



## First data on testate amoebae in speleothems of caves in igneous rocks

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### Abstract

The testate amoebae form part of the habitual troglobios in caves developed in igneous rocks (plutonic and volcanic) where the little light, the persistence of humidity, the availability of silica and organic matter allow these protozoa to develop their biological cycle. This work presents a first inventory of species of amoebae testate identified in caves in igneous rocks from different parts of the World.

**Key words:** testate, caves in igneous rocks, amorphous opal speleothems.

## INTRODUCTION

Massifs formed by igneous rocks are characterized by their low porosity and scarce solubility, so in these cases runoff normally moves on the surface. However, runoff may drain exceptionally underground through the systems of cavities related to fractures, faults or diaclasses when they are open totally or partially. When these types of caves are very large they are considered pseudokarstic systems as they are developed in non soluble rocks and are also differentiated from the karst *sensu stricto*, characteristic of calcareous massifs (VIDAL ROMANÍ & VAQUEIRO 2007; Vidal Romani *et al.*, 2110 a y b). Pseudokarst is also distinguished because water circulation is normally produced at low velocity as trickles which disperse slowly onto the floor, walls or ceiling of the caves. In physical continuity with these trickles, it is normal to find specific deposits (speleothems) of small dimensions and characterized by a varied mineralogical spectrum, the most frequent mineral species being: amorphous opal, evansite, pigotite, alophane, or even carbonates (FORTI, 2005; VIDAL ROMANÍ *et al.*, 2110 a y b). These types of speleothems were first described by Caldleugh in 1829, and during a long time it was considered that they were due to the rock weathering produced by the water (VIDAL ROMANÍ *et al.*, 1979; VIDAL ROMANÍ, 1983; VIDAL ROMANÍ & VILAPLANA, 1984; WEBB & FINLAYSSON, 1984). For this reason, these deposits were first characterized almost exclusively by their mineralogy ignoring their relationship with the activity of microorganisms which lived in these caves. Later, the introduction of the scanning electronic microscopy in the

study of these speleothems (KASHIMA *et al.*, 1987; VIDAL ROMANÍ *et al.*, 1984, 1988) has showed the existence of a close relationship between the troglotrophic activity carried out by cyanobacteria, diatoms, testate amoebae, fungi, *etc.*, and the formation of these deposits (justifying the name of biospeleothems that some authors give to them (Forti, 2001; VIDAL ROMANÍ *et al.*, 2110 a y b)). Obviously, the greater volume of water and also its greater persistence inside a cave, when compared to what takes place outside, allow to originating the necessary conditions for the development of microorganisms (BASTIAN *et al.*, 2009; SAIZ-JIMÉNEZ *et al.*, 2011) transforming these caves (given their scarce light, abundance of organic matter and availability of silica) into a suitable habitat for the testate amoebae (VIDAL ROMANÍ *et al.*, 2110 a and b). Though a significant microbiological activity exists in the karstic environment *sensu stricto* (NORTHUP & LAVOIE, 2001), the great abundance of water makes the physical processes, especially chemical, prevail with the corresponding formation of speleothems (by dissolution-precipitation), even inhibiting the processes developed by the microorganisms that need more stable and quite environments, and essentially a slower water dynamics. Perhaps the best demonstration of what happens with the microorganisms in karstic and pseudokarstic environments is the same existence of testate amoebae, characteristic of the pseudokarst, whose tests are so small and delicate that they do not resist a short transport process and are simply destroyed by the change of the humidity conditions inside the cave, undoubtedly causing the death of the protozoan and the immediate dismemberment of its test. For the specific

case of the pseudokarstic environments developed in massifs of igneous rocks, the organic activity influences on the formation of the biospeleothems in two phases: first, the destruction of the silicates of the rock which are dissolved in the infiltration water and second, the generation of speleothems, especially those containing amorphous opal

(VIDAL ROMANÍ et al., 2110 a y b), when Si dissolved in water precipitates due to the oversaturation by evaporation when the water contribution to the systems ends. In this work, we refer generically to all the igneous rocks grouped in 4 sets whose chemical and mineralogical compositions may vary substantially (Table 1).

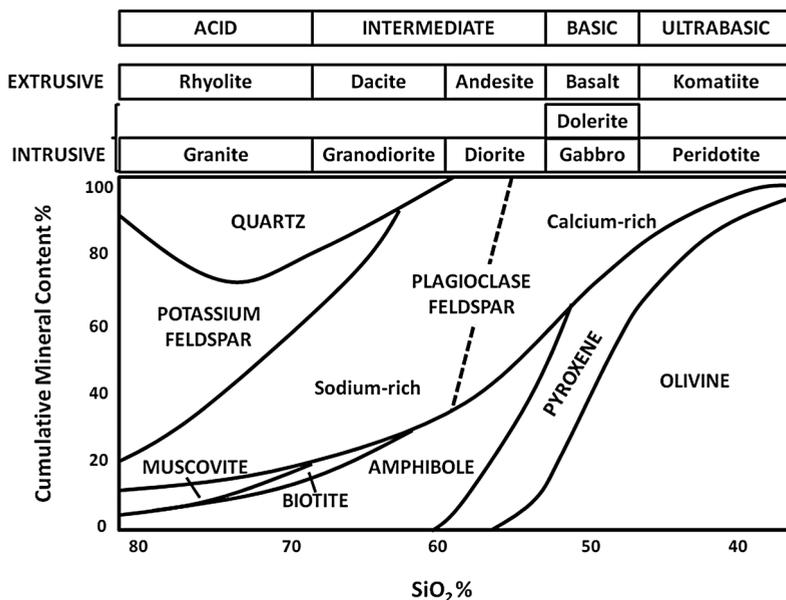


Table 1 . Types of igneous rocks (intrusive and extrusive) with their mineralogical composition and percentage of SiO<sub>2</sub>.

