Title: First evidence of testate amoebae in Lago Fagnano (54° S), Tierra del Fuego (Argentina): Proxies to reconstruct environmental changes

Article Type: Research Paper

Keywords: Tierra del Fuego, Lago Fagnano, testate amoebae, earthquake, environmental changes

Abstract: We report here the first findings of testate amoebae at high southern latitudes (54° S) from four gravity cores recovered in the Lago Fagnano (Tierra del Fuego, Argentina), where twelve taxa have been recognized. Among them, Centropyxis constricta "constricta", Centropyxis elongata, Diffugia globulus, Diffugia oblonga "oblonga", and Diffugia protaeiformis "amphoralis" are always present, while other taxa are randomly distributed. According to the sand/silt ratio in the different cores, the Total Organic Carbon content and the Carbon/Nitrogen ratio, as well as the presence/disappearance and abundance of testate amoebae from cluster analysis, we infer a correlation between major textural/granulometrical changes found in the cores and environmental changes. A seismic event occurred on 1949, which substantially modified the morphology of the eastern Lago Fagnano shoreline and the supply pattern from two main eastern tributaries of the lake, is recorded in the studied cores. This event has in part modified the distribution of testate amoebae taxa within the studied cores. Present results show that testate amoebae represent important indicators to detect changes occurring in the environment in which they live.
Dear Dr. Audemard,

Please find the revised version of the above-mentioned paper. We have remarkably changed the text, according to the reviewers requests and comments. In the new text the changes are marked in red. Below you will find the description of how we modified the text.

The main modifications made on the text are:
1) Sampling frequency on samples has been improved (smaller sampling range)
2) Details on palaeo-earthquakes in the study area have been inserted
3) Cluster analyses on testate amoebae have been performed

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Nevertheless, as the detected abrupt change is attributed to a major earthquake, a few references on this subject should be added to discuss this interpretation.

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Reviewer #2:
For the minor revisions, please refer to the comments in the attached edited manuscript file. I really hope that the author finds my comments helpful as he continues to revise the manuscript.

As far as concerns the minor edits in the text we accepted them and corrected accordingly.

Major Revisions
1- Title and Key Words
* Tierra del Fuego is divided between Argentina and Chile, and so is Lago Fagnano. In the introduction, the author states that the samples were collected from the Argentine side, so I suggest adding the name of the country to the title and the key words.

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Please refer to the following address for further correspondence:

Dr. Mauro Caffau
Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS) – Trieste (Italy)
Borgo Grotta Gigante 42/C - 34016 Sgonico (Trieste)
Fax +39-040327307; Tel +39-0402140350
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Trieste, June, 8, 2015
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Testate amoebae represent important indicators to detect changes occurring in the environment in which they live.
First evidence of testate amoebae in Lago Fagnano (54° S), Tierra del Fuego (Argentina): Proxies to reconstruct environmental changes

Mauro Caffau*, Davide Lenaz, Emanuele Lodolo, Massimo Zecchin, Cinzia Comici, Alejandro Tassone

*Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS), Trieste - Italy
*Corresponding author: mcaffau@ogs.trieste.it

Abstract
We report here the first findings of testate amoebae at high southern latitudes (54° S) from four gravity cores recovered in the Lago Fagnano (Tierra del Fuego, Argentina), where twelve taxa have been recognized. Among them, Centropyxis constricta “constricta”, Centropyxis elongata, Diffugia globulus, Diffugia oblonga “oblonga”, and Diffugia protaeiformis “amphoralis” are always present, while other taxa are randomly distributed. According to the sand/silt ratio in the different cores, the Total Organic Carbon content and the Carbon/Nitrogen ratio, as well as the presence/disappearance and abundance of testate amoebae from cluster analysis, we infer a correlation between major textural/granulometrical changes found in the cores and environmental changes. A seismic event occurred on 1949, which substantially modified the morphology of the eastern Lago Fagnano shoreline and the supply pattern from two main eastern tributaries of the lake, is recorded in the studied cores. This event has in part modified the distribution of testate amoebae taxa within the studied cores. Present results show that testate amoebae represent important indicators to detect changes occurring in the environment in which they live.

Keywords: Tierra del Fuego, Lago Fagnano, testate amoebae, earthquake, environmental changes.

1. Introduction
Testate amoebae (Protozoa: Rhizopoda) are unicellular organism characterized by enclosed, morphologically distinct tests. There are several types of tests, referred to as xenogenous
agglutinated or autogenous tests. The former are exceedingly variable, composed of particles derived from the environment such as lithic granules and sometimes organic detritus such as diatom frustules. Autogenous tests are composed of material secreted by the organism. (Charman, 2001; Scott et al., 2001; Patterson and Kumar, 2002). Testate amoebae are present in a wide range of terrestrial and aquatic environments, including freshwater lakes, brackish intertidal environment, wetlands, peatlands and, to a lesser extent, moist soil and forest litter (Charman, 2001; Scott et al., 2001, Booth, 2002, Charman et al., 2006). Testate amoebae have proven to be good environmental and paleoenvironmental indicators, both because their tests tend to be resistant to dissolution and well preserved in sediments, and because they are sensitive to a wide variety of environmental variables (Medioli and Scott, 1988; Warner and Charman, 1994; Charman 2001; Patterson and Kumar, 2002; Patterson et al., 2002) including metal and organic pollutant contamination, substrate type, salinity, levels of organics, oxygen concentration, water temperatures, water table fluctuations, humification and changes in intertidal flooding (e.g., Scott and Medioli, 1983; Patterson et al., 1996, 2002; Medioli et al., 1990; Collins et al., 1990; McCarthy et al., 1995; Asioli et al., 1996; Charman et al., 1998; Reinhardt et al., 1998; Patterson and Kumar, 2000, 2002). Most research on testate amoebae has focused on faunas found at higher latitudes (Scott and Medioli, 1983; Collins et al., 1990; Patterson et al., 1996, 2002; Tolonen et al., 1992; Kliza and Schröder, 1999). In South America testate amoebae have been reported, among others, in the wetlands of central Brazil (Green, 1975), in Lake Cocococha, Peru (Haman and Kohl, 1994), in a peat from the Puyehue National Park in Chile (Zapata et al., 2008), whereas the most southern occurrence was found in the Chilean Guamblin Island (Fernandez et al., 2012), located at about 45° S.

