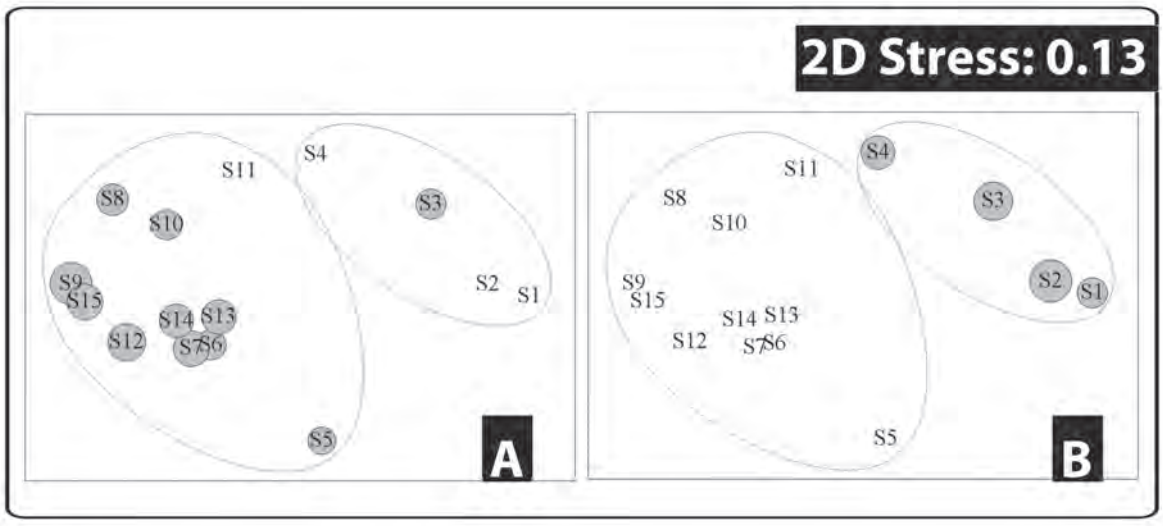

Fig. 8. Bubble plots of the two most conspicuous species making up the observed dissimilarity between both communities: **A** – *Certesella martiali* and **B** – *Difflugia globularis*. Bubble plots are superimposed from the nMDS showed in Fig. 5. Bubble size approximates relative proportion of a given morphospecies in each sampling sites (gray circles with numbers) and each community type (outlined circles).

