

Checklist, diversity and distribution of testate amoebae in Chile

Leonardo D. Fernández^{a,b,c,*}, Enrique Lara^a, Edward A.D. Mitchell^{a,d}

^aLaboratory of Soil Biology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, CH-2000, Neuchâtel, Switzerland

^bLaboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile

^cCentro de Estudios en Biodiversidad (CEBCH), Magallanes 1979, Osorno, Chile

^dBotanical Garden of Neuchâtel, Chemin du Perthuis-du-Sault 58, CH-2000 Neuchâtel, Switzerland

Abstract

Bringing together more than 170 years of data, this study represents the first attempt to construct a species checklist and analyze the diversity and distribution of testate amoebae in Chile, a country that encompasses the southwestern region of South America, countless islands and part of the Antarctic. In Chile, known diversity includes 416 testate amoeba taxa (64 genera, 352 infrageneric taxa), 24 of which are here reported for the first time. Species–accumulation plots show that in Chile, the number of testate amoeba species reported has been continually increasing since the mid-19th century without leveling off. Testate amoebae have been recorded in 37 different habitats, though they are more diverse in peatlands and rainforest soils. Only 11% of species are widespread in continental Chile, while the remaining 89% of the species exhibit medium or short latitudinal distribution ranges. Also, species composition of insular Chile and the Chilean Antarctic territory is a depauperated subset of that found in continental Chile. Nearly, the 10% of the species reported here are endemic to Chile and many of them are distributed only within the so-called Chilean biodiversity hotspot (ca. 25° S–47° S). These findings are here thoroughly discussed in a biogeographical and evolutionary context.

Keywords: Amoebozoa; Biogeography; Ecology; SAR supergroup; South America; The Antarctic

Introduction

Testate amoebae are a polyphyletic group of shelled protists present in many terrestrial, freshwater and occasionally marine habitats. Their sensitivity to slight changes in the environmental conditions has turned them into an increasingly

used group of bioindicators to monitor pollution (Nguyen-Viet et al., 2007; Meyer et al. 2012), ecosystem restoration and management (Turner and Swindles 2012; Valentine et al. 2013), cadaver decomposition (Seppey et al. 2015; Szelezcz et al., 2013) or natural ecological gradients (Opravilova and Hajek 2006; Payne 2011; Koenig et al. 2015). Their shells can be preserved for a long time in peats and sediments, and ancient communities are nowadays commonly used to monitor past environmental changes (Mitchell et al. 2008; Payne and Pates 2009). To date, testate amoebae have been assigned to two different deep eukaryotic lineages: Amoebozoa (lobose testate amoebae) and the supergroup SAR

*Corresponding author at: Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland.

E-mail addresses: limnoleo@gmail.com, leonardo.fernandez@unine.ch (L.D. Fernández).

(filose testate amoebae) (Adl et al. 2012). Past estimations of their diversity reached about 2,000 species (Meisterfeld 2002a, 2002b). However, these estimations are currently being revised due to the introduction of DNA barcoding, which revealed an important cryptic diversity (Kosakyan et al. 2012; Heger et al. 2013; Singer et al. 2015). Notably, it has been shown in several cases that allopatric speciation occurred, and several species are known to have limited geographic distributions as demonstrated with both morphological observations (Smith and Wilkinson 2007; Smith et al. 2008; Zapata and Fernández 2008) and molecular methods (Heger et al. 2013).

The study of the diversity and distribution of testate amoebae boasts a long tradition (Penard 1902; Claparède and Lachmann 1868; Ehrenberg 1853; Leidy, 1879). However, most studies on these microorganisms have been developed in the Holarctic region (Foissner 1997, 1999; Smith et al. 2008). Data from the Southern Hemisphere are still rare and sparse, indicating a limited knowledge on these protists along a considerable fraction of the terrestrial surface of the planet. Chile encompasses almost all the southwestern region of South America, countless islands and part of the Antarctic continent and covers a vast array of climatic zones. Therefore, this country has a considerable interest for testate amoebae biogeography. Despite a long history of records of testate amoebae from Chile, the data are still scattered and incomplete. Knowledge of Chilean testate amoebae began with Ehrenberg (1843), who studied a few samples collected at Cape Horn (Horn Island, Chile), the southernmost headland of Tierra del Fuego. Forty-six years later, Certes (1889) reported several new species and genera after studying samples collected from 1882 to 1883 by the French Scientific Mission to Cape Horn at Orange Bay (Hoste Island, Chilean part of Tierra del Fuego). In this manuscript, Certes described remarkable species such as *Apodera vas* (Certes, 1889) and *Certesella martiali* (Certes, 1889). Subsequently, Wailes (1913) added some new records and species from samples collected in Punta Arenas, Valparaíso and Antofagasta from austral, central and northern continental Chile, respectively. It is noteworthy that back then, all mentioned localities were important seaports, which were (or still are) part of international commercial sea routes. This probably largely determined the selection of sampling sites at that time. Later, Jung (1942a) published a paper documenting a large number of known and then unknown taxa that he observed in samples collected both in the Reloncaví Inlet and the inland territory of south-central Chile. This paper was important for the natural history of Chilean testate amoebae as it broke the trend of sampling exclusively at seaports and documented the species that were present in remote and often pristine sites. Some years later, Hoogenraad and de Groot (1951) reported some testate amoeba species that they found in moss samples collected in austral and central Chile. Then, Bonnet (1966) investigated the community composition and distribution of testate amoebae in south-central Chile. He described some new species and found

that the distribution of these organisms was mainly determined by the C:N ratio, pH and water availability in different terrestrial habitats. More recently, Smith (1978, 1985) documented the testate amoeba species composition of the Chilean Antarctic territory, revealing a pauperized diversity. Like-wise, Zapata and colleagues further documented the testate amoeba species composition of south-Central Chile (Zapata and Rudolph 1986), the Chilean Antarctic territory (Zapata and Matamala 1987) and Easter Island (Zapata and Crespo 1990). Finally, the 20th century ended with the contribution of Golemansky and Todorov (1996), who described for the first time the psammobiotic fauna from the marine littoral and supralittoral of Chile. The current century has witnessed an increase in the number of studies involving testate amoebae. Several papers describing the Chilean testate amoebae fauna and ecology as well as providing new records or describing new species have been published during the last years (Zapata et al. 2002; Zapata 2005; Zapata et al. 2007a, 2007b; Zapata and Fernández 2008; Zapata et al. 2008; De Smet and Gibson 2009; Fernández and Zapata 2011; Santibañez et al. 2011; Fernández et al. 2012; Chatelain et al. 2013; Fernández 2015).

Bringing together more than 170 years of data accumulated by numerous researchers, this paper represents the first attempt to synthesize the knowledge about Chilean testate amoebae, including both published and unpublished information. The first purpose of this study was to give an updated and comprehensive species checklist that includes nomenclature changes prompted by recent research as well as ecological information and geographical data on the distribution of each species in the whole Chilean territory including continental Chile, insular Chile and the Chilean Antarctic territory. The second purpose of this study was to investigate the diversity and spatial patterns of these organisms in Chile using qualitative and quantitative approaches including estimations of richness at different taxonomic levels, habitats surveyed, habitat preference, latitudinal patterns of distribution, levels of endemism and comparisons among the species composition recorded on insular, Antarctic and continental Chile. We expect that this manuscript will contribute to reduce the existing knowledge gap between South American and Holarctic testate amoebae and encourage the local research interest in this group.

Material and Methods

Geographical setting

Chile is a long and narrow country situated on the Pacific coast of South America, stretching over 4300 km southwards from latitude 17°30' S to Cape Horn at 56° S. Chile also has sovereignty over the Pacific islands of Easter Island and the Juan Fernández Archipelago and claims Antarctic Territory between 53° and 90° W. As a result, Chile is usually partitioned in three political divisions: continental Chile, insular

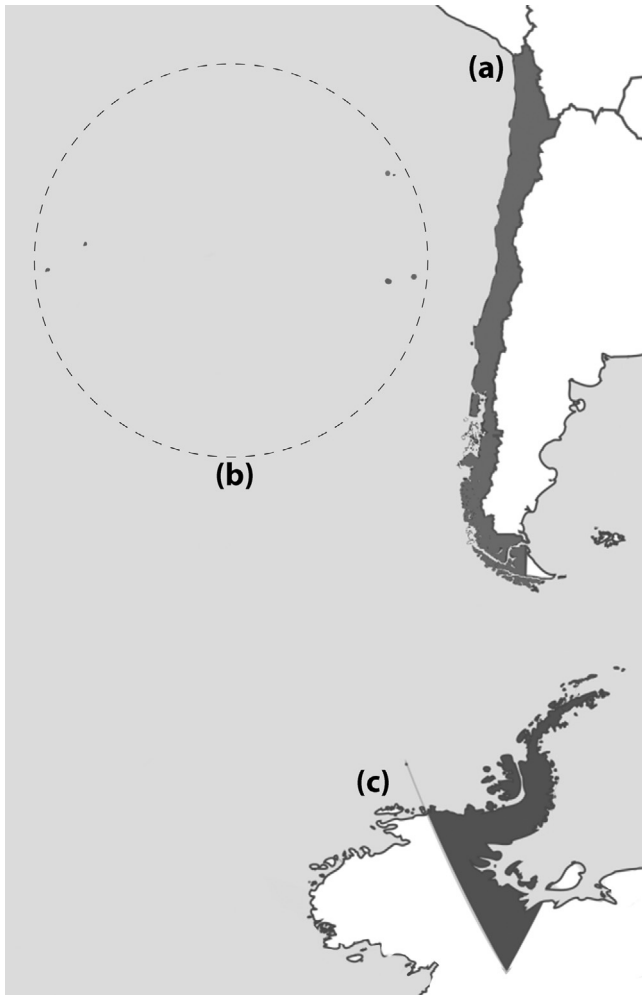


Fig. 1. The Chilean territory includes: (a) continental Chile, which stretches from 17°30' S to Cape Horn at 56° S; (b) insular Chile, which includes many continental and oceanic islands (the latter are indicated by the dotted circle); and (c) the Antarctic territory included between 53° W and 90° W.