In this paper, we present the first evidence of the presence of testate amoebae at the latitude of 54° 30’ S in Tierra del Fuego, which is the southernmost latitude at which these organisms have been found to date. In order to achieve a better understanding of sediment provenance, transport mechanisms, inputs of organic matter from terrestrial sources, and on the sensitivity of testate amoebae to environmental changes, we combined analyses of organic matter parameters (C and N) with testate amoebae assemblages, their distribution within four boreholes, and the physical properties of sediments analyzed from four boreholes taken in the eastern part of Lago Fagnano.

2. General morphology of Lago Fagnano and depositional setting

Lago Fagnano (54° 30’ S, 68° W, 26 m above sea level), covering a total area of 596 km², is located in the central part of the Tierra del Fuego, and represents the southernmost and largest ice-free lake of the Southern Hemisphere (Fig. 1A). The basin trends roughly E-W, is more than 105 km long, and its width varies from 2.8 to 9.7 km. It occupies a significant segment of the South America-
Scotia transform plate boundary, which in Tierra del Fuego is known as the Magallanes-Fagnano fault system (Lodolo et al., 2002, 2003, 2007; Tassone et al., 2005; Menichetti et al., 2008). Repeated bathymetric and seismic surveys carried out along the entire length of the basin (Waldmann et al., 2008, 2010a, 2011; Zanolla et al., 2011; Lodolo et al., 2012; Esteban et al., 2014) have delineated in detail its morphological setting and sedimentary architecture. Data show that the basin floor is divided into two principal parts, separated by a morphologically complex, shallow relief located in the central part of the lake. This suggests that the basin is composed of at least two sub-basins. The deepest depression, with a maximum water depth of 206 m, is found in the eastern sub-basin, whereas the western half of the lake has a maximum water depth of 165 m (Zanolla et al., 2011). Combining geophysical data with field observations and regional maps, Waldmann et al. (2010b) asserted that the Lago Fagnano region has experienced several phases of glacier growth and retreat since the Last Glacial Maximum. The peculiar shape and morphology of the lake, and its location within the principal displacement zone of the Magallanes-Fagnano fault system, suggests that the tectonic activity along this lineament has controlled the formation and development of the basin at least in its early times. However, the Late Quaternary glacial activity played an important role in shaping the bottom morphology and the surrounding landscape, as testified by the geometry and sedimentary setting of the deposits filling the basin, and by the widespread glacial landforms surrounding the lake coastline (Coronato et al., 2009).

Currently, the main tributary of the eastern sector of the Lago Fagnano is the Rio Turbio. The climate of this region is Alpine, with a strong winter sub-polar Antarctic influence, and is under the south-westerly wind effect, bringing moisture and humidity to the region during austral summers (Rabassa et al., 2000). The average air temperature is about +5 °C (Tuhkanen, 1992). Precipitation is essentially of orographic nature (about 550 mm mean annual precipitation), and the drainage basin belongs to the vegetation zone of the subantarctic deciduous forest (Richter et al., 2010).

Petrophysical, sedimentological and geochemical studies of a Holocene lacustrine laminated succession, performed on selected cores (Waldmann et al., 2008, 2010a, 2011; Moy et al., 2011), revealed significant variations in major and trace elements, as well as in organic content, suggesting high variability in environmental conditions. Constituents of recent sediments are both biogenic and lithogenic, consisting of fresh water lacustrine biogenic material and variable amounts of reworked organic and clastic detritus delivered by rivers. In the area of Rio Turbio estuary, the sediment supply is characterized by silty suspended particles. Since the stream provides old organic carbon from plants and grassland, soil or peat material eroded from the nearby land, Moy et al. (2011) pointed out that radiocarbon ages of bulk organic matter in core samples from the central area of the
lake are unreliable. These datings demonstrate that the first 2.5 m of sediment has been deposited during Holocene time, but the ages of samples collected from the cores are irregularly distributed and range from ca. 0.5 to 11 kyr B.P. (Moy et al., 2011). Because the cores analyzed in this study are likely more directly under riverine influence than those of the central lake due to their vicinity to the Rio Turbio mouth, it is expected that the sedimentation rate, as well as the thickness of Holocene sediments and the amount of reworked organic matter are higher, which would make any $^{14}$C date questionable. Therefore, tacking also into account that the thickness of present cores does not exceed 0.95 m, that is significantly lower than that of the cores of the central lake, we refer to the core material generally as Holocene sediment. In order to obtain an approximate age, Waldmann et al. (2008) counted the light/dark laminae couplets present in their core sediments of Lago Fagnano, considering that a couple of laminae deposited in one year.