gests that a minute size do not necessarily causes an unconstrained geographical distribution pattern, and that therefore, other 'factors' must be involved (Foissner 2007). This leads us to consider other causes to explain the low species richness observed not only in this study, but in other research on thecamoebian developed exclusively on islands (e.g. Zapata and Crespo 1990, Balik 1994, Wilkinson and Smith 2006, Heger *et al*. 2009). For instance, it seems more appropriate to explain the low species richness recorded for these organisms on islands through a merge of the 'island biogeography theory' (McArthur and Wilson 1963) and the 'niche theory' (Hutchinson 1957), which were originally proposed to explain the distribution and organization of macroscopic organisms in ecological communities. The first theory predicts a dynamic equilibrium between colonization of new species and extinction of resident species, in which the species richness is an increasing function of island size and a decreasing function of the distance to a source of potential colonizers. While the niche theory (*sensu* Hutchinson), predicts a positive relationship between species richness and the 'habitat heterogeneity' (which include the 'habitat diversity'). However, the island size, as is postulated by the island biogeography theory, probably does not have a direct influence on species richness of microorganisms (given their obvious microscopic sizes), but could indirectly influence the species richness through the reduction or the increase of the 'habitat heterogeneity,' and thus, the number of inter-specific interactions (which is in agreement with the niche theory). As a result, species-poor communities inhabiting islands will tend to be a nested subset of the species-rich ones inhabiting mainland. Nestedness of insular biotas is an extremely common pattern in macroorganisms (Wright *et al*. 1996), but has never been tested on 'true' insular microorganisms. To date, nestedness has been evaluated and confirmed only at the landscape scale on free-living microorganisms (e.g. Peay *et al*. 2007, Soininen 2008, Fernández *et al*. submitted), and the island biogeography theory *per se* has only been tested on small scale for microbial communities (e.g. proposing water-filled treeholes, engineering machines, etc. as analogous to 'islands') (Kinkel *et al*. 1987, Bell *et al*. 2005, Reche *et al*. 2005, van der Gast *et al*. 2005, Peay *et al*. 2007, Lyons *et al*. 2010), and without taking into account the habitat heterogeneity. The joint assessment of both theories and posterior confirmation of the nested pattern on 'true' insular microorganisms (i.e. number of species inhabiting mainland versus those inhabiting islands) would suggest that the processes that generate and maintain insular biogeographic patterns in macroorganisms also operate on microorganisms (e.g. extinction-colonization dynamics). This could explain why there is lower protist diversity on islands, giving a valuable precedent to the current debate on biogeographical patterns in microorganisms. Furthermore, the traditional use of morphospecies concept also appears as an appropriate cause to explain the low species richness on Guamblin Island, since this can badly underestimate protistan diversity (Heger *et al*. 2011, Kosakyan *et al*. 2012). This could be especially true for southern Chile (36°S to 56°S), a zone where the coastal margins had wide ice-free areas during periods of the Last Glacial Maximum, providing forested refugia for biota and promoting for thousands of years the local radiation of the taxa (Villagrán and Hinojosa 1997, Nuñez *et al*. 2011). This suggest that the zone in which lies Guamblin Island (44°S and 46°S) would harbor new cryptic or pseudocryptic species of testate amoebae (species which can be discriminated only by molecular techniques or by scanning electron microscopy, respectively), which in turn represent a probably hidden and endemic diversity yet not evaluated. This hypothesis is also supported by the records of arcellinids not identified to species or genera level (Table 2). These microorganisms have never been observed within continental Chile, and thus perhaps are endemics to Los Chonos Archipelago. Another precedent that suggests that the southern Chile could harbor a hidden diversity of testate amoebae is a detailed morphometric study developed by Zapata and Fernández (2008) on populations of *A. vas* belonging to this zone. These authors found that shell size of this arcellinid have a multimodal distribution within different populations from southern Chile, suggesting the existence of several cryptic species rather than one, which in turn, could have radiated as a result of the combined effects of climatic processes, tectonic activity and glaciers historically experienced by the zone south of 41°S. Nevertheless, future molecular-based studies are needed to test, and eventually confirm or reject, if this region has historically acted as a 'center of origin' of species. Further, although many of the species recorded here fall within the 'cosmopolitan' or 'ubiquitous' categories, we acknowledge that the determination of the species was not always easy using European literature. Therefore we suspect that, in fact, several of the species from Guamblin Island, and perhaps from southern Chile, do

not correspond to any of these categories or to typical European species. However, this conjecture is based merely on morphological evidence, which once again stresses the need for molecular-based studies to reveal whether we are observing the same species recorded in the northern hemisphere or not.

