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First Record of Testate Amoebae on Glaciers and Description of a New Species *Puytoracia jenswendti* nov. sp. (Rhizaria, Euglyphida)

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Summary. This study documents for the first time the presence of testate amoebae on glaciers. Three shallow firn cores of 10 m depth were obtained from the Mocho-Choshuenco and Osorno volcanoes, Southern Andes, Chile, in October and November, 2005. Euglyphid testate amoebae were detected in 28 samples that correspond to the spring-summer layers of the firn cores. Inspection of 454 collected individuals reveals the presence of four different taxa. Three of these taxa, *Trinema lineare*, *Trinema enchelys* and *Puytoracia bergeri* have previously been reported in ice-free environments. The fourth taxon corresponds to a new species *Puytoracia jenswendti* nov. sp. The observation of food content and reproductive activities in a significant fraction of specimens evidence that testate amoebae are competent to inhabit glaciers. The testate amoebae found in the firn cores display clear seasonal variations in abundance indicating that these records can provide a new and novel proxy as paleoindicator for firn/ice core dating and for estimation of past glacier mass balance.

Key words: Glacier, Firn core, Testate amoebae, Trinematidae, *Trinema*, *Puytoracia*.

INTRODUCTION

Analysis of isotopic content and chemical species in ice cores retrieved from glaciers provides one of the most detailed records of climate and past environmental conditions available to model past and future climate and environmental conditions (Lorius *et al.* 1990, Bradley 1999). However, in glaciers with high production of melt water such as in temperate glaciers, where ice is at the melting point, percolation disturbs seasonal signals precluding the access to valuable proxies (Schwikowski *et al.* 1999, Eichler *et al.* 2001, Pohjola 2002). In the Southern Andes most of the glaciers are temperate and in recent decades they have been retreating and shrinking rapidly in response to climate change (IPCC 2001, Rivera *et al.* 2000). Temperate glaciers are inhabited by extremophile biotic communities, which are supported by microalgae, and have also been reported in many parts of the world (Kol 1864; Kohshima 1984, 1987; Kohshima *et al.* 2002; Uetake *et al*. 2006; Santibañez *et al.* 2008). During the melt season (spring-summer)

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microalgae grow on the surface of temperate glaciers and are later covered by snow in the autumn-winter. Yoshimura *et al.* (2000) first reported that microalgae layers in ice cores can be used to date firn/ice cores. Further analysis of ice cores at Mocho-Choshuenco and Osorno volcanoes in the Southern Andes revealed for the first time the presence of protozoa in glaciers and showed that they provide a novel tool as paleoindicators and also to estimate past mass balance in mountain glaciers (Santibañez *et al.* 2008). The present work documents that protozoa inhabiting temperate glaciers in the Southern Andes correspond to testate amoebae, a functional group of protists (Wanner 1999) that populate a broad variety of terrestrial habitats, including ice-free Polar Regions (Beyens *et al.* 1986a, b; Smith 1992; Beyens and Chardez 1995; Vincke *et al.* 2004a, b, c; Matheeussen *et al.* 2005). This study broadens the spectrum of habitats of testate amoebae and reports a new testate amoeba taxon *Puytoracia jenswendti*. It also suggests testate amoebae as new and novel proxies as paleoindicators for firn/ice core dating and for estimation of past glacier mass balance.

MATERIALS AND METHODS

Study site

Three shallow firn/ice cores were retrieved in temperate glaciers located on volcanoes from the Cordillera de los Andes, Chile, between October and November of 2005 (Fig. 1). Two of them are from Mocho-Choshuenco Volcano (39°55′ S; 72°02′ W; 2000 m a.s.l. and 2422 m a.s.l., respectively) and the third from Osorno Volcano (41°06′ S; 72°30′ W; 2652 m a.s.l.).

The annual mean temperature at Chilean coastal stations on the Pacific at latitude of ~ 40° S is ~11°C. In combination with a normal altitudinal temperature gradient of ~ 6.5°C km^{-1} (ISO 2533:1975), the mean annual temperature at the summit of Mocho-Choshuenco and Osorno volcanoes is on the order of –5, –6°C. During the summer there is a strong melting even on the top of the volcanoes. The mean monthly temperature in the summer months at the study sites of Mocho-Choshuenco and Osorno volcanoes during the period registered by firn/ice cores was estimated directly from 850 hPa radiosonde data from Puerto Montt (El Tepual) (Table 1). Escobar (2008) found a high correlation during 2006 between El Tepual radiosonde data and meteorological data from an automatic weather station at Mocho-Choshuenco meteorological station located at 1995 m a.s.l. (39.93º S; 72.01º W). The pluviometric regime in central-south Chile (30– 40º S) is characterized by precipitation events that main-

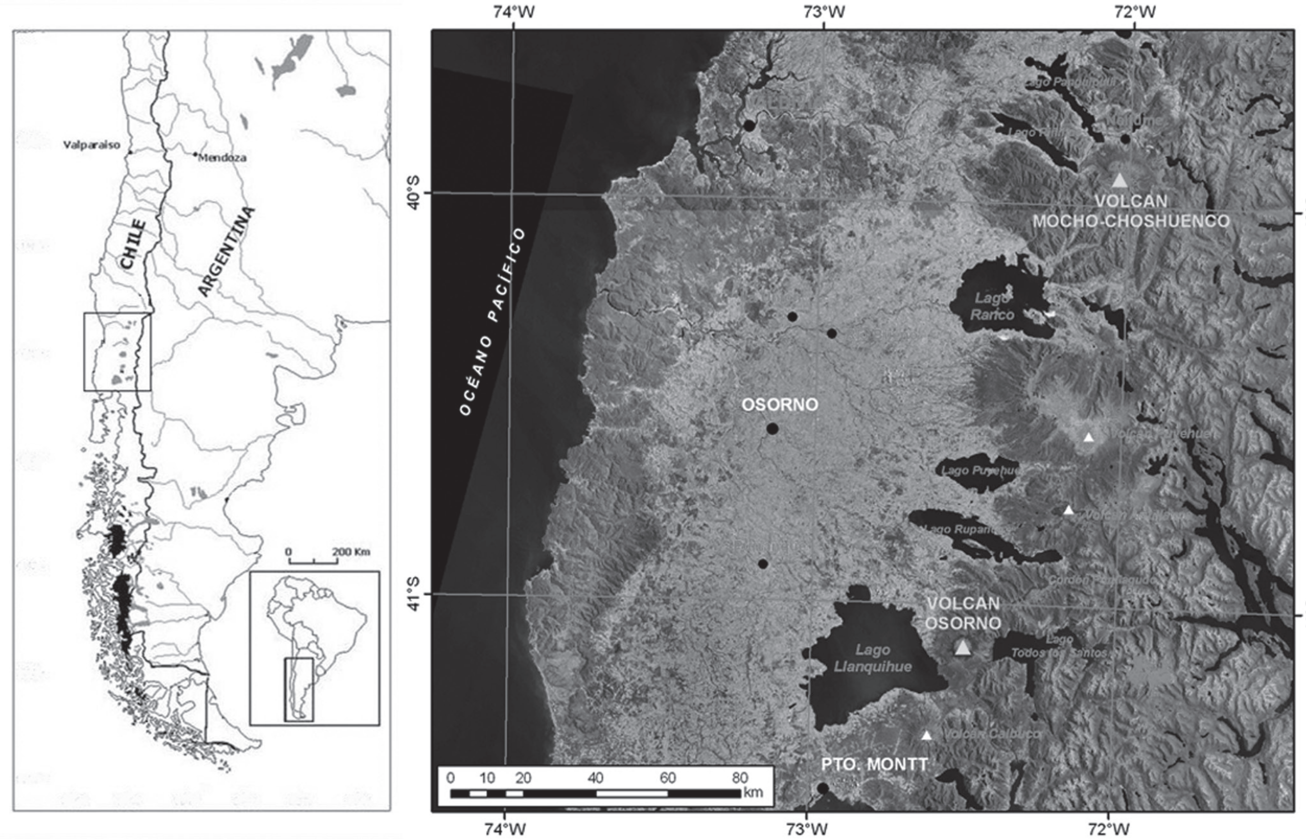

Fig. 1. Mocho-Choshuenco and Osorno volcanoes in the Chilean lake district.

Table 1. Mean monthly air temperature at 12 UTC (Coordinated Universal Time) for the study sites, during the Spring-Summer (Sp-S) signal periods recorded by firn/ice cores, obtained from radiosonde data at 850 hPa from the station Puerto Montt El Tepual (41.43º S, 73.12º W, 90 m a.s.l.).