Changes in the geochemical characteristics of lake sediments may indicate variations in the sedimentation pattern through time. These changes may indirectly occur following both strong climatic oscillations and seismic events. In the case of Lago Fagnano, a Moment magnitude = 7.5 earthquake occurred on December 17, 1949 (Lomnitz, 1970), which significantly modified the landscape, the hydrographic net, and the shoreline of the eastern Lago Fagnano (Figs. 1C; 1D). Following this event, a gravel barrier along the Lago Fagnano eastern shoreline was created, modifying the flow pattern of the two former principal tributaries represented by the Rio Turbio and Rio Tolluin (Fig. 1B). A lagoon-like area (a sag pond) was thus created immediately following the earthquake (Fig. 1C,D), and the inundation caused a dead forest of *Nothofagus pumilio*, as presently observable (Costa et al., 2006). Today, the only output of the lagoon-like area is represented by the mouth of the Rio Turbio (Fig. 1B), as seen from satellite images which show the presence of currently buried stream outputs in correspondence of the northern sector of the sag pond. Fingerprints of palaeo-earthquakes enucleated along the Magallanes-Fagnano fault system have been also recognized in the study area by seismic records acquired in the Lago Fagnano, and represented by Holocene slope failures and megaturbidites (Waldmann et al., 2011).

3. Materials and methods

The meter-length gravity cores analyzed in this paper have been recovered in the vicinity of the eastern shore of Lago Fagnano on March 2010 (Lodolo et al., 2012). Sampling included smear slide analysis to characterize sediment type and the fossiliferous fraction. For the analysis of the lithological composition, 10 cm$^3$ sample volume of wet sediment for a total of 97 sediment samples were taken at approximate 4 cm intervals from four gravity cores. The percentage content of sand, silt and organic material, including macrophytes, terrestrial plant remains, wood remains,
Gyrogonites of Characeans, seeds and testate amoebae, was determined. Layers of macrophytes, terrestrial plant remains, and dark laminations are usually present and well distributed along the four cores, although at different concentrations in the analyzed samples. To calculate the percentage content of sand and silt of the samples, wet sediments were dried in oven at 40 °C and hence weighted. Samples were subsequently disaggregated with 10 V hydrogen peroxide, washed through 0.063 mm sieve, dried and weighted. Each dried sediment was further analyzed at the optical microscope, to describe the biogenic and detrital inorganic material.

For organic content measurements, the sediment sample was freeze-dried and grounded in a mortar, then pestled and the fraction ≥ 1 mm was separated from the rest of the sample; a 1 mm mesh was used instead of 250 µm because of the coarse grain size of the sediments. Two replicates of about 8-12 mg were weighted on a microultrabalance Perkin Elmer mod. AD-4 (0.1 µg accuracy). Total Nitrogen (TN) and Total Organic Carbon (TOC) were measured using an elemental analyzer CHNO-S Costech mod. ECS 4010. Known amounts of standard Acetanilide (C₈H₉NO – Carlo Erba; Assay ≥ 99.5 %) were used to calibrate the instrument. For TOC analysis, subsamples were treated with subsequent additions of hydrochloric acid at increasing concentration (0.1 N and 1 N) to remove the carbonates (Nieuwenhuize et al., 1994). The detection limit (DL) of the instrument was 0.004 % dwt (dry weight) for TN and 0.14 % dwt for TOC.

For the analyses of the testate amoebae, 3 cm³ sample volume of wet sediment were taken at approximate 4 cm intervals throughout the cores and prepared following the method described by Scott et al. (2001). Sediment samples were examined (Leica microscope) under x20 and x80 magnification, and 200-300 testate amoebae were counted. Selected specimens were photographed on a SEM (Scanning Electron Microscope) Leica Stereoscan 430i. Testate amoebae were identified to the strain level using Burbige and Schöder-Adams (1998) and Reinhardt et al. (1998).

X-ray diffraction patterns were obtained on bulk sediment samples spread out on aluminum plates using a STOE D 500 X-ray diffractometer at room temperature. CuKα radiation was used through a flat graphite crystal monochromator. Samples were investigated in a range between 2 and 40° of 2 θ angle with a step scan of 0.1° and 2 s measure time.

R-mode cluster analysis was performed computing Bray and Curtis (1957) dissimilarity (i.e., a statistic used to quantify the compositional dissimilarity between two different sites, based on counts at each site) between variables (i.e. species), and then using complete linkage as an agglomerative algorithm. Cluster analysis was carried out using a specific software (http://www.r-project.org). No other multivariate techniques were used since only one environmental parameter (sand/silt ratio) was available for a considerable number of samples.
4. Results

4.1. Sedimentological analysis

In all the analyzed cores the sand percentage is always lower than the silt + clay fraction, with ratios ranging from 0.09 to 0.82, except for one sample (at 24 cm depth) in the core CF-1B. Notably, the amount of sand is usually higher in the upper 40 cm of all cores apart in the core CF-3A, where the amount of sand is higher in its lower part. It is also to be noted that in the two northern cores there is a concomitant increase of sand fraction (sand/silt ratio about 0.8) at 62-64 cm depth. Percentages of sand and silt + clay are shown in Figs. 2, 3, 4 and 5. Sands are mainly composed of quartz and subordinately of feldspars. Clay minerals are also present. No appreciable changes in the percentages of the recognized minerals are recorded among the cores.