Chile and the Chilean Antarctic territory (Fig. 1). Chile is regarded as a land of climatic and geographic extremes. Roughly, the climate of continental Chile is dry and hot in the north, mostly Mediterranean in the central region, and both subpolar and oceanic in the southern region. The climate of insular Chile is either subtropical or oceanic and the Chilean Antarctic territory is dominated by a polar climate. Thus, Chile overall includes many major biomes including hot and cold deserts, alpine, arid shrublands, deciduous forests, rainforests and grasslands. A considerable part of the Chilean territory also represents a biodiversity hotspot called the Chilean Winter Rainfall-Valdivian Forests Hotspot (i.e. the Chilean hotspot) (Arroyo et al. 2004). The Chilean hotspot stretches between 25° S and 47° S, including a narrow coastal strip between 19° S and 25° S, plus the Juan Fernández Archipelago.

Database construction

To construct the database used in this study, we first did an exhaustive literature search that included all published material devoted, partially or completely, to testate amoebae of Chile. Second, we performed a comprehensive review of material stored both in the private collection of Jaime Zapata (a retired Chilean protistologist) and in the protist collection of the Universidad de Los Lagos (Chile). This material consisted altogether of 250 permanent slides and 100 dry moss samples of 5–10 g each collected between 2000 and 2008 all over continental Chile. We examined individually each permanent slide to ensure that all species reported had been properly identified. For moss samples, we took a sub-sample of 3 g of dry moss, re-hydrated it in 250 ml of distilled water and sieved it through 400 and 15 µm mesh sieves. Each fraction was examined under an inverted microscope for documenting species occurrences. The identification of taxa was based on morphological characters of the shell following traditional taxonomic references. Important sources used in the identification of amoebae included those by Certes (1889); Jung (1942a, 1942b); Mazei and Tsyganov (2006) and Zapata et al. (2007a, 2007b). When possible, the amoebae observed were photographed by means of a digital camera connected to an inverted microscope and/or by scanning electron microscopy (SEM). For SEM microscopy, the individuals were cleaned by several transfers through distilled water, mounted on stubs and finally air-dried. The individuals on stubs were coated with gold and photographed with either a JEOL JSM-6380 or Philips XL30 operating between 10 and 15 kV. Third and finally, several species identified from samples collected by the first author between 2007 and 2013 in insular and continental Chile (6275 soil/moss/litter samples of ca. 10 cm³ each) were also integrated into the raw database. The analyses of these samples as well as the isolation and identification of these species followed the same process described above for dry moss samples.

As we compiled data from various sources that were generated using different methods and taxonomical approaches, we needed to correct several spelling errors, misidentifications and obsolete taxon names (e.g. synonyms, homonyms and *nomina nuda* taxa). We also sent our raw database to colleagues who also have taxonomic expertise in the group to have their opinion (see acknowledgments section). Their comments and suggestions were taken into account in the final version of the database.

We thus obtained a comprehensive and updated species checklist for the Chilean testate amoebae. Taxa identified at species level included forms indicated as *confer* (cf.) or with a question mark (?), meaning that organisms were similar to described species but not identical, thus representing probably new species. Intraspecific taxa included those taxa mentioned as subspecies, varieties or forms in the literature. Along with the record of each taxon, we also took note of: (a) its species authority; (b) synonyms used in the Chilean testate amoebae literature; (c) infraspecific taxa used in the Chilean

literature; (d) the habitat(s) where it has been recorded in Chile; (e) additional notes describing for example, the person who first reported/described the taxon in Chile, related homonyms, etc.; (f) its geographical distribution (i.e. whether the species occurs in continental Chile and/or insular Chile and/or the Chilean Antarctic territory); and (g) references (i.e. manuscripts that mention the occurrence of the taxon in the Chilean territory). This additional information was compiled from the consulted literature as well as from our personal observations.

Classification of the species at higher taxonomic levels followed Adl et al. (2012). These authors proposed a hierarchical system of classification founded on robust phylogenetic relatedness that avoid the use of formal rank designations such as “class”, “sub-class”, “super-order”, or “order”. The reason to do so has been motivated by utility, to avoid the common problem of a single change causing a cascade of changes to the system (Adl et al. 2012). The classification of those taxa not included in the revision of Adl et al. (2012) was based on other authors (e.g. Meisterfeld 2002a, 2002b; Haman 1988; Golemansky and Todorov 1996).

Diversity analyses

We used the information compiled in the final database to calculate the total number of valid testate amoeba taxa recorded so far within the whole Chilean territory including genera, species, infraspecific taxa and unidentified species. We then recalculated the total number of testate amoeba taxa only for continental Chile, insular Chile and the Chilean Antarctic territory to assess the differences in diversity among these territories. All these calculations were also done separately on amoebozoan and SAR testate amoebae. We then constructed a summary table for these data. In addition, we counted the number of species included in each amoebozoan and SAR genus to assess their contribution to the overall diversity. We also constructed species accumulation curves calculated in function of time to document the rate of species description over the years in Chile. Finally, we assessed the completeness of the inventory of Chilean testate amoeba species richness using the samples collected by the first author (see database construction) as a reference sample to compute sample-size-based rarefaction and extrapolation species richness curves (see Colwell et al. 2012). The extrapolation curve was calculated up to double the size of the reference sample. Rarefaction and extrapolation curves, as well as their 95% confidence intervals (bootstrap method, 20,000 replications), were computed using R 3.1.0 and the *iNEXT* package (Hsieh et al. 2014).

Habitats explored and habitat preference

We investigated which habitats have been explored to study the Chilean testate amoebae. To do this, we looked at our database and listed all the habitats reported there. Then, we

classified each habitat as terrestrial, aquatic and semi-aquatic. Here, we also introduced a new type of semi-aquatic habitat locally known as Mallín (Mallines in plural). A Mallín (from the Mapudungun language = flooded place) is a wetland that can be roughly described as a swampy grassland with mineral soils. This type of habitat is common in southern Chile, southern Argentina and their adjacent islands (Schlatter and Schlatter 2004). A Mallín remains wetter than the adjacent soils in summer and is commonly flooded during winter, favoring the concentration of organic matter and the development of wetland vegetation such as plants of the genera *Acaena*, *Azorella*, *Festuca* and *Juncus*. Depending on the structure of the soil, a Mallín sometimes can even harbor medium-sized native trees (Schlatter and Schlatter 2004). While a Mallín can also contain some isolated *Sphagnum* patches, it differs from a peatland in that it has a completely different soil composition and pH, among other physico-chemical characteristics. Refer to Fig. S1 for an example of a Mallín. Table S1 to see some of the physicochemical properties that characterize and differentiate a Mallín from a peatland. We finally counted the number of species that have been recorded in each habitat as a measure of habitat preference for these protists, where habitat preference is the use of some habitats over others by the organisms (Hall et al. 1997). This information was also explored for Amoebozoa and SAR and then put in a summary table.

Biogeographical aspects

To evaluate latitudinal patterns in the distributions of testate amoeba species, we divided continental Chile into 1° latitudinal bands (i.e. 39 latitudinal bands), registering the presence or absence of each species in each latitudinal band. The presence-absence data were used to construct a binary matrix with columns as latitudinal bands and rows as taxa. Maps of species distributions were created using data with a spatial resolution of 1° of latitude. Ranges were assumed to be continuous between latitudinal bands. To describe latitudinal patterns of species, we categorized species distribution ranges into three groups: (a) species with small distributions, occurring only within two latitudinal bands; (b) species with small-medium ranges of distribution, ranging from three to 10 latitudinal bands; (c) species with medium-large distributions, ranging from 11 to 19 latitudinal bands; and (d) species with large distributions, ranging from 20 to 39 latitudinal bands.

To determine if the taxa observed in Chile were characterized by a wide or more restricted geographical distribution, we classified the species as: (a) widely distributed, if they occurred in more than one continent; (b) South American, if they only occurred in South America; (c) Austral, if they only occurred in the southern part of Chile and Argentina (ca. 40° S to 56° S); and (d) endemics, if they only have been recorded in Chile.

Moreover, it has been proposed that the testate amoeba species composition of insular Chile and the Chilean

Antarctic territory is only a subset of that present in continental Chile (Smith 1985; Zapata and Matamala 1987; Zapata and Crespo 1990; Fernández et al. 2012). To test this hypothesis, we performed a nested analysis on the binary matrix mentioned above. Nested analysis predicts the existence of a highly ordered system in which taxa composition in depauperated sites tend to be simple subsets of richer ones (i.e. a nested pattern) (Wright et al. 1998; Fernández 2015). We used the method of Atmar and Patterson (1993) and the temperature metric (T°) to explore the occurrence of a nested pattern. The temperature metric evaluates the degree of disorder as estimated by the temperature of a binary matrix, where $T^\circ = 0^\circ$ indicates perfect nestedness and $T^\circ = 100^\circ$ indicates complete randomness. Before calculating the degree of nestedness, the binary matrix was sorted according to the marginal row and column sums, with common taxa placed in the upper rows and taxa-rich latitudinal bands placed in the left-hand column (Ulrich et al. 2009; Fernández 2015). Islands and the Antarctic continent were treated as having a direct borderline with continental Chile. We also calculated the degree of nestedness only for Amoebozoa and then only for SAR. The statistical significance of these analyses was evaluated with a row–column null-model (50,000 permutations, $p < 0.05$) a conservative algorithm that minimizes Type I errors (Gotelli 2000). Nestedness and null-model calculations as well as temperature matrices were done using R 3.1.0 and the *vegan* package (Oksanen et al. 2013).

