

Chlorophyll-*a* and Suspended Inorganic Material Affecting the Shell Traits of Testate Amoebae Community

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Abstract: Shell composition is considered an important functional trait in testate amoebae community. Differences in the composition of shells may represent adaptations of these organisms to changes in the availability of environmental resources. We aimed to evaluate the influence of these environmental resources on the presence of different testate amoeba species, based on observation of one important functional trait (shell composition), in floodplain environments. We hypothesised that species diversity would increase with increased availability of food (chlorophyll-*a*; a critical environmental requirement). We also predicted that a higher concentration of suspended inorganic material would contribute to the occurrence of species that have an exogenous shell. Testate amoeba samples were taken between 2002 and 2011 in plankton of ten floodplain environments. A significant positive correlation was noted between the concentration of chlorophyll-*a* and the diversity of testate amoeba species according to a simple regression analysis. Furthermore, non-metric multidimensional scaling showed distinct groups with lower and higher concentration of suspended inorganic material. The occurrence of testate amoeba species with smaller exogenous shells was the most influenced by environments with higher concentrations of suspended inorganic material. Thus, our results emphasised that the evaluated environmental resources are important as assembly factors to shell traits in testate amoebae community.

Key words: Protist testate amoebae, occupation functional, plankton, floodplain

INTRODUCTION

Investigations of environmental conditions in floodplain environments can allow an understanding of how these conditions influence organismal physiology and behaviour, and how the resulting organismal changes

influence biotic interactions and community structure (Simões *et al.* 2013). Thus, environmental conditions can be important keys to identifying species variability in freshwater habitats (Neiff 1996).

Testate amoebae community reveal the remarkable ecological functions that contribute to the structure and dynamics of aquatic environments. These organisms have shells that differ in their composition between species, and which may be of endogenous or exogenous origin (Bonnet 1976). Depending on the type of material used to build the shell, the species can have different environmental requirements (Ogden 1989).

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A functional trait can be any morphological, physiological, or life history characteristic measurable at the individual level with an impact on organismal fitness (Violle *et al.* 2007). Since functional traits are indicators of how species respond to changes in the environment and how they contribute to ecosystem functioning, these traits can be used to characterise alterations in community structure (Naeem and Wright 2003). For testate amoebae, shell composition is considered an important functional trait. Endogenous shells are secreted by the organism itself and can be constituted by proteinaceous, biosilica plates, or thick organic coating (idiosomes), whereas exogenous shells are composed of particles available in the environment and clustered into a cement matrix (xenosomes) by the organism (Beyens and Meisterfeld 2001, Mitchell *et al.* 2008). The physical characteristics of the material found in the shells are variable, and may include mineral particles (small grains quartz), frustules of diatoms, pollens, organic material, among others (Charman *et al.* 2001). The composition of exogenous shells seems to be linked to substrate characteristics in sediment species or to availability of inorganic suspended material in the water column in planktonic species (Medioli and Scott 1983).

As a crucial environmental requirement, the availability of chlorophyll-*a* can influence the distribution of species belonging to the lower levels of the food chain, such as testate amoebae (Gimenes *et al.* 2004). Indeed, species with both endogenous and exogenous shells use this resource as food (Bastidas-Navarro and Modenutti 2007). However, the availability of suspended inorganic material is essential only to the species that have exogenous shells (Rodriguez-Zaragoza 1994). Therefore, it is believed that differences in shell composition may represent adaptations linked to the availability of environmental resources, for instance, chlorophyll-*a* and suspended inorganic material, that could drive selection of functional traits in testate amoeba species (Arrieira *et al.* 2015a, 2016; Schwind *et al.* 2016a).

We aimed to evaluate the influence of these environmental resources on the presence of different testate amoeba species, based on observation of one important functional trait (shell composition), in floodplain environments. We hypothesised that higher availability of food (chlorophyll-*a*) would correlate with higher species diversity, given the importance of this resource to the occurrence of testate amoeba species with both endogenous and exogenous shells. We also hypothesised that higher concentrations of suspended inorganic ma-

terial would contribute to the higher occurrence of the species that have an exogenous shell, because such conditions would favour this type of shell.