Our analyses showed two distinct and discrete communities within Guamblin Island (Figs 4 and 5). The first one (community A) grouped testate amoebae inhabiting terrestrial and aquatic habitats located outside the forest. The second one (community B) grouped those inhabiting terrestrial and aquatic habitats, located within a forest. *C. aculeata* had the highest average and percentage contribution to the Bray–Curtis similarity in both communities (Table 3, column 2). This finding is not surprising given that centropyxids, primarily *C. aculeata*, are known to be opportunistic and capable of existing in high numbers under benign or harsh environmental conditions (Dalby *et al.* 2000, Zapata *et al*. 2002, Bamforth 2004). If we exclude this species, we can see that the other thecamoebians listed in Table 3 had a very high ratio in both communities (Sim/SD, column 3). As a result, they can be described as typical organisms of their respective communities (Clarke and Gorley 2005). For instance, *Cy. arcelloides*, *D. oblonga curvicollis* and *D. globularis* were prevalent within the treeless habitats (Table 3, column 2, community A). Testate amoebae belonging to *Cyclopyxis* has been reported as moderately abundant species in different environments (e.g. rivers, estuaries, peatlands, litter) from South America (Zapata *et al*. 2002, Zapata *et al*. 2007, Fernández and Zapata 2011). In particular, *Cy. arcelloides* is considered mainly a pedobiont and relatively generalist species (Borba 2008, Bobrov *et al.* 2010). Hence, his prevalent occurrence in this community may be due to this last feature, rather than a specific preference by treeless or aquatic environments. Moreover, low availability of mineral grains and mainly lack of water can restrict difflugids, because most of them have not cysts (Meisterfeld 2002). Therefore, in the presence of such elements difflugids will be normally found in appreciable numbers (Haman 1990). These organisms were also important in the differentiation of both communities, as were virtually absent in the forest community (Table 4, Figs 6 and 7), once again, probably due to lack of appropriate conditions. Accordingly, the occurrence of these organisms within the forest community could be attributed to migration events from source habitats (habitats outside the forest). Further, the prevalence of cyclopyxids and difflugids suggest that habitats located within the community A are subjected to unstable environmental conditions, such as frequent floods and droughts; given that, plagiostomic/hemispherical and acrostomic/cylindrical forms occur in high proportion in soils and aquatics habitats, correspondingly (Foissner 1987). This idea is also supported by the type of flora that dominates these habitats within this community (Table 2). Furthermore, its proximity to the coast suggests that these habitats are also subjected to occasional flooding from the sea, idea supported by the prevalence of *C. aculeata*, testate amoeba frequently reported in brackish waters (Scott *et al*. 2001, Zapata *et al*. 2002). Community of testate amoebae from forested habitats (community B) housed a greater abundance of wet forest soils acrostome species (Table 3, column 2, community B), which is consistent with results reported by other authors in similar environments (e.g. Krashevska *et al.* 2007, Bamforth 2010). For instance, the most prevalent species (discarding the opportunistic *C. aculeata*) were the nebelids *A. dentistoma*, *A. vas* and *C. martiali* (Table 3, column 2, community B), which are species that typically inhabit the rainforests of South America (Bonnet 1966, Krashevska *et al.* 2007, Fernández and Zapata 2011). This result is remarkable, since the last two species are frequently cited as a non-abundant species, although they can be easily found in optimal environments, with minimum sampling efforts (Zapata 2005, Zapata and Fernández 2008, Fernández and Zapata 2011). A research conducted by Zapata *et al*. (2008) in a peatland located in Puyehue National Park (Chile) (as a result, we assume pristine conditions) revealed similar results, recording to *A. vas* in great number. Likewise, both species were recorded in great number in a recent research conducted during a year on a pristine peatland located in the same National Park (Fernández and Zapata, unpublished data). Moreover, in a study developed in Podocarpus National Park (Ecuador) *A. vas* was observed with relatively low number of individuals per sample, but still, was reported within the abundant species (Krashevska *et al.* 2007). This raises a new question: Is the prevalence of these two species an indicator of pristine environmental conditions? It seems fair to think that the conditions in which the samples were stored and transported (e.g. maintaining the original moisture conditions, at air temperature and protected from the direct sunlight) may set up culture conditions that could change the relative abundances of the different taxa in the samples between

collection and counting (including the abundances of *A. vas* and *C. martiali*). Nonetheless, when the aim is to measure abundances and species composition of the active species at the time of sampling (as in this paper), then, samples must be kept under conditions similar to the sample temperature and moisture (Adl and Gupta 2006; Bamforth 2007, 2010). Instead, the common method of air-drying samples for transport can lyse cells (Adl and Gupta 2006), changing irremediably the original species composition and abundances. Another recommendation is to examine the samples as soon as possible (Adl and Gupta 2006, Bamforth 2010), but given the remote location of the Guamblin Island, we were able to check samples only after three weeks. But still, we suggest that the time elapsed do not affected in a significant way the species composition and structure, because we recorded centropyxids, cyclopyxids and difflugids in high numbers within swamps and creeks, as is commonly reported in other studies conducted on these habitats (e.g. see Asada and Warner 2009 and references cited therein). Similarly, we recorded a high proportion of acrostomic species (e.g. nebelids *sensu* Meisterfeld 2002) within forest soils, as also is usually reported in similar researches (Bonnet 1966; Bamforth 2007, 2010; Krashevska *et al*. 2007; Fernández and Zapata 2011). In other words there was not abnormality in the community parameters except for a slightly higher abundance than usual for *A. vas* and *C. martiali.*