As it may be observed in the acid and intermediate rocks, silica is one of the most abundant chemical element (ranging between 50 and 80%), which explains that the amorphous opal is the most frequent mineralogy of these speleothems, but not the only one, in caves of these types of rocks. On the contrary, the proportion of silica in the basic and ultrabasic igneous rocks is lower (ranging between 50 and 30%), and in these cases it may happen that amorphous opal speleothems

are scarce, do not form, or even coexist with calcium carbonate speleothems (WOO et al., 2008; BEINLICH & AUSTRHEIM, 2012; OKLAND et al., 2012).

**TESTATE AMOEBAE**

Amoebae are protozoa or unicellular organisms that may live either naked or protected by a test both in subaerial or subterranean environments. The test may

be formed by secretations of proteinaceous type, or by plates of calcite, amorphous silica (idiosomes), and in the agglutinated amoebae by mineral or organic particles like pollen grains, plates of other amoebae, or remains of other microorganisms (diatoms, collembolan, etc.) (Fig. 1) which share the subterranean environment. There is a lot of literature on amoebae species in calcareous caves (WOO *et al.*, 2008; BEINLICH & AUSTRHEIM, 2012; OKLAND *et al.*, 2012) though normally referred to as naked amoebae which, together with other organisms, form part of the stygobios that lives in the water mass which circulates through the cave. This work is a study of the troglobites from caves in igneous rocks,

focussed on testae amoebae that appear in the sedimentary registry (speleothems) preserved in these caves. In caves developed in igneous rocks, amoebae segregate plates of amorphous silica (idiosomes) with a specific spatial distribution pattern, size or geometry (Figs. 2 and 3). Some species develop spines on their tests perhaps to ensure their stability on the speleothem surfaces when water seeps over them (Fig. 4). Testate amoebae, also those with amorphous silica idiosomes, are not exclusive of caves in igneous rocks and are represented in subaerial environments: free continental waters (rivers, lakes, springs), soils, peat bogs or biofilms developed over tree barks (WYLEZICH *et al.*, 2002).

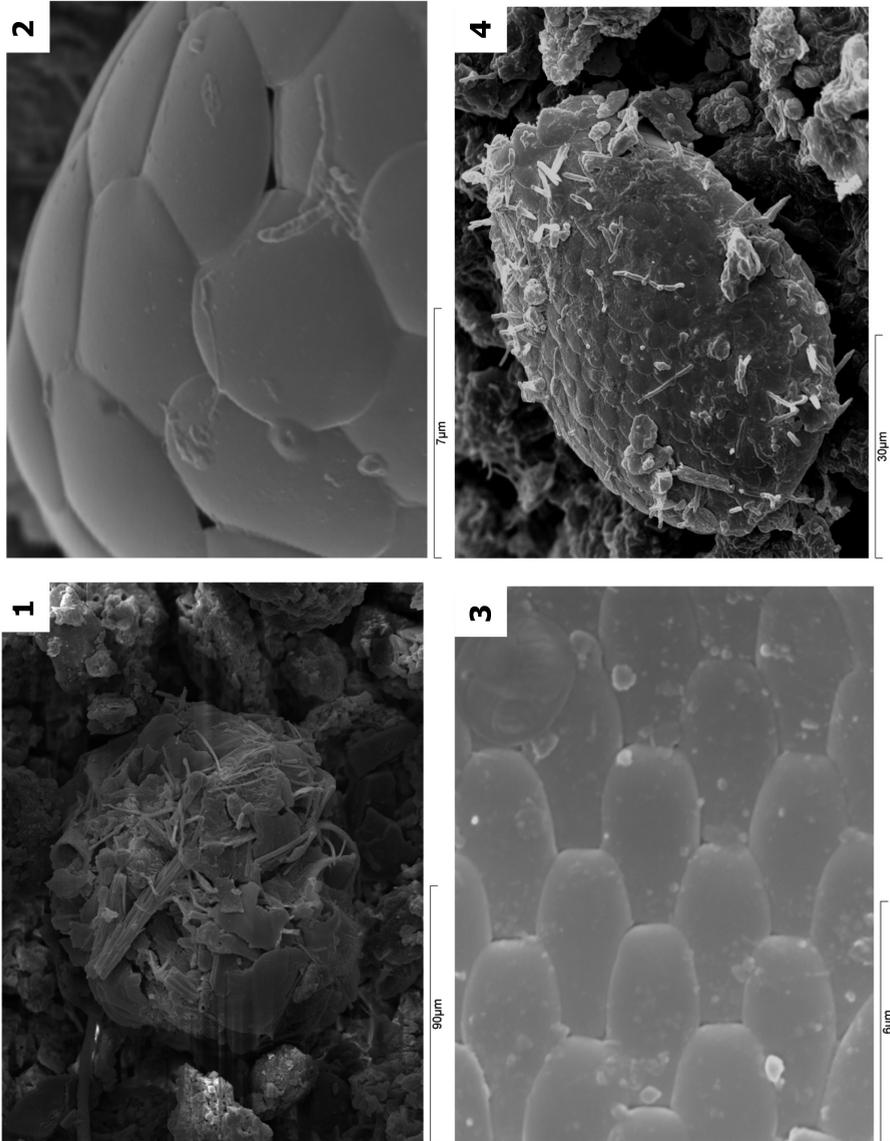


Fig.1. *Amphitrema wrightianum* (?) Berrocal del Rugidero, Badajoz, Spain. Fig. 2 Idiosomes of *Tracheteuglypha dentata*. Porteliña, Galicia, Spain. Fig. 3 Idiosomes of *Euglypha rotunda*. Peña del Hierro, Huelva, Spain. Fig. 4 *Euglypha strigosa*. Austria