Results

Analyses on testate amoeba diversity

During the analysis of the raw database, we identified two homonyms genera, transferred seven species to other genera (*combinatio nova*), synonymized 83 specific and infraspecific taxa, and identified four invalid species (*nomen nudum* taxa). All these homonyms, transferred, synonymized and invalid taxa are properly identified in the species checklist (Tables S2 and S3). After these procedures, the total number of valid testate amoeba taxa recorded in Chile reached 352 infrageneric taxa distributed in 64 genera (Table 1). From these taxa, 24 species (17 amoebozoan and 7 SAR) are here reported for the first time for Chile (Tables S2 and S3). When

we recalculated the total number of taxa for each of the three Chilean political divisions, we found that continental Chile is much more diverse than insular Chile and the Chilean Antarctic Territory (Table 1). In addition, when we calculated the number of taxa for Amoebozoa and SAR, we found that the first group is much more diverse than the second, both in the whole Chilean territory and in any of its political divisions (Table 1, Tables S2 and S3).

Among Amoebozoa, *Diffugia* and *Centropyxis* are the most diverse genera with 48 (18% of the total) and 41 (16%) taxa, respectively (Fig. 2a). Other genera such as *Arcella* (23 taxa, 9%), *Nebela* (18 taxa, 7%), *Cyclopyxis* (15 taxa, 6%), *Heleopera* (14 taxa, 6%) and *Argyria* (11 taxa, 4%) also made an important contribution to the Amoebozoa diversity (Fig. 2a). These genera together accounted for 66% of the total amoebozoan testate amoeba diversity recorded in Chile. The other 29 genera accounted for the remaining 34% of the total amoebozoan testate amoeba diversity (Fig. 2a).

Euglypha was the most diverse genus of SAR testate amoebae with 32 taxa (34% of the total) (Fig. 2b). Other species-rich genera were *Corythion* (seven taxa, 8%), *Pseudodiffugia* (seven taxa, 8%), *Sphenoderia* (six taxa, 7%) and *Trinema* (11 taxa, 5%). Together these five genera represented about 61% of the total SAR testate amoeba diversity (Fig. 2b). The other 23 genera made a low contribution to the whole diversity as together they accounted for no more than 39% of the total diversity reported in Chile (Fig. 2b).

The counting of the species richness within each genus also revealed large gaps in species identification. At least 37 testate amoeba species (11% of the total) remain unidentified. The gaps in species identification are particularly important in Amoebozoa genera such as *Arcella* (22%) and *Phryganella* (40%), as well as in SAR genera such as *Euglypha* (13%) and *Pseudodiffugia* (57%).

The assessment of the evolution of the number of species recorded as a function of time showed that the recorded and described diversity of testate amoebae in Chile has been continually increasing over time without leveling off (Fig. 3a). This analysis revealed an increase of over 20% (64 taxa) in the number of new taxa reported for Chile since the year 2000 (i.e. 15 years). This pattern was recorded for both Amoebozoa and SAR, with increases of 21 (49 taxa) and 17% (15 taxa), respectively (Fig. 3b, c). The assessment of the completeness of the inventory of Chilean testate amoeba species richness

Table 1. Total number of generic and infrageneric valid testate amoeba taxa (TA), Amoebozoa taxa (AMO) and SAR taxa recorded in Chile and its three political divisions.

Taxon	Chile			Continental Chile			Insular Chile			Antarctic territory		
	TA	AMO	SAR	TA	AMO	SAR	TA	AMO	SAR	TA	AMO	SAR
Genera	64	36	28	64	36	28	14	12	2	13	10	3
Species	255	184	71	252	181	71	32	28	4	23	14	9
Intraspecific taxa	60	46	14	57	43	14	4	4	0	0	0	0
Unidentified species	37	27	10	30	21	9	5	5	0	2	1	1

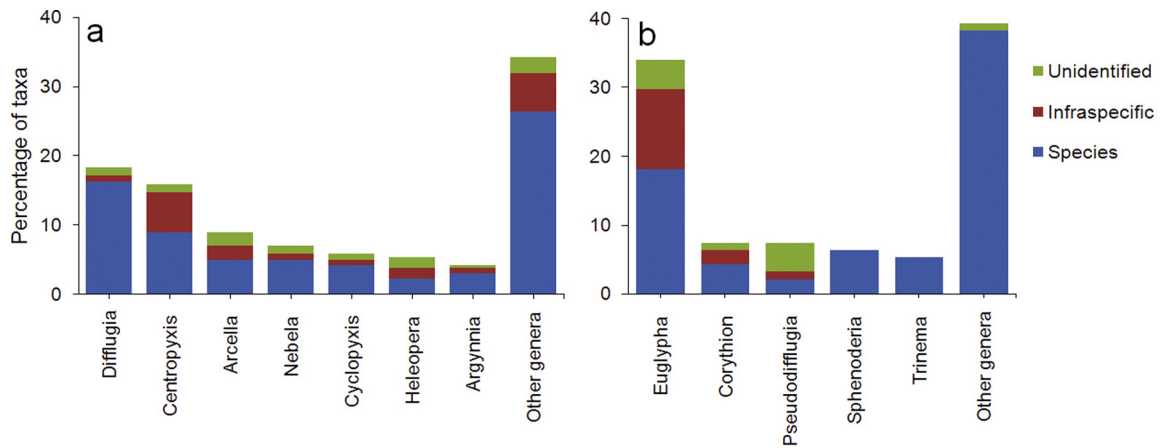


Fig. 2. Percentage of infra-generic taxa included in each (a) amoebozoan genus and (b) SAR genus in Chile. Only the most diverse genera (those that made up over 60% of the total diversity either in Amoebozoa or SAR) are detailed.



Fig. 3. Species accumulation curves calculated in function of time for: (a) all testate amoebae, (b) amoebozoan testate amoebae, and (c) SAR testate amoebae.

showed a steep slope for the rarefaction curve, indicating that the current sampling effort (i.e. the reference sample) has not been enough to detect the whole species diversity. This analysis also showed a steep slope for the extrapolation curve, suggesting that Chilean testate amoeba species diversity is far from complete, even if we double the current sampling effort (Fig. 4).

Habitats explored and habitat preference

A thorough analysis of the database constructed for this study revealed that testate amoebae were recorded from 37 different habitats (Table 2). Of these, 17 habitats were classified as terrestrial, 12 as aquatic, and six as semi-aquatic. Most testate amoeba species were found in semi-aquatic (peat-lands, 110 species) and terrestrial (rainforest soils [litter], 94 species) habitats (Table 2). Amoebozoan testate amoebae were commonly found in aquatic (pond sediments, 93 species) and semi-aquatic (peatlands, 91 species) habitats, whereas SAR testate amoebae were more often documented in terrestrial (rainforest soils [litter], 35 species) and semi-aquatic (peatlands, 20 species) habitats (Table 2, Tables S2 and S3).

Biogeographical analyses

When analyzing the latitudinal patterns of the species, we found that only a small fraction exhibit widespread distribution along continental Chile. Of the 252 testate amoebae

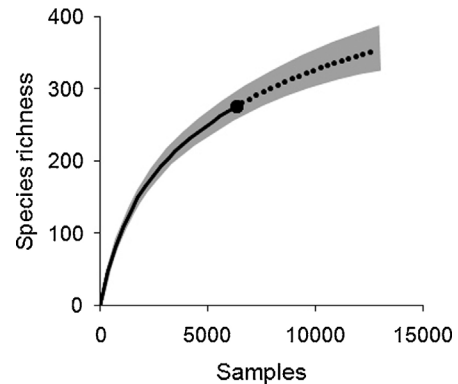


Fig. 4. Size-based rarefaction (solid curve) and extrapolation (dotted curve) with 95% confidence intervals (shaded area, based on a bootstrap method with 20,000 replications). The reference sample is indicated by a solid black dot.

Table 2. Habitats explored to study the Chilean testate amoebae (TA), including amoebozoan testate amoebae (AMO), SAR testate amoebae, and the total number of species that have been recorded in each habitat.

Type	Habitat	TA	AMO	SAR
Terrestrial	Acid mixed soils	14	6	8
	Arid shrublands	25	16	9
	Conifer forest soils (native)	9	3	6
	Humic soil lawns	26	16	10
	Humus in trunks	24	15	9
	Humus on trunks	20	14	6
	Magellanic rainforest soils	9	5	4
	Mosses and lichens covered by snow	9	0	9
	Mosses on trees (living trees)	7	3	4
	Mosses on trunks (dead trees)	15	9	6
	Muddy soils	20	15	5
	North Patagonian rainforest soils	19	19	0
	Rainforest mosses	29	21	8
	Rainforest soils (litter)	94	59	35
	Soil humus	14	11	3
	Soil mosses	85	61	24
	<i>Sphagnum</i> mosses	34	21	13
Aquatic	Estuaries	20	19	1
	Forest streams	64	50	14
	Freshwater column	10	5	5
	Glacial rivers	7	4	3
	Hot springs	6	3	3
	Lake sediments	16	13	3
	On aquatic plants	33	27	6
	On freshwater algae	13	10	3
	Pond sediments	93	77	16
	River sediments	25	24	1
	Stream sediments	52	47	5
	Volcanic crater lakes	12	7	5
Semi-aquatic	Mallines	27	22	5
	Peatlands	110	91	20
	Saltmarshes	10	9	1
	Sandy supralittoral	17	2	15
	Swamps	72	61	11
	Volcanic crater glaciers	4	0	4