CONCLUDING REMARKS

Here, we investigate for the first time the species composition and community structure of testate amoeba inhabiting an unpopulated and pristine island from the southeastern Pacific. Our results revealed low alpha diversity and a high proportion of cosmopolitan species. Nonetheless, one genus, four species and two subspecies were recorded by first time, increasing to ~ 225 species the total number of testate amoebae recorded in southwestern South America (considering both filose and lobose thecamoebians). Moreover, four morphotypes were not identified to species level, and one could not be identified to species or genera level. This suggests that in past this island could be a glacial refugium, allowing the radiation of new testate amoebae with limited capacity of dispersion, since they had never been recorded before in the next mainland, although this zone of the Southamerican continent has been extensively studied during the last 30 years. But, at the same time, our results highlight the natural-high capacity of dispersal of cosmopolitan species, given that they are occurring on a uninhabitated island, a place where (based on existing background) human influence is certainly very limited. However, the determination of these cosmopolitan species was not always easy using European descriptions. Maybe, in future, the use of the term 'cosmopolitan' would not apply to a wide fraction of the Chilean testate amoebae fauna. Upcoming molecular-based studies could help both to evaluate the genetic diversity within these microorganisms and elucidate this issue. We also hypothesized that the low diversity of species recorded on the island is due to dynamics of selective colonization-extinction, processes that determines the low species richness of insular macro-organisms. However, this hypothesis needs to be evaluated in the future. Further, we show that testate amoebae are distributed in two discrete communities along the studied transect. The first one (community A) was constrained to habitats located outside the forest and had soil and aquatic testate amoebae as typical species. The second one (community B) was restricted to habitats located within the forest and had wet forest soils testate amoebae as typical species. The suggested primary factor differentiating these both communities was the availability of appropriate habitat for the different species of testate amoeba. For instance, the lack of persistent aquatic environments within the forest probably limited the occurrences of difflugids in community B and the prevalent harsh conditions (e.g. occasional flooding from the sea) outside the forest restricted the occurrences of nebelids in community A. Finally, we stress the prevalent occurrence of *A. vas* and *C. martiali* within the Nord Patagonian rainforest. The observed occurrences of both nebelids in this island and another pristine environments suggest that the prevalence of these two species could be used as an indicator of pristine environmental conditions. Nonetheless, further studies are necessary to confirm this.

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REFERENCES

Adl M. S., Gupta V. V. S. R. (2006) Protists in soil ecology and forest nutrient cycling. *Can. J. For. Res.* **36:** 1805–1817

Arriagada A. M. (2010) Territorio patagónico chileno: ¿Qué sabemos realmente sobre su biodiversidad? *Bol. Biodivers. Chile* **3:** 1–2

Asada T., Warner B. G. (2009) Plants and testate amoebae as environmental indicators in cupriferous peatlands, New Brunswick, Canada. *Ecol. Indic.* **9:** 129–137

Bamforth S. S. (2004) Water film fauna of microbiotic crusts of a warm desert. *J. Arid Environ.* **56:** 413–423

Bamforth S. S. (2007) Protozoa from aboveground and ground soils of a tropical rainforest in Puerto Rico. *Pedobiologia* **50:** 515–525

Bamforth S. S. (2010) Distribution of and insights from soil protozoa of the Olympic coniferous rain forest. *Pedobiologia* **53:** 361–367

Balik V. (1994) On the soil testate amoebae fauna (Protozoa: Rhizopoda) of the Spitsbergen Islands (Svalbard). *Archiv. Protistenkd.* **4:** 365–372

Bell T., Duane A., Song J. I., Newman J. A., Thompson I. P., Lilley A. K., Van der Gast C. J. (2005) Larger islands house more bacterial taxa. *Science* **308:** 1884

Bobrov A. A., Mazei Y. A., Tiunov A. V. (2010) Testate amoebae of a monsoon tropical forest of south Vietnam. *Acta Protozool.* **49:** 311–325

Bonnet L. (1966) Le peuplement Thécamoebien de quelques sols du Chili (I). *Protistologica* **2:** 113–140

Borba M. (2008) Assembléias de amebas testáceas (Amoebozoa: Rhizopoda) associadas a rizosfera de *Eichhornia crassipes* (Martius) Solomons (Pontederiaceae) no Rio Cachoeira, Bahia. Thesis. Universidade Estadual de Santa Cruz

Chao A. (1984) Nonparametric estimation of the number of classes in a population. *Scand. J. Stat*. **11:** 256–270

Certes A. (1889) Protozoaires. In: Mission Scientifique du Cap Horn 1882–1883. *Zoologie* **6:** 1–53

Clarke K. R. (1993) Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.* **18:** 117–143

Clarke K. R., Gorley R. N. (2005) PRIMER v.6: User Manual/Tutorial. PRIMER–E Ltda, Plymouth, UK

Colwell R. K. (2012) EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application published at http://viceroy.eeb.uconn.edu/estimates (29 April 2012, date last accessed).