Site and altitude	Sp-S signal	December	January	February	March
Mocho-Choshuenco 2000 m a.s.l.	2004/2005	–2.2	–1.4	4.6	–2.8
Mocho-Choshuenco 2422 m a.s.l.	2004/2005	–4.8	–4.0	2.0	–5.4
	2003/2004	–7.7	–	–5.5	–2.6
	2002/2003	–4.7	–3.3	–3.3	–1.0
	2001/2002	–3.3	–2.4	–0.4	–6.4
Osorno 2652 m a.s.l.	2004/2005	–6.4	–5.7	–0.4	–7.0
	2003/2004	–9.3	–	4.3	–4.3

Table 2. Total abundance (ind mL^{-1}) of testate amoebae in firn/ice cores from temperate glaciers during the melting season (Spring-Summer). The Not identified label corresponds to individuals belong to family Trinematidae that can not be identified to species level.

Site and altitude	Sp-S signal	*T. enchelys*	*P. jenswendti*	*P. bergeri*	Not identifed	Total
Mocho-Choshuenco 2000 m a.s.l.	2004/2005	2.2	–	–	0.8	3.0
Mocho-Choshuenco 2422 m a.s.l.	2004/2005	40.5	2.5	3.1	6.7	52.7
	2003/2004	4.3	1.0	–	1.2	6.5
	2002/2003	8.8	2.0	1.8	1.0	13.7
	2001/2002	2.9	–	0.2	6.9	10.0
Osorno 2652 m a.s.l.	2004/2005	1.0	–	–	–	1.0
	2003/2004	0.8	0.8	–	–	0.8

ly occur between May and September (austral autumn-winter), due to the seasonal northward migration of the western circulation in middle latitudes. The annual mean precipitation in the coastal and piedmont Andean zone at approximately 40º S is of the order of 2–3 m, with a strong orographic effect. For example, in 1980–2004 the annual precipitation averaged 1803 mm in Puerto Montt, located on the central valley at sea level. In Punta Huano, a piedmont station located at 200 m a.s.l. 100 km north-east of Puerto Montt and 20 km east of Osorno volcano, average precipitation averaged 2972 mm in the same period.

Sampling and quantification samples

Three 10 m firn/ice cores were drilled with a fiberglass manual drill. On the glacier the firn/ice core samples were cut with a pre-cleaned stainless-steel knife into 20 cm sections, scraping off a 1 cm section of the core surface to eliminate possible contaminants. The samples were packed into pre-cleaned plastic bags which were preserved in a thermally isolated box and later melted at room temperature in a laboratory at Centro de Estudios Científicos (CECS), Valdivia, Chile, each sample being bottled in a clean plastic container of 50 mL each. All samples were then immediately stored in a freezer at –20ºC.

Biological analyses were performed at CECS. Samples were mounted and fixed inside a laminar-flow table. Filtering of 15 mL samples was performed using hydrophilic polytetrafluoroethylene (PTFE) membrane filters (JHWP013000 : 0.2 mm pore size, 13 mm diameter; Millipore, USA). Each filter was mounted and fixed in glycerol, formalin and water solution (1 : 1 : 1 volume) on a glass slide under cover-slip, sealing it later with Canada balsam. The total cell number, abundance and composition of testate amoebae species was estimated on each filter by counting the shells along nine parallel transect using a microscope (Olympus BX50WI) in bright field, at 600 × magnification.

Morphological analysis and identification

Most of the specimens found in the samples were photographed by means of a digital camera Photometrics *Cool Snap cf*, and then measured using *ImageJ* software, except for individuals with destroyed and damaged shells. Morphometric characteristics used for species identification are defined in Fig. 2. Values of descriptive statistics were calculated for each morphometric variable (Table 3). Statistical analyses were performed using the program STATISTICA ver. 7.0.

Surplus samples that contained specimens of testate amoebae, were observed by Scanning Electron Microscope (SEM; Leo electron Model 420) to obtain scale measurements and to identify better the species. These samples were fixed with Formaldehyde (3%) and filtered in the same polytetrafluoroethylene membranes. Then, the

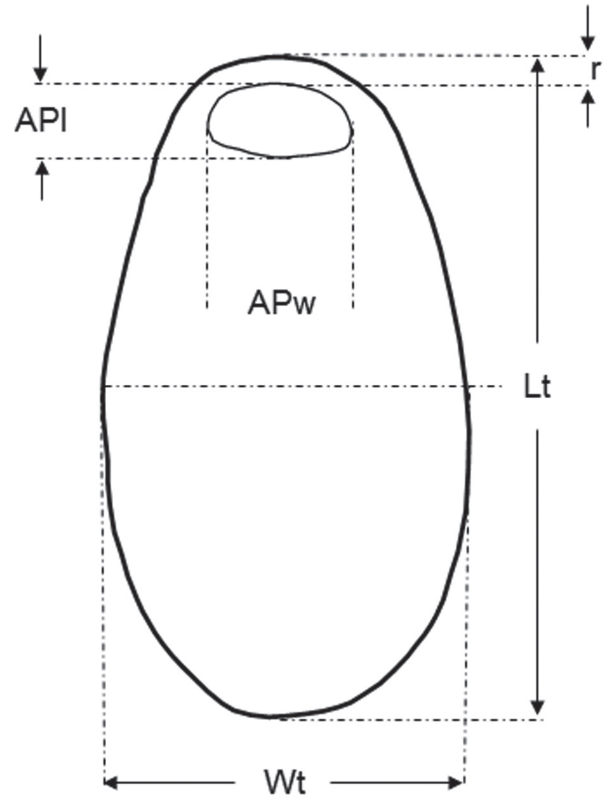

Fig. 2. Shell outline and position of measured morphometric axes of. *P. bergeri, T. enchelys* and *T. lineare* used in this study. Ventral view showing the five morphometric variables measured in three species for statistical characterization, where Lt – total shell length, Wt – shell width, APl – length of the pseudostomal aperture, APw – width of the pseudostomal aperture, r – width of the rim anterior to the pseudostomal aperture.

samples on the filters were dehydrated through an ethanol battery each 10% concentration from 10 to 100% (2 × 5 minutes in each concentration), after that, the samples were fixed on aluminum stub with an carbon conductor adhesive and coated with gold-palladium by using an Ion Coater (Model IB-2, EIKO ENGINEERING), in the same filter of polytetrafluoroethylene. All SEM measurements and observations were performed on fixed specimens. The first author carried out all analyses to guarantee consistency.

Species description was primarily carried out on specimens observed by means of the SEM. Several characteristics observed by light microscope (600 ×) were also included in the species identification (Table 3).

Morphological identification of testate amoebae was made following Harnish (1958), Grospietsch (1958), Bonnet (1970), Decloître (1981), Lüftenegger *et al.* (1988), Ogden and Hedley (1980), Meisterfeld (2000) and Nicholls (2006).

RESULTS

Species description

Preliminary analysis under bright field indicated that all protozoa present on firn/ice samples correspond to testate amoebae. Further analysis of 434 individuals in 150 samples showed that testate amoebae were present only in the summer layers. Testate amoebae species identified belong to the Class Filosea Leidy, 1879, Family Trinematidae Hoogenraad and Groot, 1940. Three of these species correspond to already known taxa *Trinema lineare* Pénard, 1890, *Trinema enchelys* (Ehrenberg, 1838) Leidy, 1878 and *Puytoracia bergeri* Bonnet, 1970. Their presence in glaciers has not been previously reported. The fourth species is new to science; it belongs to genus *Puytoracia* and has been named *Puytoracia jenswendti* (Figs 6 and 7). On average, among all 434 individuals, 78 could not be identified to the species level but all of them belong to the *Trinema* and *Puytoracia* genus. These individuals could not be identified since there is size overlap in the morphological characters that are used to differentiate these species, in fact *T. enchelys* and *T. lineare* show a test length overlap between 32–41 μm and a test width overlap between 15–21 μm. On the other hand, *P. bergeri* and *T. enchelys* present the same problem, when observing the plates is not possible, they have a test length overlap between 43–58 μm and a test width overlap between 27–40 μm. For that reason, all the individuals that possess morphological character overlaps are morphologically impossible to differentiate, and molecular biology is needed.The sample with the largest fraction of non-identified individuals correspond to the layer of the previous summer (2001/02) of the firn core drilled on the summit of Mocho-Choshuenco Volcano (34 out of 49 specimens). In the rest of the firn/ice cores the non-identified testate amoebae amounted to 0–13%. The set of non-identified individuals was excluded from further analysis. Testate amoebae were established to live on glaciers as was inferred from shell content analysis in 280 individuals that showed 57.9% to have unidentified food content (Fig. 4c), 19.6% of the individuals show clearly algae and fungi inside the shell and 22.5% presented empty shells. In addition 6.4% correspond to shells with signs of reproduction (i.e. either conjugation or cell division) (Fig. 4d).

In the following section a brief morphological description of each testate amoeba species sampled is given. The morphometric variables of each species are presented in Table 3.