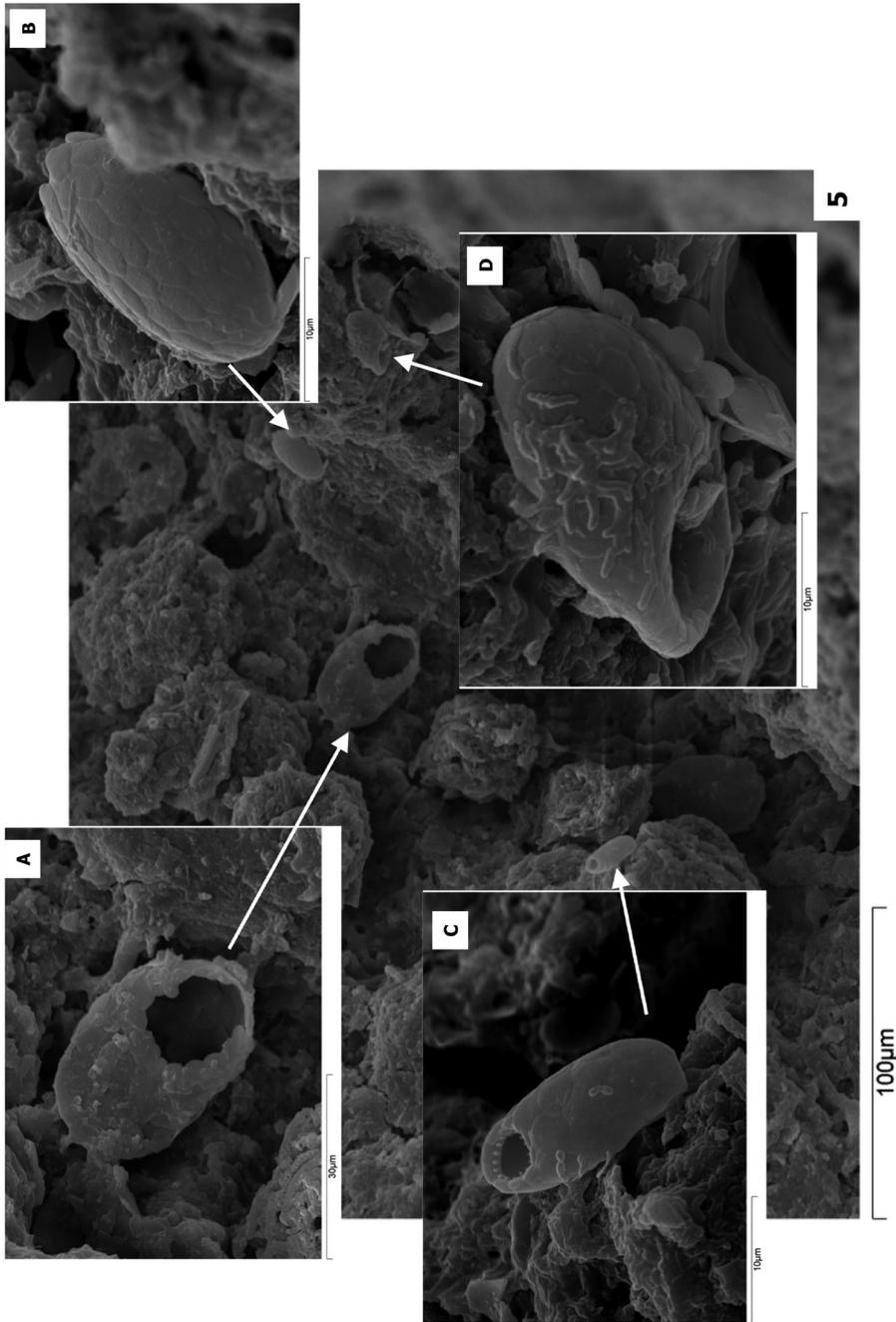


Fig. 5. Different species of testate amoebae. Portelãña, Galicia, Spain. A-C-D: *Trinema linare*; B: *Euglypha rotunda*

An amoeba's test has a flask-shaped form and is 100  $\mu\text{m}$  long and maximum 50  $\mu\text{m}$  width though the ones studied in our work are larger (Fig. 5). In one of the edges, there is an opening (pseudostome) frequently located on the narrowest part of the test with a border of very varied morphology (Figs. 6, 7 and 8) where there are the pseudopodia used to trap aliments and to move the protozoan. This pseudostome may be surrounded by modified plates which contain one or several teeth (Fig. 9).

Sedimentary environments with acid pH, either caves in igneous rocks or superficial environments, are characterized by the weak drainage energy and bad water retention during the dry period, causing very quick environmental changes from wet to dry in very short time intervals. Perhaps that is why the organisms which colonize these environments, in our case the testate amoebae, develop strategies to survive draught preserving the humidity as well as to defend themselves from other predatory organisms.

## MATERIAL AND METHODS

In this work, the results of the study of samples from caves in igneous rocks from different parts of the World (Spain, Portugal, Austria, Sweden, Argentina, Swaziland, Madagascar, Western and South Australia) are presented. In most of the study cases, specimens of testate amoebae associated to speleothems were identified. The samples were conveniently protected in bags or boxes depending on their fragility grade and dimensions to avoid their physical deterioration during their transportation to the laboratory. Once in the laboratory, they were examined, without previous treatment, at the stereoscopic microscope Nikon SMZ1500

and photographed with a Nikon DS.Fil in order to select the best specimens. For their observation under the SEM, they were prepared according to the standard protocol which consisted in a not very aggressive desiccation (they were stored during a week in a vacuum desiccator of silica-gel) so as to avoid the production of artifacts, essentially the polygonal cracking of the amorphous opal layers. After their desiccation, the samples were metalized by spattering with a thin gold layer of 50-100  $\text{\AA}$  with cathode pulverization equipment BAL-TEC SCD 004. The use of carbon spattering was avoided because the normal analytic determinations in this type of sample could have masked the carbon content of some of the very abundant organic remains (palynomorphs, mites, collembola, bacteria, etc.). The samples thus prepared were studied under the scanning electronic microscope JEOL JSM 6400 selecting different magnification levels to locate and identify the organic and mineral elements in each studied sample.