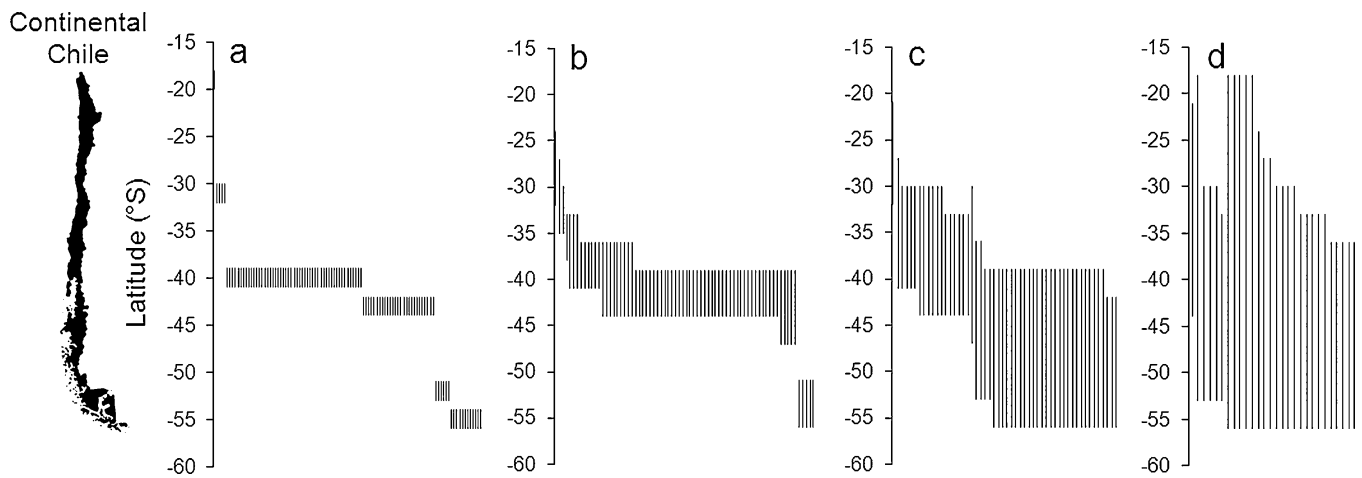


Fig. 5. Latitudinal distribution patterns recorded for 252 testate amoeba species in continental Chile. (a) 102 species (40% of the total) have small distributions; (b) 70 species (28%) exhibit small–medium ranges of distribution; (c) 52 species (21%) have medium–large distributions; and (d) 28 (11%) species have large distributions. Each vertical band represents the distribution of a single species.

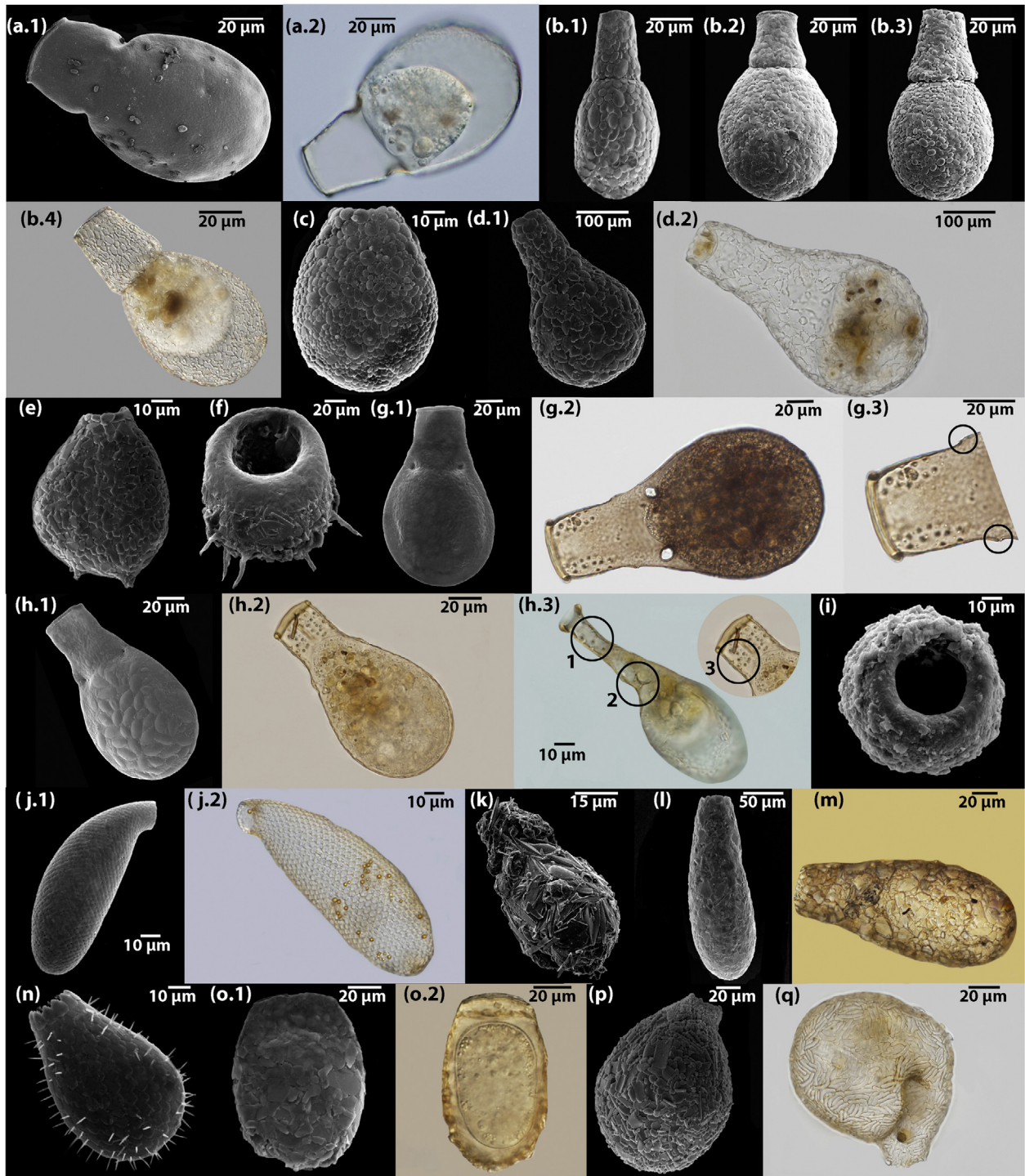


Fig. 6. Scanning electron microscopy and light micrographs of typical testate amoeba taxa found in Chile: (a) *Alocodera cockayni* (Penard, 1910): (a.1) from a peatland, TDF; and (a.2) living individual from a rainforest, SC. (b) *Apodera vas* Jung, 1942: (b.1–b.3) three different morphotypes that co-occur in peatlands of CC (from Zapata and Fernández 2008); and (b.4) living individual from a peatland, TDF. (c) *Argynnia dentistoma* (Penard, 1890): individual from a rainforest, IC (from Fernández et al. 2012). (d) *A. gertrudeana* Jung, 1942: (d.1) individual from a swamp, SC; and (d.2) living individual from a rainforest, CC. (e) *A. schwabei* Jung, 1942: from a peatland, CC. (f) *Centropyxis aculeata* (Ehrenberg, 1838): individual from a hot spring, SC. (g) *Certesella martiali* (Certes, 1889): (g.1) individual from a Mallín, CC; (g.2) living individual from a peatland, TDF; and (g.3) close-up of the neck of the previous individual, showing both the two rows of internal teeth that run along the neck (giving it a dotted appearance) and the small lateral pores that are usually present in this taxon (circles). (h) *C. certesi* (Penard, 1911): (h.1) individual from soil mosses, SC; (h.2) living individual from a peatland, TDF; (h.3) lateral view of the previous individual, showing the internal teeth of the neck (circle 1), the internal tube that connect the two big and invaginated pores that characterized the genus

species that have been recorded in continental Chile: (a) 102 species (40% of the total) exhibit restricted distributions, occurring only within two latitudinal bands (Fig. 5a); (b) 70 species (28%) exhibit small–medium ranges of distribution, ranging from three to 10 latitudinal bands (Fig. 5b); (c) 52 species (21%) exhibit medium–large distributions, ranging from 11 to 19 latitudinal bands (Fig. 5c); and (d) 28 (11%) species exhibit large distributions, ranging from 20 to 39 latitudinal bands (Fig. 5d).

Furthermore, a high number of species occurring in Chile exhibit a wide geographical distribution although an important proportion also appears as unique to southern Chile. In detail, we determined that: (a) 269 species (86% of the total) are widely distributed; (b) three species (1%) are restricted to South America; (c) 11 species (3%) are austral; and (d) 31 species (10%) are endemic to Chile. A further analysis revealed that almost all the endemic species are restricted to south-central Chile an area included within the so-called Chilean hotspot. The endemism recorded is due to the high number of unique amoebozoan and SAR testate amoeba taxa present in this region, which, respectively, exceeds 10 and 8% of the total number of species recorded in Chile. The taxa that are potentially endemic to South America, to austral South America and Chile are highlighted in the additional notes section of Tables S2 and S3. Figs 6 and 7 show SEM and light micrographs of some of these potentially endemic taxa and other typical taxa found in Chile. It is noteworthy that these figures show for the first time some species that were previously only known from poor quality drawings or photographs. These images thus confirm their existence.

The temperature metric confirmed that insular and Antarctic species composition represents a subset of that present in continental Chile at different taxonomic criteria for all testate amoebae, for amoebozoan taxa, and for SAR taxa (Table 3). However, a few exclusive taxa have also been recorded in both insular Chile and the Chilean Antarctic territory (Tables S2 and S3).

Discussion

In Chile, testate amoeba diversity is very high (Table 1), with a magnitude comparable to or even higher than in other regions of the planet. The Chilean testate amoeba diversity appears two or three times higher than that of other Neotropical countries, such as Peru, Colombia, Ecuador or Mexico (Haman and Kohl 1994; Escobar et al. 2005; Krashevskaya et al.