MATERIALS AND METHODS

Study area

This study was performed in the upper Paraná River floodplain, which is part of the Environmental Protection Area of the Floodplain Islands of the Paraná River, Brazil (22°40'–22°50' S and 53°10'–53°40' W). The sampling was carried out in ten floodplain environments (seven lakes and three rivers). These environments within the floodplain belonging to three sub-basins formed by the major river (Paraná River sub-basin) and two tributaries (Baía River and Ivinhema River sub-basins) (Figure 1).

Sampling design

Testate amoeba samples were taken during two hydrological periods, flooding (February/March) and drought (August/September), from 2002 to 2011 on the subsurface of the pelagic zone, resulting in 200 samples (two periods * 10 environments * 10 years). Six hundred litres of water were filtered per sample in a plankton net (68 µm) using a pump motor. Organisms were fixed with 4% formalin, and buffered with calcium carbonate, avoiding the destruction of the shells of the organisms. In the sample, only organisms that had an identified protoplasm stained with Rose Bengal were considered alive and counted.

Testate amoeba abundance was determined using a Sedgewick–Rafter chamber counting under an optical microscope (Olympus CX31) at a magnification of 400×. This counting was performed by sets of three sequential sub-samplings obtained by a Hensen-Stempel, and at least 50 individuals were counted per sample, counting 7.5 mL from each sample in total. Samples were fully quantified when not reaching the minimum number of individuals per sample (Bottrell *et al.* 1976).

Water samples were collected using a Van Dorn sampler (5 L) into polyethylene bottles and were refrigerated (–20°C) until further laboratory analysis. The concentration of suspended inorganic material (mg L⁻¹) was determined by gravimetry (Wetzel and Likens 1991). The concentration of chlorophyll-*a* (µg L⁻¹) was quantified by 90% acetone extraction and read using a spectrophotometer at 663 nm (Golterman *et al.* 1978).

The concentrations of suspended inorganic material data were obtained, processed and provided by the Limnology Laboratory of the Research Centre for Limnology, Ichthyology and Aquaculture – State University of Maringá.

Functional trait and environment categorization

The functional traits were calculated by summing the organisms' abundance, according to each functional trait, and expressed as individuals per m⁻³. The testate amoeba species were selected according to their shell composition – endogenous or exogenous origin (Table 1 Supplemental material).

The environment floodplains were also categorised, according to mean values of the concentrations of suspended inorganic

material, as follow: lower (EL), intermediate (EI), and higher (EH) (Table 1).

Data analysis

Species diversity was estimated using the Shannon Index (H') (Pielou 1975). We assessed the relationship between the chlorophyll-*a* concentration and the species diversity through a multiple regression analysis (Sokal and Rohlf 1991). Data were previously log-transformed. The assumptions of linearity, normality, homoscedasticity and independence were tested. This analysis was performed using the Statistica software 7.0 (Statsoft Inc. 2005).

We also carried out a non-metric multidimensional scaling (nMDS) (Clarke and Warwick 2001) in order to assess whether the concentrations of suspended inorganic material predicts the occurrence of the testate amoebae with exogenous shell in different floodplain environments. The similarity matrix was calculated with data obtained from Bray-Curtis index and distortion of the resolution was expressed by stress value. The closer to zero is the stress value, the better adjusted to the original distance of the sample data and the configuration obtained by analysis (Legendre and Legendre, 1998). We also performed a Multi-Response Permutation Procedures (MRPP) (McCune and Grace 2002), which provides a *p*-value based on permutation test using 999 randomizations. This analysis also describes an effect size (statistic "A") that describes within-group homogeneity, compared to the random expectation. The value for a ranges from zero to one. When all species are identical within groups, we can observe the highest possible value for A. If heterogeneity within groups equals expectation by chance, then A is equal to zero. On the other hand, if there is less agreement within groups than expected by chance, then $A < 0$ (McCune and Grace 2002). We used the Biodiversity R, Mass, and Vegan packages of R free software version 2.8.1 (R Core Team 2012) to conduct our analyses.

RESULTS

Species composition of the testate amoebae community was represented by 107 taxa belonging to 12 families; Diffugiidae showed the highest number of species (55 taxa) followed by Arcellidae (21 taxa), Centropyxidae (12 taxa), Lesquereusiidae (10 taxa). Other families with fewer species: Cyphoderidae (one taxon), Heleoperidae (one taxon), Hyalospheniidae (one taxon), Phryganellidae (one taxon), Plagiopyxidae (two taxa),

Trigonopyxidae (one taxon), Euglyphidae (one taxon), and Trinematidae (one taxon) (Table 1 – Supplemental material).