Core CF-3A was taken at a water depth of 19.8 m and recovered 92 cm of lacustrine deposits (Fig. 2). The sediment is thoroughly laminated, consisting of an alternation between 1.5-2 mm thick dark-gray silty laminae and 1-2 mm thick slightly lighter-colored laminae of clayey silt. A peak in sand/silt ratio is found at 62-64 cm from the top. Several layers of plant remains are also found along the core; they are up to 2-3 mm thick and are present at depths of 88, 78, 67, 56, 18, 15 and 8 cm. Other patches of organic content, less than 1 mm thick, are frequent and distributed at about 5 cm intervals along the core. Gyrogonites of Characeans are found at depth of 56 cm. A wood remain, 1.5 cm thick, was recovered at depth of 18 cm. Inorganic components mainly consist of poorly selected, rounded quartz grains.

Core CF-1B was taken at a water depth of 20.4 m and recovered 80 cm of lacustrine deposits (Fig. 3). The examined sediments consist of interlaminated silt to sandy silt, silt and clay, forming planar lamination. In detail, from the bottom up to 38 cm, the recovered material consists of gray silt with dark-gray silty clay laminae 0.5-2 mm thick. These sediments contain relatively scarce organic content, in places found in small lenses 2 to 4 mm thick, whereas scattered patches of macrophytes remains, Gyrogonites of Characeans and terrestrial plant are found at depths of 48 and 40 cm. Between these depths, sand contents average is 27.4%. A peak in sand/silt ratio is found at 62-64 cm depth. From 38 to 4 cm, the succession consists of sandy silt and silt laminae 1 to 2.5 mm thick. The abundance of lenses and layers of vegetal remains give a dark-gray color to these sediments. Abundant Gyrogonites of Characeans are found in two organic layer at depths of 24 and 8 cm.

Core CF-10 was retrieved at a water depth of 51 m and recovered 95 cm of lacustrine deposits (Fig. 4). The examined sediment consists of interlaminated clayey silt and sandy silt, forming planar lamination. An upward increase in sand/silt ratio is found starting from 64 cm depth.
In detail, from the bottom up to 64 cm, the sediment consists of dark-gray silt with black silty clay laminae 0.5-2 mm thick, apart from the lowermost part of the core (95-85 cm), where organic matter have been smeared during the core cutting, creating blackish waves. This is observed also in the 48-50 cm interval. Layers with abundant vegetal remains are present from depth of 64 cm to the top of the core. In particular, a 8 mm thick layer with abundant Characeans Gyrogonites is revealed at 30 cm depth. Other layers with abundant vegetal remains, containing some Characeans Gyrogonites, are up to 3-5 mm thick and are found at depths of 28, 20, 10 and 8 cm. Vegetal remains are present also in the upper 12 cm the core. Core CF-10 presents the lowest amount of sand, which progressively increases toward the top. The highest sand concentration, 32 wt. %, corresponds to an organic layer between 29 and 32 cm depth.

Core CF-F was retrieved at a water depth of 36 m and recovered 83 cm of lacustrine deposits (Fig. 5). The examined sediment consists of interlaminated clayey silt and sandy silt, forming planar lamination. An upward increase in sand/silt ratio is found starting from 64 cm depth. In detail, from the bottom up to 64 cm, the sediment is composed of dark-gray silt with black silty clay laminae 0.5-2 mm thick. This interval contains terrestrial plant remains that consist of wood fragments with sizes from 2 to 6 mm and seeds found in two thicker layers 14 and 5 mm thick, at depths of 75 cm and 66 cm, respectively. From 64 cm to the top of the core the sediment consists of gray sandy silt with dark-gray silt laminae, 1 to 2.5 mm thick. It contains terrestrial plant and macrophytes remains, 2-3 mm thick.

4.2. Total Organic Carbon and Nitrogen analyses

The concentration of Total Organic Carbon (TOC) in a lake is fundamental for characterizing the abundance of organic matter in the sediments (Meyers and Teranes, 2001; Meyers, 2003), as its variation and distribution serve to trace its provenance and the mechanism of sedimentation. The Carbon/Nitrogen (C/N) ratio allows to differentiate between sources of organic matter in lake sediments, whether lacustrine or terrestrial (Meyers and Lallier-Vergès, 1999; Meyers and Teranes, 2001; Meyers, 2003). Lacustrine algae are cellulose-poor and protein-rich with low C/N values, usually between 4 and 10, while terrestrials plants are cellulose-rich and protein-poor, providing higher C/N values that could reach a range between 17 and 42 (Meyers, 2003). TOC contents (percentage of dry weight) and C/N ratio values obtained from the analysis of the four cores are presented in Figs. 2, 3, 4 and 5.