Colwell R. K., Coddington J. A. (1994) Estimating terrestrial biodiversity through extrapolation. *Philos. T. Roy. Soc. B*. **345:** 101–118

Corporación Nacional del Medio Ambiente (CONAMA) (1987) Estudio de los recursos naturales presentes en el Parque Nacional Isla Guamblin. Santiago, Chile

Dalby A. P., Kumar A., Moore J. M., Patterson R. T. (2000) Preliminary survey of arcellaceans (thecamoebians) as limnological indicators in tropical lake sentani, Irian Jaya, Indonesia. *J. Foram. Res.* **2:** 135–142

Fenchel T. (2005) Cosmopolitan microbes and their 'cryptic' species. *Aquat. Microb. Ecol.* **41:** 49–54.

Fenchel T., Finlay B. (2003) Is microbial diversity fundamentally different from biodiversity of larger animals and plants? *Eur. J. Protistol*. **39:** 486–490

Fernández L. D. (2010) Foraminíferos (Protozoa: Foraminiferida) del estuario del Río Contaco (40°33′S; 73°43′O), Chile. *Bol. Biodivers. Chile* **4:** 18–62

Fernández L. D., Zapata J. (2010a). Registro tafonómico de *Ammonia beccarii* (Linné, 1758) (Protozoa: Foraminiferida) en la marisma Quillaipe (41°32′S; 72°44′O), Chile. *Lat. Am. J. Aquatic Res*. **38:** 286–291

Fernández L. D., Zapata J. (2010b). Distribución de foraminíferos bentónicos (Protozoa: Foraminiferida) en la Ensenada Quillaipe (41°32′S; 72°44′O), Chile: implicaciones para el estudio del nivel del mar. *Rev. Chil. Hist. Nat.* **83:** 567–583

Fernández L. D., Zapata J. (2011) Variación estacional en la comunidad de amebas testadas de una turbera temperada del sur de Chile. *Bol. Soc. Biol. Concepción Chile* **80:** 27–39

Foissner W. (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Progress in Protistology* **2:** 69–212

Foissner W. (2006) Biogeography and dispersal of micro-organisms: a review emphasizing protists. *Acta Protozool.* **45:** 111–136

Foissner W. (2007) Dispersal and biogeography of protists: recent advances. *Jpn. J. Protozool.* **40:** 1–16

Foissner W. (2008) Protist diversity and distribution: some basic considerations. *Biodivers Conserv.* **17:** 235–242

Foissner W., Hawksworth D. L. (2009) Protist diversity and geographical distribution. Springer, Berlin, Germany

Fontaneto D. (2011) Biogeography of microscopic organisms: Is everything small everywhere? Cambridge University Press, Cambridge, UK

Haberle S. G., Bennett K. D. (2004) Postglacial formation and dynamics of north patagonian rainforest in the Chonos Archipelago, southern Chile. *Quaternary Sci. Rev.* **23:** 2433–2452

Haman D. (1990) Living thecamoebinid distribution, biotopes and biofacies, in an upper deltaic plain lacustrine subenvironment, Lac des Allemands, Louisiana. *Rev. Esp. Micropal*. **22:** 87–100

Heger T. J., Mitchell E. A. D., Ledeganck P., Vincke S., Van de Vijver B., Beyens L. (2009) The curse of taxonomic uncertainty in biogeographical studies of free-living terrestrial protists: a case study of testate amoebae from Amsterdam Island. *J. Biogeogr*. **36:** 1551–1560

Heger T. J., Pawlowski J., Lara E., Leander B. S., Todorov M., Golemansky V., Mitchell E. A. D. (2011) Comparing potential COI and SSU rDNA barcodes for assessing the diversity and phylogenetic relationships of cyphoderiid testate amoebae (Rhizaria: Euglyphida). *Protist* **162:** 131–141