Table 3. Descriptive statistics for five morphological and two biometrical variables of species found in glaciers of the south of Chile. All measurements in µm. Defined as follows: Lt – length test, Wt – width test, APw – width of pseudostomal aperture, APl – length of pseudostomal aperture, r – the distance from the top of aperture to the oral end of the test. n – number of tests, Mean – arithmetic mean, M – median, SD – standard deviations, SE – standard error of mean, CV – coefficient of variation, Min – minimum, Max – Maximum.

Species/Character	n	Mean	M	SD	SE	CV	Min	Max
P. bergeri								
Lt	29	54.4	55.2	4.0	0.74	7.3	44.3	62.1
Wt	23	26.2	26.5	2.8	0.58	10.7	20.7	32.5
APw	16	10.7	10.5	1.2	0.30	11.0	8.1	12.7
APl	17	6.4	6.6	0.8	0.20	13.0	4.2	7.3
r	16	2.3	2.3	0.3	0.08	13.5	1.6	2.8
Wt/Lt	22	0.49	0.48	0.08	0.02	16.86	0.33	0.67
APl/r	16	2.9	2.9	0.6	0.14	19.4	1.5	4.0
P. jenswendti								
Lt	32	83.1	82.8	1.2	7.0	8.5	67.9	96.1
Wt	24	22.7	22.5	0.7	3.4	14.8	16.5	30.5
APw	22	10.5	10.0	0.3	1.3	12.7	8.9	14.8
APl	22	7.9	7.8	0.3	1.5	19.1	5.9	11.9
r	22	2.7	2.8	0.1	0.4	13.4	2.1	3.3
Wt/Lt	23	0.27	0.27	0.01	0.04	15.46	0.20	0.34
APl/r	22	3.0	2.9	0.2	0.7	23.4	1.9	4.5
T. enchelys								
Lt	206	57.1	56.2	10.1	0.7	17.7	36.3	115.9
Wt	130	23.3	22.9	4.8	0.4	20.4	11.2	47.5
APw	114	10.8	10.7	2.5	0.2	23.1	5.4	21.0
APl	115	6.9	6.5	1.8	0.2	25.5	4.1	15.1
r	111	2.2	2.2	0.5	0.0	21.9	1.3	4.1
Wt/Lt	129	0.40	0.40	0.07	0.01	17.11	0.23	0.60
APl/r	111	3.2	3.0	0.8	0.1	24.3	1.9	5.5
T. lineare								
Lt	6	37.3	38.3	3.7	1.5	10.0	30.4	41.4
Wt	6	19.1	20.3	3.8	1.6	20.1	13.7	23.4
APw	4	8.4	8.5	0.4	0.2	5.2	7.7	8.7
APl	3	4.6	4.7	0.4	0.3	9.6	4.1	4.9
r	4	2.0	2.1	0.4	0.2	21.1	1.5	2.4
Wt/Lt	5	0.54	0.55	0.03	0.01	5.21	0.50	0.57
APl/r	3	2.1	2.0	0.1	0.1	6.4	2.0	2.2

Genus *Trinema* Dujardin, 1841

Trinema lineare Pénard, 1890

The shell is ovoid, circular to oval in transverse section (Fig. 3). The pseudostome is circular, sub terminal, invaginated and toothed. The shell exhibits two silica body scale types: large circular and small elliptical scales. Scales are not arranged in a regular manner. Circular plates dominate the body and have diameters ranging 3.1–5.0 µm. Smaller elliptical scales (long axis: 1.3–2.2 µm; minor axis of 0.9–1.2 µm are observed in the aperture and interstices). The shell width/length ratio ranged 0.50 and 0.57. The ratio between the small diameter of the aperture and the distance from the top of aperture to the oral end of the test ranged between 2.0–2.2. The most variable character in *T. lineare* in glaciers is shell width (CV: 20.1%) and the most stable is pseudostome width axis (CV: 5.2%; Table 3). Morphological features of *T. lineare* on glaciers are similar to those reported for this taxon in soil, moss and aquatic habitats (Grospietsch 1958, Decloître 1981, Ogden and Hendley 1980).

Trinema enchelys (Ehrenberg, 1838) Leidy 1878

This species is morphologically similar to *T. lineare*. The difference is the size. The test length 36–116 µm and width 11–48 µm (Fig. 4a, b). The most variable characters in individuals from these firn/ice cores are pseudostome length and width axes (CV: 25.5% and 23.1%). The most stable character is width/length ratio (CV: 24.3%; Table 3). Most morphological features of these specimens correspond to *T. enchelys* as described

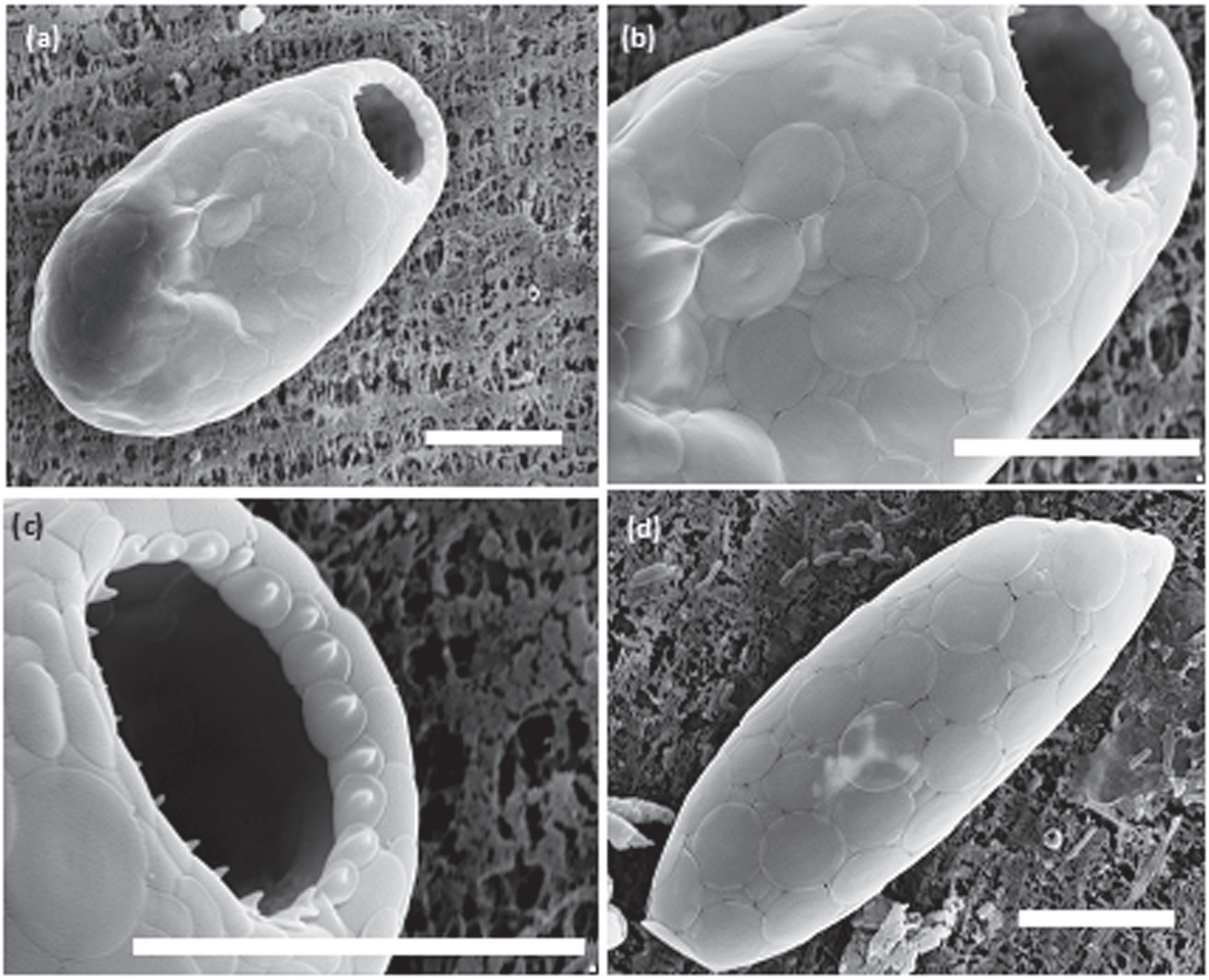

Fig. 3. SEM images of *Trinema lineare*, (a)–(c) the same specimen. **(a)** Ventral view; **(b)** detail of the test, large circular and small elliptical scales on the test surface; **(c)** the pseudostomal aperture ornamented with toothed scales; **(d)** lateral view of another specimen. Scale bars: 10 μm.

by Grospietsch (1958), Ogden and Hendley (1980), Decloître (1981), Lüftenegger *et al*. (1988) and Meisterfeld (2000). However, here shell width values (Table 3) are smaller (11.2 μm) than the published value of 15.0 μm (Ogden and Hendley 1980, Decloître 1981) and pseudostome larger (21.0 μm) than those previously reported for this taxon (20.0 μm in Ogden and Hendley 1980 and Decloître 1981).