2007; Bobrov and Krasilnikov 2011). The testate amoeba diversity recorded in Chile even exceeded that recorded in Argentina or China, countries that historically have experienced a sampling effort comparable to that observed in Chile (Vucetich and Lopretto 1995; Qin et al. 2011). However, none of these countries have been systematically sampled through all its biomes in the way we sampled Chile. Still, when compared with well-studied regions of the world, Chilean testate amoeba diversity stands high. A recent revision, (Smith et al. 2008) shown that so far 229 species have been recorded in North and Central America, 428 in Australia, 648 in Africa and 1031 in Europe. Thus, the Chilean diversity is exceeded only by that recorded in Australia, Africa and Europe. Note, however, that we are comparing here the diversity observed in Chile with magnitudes of diversity recorded in whole continents. Moreover, we do not know to what extent these comparisons could be valid because as stressed by Smith et al. (2008), the higher diversity recorded for some regions might be only a reflection of intensity of sampling, rather than true higher diversity. Chile, for instance, has only started to be sampled systematically in the middle of the twentieth century, while European countries have been surveyed during more than two centuries (Foissner 1997, 1999). In turn, it is probable, for instance, that countries such as Colombia that are acknowledged to contain diversity hotspots for many (macroscopic) taxa (Myers et al. 2000) harbor an even larger diversity than Chile. However, a representative area of Chile also is regarded as a biodiversity hotspot (Arroyo et al. 2004), and some studies have highlighted the independent origin of its diversity (Segovia and Armesto 2015). Further surveys will perhaps confirm whether Chilean diversity surpasses other under-sampled regions. In any case, the number of species recorded in Chile has increased over the time. Our study indeed suggests that the number of species recorded for Chile will continue increasing, as the evolution of the species number recorded as a function of time have not yet reached a plateau (Fig. 3). Likewise, sample-size-based rarefaction and extrapolation curves show that previous studies have overlooked a large fraction of testate amoeba species diversity and that many species still remains undiscovered (Fig. 4).

The diversity of testate amoebae was much higher in continental Chile than in the Chilean Antarctic territory and insular Chile (Table 1). This finding is in agreement with the observation of several authors who have reported that the species composition found in both the Chilean Antarctic territory and insular Chile is only a depauperated subset of that found in

Certesella (circle 2), and unusual internal lateral teeth (circle 3). (i) *Cyclopyxis eurystoma* Deflandre, 1929: individual from an estuary, CC. (j) *Cyphoderia ampulla* (Ehrenberg, 1840): (j.1) individual found in a pond, CC; (j.2) living individual from soil mosses, SC. (k) *Diffugia bacillifera* Penard, 1890: individual from a peatland, CC. (l) *D. lanceolata* Penard, 1890: individual from a stream, IC (from Fernández et al. 2012). (m) *D. oblonga* Ehrenberg, 1838: living specimen found in a peatland, SC. (n) *Euglypha strigosa* (Ehrenberg, 1871): individual from an arid shrubland, NC. (o) *Heleopera petricola* Leidy, 1879: (o.1) individual of *H. petricola major* Cash, 1909 found in a rainforest, CC; and (o.2) a representative of the typical form found in a peatland, Tierra del Fuego. (p) *Lesquereusia modesta* Rhumbler, 1895: found in a peatland, CC. (q) *L. spiralis* (Ehrenberg, 1840): found in a pond, SC. Figures (d) and (e) are the first pictures ever taken for these taxa. NC: northern Chile; CC: central Chile; SC: southern Chile; TDF: Tierra del Fuego; IC: insular Chile.

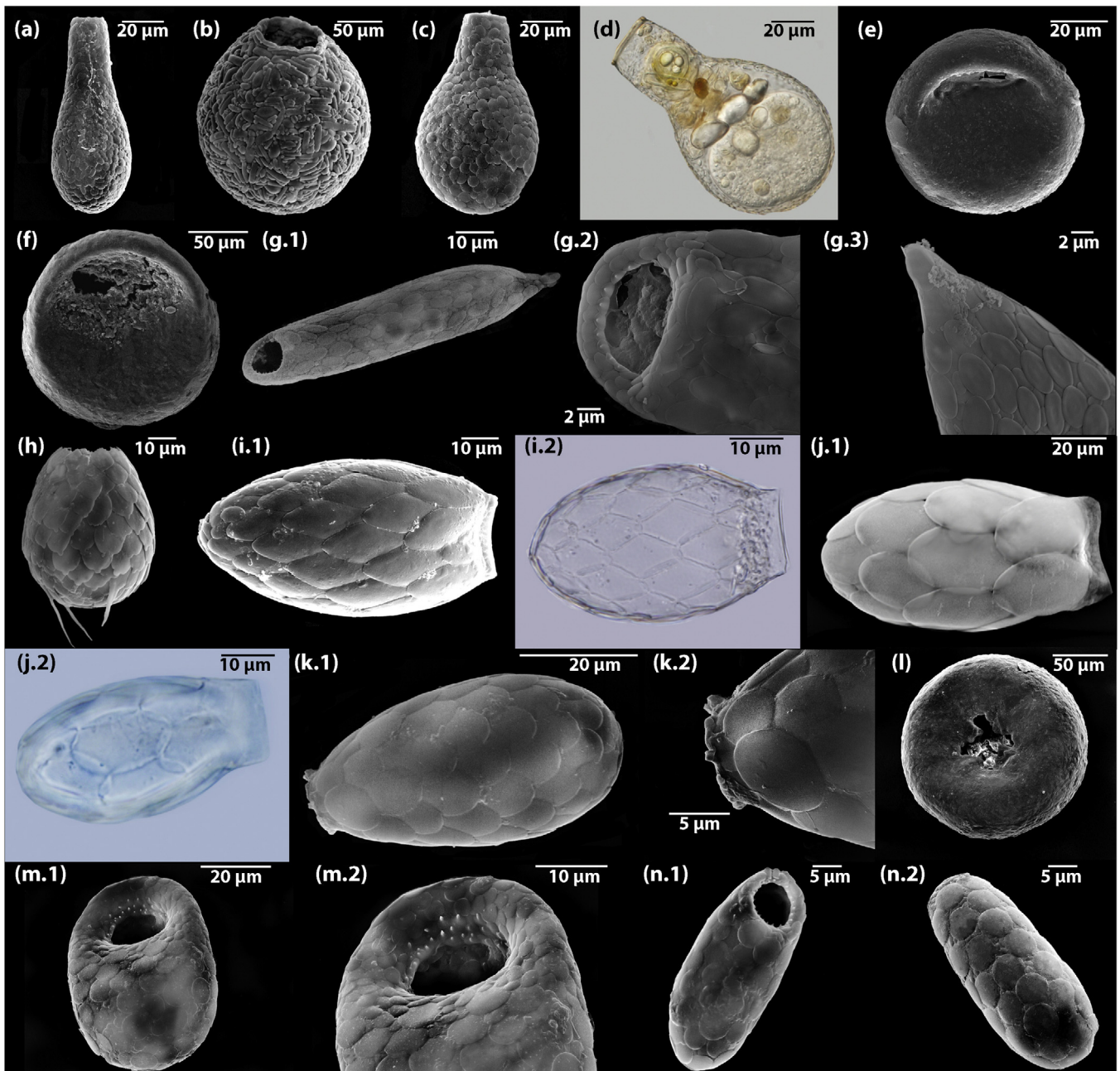


Fig. 7. Scanning electron microscopy and light micrographs of typical testate amoeba taxa found in Chile: (a) *Nebela barbata psilonata* Jung, 1942: from a rainforest, IC (from Fernández et al. 2012). (b) *Netzelia wailesi* (Ogden, 1980): from an arid shrubland, NC (from Fernández 2015). (c) *Padaungiella lageniformis* (Penard, 1902): from a rainforest, IC (from Fernández et al. 2012). (d) *P. wailesi* (Deflandre, 1936): living specimen from soil humus, CC. (e) *Plagiopyxis callida* Penard, 1910: from soil mosses, CC. (f) *Plabiata* Penard, 1911: found in humus, CC. (g) *Puytoracia jenswendti* Santibañez et al., 2011: (g.1) individual from a volcanic crater glacier, SC; (g.2) oral aperture; and (g.3) detail of the aboral horn or spine (from Santibañez et al. 2011). (h) *Scutiglypha cabrolae* De Smet and Gibson, 2009: from an arid shrubland, NC (from Fernández 2015). (i) *Sphenoderia rhombophora* Bonnet, 1966: (i.1) and (i.2) show the same individual which was found in a forest dominated by *Pilgerodendron uviferum*. (j) *S. valdiviana* Chatelain et al., 2013: (j.1) individual from a rainforest, SC; (j.2) living individual from soil mosses, SC. (k) *Tracheleuglypha dentata* (Penard, 1890): (k.1) individual from an arid shrubland, NC; and (k.2) detail of the oral aperture. (l) *Trigonopyxis arcula* (Leidy, 1879): from an estuary, SC. (m) *Trinema complanatum* Penard, 1890: (m.1) individual from an arid shrubland, NC (from Fernández 2015); and (m.2) detail of the oral aperture. (n) *Trinema lineare* Penard, 1890: individual found in soil mosses, (n.1) frontal view; and (n.2) posterior view. Figure (i), is the first SEM picture ever taken for this taxon. NC: northern Chile; CC: central Chile; SC: southern Chile; TDF: Tierra del Fuego; IC: insular Chile.

Table 3. Degree to which species-poor communities inhabiting insular Chile and the Chilean Antarctic territory are subsets of species-rich communities located in continental Chile. Nestedness was based on a presence–absence data matrix ordered according to the marginal row/column sums. Below are shown the observed and expected values of the temperature metric for all testate amoebae, for testate amoebae belonging to the Amoebozoa and for testate amoebae belonging to the SAR. Statistical inference was based on the lower and upper confidence limits (L95% CL and U95% CL, respectively) of the null distribution of 50,000 randomized matrices.