Hromic T. (2002) Foraminíferos bentónicos de Bahía Nassau, Cabo de Hornos, Chile. Comparación con Foraminíferos del Cono Sur de América, Antártica e Islas Malvinas. *An. Inst. Pat. Ser. Cs. Nat.* **30:** 95–108

Hromic T. (2009) Distribución batimétrica de foraminíferos bentónicos (Protozoa: Foraminiferida) al sur del Estrecho de Magallanes (52°–56°S), Chile. *An. Inst. Pat. Ser. Cs. Nat.* **37:** 23–38

Hutchinson G. E. (1957) Concluding remarks. *Cold Spring. Harb. Sym*. **22:** 415–427

Jung W. (1942) Südchilenische Thekamöeben. *Archiv. Protistenkd.* **95:** 253–356

Kinkel L. L., Andrews J. H., Berbee F. M., Nordheim E. V. (1987) Leaves as islands for microbes. *Oecologia* **71:** 405–408

Kosakyan A., Thierry J. H., Leander B. S., Todorov M., Mitchell E. A. D., Lara E. (2012) COI barcoding of nebelid testate amoebae (Amoebozoa: Arcellinida): extensive cryptic diversity and redefinition of the Hyalospheniidae Schultze. *Protist* **163:** 415–434

Krashevska V., Bonkowski M., Maraun M., Scheu S. (2007) Testate amoebae (Protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia* **51:** 319–331

Kreutzweiser D. P., Capell S. S., Good K. P. (2005) Macroinvertebrate community responses to selection logging in riparian and upland areas of headwater catchments in a northern hardwood forest. *J. North. Amer. Benthol. Soc.* **24:** 208–222

Lahr D. J. G., Bergmann P. J., Lopes S. G. B. (2008) Taxonomic identity in microbial eukaryotes: a practical approach using the testate amoeba *Centropyxis* to resolve conflicts between old and new taxonomic descriptions. *J. Eukaryot. Microbiol.* **55:** 409–416

Lara E., Heger T. J., Scheihing R., Mitchell E. A. D. (2011) COI gene and ecological data suggest size-dependent high dispersal and low intra-specific diversity in free-living terrestrial protists (Euglyphida: Assulina). *J. Biogeogr.* **38:** 640–650

Lyons M. M., Ward J. E., Gaff H., Hicks R. E., Drake J. M., Dobbs F. C. (2010) Theory of island biogeography on a microscopic scale: organic aggregates as islands for aquatic pathogens. *Aquat. Microb. Ecol.* **60:** 1–13

MacArthur R. H., Wilson E. O. (1963) An equilibrium theory of insular zoogeography. *Evolution* **17:** 373–387

Meisterfeld R. (2002) Order Arcellinida. In: An Illustrated guide to the Protozoa, Vol. 2, Society of Protozoologists, (Eds. J. J. Lee, G. F. Leedale, P. Bradbury). Kansas, USA, 827–860

Mitchell E. A. D., Meisterfeld R. (2005) Taxonomic confusion blurs the debate on cosmopolitanism versus local endemism of free-living protists. *Protist* **156:** 263–267

Nuñez J. J., Wood N. K., Rabanal F. E., Fontanella F. M., Sites J. W. Jr. (2011) Amphibian phylogeography in the Antipodes: Refugia and postglacial colonization explain mitochondrial haplotype distribution in the Patagonian frog *Eupsophus calcaratus* (Cycloramphidae). *Mol. Phylogenet. Evol.* **58:** 343–352

Peay K. G., Bruns T. B., Kennedy P. G., Bergemann S. E., Garbelotto M. (2007) Astrong species-area relationship for eukaryotic soil microbes: island size matter for ectomycorrhizal fungi. *Ecol. Lett.* **10:** 470–480

Reche I., Pulido-Villena E., Morales-Baquero R., Casamayor E. O. (2005) Does ecosystem size determine aquatic bacterial richness? *Ecology* **86:** 1715–1722

Scott D. B., Medioli F. S., Schafer C. T. (2001) Monitoring in coastal environments using foraminifera and thecamoebian indicators. Cambridge University Press, Cambridge, UK

Schrödl M., Grau J. H. (2003) Nudibranchia from the remote southern Chilean Guamblin and Ipún islands (Chonos Archipelago, 44–45°S), with re-description of *Rostanga pulchra* MacFarland, 1905. *Rev. Chil. Hist. Nat.* **79:** 3–12