Genus *Puytoracia* Bonnet, 1970

Puytoracia bergeri Bonnet, 1970

Shell is ovoid; and exhibits a flat, elliptical transverse section (Fig. 5a; Table 3). The pseudostome is circular, subterminal, invaginated and toothed. The shell exhibits large and small elliptical plates between the large ones which are not arranged in regular fashion. Smaller elliptical scales dominate in the aperture and interstices (Fig. 5a,b). The most variable characters in individuals from these firn/ice cores are pseudostome length and width axes (Table 3). The most stable character is shell length (CV: 7.3%; Table 3). The *P. bergeri* specimens analyzed have morphological features that correspond to those described by Nicholls (2006) for this taxon. However, shell width values are smaller (20,7 μm) than the published value of 15.0 μm, pseudostome diameter values are smaller (4.2 μm) than the published value of

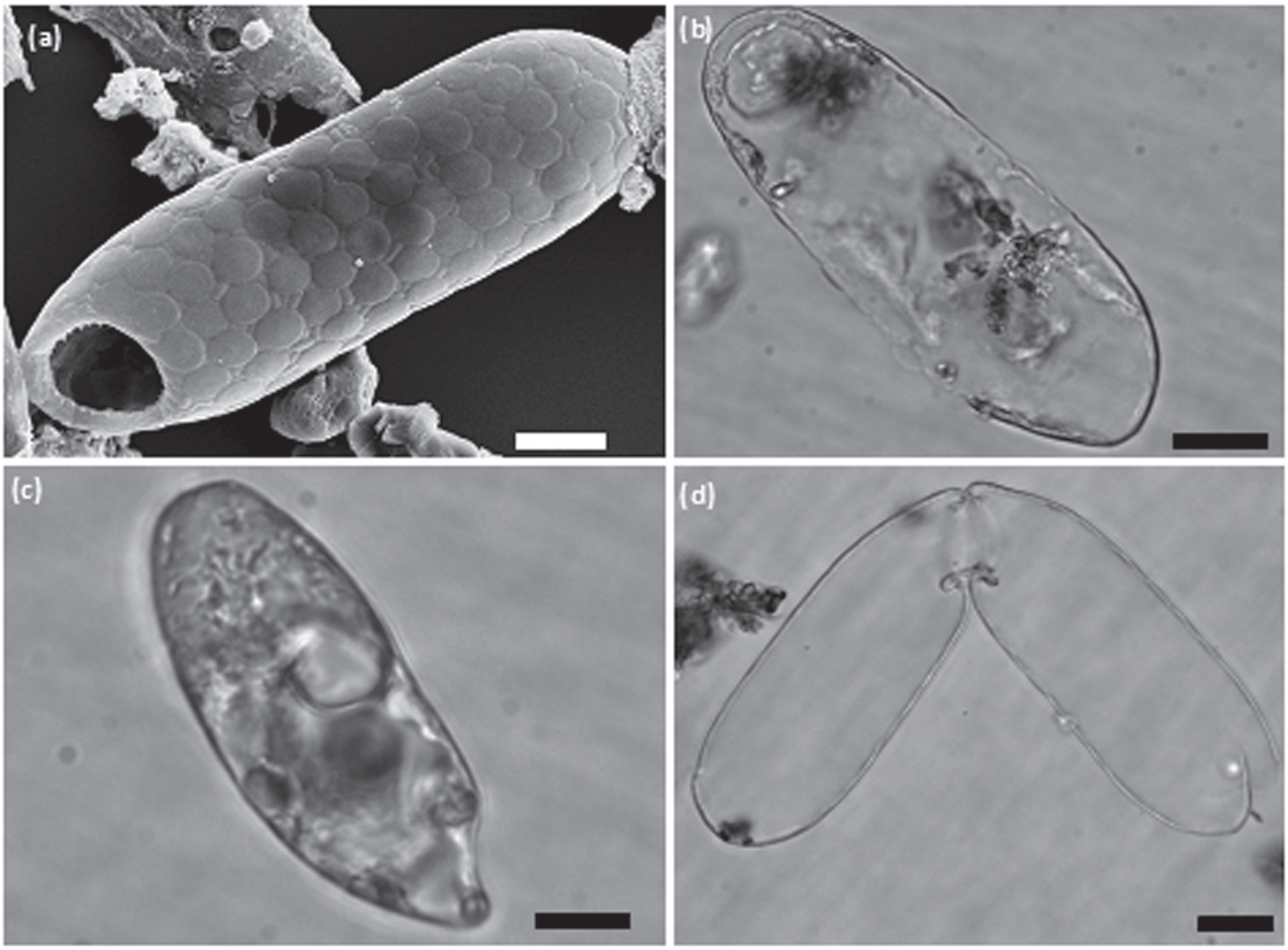

Fig. 4. *Trinema enchelys* images, (a)–(b) ventral view of the test. **(a)** the test showing scales **(c)** lateral view; **(d)** ventral view, reproduction of testate amoebae on the glacier. (a)–(b) acquired by SEM and (c)–(d) acquired by bright field microscopy (BFM). Scale bars: 10 µm.

6 µm and shell length values are larger (62.1 µm) than the published value of 58 µm previously described for this taxon by Nicholls (2006).

***Puytoracia jenswendti* nov. sp.**

Classification summary: We follow the scheme proposed by Adl *et al.* (2005) for the classification: Eukaryota; Rhizaria, Cavalier-Smith, 2002; Cercozoa, Cavalier-Smith, 1998, emend. Adl, 2005; Silicofilosea Adl *et al*., 2005; Euglyphida, Copeland, 1956, emend. Cavalier-Smith, 1997; Trinematidae Hoogenraad and De Groot, 1940; *Puytoracia*, Bonnet, 1970. According to traditional schemes (Corliss 1994) which should nolonger be used the classification would be Phylum, Rhizopoda von Siebold, 1845; Class, Filosea Leidy, 1879; Family, Trinematidae Hoogenraad et Groot, 1940; Genus, *Puytoracia* Bonnet, 1970.

Type locality: Surface of temperate mountain glaciers from Mocho-Choshuenco (39°55′ S; 72°02′ W; 2422 m a.s.l.) and Osorno (41°06′ S; 72°30′ W; 2652 m a.s.l.) volcanoes, Cordillera de los Andes, Chile; melting season (spring-summer).

Type specimen: Type specimen mounted in Canada balsam on a glass slide deposited in extremophiles collection of Centro de Estudios Científicos, Valdivia Chile and National Museum of Natural History under catalogue code MNHNCL PROT- Nº 11850.

Etymology: This species has been named *"jenswendti"* after Dr. Jens Wendt, a German Geodesist at Centro de Estudios Científicos who perished in a plane crash while carrying out glaciological explorations in the Southern Andes in 2009. Dr. Wendt was a distinguished specialist in satellite geodesy, who developed innovative studies on the deformation of the Earth's