In core CF-3A, TOC content ranges between 0.9 and 1.3 %, with an increment at the top of the sample, where it reaches a value of 1.6 % dwt. Overall, TOC shows an increase upward (Fig. 2). The C/N values are more or less constant, ranging from 11 to 14. To note that the lowest values of
TOC (0.9 %) and C/N ratio (10) are found at 8 cm depth (Fig. 2). Core CF-1B shows TOC values ranging between 1.2 and 1.7 %, slightly higher than in the previous core. Overall, TOC increases upward up to 15 cm depth and then decreases (Fig. 3). The C/N ratio is between 14 and 16, and only at the top it decreases to 12 (Fig. 3). In core CF-10, TOC is usually lower than in cores CF-3A and CF-1B, and shows an overall upward increase (Fig. 4). Very low TOC values, 0.5 to 0.7 %, are reported in the lower part of the core, from 74 to 93 cm depth, while a value of 0.8 % is found at 40 and 20 cm depth. Along the rest of the core, the value of TOC is in the range 1.1 - 2.1% dwt, the latter corresponding to a layer with a high concentration of vegetable remains at 29 cm depth. C/N ratios are low (8-11) from the bottom to 74 cm depth, while are higher (12-18) from 74 cm to the top, with the exception of a ratio equal to 10 at 20 cm depth. This is the only core where it is possible to find C/N values below 10, which is indicative of poor to absent terrestrial contribution (Fig. 4). The first peak in both C/N and TOC in core CF-10 is recorded at 64 cm depth (Fig. 4). TOC content in core CF-F does not show relevant variability, being comprised between 2 and 2.3 % dwt (Fig. 5). Slightly lower values are recorded towards the bottom (1.6 % dwt; 48 cm depth) and at the top (1.9 % dwt, 1 cm depth). Notably, these higher concentrations are determined by a higher volume of organic matter in the sediment. Similarly, C/N ratio variations are limited and comprised between 14 and 16 (Fig. 5).

4.3. Age estimation
The approximate time interval represented by the core sediments was estimated by counting the light/dark coupled laminae in the cores, according to the method applied by Waldmann et al. (2008) for cores recovered in the central part of the Lago Fagnano. The laminae counts suggest a mean sedimentation rate in the order of 1 cm/yr, suggesting that the studied core sediments accumulated during the last century.

4.4. Testate amoebae distribution in the cores
Species diversity of the testate amoebae is generally low, with 12 taxa identified from the four examined cores (Fig. 6 and Table 1, 2, 3 and 4). Preservation of tests was generally good in all the cores.

4.4.1. Core CF-3A
Only four species, *Centropyxis elongata*, *Diffugia oblonga* “oblonga”, *Diffugia protaeiformis* “amphoralis” and *Diffugia viscidula*, are present in all the collected samples (Table 1).
The relative abundance of *D. protaeiformis* “amphoralis” is present in a range of 25.0 to 36.4 % from the bottom of the core up to 60 cm depth and then reaches a maximum value of 43.6 % at 56 cm depth. Subsequently it decreases at 40 cm with a minimum of 9.5 %, maintaining values below 16.1 % up to 28 cm. A trend of increasing values of its relative abundance is seen in the upper part of the core from 20.9 % at 24 cm up to 34.6 % at the top of the core. The abundance of *D. oblonga* “oblonga” is found below 17.2 % from the bottom of the core up to 44 cm depth, including a lower range of 3.7 to 6.8% from 80 to 56 cm, and subsequently increases to a maximum value of 28.6 % at 40 cm. At 28 cm there is another peak of 25%, followed by a range of 23.1 to 13.5 % up to the top of the core. Similarly, the relative abundance of *Diffugia oblonga* “linearis” presents a peak at 40 and 36 cm depth, although its overall abundance is very low, and the *Diffugia oblonga* “glans” is mainly present between 40 and 20 cm depth. The relative abundance of *D. viscidula* decreases at 40 and 20 cm depth with values of 4.8 and 3.9 %, respectively. There is a trend of decreasing values from 16.7 to 7.4% of the relative abundance of *C. elongata* from the bottom to a depth of 72 cm. Above this depth its abundance is similar, showing slight fluctuations. *Lagenodifflugia vas* appears at 64 cm depth and is always present in the upper part of the core, showing relative abundances between 12.9 and 30.8 %. Among the other taxa, *Diffugia urceolata* “lageniformis” is mainly present below 40 cm depth and is very low between 32 and 28 cm depth. The relative abundance of *Cyclopyxis kahl* is low and it is present from 80 to 60 cm and from 16 to 8 cm depth. Similarly, the abundance of *Diffugia globulus* is very low and is mainly present in the lower part of the core and at 28 cm depth. *Centropyxis constricta* “constricta” shows a decreasing trend from the bottom towards the top of the core and disappears at 16 cm depth.

**4.4.2. Core CF-1B**

As in the previous core, only four species *C. elongata*, *D. oblonga* “oblonga”, *D. protaeiformis* “amphoralis” and *D. viscidula* are present in all the collected samples (Table 2). The most common taxon is *D. protaeiformis* “amphoralis”, with slightly variations from the bottom to 24 cm depth, with a peak of 24.1% at a depth of 44 cm. Above 20 cm up to the top of the core there is an abrupt increase of its abundance ranging from 30.0 to 34.8 %. The second most abundant species is *D. oblonga* “oblonga”. Two intervals may be distinguished, a low abundance from the bottom of the core up to 48 cm with a range of 8.7 to 14.3 % and a higher abundance from 44 cm up to the top, ranging between 16.0 to 24.5 %. The distribution of *D. viscidula* is preserved with slightly variations between 11.1% and 18.2% along the core, with a peak of 20.3% at 50 cm depth and two minimal values of 7.7% and 8.2% at 32 and 20 cm respectively. *Centropyxis constricta* “constricta” and *C. elongata* are decreasing in abundance towards the top of the core, showing a reduction trend
from the bottom to the top. As in core CF-3A, *L. vas* appears at 64 cm depth with a low abundance that maintains also at 62 cm, and subsequently shows an increasing trend with slight variations up to the top of the core. *Cyclopyxis kahli*, *D. globulus*, *D. oblonga* “glans”, *D. oblonga* “linearis” and *D. urceolata* “lageniformis” occur discontinuously along the core. They are usually absent near the top of the core.