The sedimentary mineralogy, texture and structure of the samples were taken into consideration only as complementary information to define the dynamics and biogeochemical conditions of the environment. An inventory of the different species of amoebae identified in the speleothems is presented herein. The aspect of these protozoa is very much influenced by the time elapsed between the cave water appearance and the observation moment, obviously better preserved when the sample was taken during or immediately after the wet period of the cave.

The amoebae were located on the external surface of the speleothems protected in small depressions or in the open voids of the porous fabric of the speleothem (Figs. 10 and 11). Their preservation state depends on

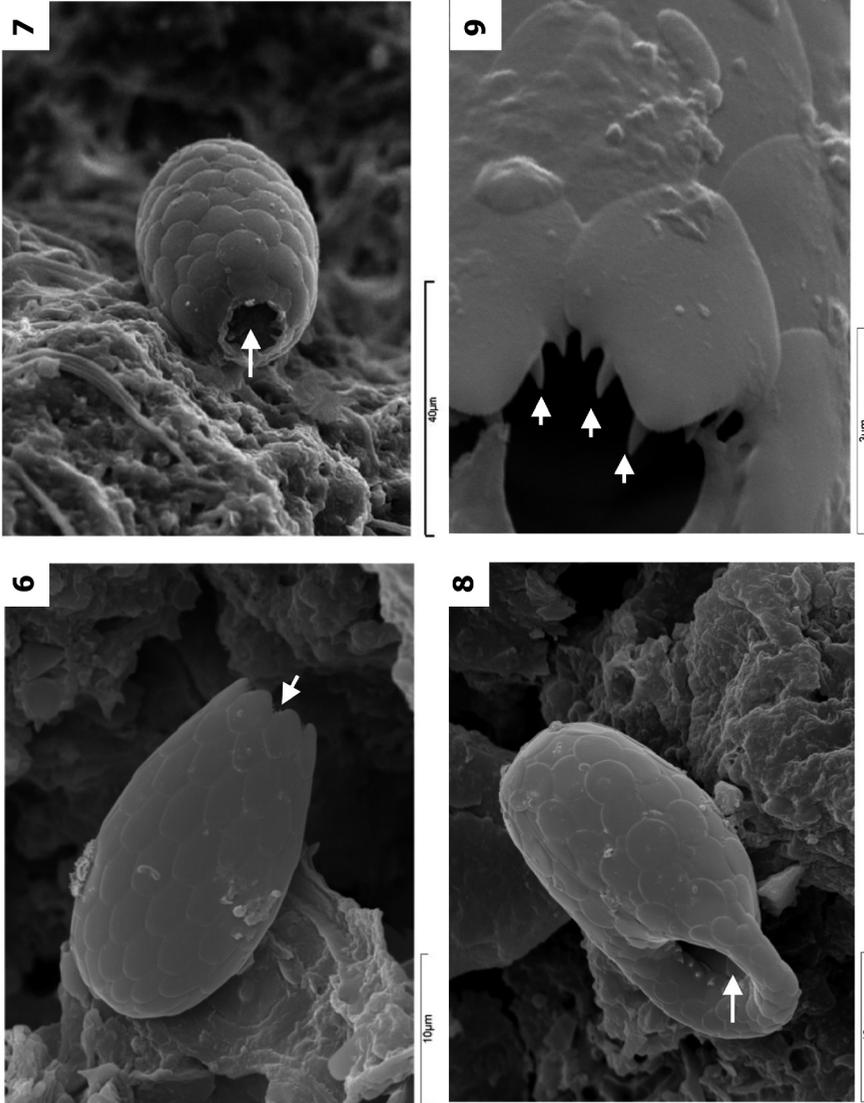


Fig. 6. *Euglypha rotunda*, Portelã, Galicia, Spain. Arrow: pseudostome. Fig. 7 *Tracheleuglypha dentata*, Ávila, Spain. Arrow: pseudostome. Fig. 8 *Trinema complanatum*, Portelã, Galicia, Spain. Arrow: pseudostome. Fig. 9 Pseudostome with toothed idiosomes of *Euglypha rotunda* (arrows) Portelã, Galicia, Spain

whether the sample was collected during the wet stage when all the amoebae were alive and the test was intact, or during the dry stage when the test could be disintegrated totally or partially. In the transition stage from dry to wet, it was observed the colonization, in different grades, of the test by cyanobacteria which could cover them completely (Figs. 12, 13 and 14). According to this interpretation, different transformation states are distinguished: intact located on the speleothem surface (Figs. 4, 6, 7 and 8) or in different destruction phases with the test partially collapsed by flattening (Figs. 15 and 16) or totally dismantled with release of idiosomes, which are gradually

incorporated into the speleothem as elements of its sedimentary fabric (Fig 17). They may appear as isolated individuals, in sets of several specimens (not necessarily of the same specie) (Figs. 5 and 18), sometimes of different sizes (Fig. 5) and also physically associated apparently (Fig. 13 and 19). In caves developed in igneous rocks, two big types of speleothems are distinguished: flowstone (Fig. 20) and cylindrical (Figs. 21 and 22) (VIDAL ROMANÍ et al., 2010 a and b) which will deposit indistinctly on the ceiling, wall or floor of the caves. Specimens of testate amoebae were found in both types of speleothems, though in greater quantity in the cylindrical ones (Figs. 21 and 22).