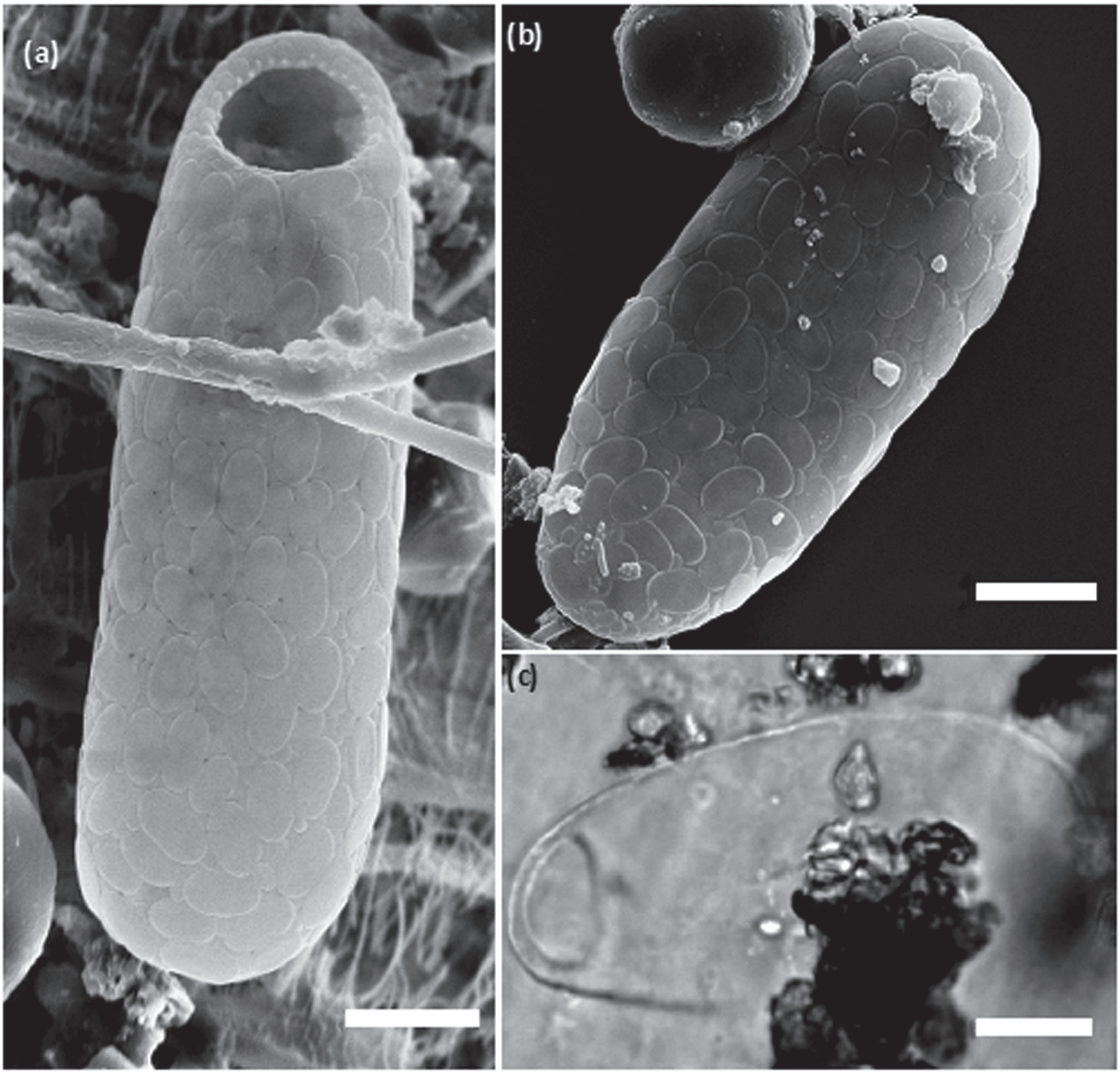

Fig. 5. *Puytoracia bergeri* images. **(a)** ventral view of shell; **(b)** dorsal view, elliptical idiosomes; **(c)** ventral view. (a)–(b) SEM images and (c) BFM image. Scale bars: 10 μm.

crust and ice dynamics within Germany, the southern Andes and Antarctica.

Morphology of the shell: The shell is cylindrical with a tapering towards the pseudostome, and this corresponds to a plagiostome with a visor test type. The superior extreme is rounded and the inferior extreme terminates in a horn or tip (Figs 6 and 7b, d).

The shell length is 67.9–96.1 μm; the shell width is 16.5–30.5 μm (Fig. 7a). The pseudostome is oval, sub terminal, invaginated and toothed. The central axis width is 8.9–14.8 μm; the central axis length 5.911.9 m. The pseudostomal rim aperture has a row with denticulate plates with 23–28 units (Fig 7c). The shell has two sizes of elliptical silica body scales: large and smaller scales are inserted between in the interstices among the large ones. Large scales are arranged parallel to the longitudinal body axis. The large elliptical plates dominate the body (1.87–3.5 × 4.9–6.4 μm). The small elliptical

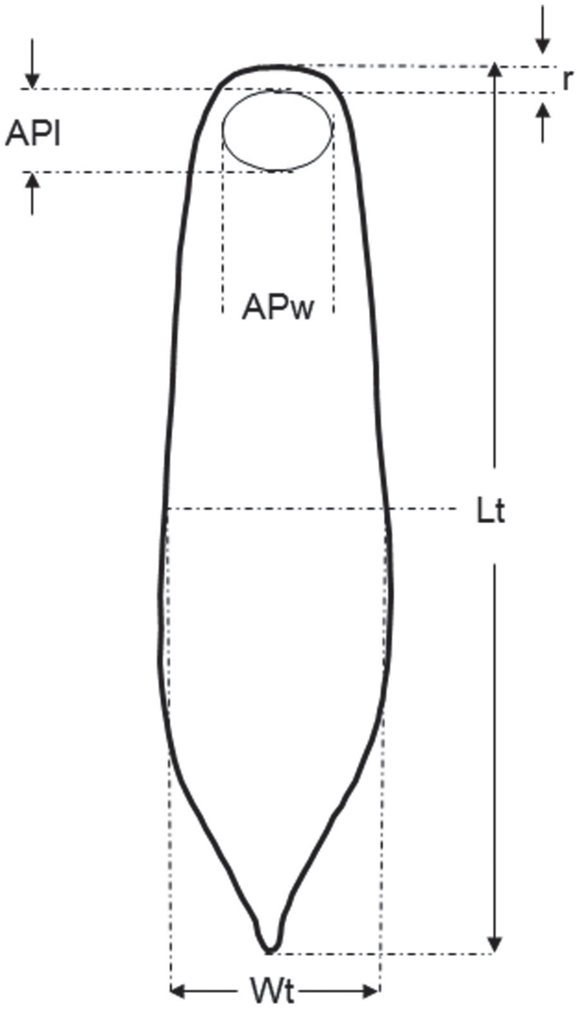

Fig. 6. Shell outline and position of measured morphometric axes of. *P. jenswendti*. Ventral view showing the five morphometric variables measured for statistical characterization, where Lt – total shell length, Wt – shell width, APl – length of the pseudostomal aperture, APw – width of the pseudostomal aperture, r – length of the rim anterior to the pseudostomal aperture.

scales are more rectangular than the large scales (0.8–1.5 x 1.8–2.6 μm) and they dominate on the aperture and interstices.

Biometry: Table 3 shows the morphometric characteristics of *P. jenswendti*. Values of coefficient of variation for morphometric characteristics are similar to those presented by *P. bergeri* and *P. bonneti*. Shell length, shell width, length and width of pseudostomal aperture, width of rim anterior to the pseudostomal aperture and Wt/Lt ratio are relatively constant with a CV 8.5–15.5%. The exception is the APl/r ratio which presents a comparatively higher variability (CV 23.4; Table 3). All variables are normally distributed, indicating that *P. jenswendti* is a size-monomorphic species (Fig. 8a, b). As documented in Table 4 slightly positive correlation were only found between shell length and pseudostomal aperture width (r = 0.41; n = 26, $p < 0.05$). In turns shell width was slightly correlated with pseudostomal aperture width (r = 0.41; n = 26, $p < 0.0001$) indicating suggesting that shell dimensions vary independently.

Composition and abundance of species

In what follows, information of testate amoebae abundance in summer layers in each site is given.

Mocho-Choshuenco Volcano (2000 m a.s.l.).

Bright field analyses of firn cores revealed only one summer layer between 8.4 and 10 m. It corresponds to the 2004/05 period. Here, only *T. enchelys* was identified with a total abundance of 2.2 ind mL^{-1} and comprising 73.3% of all individuals (Table 2). SEM analysis of a few of the non-identified individuals suggested the presence of *T. lineare*.

Mocho-Choshuenco Volcano summit (2422 m a.s.l.).

Biological analyses of this core showed four layers at 5.4–6.4, 6.8–7.4, 7.6–8.4 and 8.6–9.2 m that corresponds to the periods 2004/05, 2003/04, 2002/03 and 2001/02, respectively. In the 2004/05 layer three species were identified; *T. enchelys*, *P. jenswendti* and *P. bergeri*, with total abundance of 40.5, 2.5 and 3.1 ind mL^{-1}, respectively. This layer presented the highest abundance of testate amoebae among all cores (52.7 ind mL^{-1}) in all periods, with *T. enchelys* displaying the highest abundance (Table 2). In the 2003/04 period two species were observed; *T. enchelys and P. jenswendti*. Their total abundances were 4.3 and 1.0 ind mL^{-1}, re-

Table 4. Correlation coefficients between shell measurements of the *Puytoracia jenswendti* nov. sp on glaciers from Mocho-Choshuenco and Osorno volcanoes. Significant relationship, Student's t-test; *** P < 0.001, * 0.05 > P > 0.01, NS – not significant.