According to the multiple regression analysis, species diversity of the testate amoebae community was significant ($t = 11.27$; $p < 0.01$) and positively affected by the concentration of chlorophyll-*a*. The following model containing these variables explained 25% of data variability: $\text{Log}_{10}(\text{diversity}) = 3.41 + 0.12 \times \text{Log}_{10}(\text{Chlo})$.

The results of nMDS ordination (stress = 0.17) showed two distinct groups in the spatial gradient corresponding to environments with lower (EL) and higher (EH) concentrations of suspended inorganic material. Furthermore, the MRPP results (999 random permutations) showed a significant differences ($A = 0.16$; $p < 0.01$) between concentrations of suspended inorganic material and distinct groups of testate amoeba

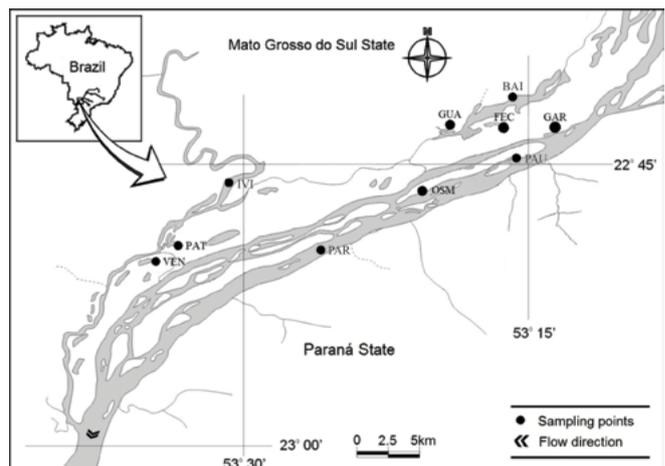


Fig. 1. Study area and location of the sampling stations in the upper Paraná River floodplain: PAR – Paraná River, OSM – Osmar Lake, PAU – Pau Vêio Backwater, GAR – Garças Lake, BAI – Baía River, GUA – Guaraná Lake, FEC – Fechada Lake, IVI – Ivinhema River, PAT – Patos Lake, VEN – Ventura Lake.

Table 1. Minimum, maximum, and mean concentrations of suspended inorganic material for environment floodplain groups investigated. Measures are given in mg L^{-1} .

	Environment floodplain groups								
	EL			EI			EH		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Suspended inorganic material	0.03	0.51	0.31	0.52	4.50	1.55	4.58	8.75	6.65

species. Most species with exogenous shells and all species with endogenous shells were associated with EL; however, other exogenous species: *Heleopera petricola*, *Nebela penardiana*, *Phryganella hemisphaerica*, *Hoogenraadia criptostoma*, *Plagiopyxis callida*, *Cyclopyxis kahli*, and *Trinema lineare*, were associated with EH. The environments with intermediate concentrations of inorganic suspended material showed no distinct groups in the spatial gradient ordination (Figure 2).

DISCUSSION

The families with the highest species composition in this study were Diffugiidae, Arcellidae, Centropyxidae and Lesquereusiidae. Several other studies have also highlighted the high diversity of species within these families in floodplain environments (Lansac-Tôha *et al.* 2009, 2014; Alves *et al.* 2012; Arrieira *et al.* 2015b, Schwind *et al.* 2016b). On the other hand, species