Simonetti J. A., Arroyo M. T. K., Spotorno A. E., Lozada E. (Eds.) (1995) Diversidad biológica en Chile. Comisión Nacional de Ciencia y Tecnología, Santiago, Chile

Soberón J., Llorente J. (1993) The use of species accumulation functions for the prediction of species richness. *Conserv. Biol.* **7:** 480–488

Soininen J. (2008) The ecological characteristics of idiosyncratic and nested diatoms. *Protist* **59:** 65–72

Szeicz J. M., Haberle S. G., Bennett K. D. (2003) Dynamics of North Patagonian rainforests from fine-resolution pollen, charcoal and tree-ring analysis, Chonos Archipelago, Southern Chile. *Austral. Ecol*. **28:** 413–422

Ugland K. I., Gray J. S., Ellingsen K. E. (2003) The species-accumulation curve and estimation of species richness. *J. Anim. Ecol.* **72:** 888–897

van der Gast C. J., Lilley A. K., Ager D., Thompson I. P. (2005) Island size and bacterial diversity in an archipelago of engineering machines. *Environ. Microbiol.* **7:** 1220–1226

Villagrán C., Hinojosa L. F. (1997) Historia de los bosques del sur de Sudamérica II: análisis fitogeográfico. *Rev. Chil. Hist. Nat.* **70:** 241–267

Wailes G. (1913) Freshwater Rhizopoda from North and South America. *J. Linn. Soc.* **32:** 201–218

Wilkinson D. M. (2001) What is the upper size limit for cosmopolitan distribution in free-living microorganisms? *J. Biogeogr.* **28:** 285–291

Wilkinson D. M., Smith H. G. (2006) An Initial Account of the Terrestrial Protozoa of Ascension Island. *Acta Protozool.* **45:** 407–413

Wilkinson D. M., Koumoutsaris S., Mitchell E. A. D., Bey I. (2012) Modelling the effect of size on the aerial dispersal of microorganisms. *J. Biogeogr.* **39:** 89–97

Wright D. H., Patterson G. M., Cutler A., Atmar W. (1996) A comparative analysis of nested subset patterns of species composition. *Oecologia* **113:** 1–20

Yang J., Smith H. G., Sherratt T. N., Wilkinson D. M. (2010) Is there a size limit for cosmopolitan distribution in free-living microorganisms? A biogeographical analysis of testate amoebae from polar areas. *Microbial Ecol.* **59:** 635–645

Zapata J. (1999) Foraminíferos bentónicos recientes de Bahía Cumberland (33°41′S; 78°50′W) Archipiélago de Juan Fernández, Chile: Aspectos zoogeográficos. *Bol. Soc. Biol. Concepción Chile* **70:** 21–35

Zapata J. (2005) Tecamebas (Protozoa, Rhizopoda) de la turbera Rucapihuel (40°34′42.5″S; 73°34′31.4″W), Chile. *Bol. Soc. Biol. Concepción Chile* **76:** 39–56

Zapata J., Crespo J. (1990) Tecamebas del volcán RanoKau, Isla de Pascua (27°10′S; 109°26′W). *Biota* **6:** 53–59

Zapata J., Álvarez P., Cea C. (2002) Tecamebas del Río Contaco (40°33′12″S; 73°43′00″W), Osorno, Chile. *Bol. Soc. Biol. Concepción Chile* **73:** 17–35.

Zapata J., Toledo R., Rojas M. (2007) Clave ilustrada para los géneros de tecamebas (Protozoa, Rhizopoda) de la laguna "Mallín" (Parque Nacional Puyehue: 40°45.0′S, 72°18.7′O), Chile. *Bol. Soc. Biol. Concepción Chile* **78:** 67–76

Zapata J., Fernández L. D. (2008) Morphology and morphometry of *Apodera vas* (Certes, 1889) (Protozoa: Testacea) from two peatlands in southern Chile. *Acta Protozool.* **47:** 389–395

Zapata J., Yáñez M., Rudolph E. (2008) Tecamebianos (Protozoa: Rhizopoda) de una turbera del Parque Nacional Puyehue (40°45′S; 72°19′W), Chile. *Gayana* **72:** 9–17

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