Characters	Lt	Wt	APw	r
Lt	–			
Wt	0.28NS	–		
APw	**0.41***	**0.75*****	–	
API	0.27	–0.01NS	0.03NS	
r	0.03	0.34NS	0.06NS	–

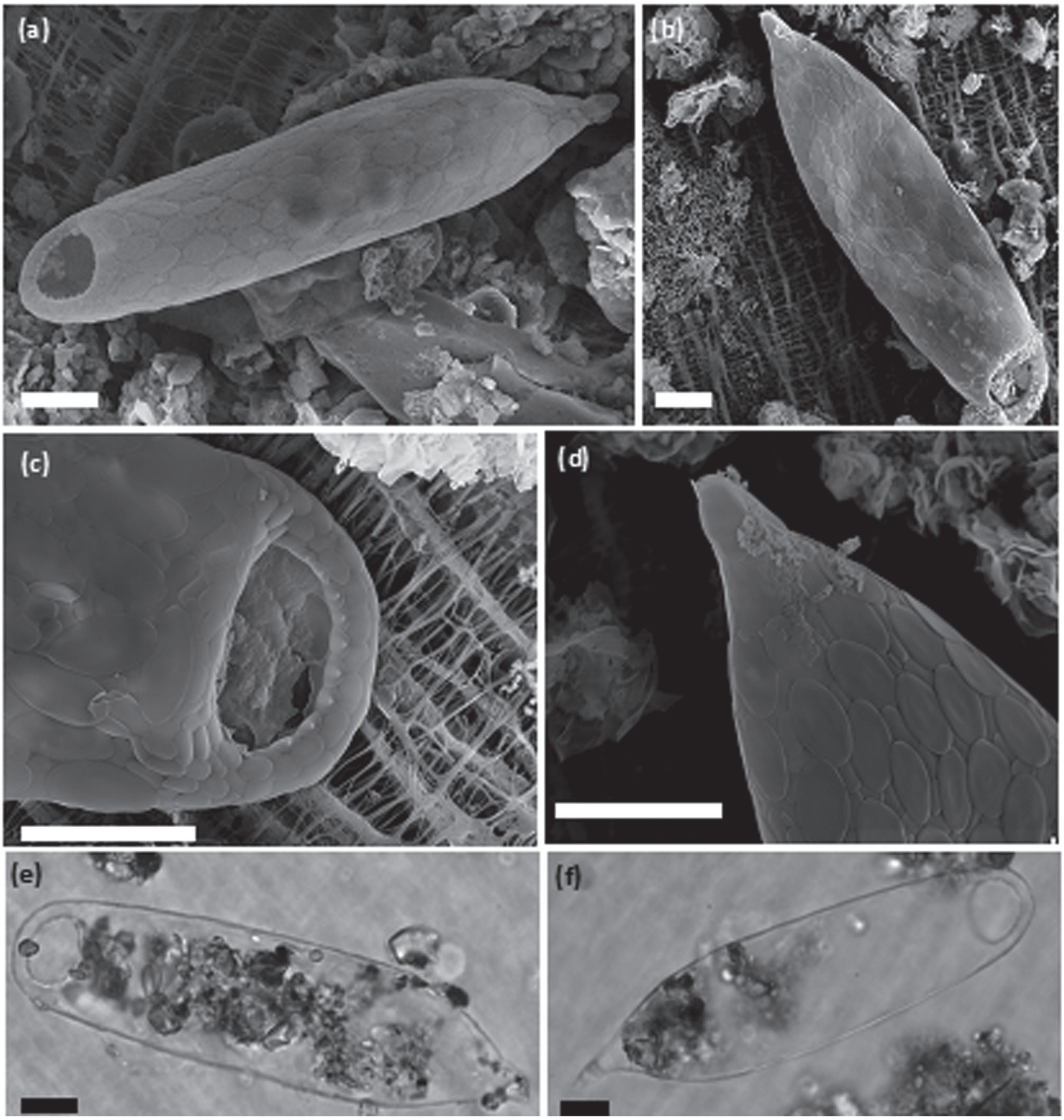

Fig. 7. *Puytoracia jenswendti* photographs **(a)–(b)** ventral view and idiosomes, both large and small elliptical scales on the test surface; **(c)** the pseudostomal aperture; **(d)** terminating in a horn or tip; **(e)–(f)** ventral views. (a)–(d) SEM images and (e)–(f) BFM images. Scale bars: 10 µm.

spectively. Here *T. enchelys* presented the highest abundance (Table 2).

During 2002/03, three species were observed; *T. enchelys*, *P. jenswendti* and *P. bergeri*, with total abundance of 8.8, 2.0 and 1.8 ind mL^{-1} a. *T. enchelys* was the most abundant species (Table 2).

In the last summer layer 2001/02, *T. enchelys* and *P. bergeri* were observed with total abundance of 2.9 and 0.2 ind mL^{-1}. SEM random analysis corroborated the presence in this layer of only the above-mentioned species.

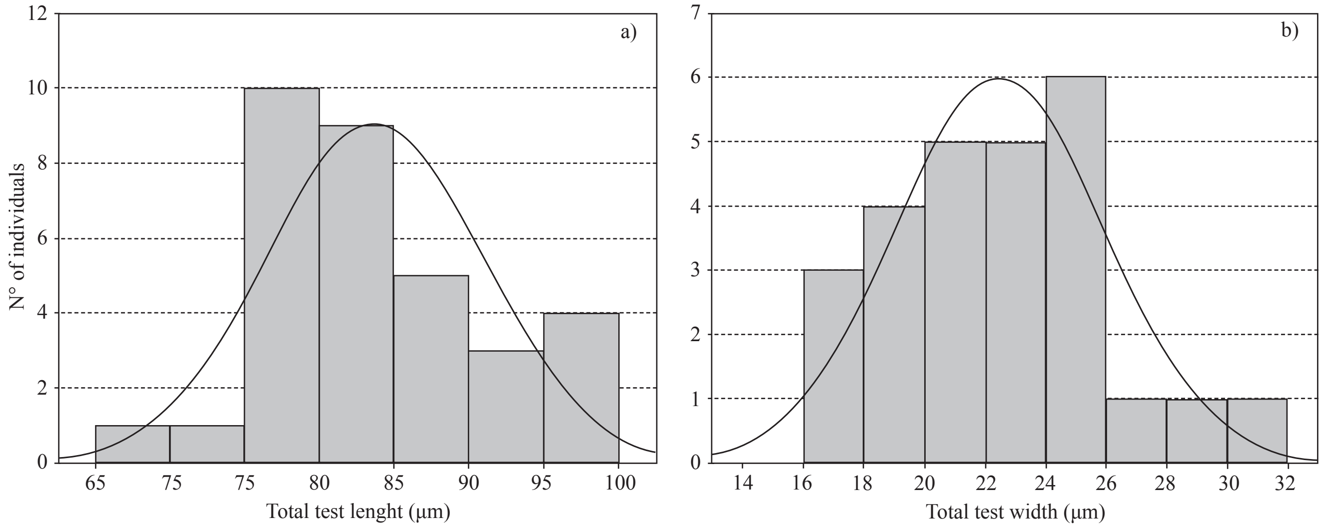

Fig. 8. Frequency plots of body dimensions in *Puytoracia jenswendti*. **(a)** The histogram shows the size frequency of total length and **(b)** the size frequency of shell width. The curves are fitted by Kolmogorov-Smirnov goodness for fit test.

Summit of Osorno Volcano (2652 m a.s.l.)

In this core two summer layers at 5.6–7.2 m and 8.2–10.0 m that correspond to the melting periods of 2004/05 and 2003/04 were identified. In the layer 2004/05 only *T. enchelys* was observed *with* an abundance of 1.0 ind mL^{-1} (Table 1). During 2003/04, two species were observed; *T. enchelys* and *P. jenswendti* at equal abundance (0.82 ind mL^{-1}; Table 1). Random SEM analysis corroborated that only these two species were present.

DISCUSSION

The presence of protozoa in glaciers was first recorded by Santibáñez *et al*. (2008) after performing biological analysis of firn cores from Mocho-Choshuenco and Osorno volcanoes in the southern Andes. Further analysis revealed unambiguously that protozoa present in the firn cores correspond to testate amoebae, a polyphyletic artificial group with extensive intraspecific phenotypic plasticity (Wanner 1999). At present this group is formed by ca. two thousand species distributed into three unrelated taxonomic levels, one Phylum (Granuloreticulosa) and two orders (Arcellinida and Euglyphida, Mitchell *et al*. 2008). The order Euglyphida is ubiquitous and characterized by filose pseudopodia. It is distributed world wide, including ice-free Arctic and Antarctic regions and mountains, particularly the genus *Trinema* has been reported (Lousier 1976; Balik 1994; Beyens and Chardez 1995; Todorov 1998; Trappeniers *et al.* 1999, 2002; Van Kerckvoorde *et al.* 2000; Matheeussen *et al.* 2005; Vincke *et al*., 2006a b, 2007). This genus comprise species that are early colonizers of new environments such as volcanic tephra (Smith 1985), pavement biofilm (Coupe *et al.* 2003), coal mining tailing and forest fire soil environments (Wanner and Dunger 2002, Wanner and Xylander 2003). It also includes species proposed to possess facultative psychrophile characteristics (Smith *et al*. 2008). On such grounds, it does not seem surprising that the most abundant protozoa found in glaciers belong to the genus *Trinema*, which was indeed recorded in all firn cores analyzed. *Trinema* is known to display a marked phenotypic variability in natural habitats and clonal cultures (Schönborn 1992, Meisterfeld 2000, Bobrov and Mazei 2004). This represents a challenge for taxonomic description and identification to the species level and the genus *Trinema* has been defined as a problematic group. Phenotypic variability can be produced by genetic differences, environmental influence and stochastic events of development (Vogt *et al.* 2008). In general *Trinema* is characterized by a plagiostome-type shell and circular scales. Identification to the species level rests essentially on body size, pseudostoma shape and biometric relations like length/wide ratio. The two species identified in glaciers correspond to *T. enchelys* and *T. lineare* which previously have been recorded in aquatic moss and soils habitats, including mountains and ice-free polar regions. It is worth mentioning that some authors have objected the identity of these two species (Meisterfeld 2000). Nevertheless, recent molecular analysis supports the notion that they actually correspond to different taxa (Lara *et al.* 2007). In the firn cores analyzed here, *T. enchelys* was dominant among the four identified testate amoebae species.