### 4.4.3. Core CF-10

This core contains the highest number of taxa (Table 3). In fact, besides the species mentioned above for the previous cores, in this core occur the *D. oblonga* “tenuis”, although it is present only from 42 to 20 cm depth. The testate amoebae in this core are found only above 62 cm depth.

*Centropyxis constricta* “constricta” and *C. elongata* are the most abundant species. Two intervals may be distinguished, one of higher abundance: from 62 cm to 36 cm or 40 cm for both taxa respectively; and another with lower abundance up to the top of the core. *Cyclopyxis kahli*, *D. oblonga* “glans” and *D. urceolata* “lageniformis” are present in very low amounts (Table 3). *Diffugia oblonga* “oblonga” and *L. vas* appear at 36 cm whereas *D. viscidula* occurs at 32 cm depth and the relative abundance of these three taxa show a discontinuous trend. *Diffugia globulus*, *D. oblonga* “linearis” and *D. protaeiformis* “amphoralis” also show irregular or discontinuous trend, with relative abundance values well below 18.8% (registered at 8 cm depth for *D. protaeiformis* “amphoralis”), except for a peak of 22.4% at 42 cm depth for *D. globulus*.

### 4.4.4. Core CF-F

Five species, in particular *C. constricta* “constricta”, *C. elongata*, *D. globulus*, *D. oblonga* “oblonga”, and *D. protaeiformis* “amphoralis”, are present in all the collected samples (Table 4). The most abundant species is *D. oblonga* “oblonga”, the distribution of which shows a trend similar to that of TOC (Fig. 5). The second most abundant species is *D. protaeiformis* “amphoralis”, showing an increase from 48 cm depth to the top. *Centropyxis constricta* “constricta”, shows a variable abundance, being comprised between 13.6 and 16.7%, with a marked decrease at 64 cm depth, where its abundance is 10.7%. The abundance of *C. elongata* decreases from the bottom (about 10.5%) to 48 cm depth (6.3%), and then increases up to 11.1%. *Diffugia globulus* shows variable abundances and is less frequent at 48 cm depth. *Lagenodiffugia vas* appears at 64 cm (3.6%) and increases up to 10.4% at 48 cm depth. *Diffugia oblonga* “glans”, *D. oblonga* “linearis” *D. oblonga* “tenuis”, *D. urceolata* “lageniformis” and *D. viscidula* occur discontinuously along the core. Among these, *D. oblonga* “tenuis” is the only species present at the top of the core. As in core
CF-3A, *L. vas* appears at 64 cm depth with a low abundance that maintains also at 62 cm, and subsequently shows an increasing trend with slight variations up to the top of the core.

### 5. Discussion

From the studied sedimentary cores collected in the eastern Lago Fagnano, it is possible to discriminate between the two cores that are closer to the coast (i.e., the northern cores CF-3A and CF-1B) and those that are more distant (i.e., the southern cores CF-F and CF-10) (Fig. 1B). The latter show a very similar trend, with an average upward sand content increase (Figs. 4 and 5). The northern cores show a similarity in their lowest part, with a peak of the sand/(silt + clay) ratio at 62-64 cm depth (Figs. 2 and 3). A minor sand content increment at this depth is found also in cores CF-F and CF-10 (Figs. 4 and 5). An average upward sand content increment above 50 cm depth characterizes also core CF-1B (Fig. 3), whereas this increment does not occur in core CF-3A (Fig. 2).

In general, TOC content shows an overall slight upward increment in cores CF-3A, CF-1B and CF 10, with a decrease in the uppermost part of core CF-1B, whereas a clear trend is not apparent in core CF-F (Figs. 2-5). The C/N ratio, instead, does not show a trend in the cores except in core CF-10, where it exhibits an overall upward increment, although discontinuous (Figs. 2-5). It is noteworthy that core CF-10 records the first input of terrestrial material at 64 cm depth, as the C/N ratio increases from about 8 to 15, and this is accompanied by an increase in TOC values at the same depth (Fig. 4).

A positive correlation is found between the percentage abundance of testate amoebae species indicative of abundant organic matter, such as *D. oblonga “oblonga”* and *D. protaeiformis “amphoralis”* (Collins et al., 1990; Lorencová, 2009), and TOC content. In fact, the overall upward TOC increment in cores CF-3A, CF-1B and CF-10 is accompanied by an overall upward increment of these species, although less evident in core CF-3A (Figs. 2-4). In contrast, a negative correlation is found between the percentage abundance of testate amoebae species that tolerate a low organic matter content, such as *C. constricta “constricta”* and *D. urceolata “lageniformis”* (Patterson et al., 1985, 1996), and TOC in the same cores, excluding the lower part of core CF-10 that does not contain testate amoebae (Figs. 2-4). The same trend is exhibited also by *C. elongata* (Fig. 2-4). *Centropyxis constricta “constricta”* and *D. urceolata “lageniformis”*, however, are also characteristic of cold climate conditions (Collins et al., 1990; Patterson et al., 1996). Tacking into account that the temperature of the study area rose during the last century (Rabassa, 2010), the observed upward decrease in percentage abundance of these two species, and their disappearance in
the upper part of almost all cores (Figs. 2-5), might be related to either overall warming or TOC content trend, or both. Also *D. globulus* is an indicator of cold climate (Schönborn, 1984; Collins et al., 1990; Asioli et al., 1996) and this species is more abundant in the lower part of all cores excepting for core CF-10 (Figs. 2-5).