	Temperature metric		L95% CL	U95% CL	<i>p</i> -value
	Observed	Expected			
Testate amoebae	1.74	6.83	1.97	12.73	0.001
Amoebozoa	0.95	5.32	2.53	10.64	0.001
SAR	0.82	6.27	3.12	11.52	0.001

continental Chile (Smith 1985; Zapata and Matamala 1987; Zapata and Crespo 1990; Fernández et al. 2012). Our nested analyses underpinned this trend by showing the existence of a nested pattern of species composition between these three territorial divisions (Table 3). However, this does not mean that exclusive, and thus, potentially endemic species are absent in the Antarctic and insular territories. Indeed, several species have only been recorded in these regions while others could not be identified to species level, suggesting that these territories could harbor an unexplored diversity (Fernández et al. 2012). The low diversity recorded in the Antarctic can be attributed mainly to the existence of hostile environmental conditions and lack of suitable habitat for the establishment of viable populations (Smith 1985; Zapata and Matamala 1987). However, this cause cannot be used to explain the low diversity in insular Chile. Chilean islands include habitats similar to those found in continental Chile, which in turn are potentially suitable for the establishment of testate amoebae (Zapata and Crespo 1990; Fernández et al. 2012).

Among the possible causes for the low insular diversity are the remoteness and the area of the islands. According to the theory of island biogeography (MacArthur and Wilson 1963), taxa diversity is a decreasing function of the isolation of the island and an increasing function of the area of the island. Thus, taxa composition in insular Chile must be a pauperized subset of that recorded in the continent because first, only good dispersers may colonize islands; and second, the area of the islands represents a small fraction of the continent. However, while the area per se probably does not directly determine the protist species richness of islands, it probably determines the heterogeneity of habitat (e.g. pH, temperature and moisture ranges) and thus, the number of inter-specific interactions that occur on islands (Hutchinson 1957). Besides the above prediction, the airborne dispersal hypothesis (Wilkinson 2001; Lara et al. 2011; Wilkinson et al. 2012) predicts that the small species (those having a size of below a certain threshold, such as 100 or 20 μ m) are more likely to be passively dispersed over long distances than large species. Although the mechanism proposed by this hypothesis could be a surrogate of other physiological traits of protists (e.g. perhaps, larger species are worse dispersers because they have more labile cysts) (Fernández et al. 2012) it arises as a

potential cause in our study. Continental islands (i.e. islands that lie on the continental shelf) are closer to continental Chile and indeed contain several large taxa (Fernández et al. 2012), while oceanic islands (i.e. islands that do not sit on the continental shelf) are farther from the continent and are almost free of large taxa (Zapata and Crespo 1990).

Moreover, when compared with continental Chile, the diversity of insular Chile and the Chilean Antarctic territory is abysmally low (Table 1). We suggest that this pattern is, in part, an artifact of the method used to identify the species in these territories. There, species have been historically identified with a morphological approach. This approach does not allow discriminating between pseudocryptic and cryptic species and therefore may underestimate protist diversity. We think that the use of molecular approaches to identify species in these territories would help to detect various pseudocryptic and cryptic species, increasing the known number of species for insular Chile and the Chilean Antarctic territory. However, by saying this we are not asserting that the use of molecular approaches will show that these territories are equally or more diverse than continental Chile. Most likely, diversity in these territories would still be largely a subset of that found in continental Chile (e.g. as predicted by the theory of island biogeography). Nonetheless, the use of molecular approaches certainly would show that these territories also harbor endemic species that do not co-occur in continental Chile and that they are not as poor in species as shown by the current data.

As a general rule, we recorded higher testate amoeba diversity within the Amoebozoa than within the SAR in Chile and all its political divisions (Table 1). Amoebozoan testate amoebae mainly include large taxa (e.g. *Certesella*, *Nebela*), while SAR testate amoebae are mostly represented by very small taxa (e.g. *Trinema*, *Sphenoderia*) (Meisterfeld 2002a, 2002b). Thus, it is not clear whether this is a real trend (e.g. Amoebozoa exhibits higher diversification rates) or only a bias introduced by the fact that very small species are often overlooked or hardly differentiated. In support for the latter explanation, a recent environmental DNA survey revealed in forest litter samples the presence of Paulinellidae; a taxon of SAR largely overlooked by conventional methods in terrestrial habitats (Tarnawski and Lara 2015). Likewise, detailed (SEM) morphological analyses have shown that

small difference in scaling patterns can discriminate sometimes quite genetically divergent species of SAR (Wylezich et al. 2002; Chatelain et al. 2013).

Our analyses showed that Chilean testate amoebae are very diverse in semi-aquatic or terrestrial habitats such as peatlands and rainforest soils (Table 2). These habitats are quite common and abundant at mid-latitudes (Gajardo 1994; Schlatter and Schlatter 2004), coinciding with areas where there is a constant tradeoff between water and solar energy inputs throughout the year (i.e. temperate areas) (Arroyo et al. 2004). The ecological conditions that prevail in these areas certainly favor the occurrence of a high diversity, since water availability and mild temperatures are particularly critical for the survival and reproduction of testate amoebae (Meisterfeld 2002a, 2002b; Fernández 2015). In fact, the geographical distribution of most species is restricted to the temperate zone, and few species extend its geographical distribution beyond mid-latitudes (Fig. 5). Moreover, our analyses showed that some representatives of these protists can also occur in hot and dry habitats or cold and dry habitats such as arid shrub-lands and glaciers (Table 2). These habitats are common at low and high latitudes (Gajardo 1994; Zapata and Matamala 1987), where there is a constant deficit of water or solar energy inputs over the year, respectively (Arroyo et al. 2004). Because of these characteristics, these habitats are considered suboptimal for testate amoebae (Smith 1985; Zapata and Matamala, 1987; Fernández 2015). This lead us to suggest that populations living in these habitats are not self-sustaining and therefore, are supported by continual immigration of individuals coming from optimal habitats (temperate areas), where populations are actively growing and reproducing (i.e. a source-sink dynamics at biogeographical scale, see Fernández 2015). The fact that most of the species that occurs at low and high latitudes also occur at mid-latitudes supports this idea (i.e. protist species composition at low and high latitudes are mainly a subset of that found at mid-latitudes)(Fig. 5).

Our study also revealed that one-third (33%) of testate amoeba species recorded in Chile are widely (20–38 latitudinal bands) or relatively widely (11–19 latitudinal bands) distributed all along continental Chile while other were reported over shorter latitudinal ranges (Fig. 4). Species exhibiting vast geographical distributions all along Chile are often also generalists with wide ecological tolerances to various environmental factors, such as *Arcella vulgaris* or *Centropyxis aculeata*. The first species can tolerate high salt concentrations due to leaching from de-iced roads (Roe and Patterson 2014), and can live in activated sludge (Jaromínglen et al. 2013) as well as in acidic *Sphagnum* mosses environment (Mieczan 2007). The second occurs from alkaline lakes (Qin et al. 2013) to Amazonian peatland pools (Swindles et al. 2014), tolerates also well organic pollution (Dorgham et al. 2013) and can survive in arid soils that are dry for much of the year (Fernández 2015). Because of their tolerance, they have a high colonization potential; these species are considered as cosmopolitan.

However, most widespread species have never been studied with molecular methods. An exception here is *Nebela collaris*, which has been found to be rather a complex of species than a single entity, encompassing nowadays at least eight “morpho-phylogenetic species” (Singer et al. 2015). It is therefore possible that seemingly widespread species comprise several entities with more restricted distributions. A similar case occurs with some relatively widespread taxa such as *Apodera vas* and *Certesella* spp. These taxa are consistently distributed in southern and central Chile but their latitudinal distribution is abruptly cut northward by the so-called ‘South American arid diagonal’, where climatic conditions become drier (ca. 30° S; southern edge of the Atacama Desert). They exhibit high morphological variation within and between populations along their latitudinal distributions, and morphological parameters measured show significant discontinuities (Zapata and Fernández 2008; Fig. 6). This suggests that these taxa represent species complexes consisting of several closely related species with more restricted distributions than *A. vas* and *Certesella* spp. sensu lato. Moreover, species exhibiting restricted geographical distributions in Chile are both very rare and medium- to large-sized taxa (ca. 70–250 µm) that inhabit specific habitats, such as *Argyria schwabei*, *A. gertrudeana*, *Puytoracia jenswendti* and *Scutiglypha cabrolae* (Figs 6 and 7). The first two species are only found within habitats included in the southern rainforest biome (Zapata 2005; Fernández and Zapata 2011), the third has been found only on the surface of a glacier (Santibañez et al. 2011) and the fourth is only known from an isolated high altitude volcanic lake in the Andes Range (De Smet and Gibson 2009) and extremely arid soils of the southern edge of the Atacama Desert (Fernández 2015). Most of the species with restricted geographical distributions are also endemic to Chile and their distributions are within the Chilean biodiversity hotspot. The value of endemism recorded so far for Chile reaches close to 10% of the total diversity. Some researchers believe that a value close to 10% is sufficient for an area to qualify as a geographical province, at least for macroscopic organisms (Briggs and Bowen 2012). Therefore, the Chilean biodiversity hotspot could potentially host a unique micro-eukaryotic diversity and represent an area of evolutionary innovations or refuges where an older and still understudied microbiota persists. We trust that future molecular-based studies and phylogeographic analyzes will enable us to test this hypothesis.

Conclusions

The Chilean territory represents an area of high diversity for testate amoebae. In this country, testate amoeba diversity has exponentially increased in recent years and this trend will continue over time. This conjecture is supported by the emerging use of molecular techniques, which promise to detect a cryptic diversity largely overlooked by traditional approaches. Testate amoebae also comprise a widespread

group in Chile that occurs in many habitats, though they are more diverse in peatlands and rainforest soils. However, this pattern may be an artifact of uneven sampling effort, since in Chile the latter habitats have been more sampled than others. This calls for studying other less explored habitats (e.g. hot and cold deserts, glaciers) to expand our view on its ecology thus allowing us to use them as bioindicators of the past and present-day environmental conditions in this region of the planet.

Testate amoebae are not randomly distributed in Chile. Species composition of Insular Chile and the Chilean Antarctic territory is mainly a depauperated subset of that found in continental Chile. This nested pattern of species composition has been also found for several metazoans and has been explained in terms of the theory of island biogeography. We suggest that the occurrence of this pattern also is driven by the same mechanisms proposed by this theory (i.e. selective extinction and selective colonization).