The genus *Puytoracia* was first described by Bonnet in 1970. Until now this genus includes only two species, *P. bergeri*, found in forest boreal wetlands in Quebec, Canada and soils in the Himalaya region of Nepal (Bonnet 1970, 1977), and *P. bonneti* reported in forests bogs in Canada (Nicholls 2006). More recently Krashevska (2007) documented for the first time the presence of *Puytoracia* in the Southern Hemisphere after recording *P. bergeri* over a 2000–3000 m altitudinal gradient in soil and trees in the rainforest of the tropical mountains in southern Ecuador. The *Puytoracia* genus is distinguished by the presence of elliptical scales. This distinct trait allowed to detecting the presence of specimens of this genus in ice cores. The two species that until now comprise this genus can be distinguished on the basis of pseudostome shape, which is deeply invaginated and double toothed in *P. bonneti* (Nicholls 2006) while *P. bergeri* displays a single-tootheed plagiostome-type pseudostome. On this basis, careful SEM scrutiny allowed the unambiguous detection of *P. bergeri* in three summers in one ice core from the summit of Mocho-Choshuenco Volcano. In addition, bright field analysis indicated that a set of testate amoebae specimens, present only in ice cores from the summit of Mocho-Choshuenco and Osorno volcanoes, are conspicuous in that they display larger body sizes and a characteristic tipped-ended inferior extreme that somehow resembles that seen in *Playfairina caudatum* which, however, displays circular instead of the elliptical scales that characterize the *Puytoracia* genus (Nicholls 2006). The set of specimens found only in firn cores from the summit of volcanoes have much larger large elliptical scales than those of *P. bergeri* such that they can be discerned under the bright field microscope. This testate amoeba taxon is also characterized by a smaller width/length ratio than all known testate amoebae species of the Trinematidae family and, in contrast with *P. caudatum*, it exhibits an invaginated aperture. The above features clearly distinguish this testate amoeba as a new *Puytoracia* species which we named *Puytoracia jenswendti*.

Individuals feeding and reproducing were recorded in all firn cores and periods, which indicate that the specimens were active on the glaciers. Although no ecological parameters were recorded in this study, it is possible to suggest that the testate amoebae abundance on Mocho-Choshuenco Volcano summit was higher since the temperature is higher than at Osorno Volcano summit.

Because of their diversity, shell conservation, short generation times, strong correlation of community structure with abiotic factors and their extended fossil record, testate amoebae provide an attractive tool for palaeoecological, evolutionary and palaeoclimate studies in different environments (Booth 2001, Hendon *et al.* 2001, Mitchell *et al.* 2001, Patterson and Kumar 2002, Wilmshurst *et al.* 2003, Bobrov *et al.* 2004, Payne and Mitchell 2007, Charman *et al.* 2007, Nguyen-Viet *et al.* 2007). Recently, the successful use of testate amoebae as seasonal markers in firn/ice cores from temperate glaciers has been documented (Santibáñez *et al.* 2008). Moreover, isotope analysis of testate amoeba shells might provide a unique tool to gather currently unavailable information on past temperature conditions from middle latitudes. Indeed, oxygen isotope analysis of diatom frustules has been broadly used in palaeoenvironmental reconstructions from lake sediments as an indicator of changes in the precipitation to evaporation ratio (Leng and Barker 2006).

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REFERENCES

Adl S. M., and many others (2005) The new higher level classification of Eukaryotes with emphasis on the taxonomy of Protists. *J. Eukaryot. Microbiol.* **52:** 399–451

Balik V. (1994) On the soil testate amoebae fauna (Protozoa: Rhizopoda) of the Spitsbergen Island (Svalbard). *Arch. Protistenkd.* **144:** 365–372

Beyens L., Chardez D., De Lantsheer R., with collaboration of De Bock P., Jacques E. (1986a) Testate amoebae populations from moss and lichen habitats in the Artic. *Polar. Biol.* **5:** 165–173

Beyens L., Chardez D., De Landtsheer R., with collaboration of De Baere D. (1986b) Testate amoebae communities from aquatic habitats in the Artic. *Polar. Biol.* **6:** 197–205

Beyens L., Chardez D. (1995) An annotated list of testate amoebae observed in the Arctic between the longitudes 27°E and 168°W. *Arch. Protistenkd*. **146:** 219–233

Bradley R. (1999) Paleoclimatology: reconstructing climates of the Quaternary. Harcourt. academic press, San Diego

Bobrov A., Mazei Y. (2004) Morphological variability of testate amoebae (Rhizopoda: Testacealobosea: Testaceafilosea) in natural populations. *Acta Protozool*. **43:** 133–146

Bonnet L. (1970) Nouveaux thécamoebiens su sol (VI). *Bull. Soc. Hist. Nat. Toulouse* **106:** 328–333

Bonnet L. (1977) Le peuplement thécamoebien des sols du Népal et son interet bogeographique. *Bull. Soc. Hist. Nat. Toulouse* **113:** 331–348

Booth R. K. (2001) Ecology of testate amoebae (Protozoa) in two lake superior coastal wetlands: implications for paleoecology and environmental monitoring. *Wetlands.* **21:** 564–576

Cavalier-Smith T. (2003) Protist phylogeny and the high-level classification of Protozoa. *Eur. J. Protistol.* **39:** 338–348

Charman A. J., Blundell A., ACCROTELM members. (2007) A new European testate amoebae transfer function for palaeohydrological reconstruction on ombrotrophic peatlands. *J. Quaternary Sci.* **22:** 209–221

Corliss J. O. (1994) An interim utilitarian ("user-friendly") hierarchical classification and characterization of the protists. *Acta Protozool*. **33:** 1–51

Coupe S. J., Smith H. G., Newman A. P., Puehmeier T. (2003) Biodegradation and microbial diversity within permeable pavements. *Eur. J. Protistol.* **39:** 495–498

Decloitre L. (1981) Le Genre *Trinema* DUJARDIN, 1841, Révision à jour au 31. XII. 1979. The genus *Trinema* DUJARDIN, 1841. *Arch. Protistenkd.* **124:** 193–218

Eichler A. M., Schwikowski M., Gäggeler H. W. (2001) Melt water induced relocation of chemical species in Alpine firn. *Tellus* **53**B: 192–203

Escobar I. (2008) Comportamiento de la precipitación y temperatura en la zona de los lagos de Chile y su relación con el retroceso de glaciares. Thesis. Facultad de Ciencias, Departamento de Física y Meteorología. Universidad de Valparaíso

Grospietsch T. (1958) Wechseltierchen (Rhizopoden). Kosmos Verlag, Stuttgart

Harnish O. (1958) II Klasse: Wurzelfüssler, Rhizópoda. In: Die Tierwelt Mitteleuropas, Band 1, Urtiere-Hohltiere-Würmer, Lieferung, (Eds. P. Brohmer, P. Ehrmann, G. Ulmer). Quelle & Meier, Leipzig, 1–26

Hendon D., Charman D. J., Kent M. (2001) Palaeohydrological records derived from testate amoebae analysis from peatlands in northern England: within-site variability, between-site comparability and palaeoclimatic implications. *The Holocene* **11:** 127–148

IPCC. (2001) Climate Change 2001: The Scientific Basis. Contribution of Working Group. In: the Third Assessment Report of the Intergovernmental Panel on Climate Change, (Eds. J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, D. J. Van der Linden, X. Dai, K. Maskell, C. A. Johnson). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

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

Kohshima S. (1984) A novel cold-tolerant insect found in a Himalayan glacier. *Nature.* **310:** 225–227

Kohshima S. (1987) Glacial biology and biotic communities. In: Evolution and coadaptation in biotic communities, (Eds. S. Kawano, J. H. Connell, T. Hidaka). Kyoto University, Faculty of Science, Kyoto, 77–92

Kohshima S., Yoshimura Y., Takeuchi N. (2002) Glacier ecosystem and biological ice-core analysis. In: The Patagonian Ice Fields: a unique natural laboratory for environmental and climate change studies, (Eds G. Casassa, F. Sepúlveda, R. Sinclair). Kluwer Academic/Plenum Publishers, New York, 1–8

Kol E. (1968) Kryobiologie. Biologie und Limnologie des Schnees und Eises. In: Kyovegetation. Die Binnengewäser. Thienemann, Vol. 24, (Eds. H. Elster, W. Ohle, E. Suttgart). Schweizerbart'sche Verlagsbuchhandlung