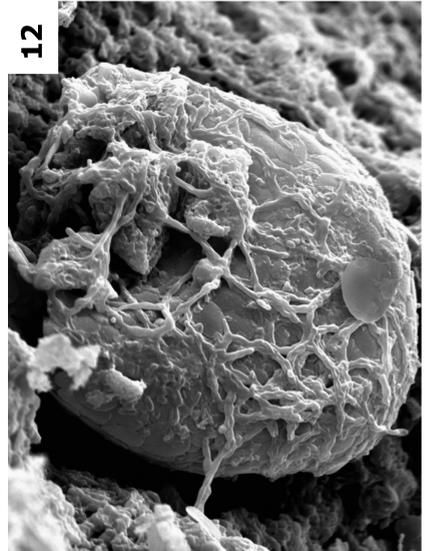
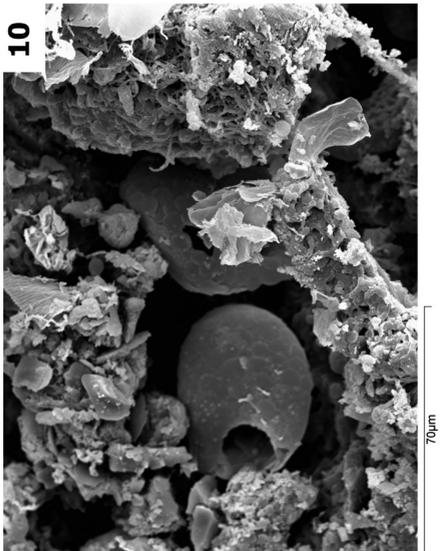
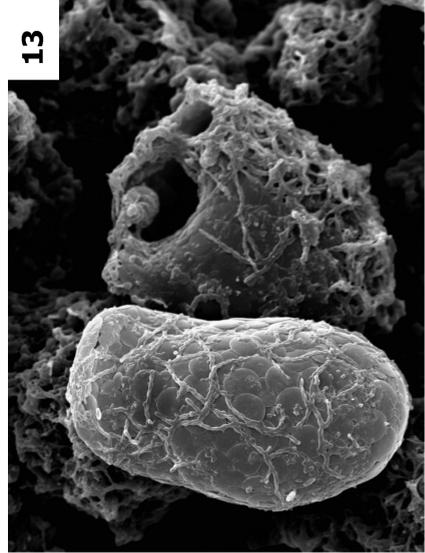
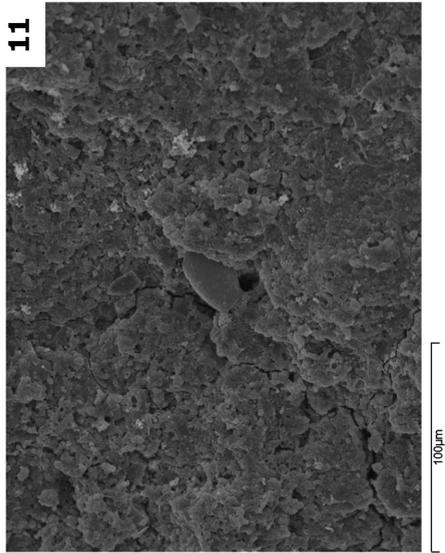


Fig. 10. *Trinema encheus* protected in holes of the porous granular fabric. Trapa, Galicia, Spain. Fig. 11 *Corythion dubium* included in the polymineral granular material. Hölick Grotta, Sweden. Fig. 12 *Trinema sp.* with cyanobacteria covering its test. Trapa, Galicia, Spain with cyanobacteria covering its test. Trapa, Galicia, Spain