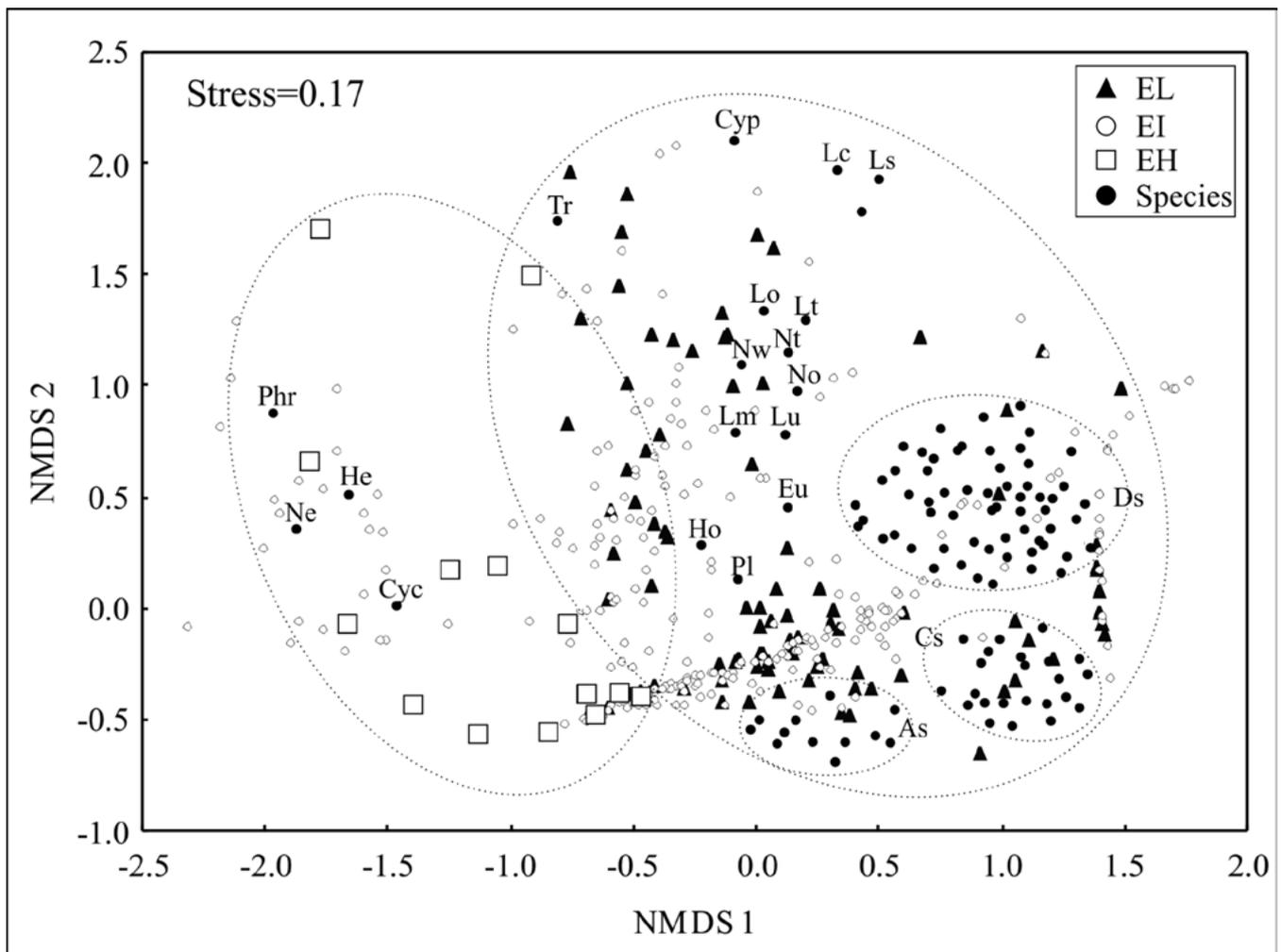


Fig. 2. Ordination results of the non-metric multidimensional scaling to combination between environments and testate amoeba species. Symbols represent the environments with lower (EL), intermediate (EI), and higher (EH) concentration of inorganic suspended material. Species: Cyp – *Cyphoderia cf. ampulla*, Eu – *Euglypha acanthophora*, Lm – *Lesquereusia mimetica*, Lt – *L. modesta*, Lu – *L. modesta* var. *caudata*, Lo – *L. ovalis*, Ls – *L. spiralis*, Lh – *L. spiralis* var. *hirsuta*, Lc – *L. spiralis* var. *caudata*, No – *Netzelia oviformis*, Nt – *N. tuberculata*, Nw – *N. walesi*, He – *Heleopera petricola*, Ne – *Nebela penardiana*, Phr – *Phryganella hemisphaerica*, Ho – *Hoogenraadia criptostoma*, Pl – *Plagiopyxis callida*, Cyc – *Cyclopyxis kahli*, Tr – *Trinema lineare*. Arcellidae species (As), Centropyxidae species (Cs), and Diffugiidae species (Ds) were grouped to more easily identify the relationships between species and environments.

richness was lower within Nebelidae, Plagiopyxidae, Phryganellidae, Cyphoderidae, Euglyphidae, Trigonopyxidae, and Trinematidae. These families have a restricted distribution and low occurrence in planktonic compartments (Velho *et al.* 2000). However, these species are important in ecological studies because they have specific requirements that can make them useful as bioindicators of environmental conditions in aquatic habitats (Scott *et al.* 2001). Some testate amoeba species for example, can restrict by higher phosphorus concentrations and, therefore, can be considered as bioindicator of environments with higher concentrations of phosphorus (Mieczan *et al.* 2012).