The lack of a clear trend in TOC content in core CF-F is reflected by a relatively erratic abundance of *D. oblonga* “oblonga” and *C. constricta* “constricta”, whereas the percentage abundance of *D. protaeiformis* “amphoralis” overall increases upward, as observed in the other cores, and that of *D. urceolata* “lageniformis” decreases upward (Fig. 5). The reason for the upward increase of percentage abundance *D. protaeiformis* “amphoralis” in core CF-F is unclear, whereas the upward decrease in the same core of *D. urceolata* “lageniformis”, as well as of *D. globulus*, probably reflects the overall temperature increase during the last century. All these evidence suggest that, between the two species that record changes of both TOC content and temperature, *C. constricta* “constricta” is probably more sensitive to TOC, and less sensitive to temperature changes than *D. urceolata* “lageniformis”, as only the latter shows an upward decrease of percentage abundance in core CF-F (Fig. 5). In contrast to the species mentioned above, *C. kahli*, *D. oblonga* “glans”, *D. oblonga* “linearis”, *D. viscidula* and *L. vas* do not exhibit a clear relationship with TOC content (Figs. 2-5), possibly due to either a minor sensitivity to environmental changes or a more complex sensitivity to a combination of several variables.

The reason for the lack of testate amoebae in the lower part of core CF-10 is unclear. Some testate amoebae species (*C. constricta* “constricta”, *C. elongata*, *C. kahli*, *D. globulus*, *D. oblonga* “linearis”, *D. protaeiformis* “amphoralis”) appear at 62 cm from the top, and both *C. constricta* “constricta” and *C. elongata* dominate in the central part of this core (Fig. 4). In cores CF-1B and CF-3A (Figs. 2 and 3), a greater concentration of sand is recorded just at depth of 62-64 cm, whereas a minor increase in sand content is found in core CF-F (Fig. 5). Taking into account a mean sedimentation rate of 1 cm/yr for the studied sediments (see Section 4.3), the layer at 62-64 cm depth accumulated about 62-64 years ago. This marked change in the cores might be related to a large amount of both sand and suspended organic material made available just after the earthquake event occurred on 1949 (Lomnitz, 1970) and transported far from the shoreline by currents.

This textural variation in sediment cores, which occurred just after the earthquake as a consequence of a change in the river input (increment in the sand content at a 62-64 cm depth in the cores), has also had an influence on the distribution and abundance of testate amoebae. This is seen especially in cores CF-10 and CF-F, which are the most southern and more distant from the coast of Lago Fagnano, while in the two cores CF-3A and CF-1B this variation in the distribution of taxa
was milder (see Figs. 2 to 5). This might reflect a particular sensitivity of this species to the seismic event.

Cluster analyses performed on testate amoebae assemblages, has shown that taxa can be grouped into three clusters (Fig. 7). The first cluster is characterized by the presence of *D. oblonga* “tenuis”, *D. oblonga* “glans” and *D. oblonga* “linearis”. The second cluster is represented by *C. kahli, D. globulus* and *D. urceolata* “lageniformis”. The third one collects all the other species. The first and the second cluster are represented by species that are usually present in low amounts and where species are alternatively present, i.e. when *C. kahli, D. globulus* and *D. urceolata* are present *D. oblonga* “tenuis”, *D. oblonga* “glans” and *D. oblonga* “linearis” are missing and *vice versa*.

6. Conclusions

The conclusions of this study can be summarized as follows:

(1) Here is reported the first findings of testate amoebae in the sediments of four gravity cores (CF-3A, CF-1B, CF-10, CF-F), acquired in the eastern part of the Lago Fagnano (Tierra del Fuego) at latitude of 54° S. This is the first evidence of these microorganism at those southern latitudes.

(2) Twelve taxa have been recognized. Among them *C. constricta* “constricta”, *C. elongata, D. globulus, D. oblonga* “oblonga”, and *D. protaeiformis* “amphoralis” are always present. *D. oblonga* “tenuis” is present only in the southern cores, while *C. kahli* misses in the southernmost core.

(3) An increase in sand/silt ratio at 62-64 cm depth is recognizable in all cores. Taking into account a mean sedimentation rate of 1 cm/yr for the studied sediments, the event found at 62-64 cm depth is inferred to be related to both sand and suspended organic material made available just after a 7.5 magnitude earthquake occurred on 1949, which severely modified the morphology of the eastern shoreline of the lake.

(4) The content of Total Organic Carbon (TOC) shows an overall, although discontinuous, upward increase in some cores (CF-3A, CF-1B and CF 10); The Carbon/Nitrogen (C/N) ratio, instead, does not show a clear trend excepting in core CF-10, where it exhibits an overall upward increase with values lower than about 10 (i.e. lacustrine) below 64 cm and higher than 11 upwards (i.e. terrestrial).