Moreover, in continental Chile only generalist species are widely distributed (ca. 11% of the total), occurring from temperate to extremely hot and cold biomes. In contrast, the remaining 89% of the species have a much more restricted distribution, occurring mainly in the temperate zone where there is a constant tradeoff between water and mild temperatures inputs over the year. At least the 10% of these species are potentially endemic to Chile and occur exclusively in temperate biomes of the so-called Chilean biodiversity hotspot. These diversity patterns have been repeatedly found for Chilean metazoans and have been explained in terms of ecological, historical and evolutionary causes. For example, the lift of the Andes and the Pleistocene glaciations changed the climate in this region, creating hot and cold climates at high and low latitudes, respectively. These changes forced the species to flee into mid-latitudes (temperate areas), where they undergone evolutionary events that resulted in new species. Then, since hot and cold climate conditions still prevail at low and high latitudes, very few species (mainly generalist species) have been able to re-colonize these areas (Arroyo et al. 2004). We suggest that these processes also determined the spatial distribution of these protists in continental Chile.

Finally, the occurrence of the aforementioned diversity patterns suggests that, in Chile, testate amoebae and metazoans exhibit analogous diversity patterns driven by similar underlying mechanisms. Future biogeographical, macroecological and phylogeographic analyses may validate this idea.

Acknowledgements

L.D.F. was supported by CONICYT (doctoral scholarships No. 21110037 and 78130011) and the Universidad de Concepción (Dirección de Postgrado). Field expeditions conducted by L.D.F. in Chile were funded by the following institutions (in alphabetic order): Ministerio del Medio Ambiente del Gobierno de Chile (FPA project N°

11-007-08), the Wildlife Conservation Society (Karukinka grant No 2012), Universidad de Los Lagos (project No. 2008: Foraminíferos de marismas salobres del Sur de Chile), and University of Neuchâtel (mobility grant, IDPOB). We thank Franziska Sorge and Ildiko Szelezcz for helping with German translations; Pamela Santibañez and Centro de Estudios Científicos-Chile for sharing with us SEM pictures of *P. Jenswendti*; Lorena and Mónica from Naturalis (FCNYM, UNLP-Argentina); Julio Pascual (Biblioteca IGME, Spain) for sharing specialized literature; and two anonymous referees for their helpful comments. Special thanks to Krzysztof Wiackowski for authorizing the use of figures previously published in *Acta Protozool.*; Jaime Zapata for providing his private collection of permanent slides; Ralf Meisterfeld and Yuri Mazei for kindly reviewing the taxonomic information in our database; and Fabiola Barrientos-Loebel for performing the artwork of Figs 5 and 6.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ejop.2015.07.001>.

References

- Adl, S.M., Simpson, A.G., Lane, C.E., Lukeš, J., Bass, D., Bowser, S.S., Brown, M.W., Burki, F., Dunthorn, M., Hampl, V., Heiss, A., Hoppenrath, M., Lara, E., Le Gall, L., Lynn, D.H., McManus, H., Mitchell, E.A.D., Mozley-Stanridge, S.E., Parfrey, L.W., Pawlowski, J., Rueckert, S., Shadwick, R.S., Schoch, C.L., Smirnov, A., Spiegel, F.W., 2012. The revised classification of eukaryotes. *J. Eukaryot. Microbiol.* 59, 429–493.
- Arroyo, M.T.K., Marquet, P.A., Marticorena, C., Simonetti, J.A., Cavieres, L., Squeo, F., Rozzi, R., 2004. Chilean winter rainfall-Valdivian forests. In: Mittermeier, R.A., Gil, P.R., Hoffmann, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreux, J., da Fonseca, G.A.B. (Eds.), *Hotspots Revisited: Earth's Biologically Wealthiest and most Threatened Ecosystems*. CEMEX, México D.F, pp. 99–103.
- Atmar, W., Patterson, B.D., 1993. The measure of order and disorder in the distribution of species in fragmented habitat. *Oecologia* 96, 373–382.
- Bobrov, A.A., Krasilnikov, P.A., 2011. Testate amoebas of pine forests in Mexico. *Biol. Bull.* 38, 470–476.
- Bonnet, L., 1966. Le peuplement Thécamoebien de quelques sols du Chili. *Protistologica* 2, 113–140.
- Briggs, J.C., Bowen, B.W., 2012. A realignment of marine biogeographic provinces with particular reference to fish distributions. *J. Biogeogr.* 39, 12–30.
- Chatelain, A.P., Meisterfeld, R., Roussel-Delif, L., Lara, E., 2013. Sphenoderiidae (fam. nov.), a new clade of euglyphid testate amoebae characterized by small, round scales surrounding the aperture. *Protist* 164, 782–792.
- Claparède, E., Lachmann, J. 1868. *Etudes sur les Infusoires et les Rhizopodes*, Geneve, Switzerland.
- Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.Y., Mao, C.X., Chazdon, R.L., Longino, J.T., 2012. Models and estimators linking

- individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *J. Plant Ecol.* 5, 3–21. Certes, A., 1889. Protozoaires. In: *Mission Scientifique du Cap Horn 1882–1883. Zoologie* 6, 1–53.
- De Smet, W.H., Gibson, J.A.E., 2009. On a new species of euglyphid testate amoebae, *Scutiglypha cabrolae*, from the Licancabur Caldera Lake, Central Andes. *Acta Protozool.* 48, 119–126.
- Dorgham, M.M., El-Tohamy, W.S., Aziz, N.E.A., El-Ghobashi, A., Qin, J.G., 2013. Protozoa in a stressed area of the Egyptian Mediterranean coast of Damietta, Egypt. *Oceanologia* 55, 733–750.
- Ehrenberg, C.G., 1843. Verbreitung und Einfluss des mikroskopischen Lebens in Süd-und Nord Amerika. Königliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen.
- Ehrenberg, C.G., 1853. Über das jetzige mikroskopische Süßwasser der Galapagos-Inseln. Bericht über die zur Bekanntmachung geeigneten Verhandlungen der Königl. Preussischen Akademie der Wissenschaften zu Berlin.
- Escobar, J., Martínez, J.I., Parra, L.N., 2005. Thecamoebians (Testaceous Rhizopods) from a tropical lake: La Fe Reservoir, Antioquia, Colombia. *Caldasia* 27, 293–298.
- Fernández, L.D., 2015. Source-sink dynamics shapes the spatial distribution of soil protists in an arid shrubland of northern Chile. *J. Arid Environ.* 113, 121–125.
- 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* 80, 27–39.
- Fernández, L.D., Zapata, J., Meisterfeld, R., Baessolo, L., 2012. First records and community patterns of Arcellinida inhabiting a pristine and remote island from southeastern Pacific. *Acta Protozool.* 51, 139–154.
- Foissner, W., 1997. Global soil ciliate (Protozoa, Ciliophora) diversity: a probability-based approach using large sample collections from Africa, Australia and Antarctica. *Biodivers. Conserv.* 6, 1627–1638.
- Foissner, W., 1999. Protist diversity: estimates of the near-imponderable. *Protist* 150, 363–368.
- Gajardo, R., 1994. La Vegetación Natural de Chile. Clasificación y Distribución Geográfica. Editorial Universitaria, Chile.
- Gotelli, N.J., 2000. Null model analysis of species co-occurrence patterns. *Ecology* 81, 2606–2621.
- Golemansky, V., Todorov, M., 1996. Interstitial rhizopods (Rhizopoda: Testacea and Foraminiferida) from the Antarctic Region of Chile and Valparaíso in the Pacific. *Res. Life Sci.*, 62–69.
- Haman, D., 1988. Annotated lexicon of testate lobosid and filosid amoebian genera, subgenera and type species (Sarcodina: Protozoa: Thecamoebians). *Rev. Esp. Micropaleontol.* 20, 205–244.
- Haman, D., Kohl, B., 1994. A thecamobinid assemblage from lake Cocococha, Tambopata Reserve, Madre de Dios Province, Southeastern Peru. *J. Foramin. Res.* 24, 226–232.
- Hall, L.S., Krausman, P.R., Morrison, M.L., 1997. The habitat concept and the plea for standard terminology. *Wildl. Soc. Bull.* 25, 173–182.
- Heger, T.J., Mitchell, E.A.D., Leander, B.S., 2013. Holarctic phylogeography of the testate amoeba *Hyalosphenia papilio* (Amoebozoa: Arcellinida) reveals extensive genetic diversity explained more by environment than dispersal limitation. *Mol. Ecol.* 22, 5172–5184.
- Hoogenraad, H.R., de Groot, A.A., 1951. Thekamoebe Moss-rhizopoden aus Südamerika. *Arch. F. Hydrobiol.* 45, 346–366.
- Hsieh, T.C., Ma, K.H., Chao, A., 2014. iNEXT: iNterpolation and EXTrapolation for species diversity. R package version 2.0. Available at <http://chao.stat.nthu.edu.tw/blog/software-download>
- Hutchinson, G.E., 1957. Concluding remarks. *Cold Spring. Harb. Sym.* 22, 415–427.
- Jaromin-Glen, K., Babko, R., Lagod, G., Sobczuk, H., 2013. Community composition and abundance of protozoa under different concentration of nitrogen compounds at Hajdow wastewater treatment plant. *Ecol. Chem. Eng. S* 20, 127–139.
- Jung, W., 1942a. Südchilenische Thekamöben. *Arch. Hydrobiol.* 95, 253–356.
- Jung, W., 1942b. Illustrierte Thekamöben-Bestimmungstabellen. I. Die Systematik der Nebelinen. *Arch. Protistenkd.* 95, 357–390.
- Koenig, I., Feldmeyer-Christe, E., Mitchell, E., 2015. Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four *Sphagnum* peatlands along an altitudinal gradient in Switzerland. *Ecol. Indic.* 54, 48–59.
- Kosakyan, A., Heger, T.J., 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.
- Krashevskaya, V., Bonkowski, M., Maraun, M., Scheu, S., 2007. Testate amoebae (protista) from an elevational gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia* 51, 319–331.
- Lara, E., Heger, T.J., Scheihsing, 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.
- Leidy, J., 1879. Freshwater Rhizopods of North America. Vol. Vol. 12, United States Geological Survey of the Territories, Washington, Government Printing Office.
- Mazei, Y., Tsyganov, A., 2006. Freshwater Testate Amoebae. KMK, Moscow (In Russian).
- MacArthur, R.H., Wilson, E.O., 1963. An equilibrium theory of insular zoogeography. *Evolution* 17, 373–387.
- Meisterfeld, R., 2002a. Order Arcellinida Kent, 1880. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), *An illustrated guide to the protozoa*, 2, second ed. Society of Protozoologists, Lawrence, Kansas, USA, pp. 827–860.
- Meisterfeld, R., 2002b. Testate Amoebae with Filopodia. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), *An illustrated guide to the protozoa*, 2, second ed. Society of Protozoologists, Lawrence, Kansas, USA, pp. 1054–1084.
- Meyer, C., Gilbert, D., Gillet, F., Moskura, M., Franchi, M., Bernard, N., 2012. Using bryophytes and their associated testate amoeba microsystems as indicators of atmospheric pollution. *Ecol. Indic.* 13, 144–151.
- Mieczan, T., 2007. Epiphytic protozoa (testate amoebae and ciliates) associated with *Sphagnum* in peatbogs: relationship to chemical parameters. *Pol. J. Ecol.* 55, 79–90.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.
- Mitchell, E.A.D., Payne, R.J., Lamentowicz, M., 2008. Potential implications of differential preservation of testate amoeba shells for paleoenvironmental reconstruction in peatlands. *J. Paleolimnol.* 40, 603–618.