Our results showed a positive correlation between chlorophyll-*a* concentration and species diversity. This could reflect the direct or indirect dependence of testate amoeba species on this variable as a food resource. Availability of chlorophyll-*a* influences both the testate amoeba species that feed directly on algae (Chardez 1964, Gilbert *et al.* 2000) and those that feed on other organisms that use chlorophyll-*a* as resource (Couteaux and Pussard 1983, Torres and Jebram 1993). Higher availability of chlorophyll-*a* increases the biomass of ciliates, bacteria, flagellates, and small metazoans (Auer *et al.* 2004), all of which may be food items in the diet of various testate amoebae (Gilbert *et al.* 2003). As a consequence, the direct relationship of chlorophyll-*a* with these groups of microorganisms means that an increase in chlorophyll-*a* provides an increase in the amount and variety of food available to all testate amoeba species with endogenous and exogenous shells (Bastidas-Navarro and Modenutti 2007).

The ordination results highlighted the varying influence of the concentration of suspended inorganic material on the occurrence of functional traits related to shell composition. These results are in accordance with those observed by du Châtelet *et al.* (2015), who also verified that testate amoebae select grains according to size, from those available within their immediate environment. The occurrences of some exogenous species were more influenced by environments with higher concentrations of suspended inorganic material, as observed for *Nebela penardiana*, *Heleopera petricola*, *Phryganella hemisphaerica*, and *Cyclopyxis kahli*. These species have a reduced size and use smaller mineral particles to build their shells (Souza 2008). The size and mineralogical composition of the particles that comprise the suspended inorganic material in the water column can affect the species abundance within a testate amoebae community (Dalby *et al.* 2000). Fur-

thermore, the particulate material in the pelagic region has a smaller size when compared to the inorganic particles from the sediment compartment (du Châtelet *et al.* 2010). Thus, we can infer that the concentration and composition (small versus large particles) of suspended inorganic material strongly influenced the species that use smaller particles to build exogenous shells, which favoured their occurrence in the aquatic environment.

In contrast, we observed that some testate amoeba species with exogenous shells, mainly Centropyxidae and Diffugiidae species, were more abundant in environments with lower concentrations of suspended inorganic material. It is known that these species are usually larger when compared to other exogenous species (Velho *et al.* 2000, 2001). Thus, we can infer that these bigger testate amoeba species are adapted to using the larger particles from the sediment to build their shells. This could be related to a possible lack of larger particles in the water column (Medioli *et al.* 1987).

The occurrences of endogenous species were more influenced by environments with lower concentrations of suspended inorganic material, specifically: *Cyphoderia* cf. *ampulla*, *Euglypha acanthophora*, *Lesquereusia spiralis*, *L. spiralis* var. *hirsuta*, *L. spiralis* var. *caudata*, *Hoogenraadia criptostoma*, *Plagiopyxis callida*, *Trinema lineare*, and Arcellidae species. This suggests that this environmental requirement is not a limiting factor for these species (Reid 1961), which produce their shells regardless of the presence or absence of inorganic particles.

Thus, the positive relationship observed between the concentration of chlorophyll-*a* and species diversity highlighted the importance of this environmental requirement as food for the entire community of testate amoebae, as predicted. Furthermore, higher concentrations of suspended inorganic material predicted the distribution of some exogenous testate amoebae in the environments evaluated. However, our prediction was supported only in part, because the suspended inorganic material contributed to the occurrence of testate amoeba species with smaller exogenous shells (and not to all species with exogenous shells). Our results emphasised the role of chlorophyll-*a* and suspended inorganic material as important environmental resources in the occurrence of shell traits in testate amoebae community.

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SUPPLEMENTAL MATERIAL

Table 1. List of species and functional trait related to the shell composition.