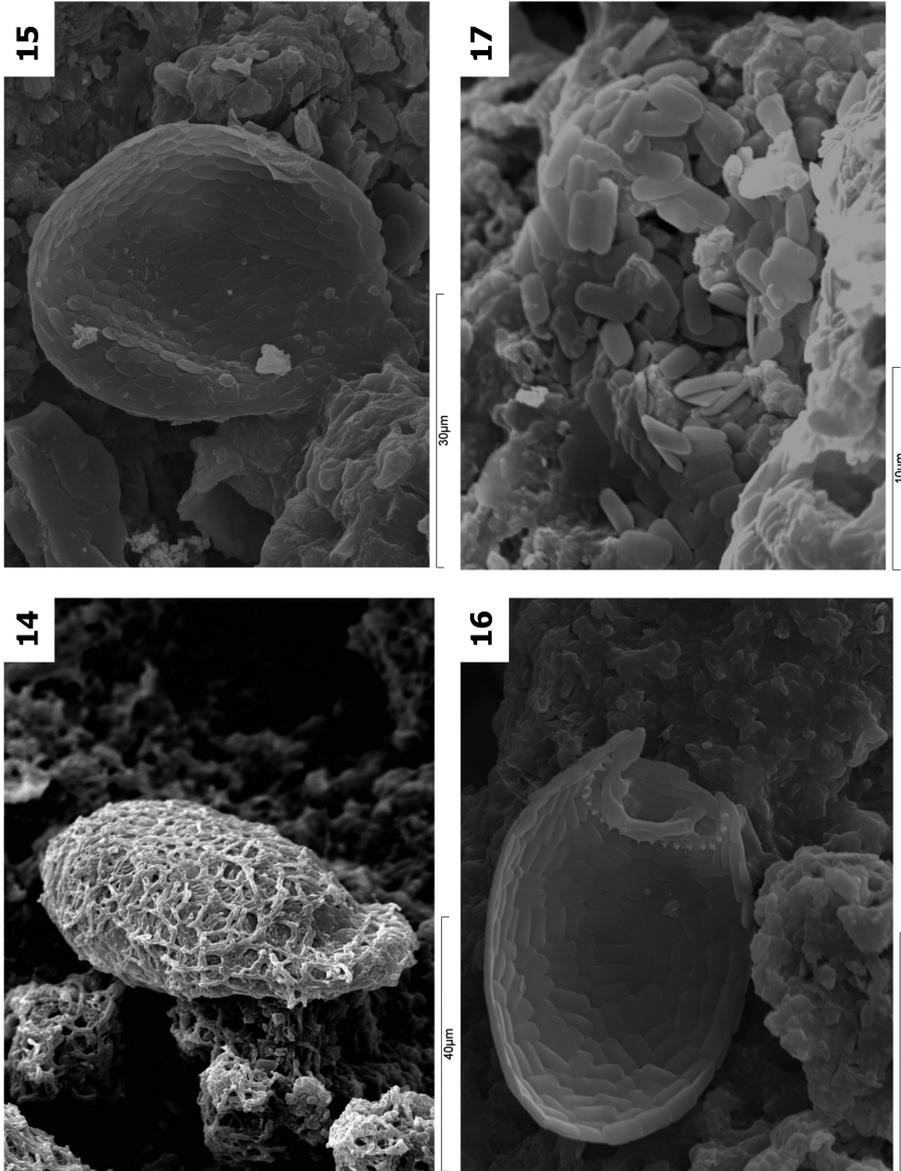


Fig. 14. *Trinema* sp. with cyanobacteria covering its test. Trapa, Galicia, Spain. Fig. 15 *Assulina muscorum*. Peña del Hierro, Huelva, Spain Fig. 16 *Corythion dubium*. Castelo das Furnas, Portugal. Fig. 17 Idiosomes of *Corythion* sp. Castelo das Furnas, Portugal

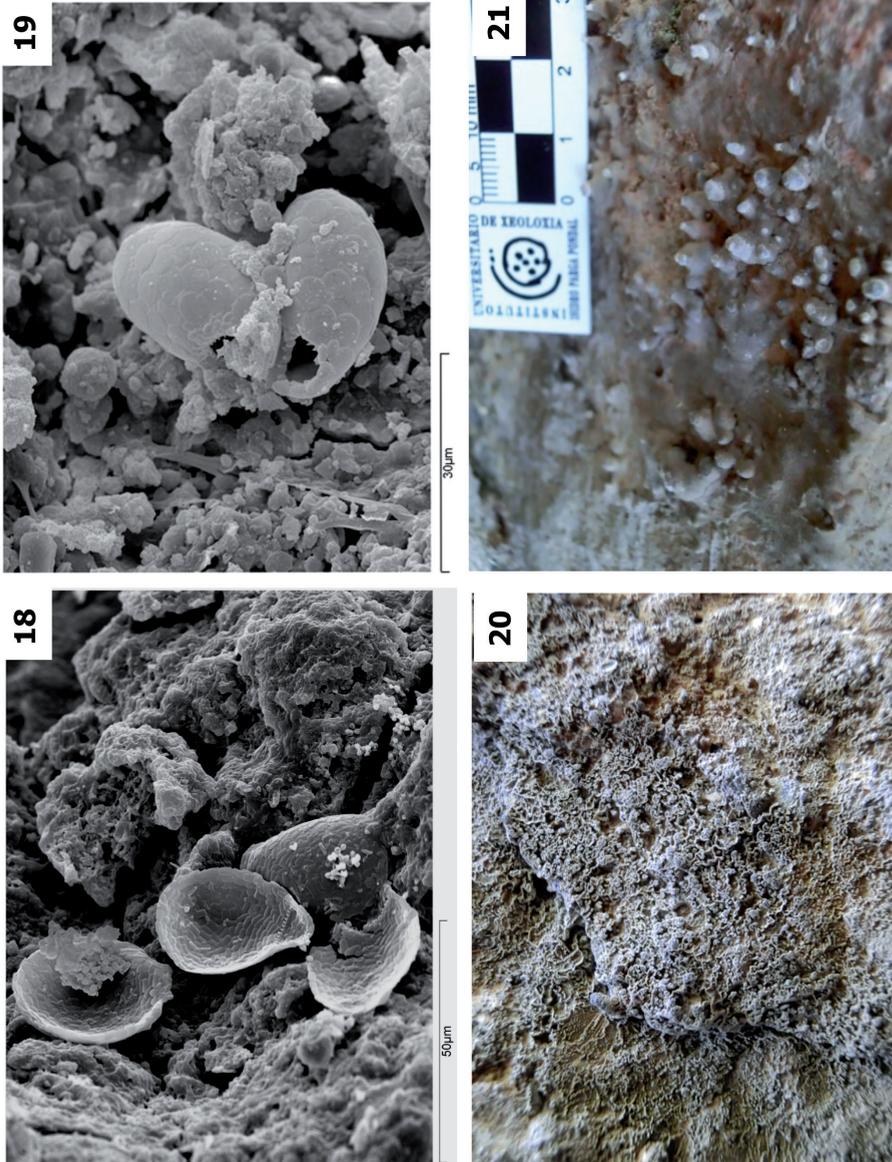


Fig. 18 Set of *Corythion dubium*. Hölick Grotta, Sweden. Fig. 19 *Trinema lineare*. Western Australia Fig. 20 Flowstone. Las Jaras, Córdoba, Spain Fig. 21 Cylindrical speleothem. Las Jaras, Córdoba, Spain