Lara E., Heger T. J., Mitchell E. A. D., Meisterfeld R., Ekelund F. (2007) SSU rRNA reveals a sequential increase in shell complexity among the Euglyphid testate amoebae (Rhizaria: Euglyphida). *Protist* **158:** 229–237

Leng M. J., Barker P. A. (2006) A review of the oxygen isotope composition of lacustrine diatom silica for palaeoclimate reconstruction. *Earth-Sci. Rev*. **75:** 5–27

Lorius C., Jouzel J., Raymaud D., Hansen J., Le Treut H. (1990) The ice-core record: climate sensitivity and future greenhouse warming. *Nature* **347:** 139–145

Lousier J. (1976) Testate amoebae (Rhizopoda, Testacea) in some Canadian Rocky Mountain soils. *Arch. Protistenkd.* **118:** 191–201

Lüftenegger G., Petz W., Berger H., Foissner W., Adam H. (1988) Morphologic and biometric characterization of twenty-four soil testate amoebae (Protozoa, Rhizopoda). *Arch. Protistenkd.* **136:** 153–189

Mattheeussen R., Ledeganck P., Vincke S., Van de de Vijver B., Nijs I., Beyens L. (2005) Habitat selection of aquatic testate amoebae communities on Qeqertarsuaq (Disko Island), West Greenland. *Acta Protozool*. **44:** 253–263

Meisterfeld R. (2000) Testate amoebae with filopodia. In: An Illustrated guide to the Protozoa, Vol. 2, Society of Protozoologists, (Eds. J. J. Lee, G. F. Leedale, P. Bradbury). Kansas, USA, 1054–1084

Mitchell E. A. D., Van der Knaap W. O., van Leeuwen J. F. N., Buttler A., Warner B. G., Gobat J-M. (2001) The palaeoecological history of the Praz Rodet bog (Swiss Jura) based on pollen, plant macrofossils and testate amoebae (Protozoa). *The Holocene* **11:** 65–80

Mitchell E. A. D. (2002) The identification of *Trinema* species living in *Sphagnum* and soils, University of Alaska Anchorage, 3 pages

Mitchell E. A. D., Charman D. J., Warner B. G. (2008) Testate amoebae analysis in ecological and paleoecological studies of wetlands: past, present and future. *Biodivers Conserv*. **17:** 2115–2137

Nicholls K. H. (2006) Form variation in *Puytoracia bergeri* Bonnet and a description of *P.bonneti* n. sp. (Rhizopoda, Filosea). *Eur. J. Protistol.* **42:** 155–163

Nguyen-Viet H., Bernard N., Mitchell E. A. D., Cortet J., Badot P. M., Gilbert D. (2007) Relationships between Testate Amoebae (Protist) Communities and atmospheric heavy metals accumulated in *Barbula indica* (Bryophyta) in Vietnam. *Microbiol. Ecol*. **53:** 53–65

Ogden C. G., Hedley R. H. (1980) An atlas to freshwater testate amoebae. Oxford University Press, Oxford

Patterson R. T., Kumar A. (2002) A review of current testate rhizopod (thecamoebian) research in Canada. *Palaeogeogr. Palaeocl.* **180:** 225–251

Payne R., Mitchell E. A. D. (2007) Ecology of testate amoebae from Mires in the Central Rhodope Mountains, Greece and development of a transfer function for palaeohydrological reconstruction. *Protist.* **158:** 159–171

Pohjola V. A. (2002) On the potential to retrieve climatic and environmental information from ice core sites suffering periodic melt, with specific assessment of the Southern Patagonia Ice Field. In: The Patagonian Ice Fields: a unique natural laboratory for environmental and climate change studies, (Eds. G. Casassa, F. Sepúlveda, R. Sinclair). New York, Kluwer Academic/Plenum Publishers, 125–138

Rivera A., Casassa G., Acuña C., Lange H. (2000) Variaciones recientes de glaciares en Chile. *Invest. Geogr. Chile* **34:** 29–60

Santibañez P., Kohshima S., Scheihing R., Jaramillo J., Shiraiwa T., Matoba S., Kanda D., Labarca P., Casassa G. (2008) Glacier mass balance interpreted from biological analysis of firn cores in the Chilean lake district. *J. Glaciol*. **54:** 452–462

Schönborn W. (1992) Adaptive polymorphism in soil-inhabiting testate amebas (Rhizopoda) – its importance for delimitation and evolution of asexual species. *Arch. Protistenkd.* **142:** 139–155

Schwikowski M., Brütsch S., Gäggeler H. W., Schotterer U. (1999) A high resolution air chemistry record from an Alpine ice core: Fiescherhorn glacier, Swiss Alps. *J. Geophys. Res*. **104:** 13, 709–719

Smith H. G. (1985) The colonization of volcanic tephra on Deception Island by Protozoa: long-term trends. *Brit. Antarct. Surv. B* **66:** 19–33

Smith H. G. (1992) Distribution and ecology of the testate rhizopod fauna of the continental Antarctic zone. *Polar. Biol*. **12:** 629–634

Smith H. G., Bobrov A., Lara E. (2008) Diversity and biogeography of testate amoebae. *Biodivers Conserv*. **17:** 329–343

Todorov M. (1998) Observation on the soil and moss testate amoebae (Protozoa: Rhizopoda) from Pirin Mountain (Bulgaria). *Acta Zool. Bulgar*. **50:** 19–29

Trappeniers K., Kerckvoorde A., Chardez D., Nijs I., Beyens L. (1999) Ecology of testate amoebae communities from aquatic habitats in the Zackenberg area (Northeast Greenland). *Polar. Biol.* **22:** 271–278

Trappeniers K., Van Kerckvoorde A., Chardez D., Nijs I., Beyens L. (2002) Testate amoebae assemblages from soils in the Zackenberg area, Northeast Greenland. *Arct. Antarct. Alp. Res.* **34:** 94–101

Uetake J., Kohshima S., Nakazawa F., Suzuki K., Kohno M., Kameda T., Arkhipov S., Fujii Y. (2006) Biological ice-core analysis of Sofisyskiy glacier in the Russian Altai. *Ann. Glaciol.* **43:** 70–78

Van Kerckvoorde A., Trappeniers K., Chardez D., Nijs I., Beyens L. (2000) Testate amoebae communities from terrestrial moss habitats in the Zackenberg area (North-East Greenland). *Acta Protozool.* **39:** 27–33

Vincke S., Ledeganck P., Beyens L., Van de Vijver B. (2004a) Soil testate amoebae from sub-Antarctic Île Crozet. *Antarct. Sci.* **16:** 165–174

Vincke S., Beyens L., Van de Vijver B. (2004b) Freshwater testate amoebae communities from Île de la Possession, (Crozet Archipelago, Subantarctica). *Arct. Antarct. Alp. Res.* **36:** 584–590

Vincke S., Gremmen N., Beyens L., Van de Vijver B. (2004c) The moss dwelling testacean fauna of Île de la Possession. *Polar. Biol*. **27:** 753–766

Vincke S., Van de Vijver B., Gremmen N., Beyens L. (2006a) The moss dwelling testacean fauna of the Stromness Bay (South Georgia). *Acta Protozool.* **45:** 65–75

Vincke S., Van de Vijver B., Nijs I., Beyens L. (2006b) Changes in the testacean community structure along small soil profiles. *Acta Protozool.* **45:** 395–406

Vincke S., Van de Vijver B., Ledeganck P., Nijs I., Beyens L. (2007) Testacean communities in perturbed soils: the influence of the wandering albatros. *Polar. Biol.* **30:** 395–406

Vogt G., Huber M., Thiemann M., van den Boogaart G., Schmitz O. J., Schubart C. D. (2008) Production of different phenotypes from the same genotype in the same environment by developmental variation. *J. Exp. Biol*. **211:** 510–523

Wanner M. (1999) A review on the variability of testate amoebae: Methodological approaches, environmental influences and taxonomical implications. *Acta Protozool*. **38:** 15–29

Wanner M., Dunger W. (2002) Primary immigration and succession of soil organisms on reclaimed opencast coal mining areas in eastern Germany. *Eur. J. Soil. Biol*. **38:** 137–143

Wanner M., Xylander WER. (2003) Transient fires useful for habitat-management do not affect soil microfauna (testate amoebae)-a study on an active military training area in eastern Germany. *Ecol. Eng.* **20:** 113–119

Wilmshurst J. M., Wiser S. K., Charman D. J. (2003) Reconstructing Holocene water tables in New Zealand using testate amoebae: differential preservation of tests and implications for the use of transfer functions. *The Holocene* **13:** 61–72

Yoshimura Y., Kohshima S., Takeuchi N., Seko K., Fujita K. (2000) Himalayan ice-core dating with snow algae. *J. Glaciol.* **46:** 335–340

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