(5) Some testate amoebae show a relation with the variations of TOC. In particular, where TOC displays an overall increase, the percentage abundance of *D. oblonga* “oblonga” and *D. protaeiformis* “amphoralis” tend to increase, whereas that of *C. constricta* “constricta” and *D. urceolata* “lageniformis” dent to decrease. The latter two species, together with *D. globulus*, are also known to be characteristic of cold climate, and their percentage abundance exhibits a
marked upward decrease, very probably related to the overall increase of the mean temperature experienced by the study area in the last century.

(6) The appearance and disappearance of certain species as well as the change in sand/silt ratio found at 62-64 cm depth in the cores suggest that the earthquake event produced some environmental changes in all locations of the eastern Lago Fagnano.

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References


**FIGURE CAPTIONS**
Figure 1. (A) Shaded-relief map showing the general morphology of Tierra del Fuego, crossed by the Magallanes-Fagnano transform system (MFS). MS indicates the Magallanes Strait. White box indicates the easternmost sector of the Lago Fagnano where data have been acquired. (B) Google Earth® image of the eastern Lago Fagnano area, with the location of the gravity cores (black stars) used in this study. Bathymetric contours (in blue) every 10 m (Zanolla et al. 2011). The blue area indicates the lagoon nearby the estuary of the Rio Turbio, and the yellow area indicates the buried streams of the Rio Tolhuin. (C) Photographs of the lagoon at the eastern shoreline of the Lago Fagnano, taken before the 1949 earthquake, compared with the present-day shoreline (D).

Figure 2. Lithology, sand/silt percentage, TOC percentage, C/N ratio and relative abundance of testate amoebae in core CF-3A.

Figure 3. Lithology, sand/silt percentage, TOC percentage, C/N ratio and relative abundance of testate amoebae in core CF-1B.

Figure 4. Lithology, sand/silt percentage, TOC percentage, C/N ratio and relative abundance of testate amoebae in core CF-10.

Figure 5. Lithology, sand/silt percentage, TOC percentage, C/N ratio and relative abundance of testate amoebae in core CF-F.

Figure 6. Scanning electron micrographs of selected testate amoebae from the eastern Lago Fagnano: (1) Centropyxis constricta “constricta” from core CF-3A; (2-3) Centropyxis constricta “constricta”, from core CF-10; (4) Centropyxis elongata from core CF-F; (5-6) Centropyxis elongata ventral and dorsal view from core CF-10, respectively; (7-8) Cyclopyxis kahli from core CF-10; (9-10) Diffugia globulus from core CF-F; (11-12) Diffugia oblonga “glangs” from core CF-1B; (13-14) Diffugia oblonga “oblonga” from core CF-3A and CF-F, respectively; (15-16) Diffugia oblonga “linearis” from core CF-F and CF-10, respectively; (17-18) Diffugia oblonga “tenuis” from core CF-F and CF-10, respectively; (19-20) Diffugia protaeiformis “amphoralis” from core CF-3A and CF-F, respectively; (21-22) Diffugia urceolata “lageniformis” from core CF-3A and CF-F, respectively; (23) Diffugia urceolata “lageniformis” from core CF-10; (24-25) Diffugia viscidula from core CF-3A and CF-1B, respectively; (26-27) Lagenodifflugia vas from core CF-1B and CF-10, respectively.

Figure 7. Cluster analysis on testate amoebae. ccc = Centropyxis constricta “constricta”; ce = Centropyxis elongata; ck = Cyclopyxis kahli; dg = Diffugia globulus; do = Diffugia oblonga “glangs”; doo = Diffugia oblonga “oblonga”; dol = Diffugia oblonga “linearis”; dot = Diffugia oblonga “tenuis”; dpa = Diffugia protaeiformis “amphoralis”; dul = Diffugia urceolata “lageniformis”; dv = Diffugia viscidula; lv = Lagenodifflugia vas.

Table 1. Percentage abundance and total number of individuals counted of testate amoebae in core CF-3A.

Table 2. Percentage abundance and total number of individuals counted of testate amoebae in core CF-1B.
**Table 3.** Percentage abundance and total number of individuals counted of testate amoebae in core CF-10.

**Table 4.** Percentage abundance and total number of individuals counted of testate amoebae in core CF-F.
Figure 1

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Figure 4

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Legend:
- Gyrolites of Characinae
- Vegetation remnants
- L1 peat
- L2 clayey silt
- L3 sandy silt
Figure 5
Click here to download high resolution image
Figure 7
Cluster Dendrogram

hclust (*, "complete")
### Table 1

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**Percentage abundance and total number of individuals counted of testate amoebae in core CF-3A.**
### Table 2

Percentage abundance and total number of individuals counted of testate amoebae in core CF-1B.

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Total number of individuals counted 161 150 224 160 273 126 224 182 392 168 84 49 37 250.

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Total number of individuals counted 63 64 106 53 51 44 60 0 0 0 0 0 0 0 0 0 0

Table 3. Percentage abundance and total number of individuals counted of testate amoebae in core CF-10.
### Table 4

#### CORE CF-F water depth 36m

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#### Table 4.

Percentage abundance and total number of individuals counted of testate amoebae in core CF-F.