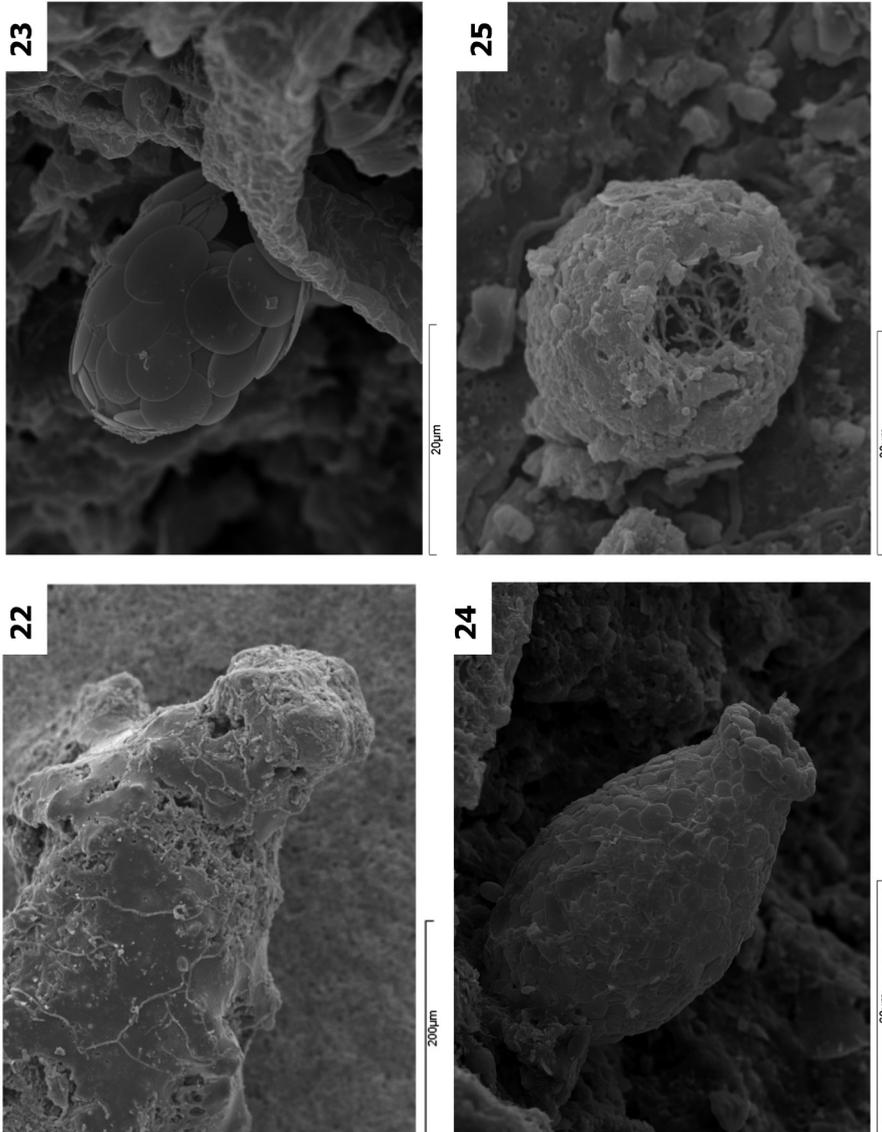


Fig. 22. Cylindrical speleothem. Skälberguet, Sweden. Fig. 23 *Sphenoderia lenta*. Porteliña, Galicia, Spain. Fig. 24. *Physochila (Nebela) griseola*. Porteliña, Galicia, Spain. Fig. 25 *Centropyxis* sp. Castelo das Furnas, Portugal

## RESULTS AND DISCUSSION

The direct observations under the scanning electronic microscope and the later study of the obtained graphic documentation allowed us to identify the different species of the testate amoebae present. The habitual genera of testate amoebae from caves in igneous rocks correspond to *Trinema*, *Euglypha*, *Corythion* and *Centropyxis* with the distinct feature of having flattened morphologies and small size. Apparently, the genera inherent to edaphic environments, according to Lousier (1974, 1982), coincide with the ones found in our caves.

The following species of testate amoebae were identified:

Species from the Family *Euglyphidae* (Wallich, 1864): *Euglypha rotunda* (Wailes, 1911) (Fig. 6), *Euglypha strigosa* (Ehren-

berg, 1872) (Fig. 4), *Sphenoderia lenta* (Schlumberger, 1845) (Fig. 23), *Tracheleuglypha dentata* (Moniez, 1888) (Fig. 7), *Assulina muscorum* (Greeff, 1888) (Fig. 15).

Species of the family *Trinematidae* (Hoogenraad & De Groot, 1940): *Trinema complanatum* (Penard, 1890) (Fig. 8), *Trinema enchelys* (Ehrenberg, 1838) (Fig. 10), *Trinema lineare* (Penard, 1890) (Fig.19), *Corythion dubium* (Taraneck, 1881) (Fig.16).

Species from the Family *Nebelidae* (Taraneck, 1882): *Physochila (Nebela) griseola* (Wailes & Penard, 1911) (Fig.24).

Some specimens provisionally assigned to the Family *Centropyxidae* (Jung, 1942) (Fig.25) were identified.

In the following table we can observe how the distribution of species of the analysed samples is presented:

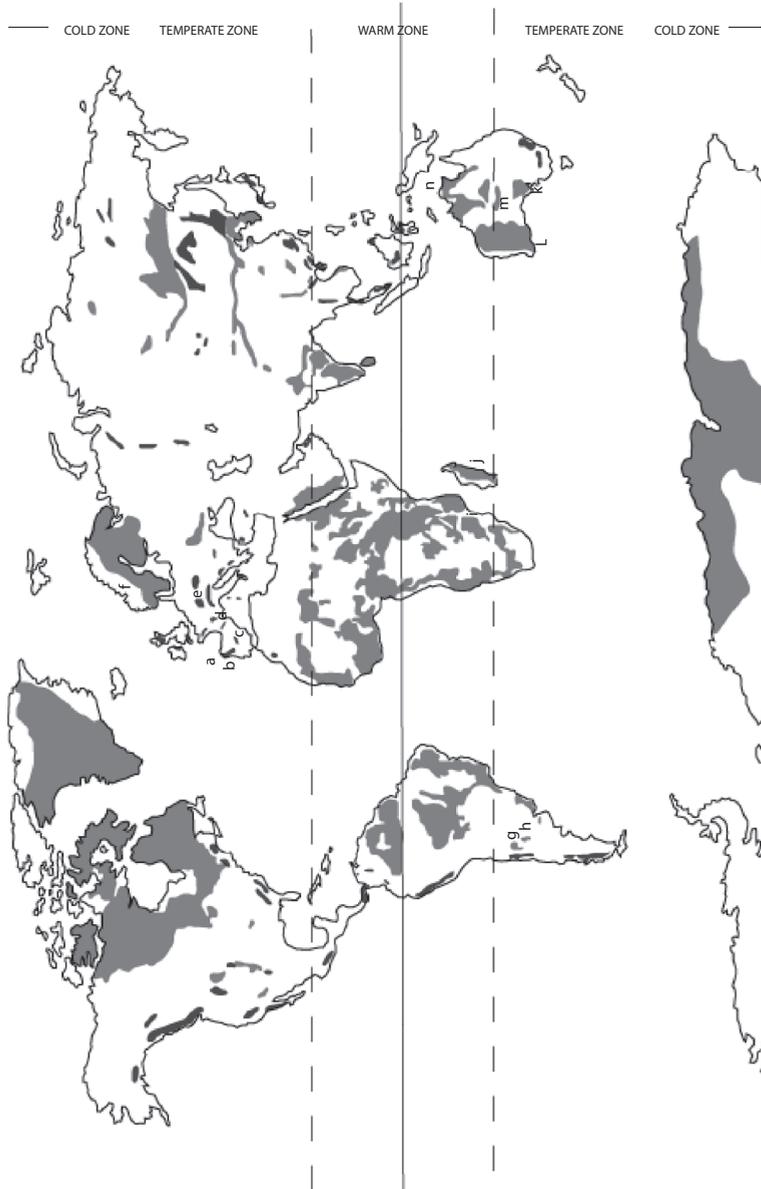
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Euglypha rotunda</i> Wailes	x								x	x	x			
<i>Euglypha strigosa</i> Ehrenberg		x	x											
<i>Sphenoderia lenta</i> Schlumberger									x					
<i>Tracheleuglypha dentata</i> Moniez				x					x					
<i>Assulina muscorum</i> Greeff							x			x				
<i>Trinema complanatum</i> Penard	x	x					x		x					
<i>Trinema enchelys</i> Ehrenberg			x				x	x						
<i>Trinema lineare</i> Penard			x		x	x			x					x
<i>Corythion dubium</i> Taraneck		x			x		x			x	x	x	x	
<i>Nebela griseola</i> Penard									x					

Table 2. Distribution of species identified in the different locations geographically marked with a number: (1) Swaziland, Gobholo; (2) Austria; (3) Western Australia and South Australia; (4) Spain, Ávila; (5) Spain, Córdoba, Las Jaras; (6) Spain, Badajoz, Berrocal del Rugidero; (7) Spain, Galicia, A Trapa; (8) Spain, Galicia, Louro; (9) Spain, Galicia, Porteliña; (10) Spain, Huelva, Peña del Hierro; (11) Portugal, Castelo da Furna; (12) Sweden, Falkberget; (13) Sweden, Hölick Grotta; (14) Sweden, Tröllhallet.

The observations made in different caves in igneous rocks (Table 2) allowed us to elaborate a quite varied inventory of species where a few are exclusive of a location. Though the work was carried out on a geographically diverse sample (with clear prevalence of samples from Spain and Sweden) (Mapa 1), a great homogeneity in the distribution of the identified amoebae species may be observed. This indicates that, in spite of the different bioclimatic conditions of the external environment of the cave, the pseudokarstic microsystem is very similar inside; thus, in our opinion, it explains the similarity of the spectrum of the identified species. It is obvious that a greater sampling should be studied, both in the number of studied speleothems and the origin zones.

Some authors (AOKI et al. 2007) assign the testate amoebae a very important role in the silica cycle based on the fact that the high solubility of the amorphous (or biogenic) opal, main mineral of the test, provides a great mobility to the silica. However, considering the scarce volume of speleothems in caves in igneous rocks and the

scarce abundance of tests that are in them, this affirmation seems to be not very plausible from the sedimentary point of view. It is convenient to take into account that most of the speleothems are formed by mineral clasts of quartz, feldspar and mica coming from the granular disaggregation of the rock due to physical weathering. The biogenic fraction (and more specifically, the amorphous opal) is reduced to a superficial film of some few micra thick that in some cases covers the whole surface of the speleothems being either cylindrical or planar, and only in cases of repeated re-dissolution gives rise to the formation of not very thick rhythmical structures. The tests will disperse over this thin film of amorphous opal (Fig. 22) but never in large concentrations of individuals. Possibly at global scale and considering the large water volumes that move in the terrestrial surface, large rates of the silica mobilization may be quantified. But at the scale of speleothems from caves in igneous rocks studied in this work, the importance of this mineral (amorphous opal) or of the silica is practically symbolic.



Map 1. World's climatic zones with the main granites and granitoids areas in grey. Locations from which the samples were taken: a. Galicia - Northwestern Spain; b. Minho - Northern Portugal; c. Guadarrama Sierra - Central Spain; d. Girona- Northeastern Spain; e. Austria; f. Central Sweden; g. Anillaco, La Rioja - Argentina; h. Sierra Grande de Córdoba - Argentina; i. Gobholo, Swaziland - Southeastern Africa; j. Andringintra Massif - Madagascar; k. Eyre Peninsula - Southern Australia - Australia; l. Hyden Rock - Western Australia - Australia; m. Devil's Marbles - Northern Territory - Australia; n. Darwin - Northern Territory - Australia

## CONCLUSIONS

Testate amoebae with amorphous opal idiosomes are common microorganisms in pseudokarstic environments developed in massifs of igneous rocks. There is a great homogeneity in the spectrum of species represented that appear to have a great independency from the weather conditions existing outside the cave and also from the type of igneous rock in which they have developed. The determinant factors seem to be the following: first, the humidity (independently from the frequency and persistence of the wet period) in the caves; second, the availability of silica in the cavity. The preservation state of the tests is a reliable microenvironmental indicator of the underground environment that allows knowing the humidity grade existing therein at the time the sample was collected: well-preserved tests in humid period, bad preserved tests in dry period, tests colonized by cyanobacteria in the re-establishment of the humidity conditions in the cave.

## ACKNOWLEDEMENTS

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