- Nguyen-Viet, H., Bernard, N., Mitchell, E.A.D., Cortet, J., Badot, P.-M., Gilbert, D., 2007. Relationship between testate amoeba (protist) communities and atmospheric heavy metals accumulated in *barbulaindica* (bryophyta) in Vietnam. *Microb. Ecol.* 53, 53–65.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. *Vegan: Community Ecology Package*. R package version 2.0. Available at <http://CRAN.R-project.org/package=vegan>
- Opravilova, V., Hajek, M., 2006. The variation of testacean assemblages (Rhizopoda) along the complete base-richness gradient in fens: a case study from the Western Carpathians. *Acta Protozool.* 45, 191–204.
- Payne, R.J., 2011. Can testate amoeba-based palaeohydrology be extended to fens? *J. Quaternary Sci.* 26, 15–27.
- Payne, R.J., Pates, J.M., 2009. Vertical stratification of testate amoebae in the Elatia Mires, northern Greece: palaeoecological evidence for a wetland response to recent climatic change, or autogenic processes? *Wet. Ecol. Manag.* 17, 355–364.
- Penard, E., 1902. *Faune rhizopodique du bassin du Léman* Genève, Kündig.
- Qin, Y., Xie, S., Smith, G.H., Swindles, G., Gu, Y., 2011. Diversity, distribution and biogeography of testate amoebae in China: implications for ecological studies in Asia. *Eur. J. Protistol.* 47, 1–9.
- Qin, Y.M., Fournier, B., Lara, E., Gu, Y.S., Wang, H.M., Cui, Y.D., Zhang, X.K., Mitchell, E.A.D., 2013. Relationships between testate amoeba communities and water quality in Lake Donghu, a large alkaline lake in Wuhan, China. *Front. Earth Sci.* 7, 182–190.
- Roe, H., Patterson, R.T., 2014. Arcellacea (testate amoebae) as bio-indicators of road salt contamination in lakes. *Microb. Ecol.* 68, 299–313.
- Santibañez, P.A., Kohshima, Y., Scheihing, R.A., Silva, R., Jaramillo, J.I., Labarca, P.J., Casassa, G., 2011. First record of testate amoebae on glaciers and description of a new species *Puytoracia jenswendi* nov. sp. (Rhizaria, Euglyphida). *Acta Protozool.* 50, 1–14.
- Segovia, R.A., Armesto, J.J., 2015. The Gondwanan legacy in South American biogeography. *J. Biogeogr.* 42, 209–217.
- Schlatter, R.P., Schlatter, J.E., 2004. Los turbales de Chile. In: Blanco, D.E., Balze, V.M. (Eds.), *Los Turbales de la Patagonia, Bases para su inventario y la conservación de su biodiversidad*, Publicación No. 19: 75–80. Wetlands International-América del Sur, Buenos Aires, Argentina.
- Singer, D., Kosakyan, A., Pillonel, A., Mitchell, E.A.D., Lara, E., 2015. Eight species in the *Nebela collaris* complex: *Nebela gimlii* (Arcellinida, Hyalospheniidae), a new species described from a Swiss raised bog. *Eur. J. Protistol.* 51, 79–85.
- Seppey, C.V.W., Fournier, B., Szelez, I., Singer, D., Mitchell, E.A.D., Lara, E., 2015. Response of forest soil euglyphid testate amoebae (Rhizaria: Cercozoa) to pig cadavers assessed by high-throughput sequencing. *Int. J. Legal Med.*, <http://dx.doi.org/10.1007/s00414-015-1149-7>.
- Smith, H.G., 1978. The distribution and ecology of terrestrial protozoa of sub-Antarctic and maritime Antarctic islands. In: *British Antarctic Survey 95*, Cambridge, UK.
- Smith, H.G., 1985. The colonization of volcanic tephra on Deception Islands by protozoa: long-term trends. *Brit. Antarct. Bull.* 66, 19–33.
- Smith, H.G., Wilkinson, D.M., 2007. Not all free-living microorganisms have cosmopolitan distributions-the case of *Nebela* (*Apodera*) *vas Certes* (Protozoa: Amoebozoa: Arcellinida). *J. Biogeogr.* 34, 1822–1831.
- Smith, H.G., Bobrov, A., Lara, E., 2008. Diversity and biogeography of testate amoebae. *Biodivers. Conserv.* 17, 329–343.
- Swindles, G.T., Reczuga, M., Lamentowicz, M., Raby, C.L., Turner, T.E., Charman, D.J., Gallego-Sala, A., Valderrama, E., Williams, C., Draper, F., Coronado, E.N.H., Roucoux, K.H., Baker, T., Mullan, D.J., 2014. Ecology of testate amoebae in an amazonian peatland and development of a transfer function for palaeohydrological reconstruction. *Microb. Ecol.* 68, 284–298.
- Szelez, I., Fournier, B., Seppey, C., Amendt, J., Mitchell, E., 2013. Can soil testate amoebae be used for estimating the time since death? A field experiment in a deciduous forest. *Forensic Sci. Int.* 236, 90–98.
- Tarnawski, S.E., Lara, E., 2015. From environmental sequences to morphology: observation and characterisation of a paulinellid testate amoeba (Euglyphida, Paulinellidae) from soil using fluorescent in situ hybridization. *Protist*, <http://dx.doi.org/10.1016/j.protis.2015.04.002>.
- Turner, T.E., Swindles, G.T., 2012. Ecology of Testate Amoebae in Moorland with a complex fire history: implications for ecosystem monitoring and sustainable land management. *Protist* 163, 844–855.
- Ulrich, W., Almeida-Neto, M., Gotelli, N.G., 2009. A consumer's guide to nestedness analysis. *Oikos* 118, 3–17.
- Valentine, J., Davis, S.R., Kirby, J.R., Wilkinson, D.M., 2013. The use of testate amoebae in monitoring peatland restoration management: case studies from North West England and Ireland. *Acta Protozool.* 52, 129–145.
- Vucetich, M.C., Lopretto, E.C., 1995. Rhizopoda: Amebas Testaceas. In: de Castellanos, Z.A., Coscarón, S., Miquel, S. (Eds.), *Fauna de agua dulce de la República Argentina 3*, Protozoa. Facultad de Ciencias Naturales y Museo, La Plata, Argentina, pp. 5–54.
- Wailes, G., 1913. Freshwater rhizopoda from North and South America. *Zool. 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., 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, B.D., Mikkelsen, G.M., Cutler, A., Wirt Atmar, 1998. A comparative analysis of nested subset patterns of species composition. *Oecologia* 113, 1–20.
- Wylezich, C., Meisterfeld, R., Meisterfeld, S., Schlegel, M., 2002. Phylogenetic analyses of small subunit ribosomal RNA coding regions reveal a monophyletic lineage of euglyphid testate amoebae (Order Euglyphida). *J. Eukaryot. Microbiol.* 49, 108–118.
- Zapata, J., 2005. Tecamebas (Protozoa, Rhizopoda) de la turbera Rucapihue (40(34'42.5" S; 73(34'31.4" W), Chile. *Bol. Soc. Biol. Concepción* 76, 39–56.
- Zapata, J., Rudolph, E., 1986. Tecamebas del río Damas (40(34' S; 73(08' W), Chile. *Biota* 2, 65–82.
- Zapata, J., Matamala, M., 1987. Tecamebianos del lago Kitiash (Isla Rey Jorge, Antártica). *Biota* 3, 59–71.
- Zapata, J., Crespo, J., 1990. Tecamebas del volcán Rano Kau, 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 73, 17–35.
- Zapata, J., Muñoz, A., Rojas, M., 2007a. Tecamebas Euglyphida (Protozoa, Cercozoa, Silicofilosea) del volcán Casablanca (40(16'17" S; 72(00'00" O), Parque Nacional Puyehue, Chile. Bol. Soc. Biol. Concepción 78, 99–105.
- Zapata, J., Toledo, R., Rojas, M., 2007b. 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 78, 67–76.
- Zapata, J., Fernández, L., 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.