	Endogenous	Exogenous
ARCELLINIDA		
ARCELLIDAE		
<i>Arcella arenaria</i> Greef, 1866	X	
<i>A. brasiliensis</i> Cunha, 1913	X	
<i>A. catinus</i> Penard, 1980	X	
<i>A. conica</i> (Playfair, 1917)	X	
<i>A. costata</i> Ehrenberg, 1847	X	
<i>A. crenulata</i> Deflandre, 1928	X	
<i>A. dentata</i> Ehrenberg, 1838	X	
<i>A. discoides</i> Ehrenberg, 1843	X	
<i>A. gibbosa</i> Penard, 1890	X	
<i>A. gibbosa mitriformis</i> Deflandre, 1928	X	
<i>A. hemisphaerica</i> Perty, 1852	X	
<i>A. hemisphaerica</i> f. <i>undulata</i> Deflandre, 1928	X	
<i>A. megastoma</i> Penard, 1902	X	
<i>A. mitrata</i> Leidy, 1879	X	
<i>A. mitrata</i> var. <i>spectabilis</i> Deflandre, 1928	X	
<i>A. nordestina</i> Vucetich, 1973	X	
<i>A. rota</i> Daday, 1905	X	
<i>A. vulgaris</i> Ehrenberg, 1830	X	
<i>A. vulgaris</i> f. <i>elegans</i> Deflandre, 1928	X	
<i>A. vulgaris penardi</i> Deflandre, 1928	X	
<i>A. vulgaris</i> f. <i>undulata</i> Deflandre, 1928	X	
CENTROPYXIDAE		
<i>Centropyxis aculeata</i> (Ehrenberg, 1838)		X
<i>C. aerophila</i> Deflandre, 1929		X
<i>C. aerophila</i> var. <i>sphagnicola</i> Deflandre, 1929		X
<i>C. cassis</i> (Wallich, 1864)		X
<i>C. constricta</i> (Ehrenberg, 1841)		X
<i>C. discoides</i> (Penard, 1890)		X
<i>C. ecornis</i> Deflandre, 1929		X
<i>C. gibba</i> Deflandre, 1929		X
<i>C. hirsuta</i> Deflandre, 1929		X
<i>C. marsupiformis</i> (Wallich, 1864)		X
<i>C. platystoma</i> (Penard, 1902)		X
<i>C. spinosa</i> (Cash, 1905)		X
<i>Cucurbitella crateriformis</i> G.L. & Th., 1960		X
<i>Cu. dentata</i> G.L. & Th., 1960		X
<i>Cu. dentata</i> f. <i>crucilobata</i> G.L. & Th., 1960		X
<i>Cu. dentata</i> f. <i>quinelobata</i> G.L. & Th., 1960		X
<i>Cu. dentata</i> f. <i>trilobata</i> G.L. & Th., 1960		X

<i>Cu. madagascariensis</i> G.L. & Th., 1960		X
<i>Cu. mespiliformis</i> Penard, 1902		X
<i>Cu. mespiliformis</i> var. <i>africana</i> G.L. & Th., 1960		X
<i>Cu.</i> var. <i>africana</i> f. <i>trilobata</i> G.L. & Th., 1960		X
CYPHODERIDAE		
<i>Cyphoderia</i> cf. <i>ampulla</i> (Ehrenberg, 1840)	X	
DIFFLUGIIDAE		
<i>Diffflugia acuminata</i> Ehrenberg, 1838		X
<i>D. acuminata</i> var. <i>inflata</i> Penard, 1899		X
<i>D. acutissima</i> Deflandre, 1931		X
<i>D. amphoralis</i> var. <i>globosa</i> G.L. & Th., 1958		X
<i>D. amphoralis</i> var. <i>cornuta</i> Hopkinson, 1909		X
<i>D. angulostoma</i> G.L. & Th., 1958		X
<i>D. bicruris</i> G.L. & Th., 1958		X
<i>D. capreolata</i> Penard, 1902		X
<i>D. corona</i> Wallich, 1864		X
<i>D. corona</i> var. <i>ecornis</i> (G. L. & Th., 1958)		X
<i>D. corona</i> f. <i>tuberculata</i> Vucetich, 1973		X
<i>D. correntina</i> Vucetich, 1978		X
<i>D. curvicaulis</i> Penard, 1899		X
<i>D. difficilis</i> Thomas, 1955		X
<i>D. elegans</i> Penard, 1890		X
<i>D. fragosa</i> Hempel, 1898		X
<i>D. globularis</i> Wallich, 1864		X
<i>D. globulosa</i> Dujardim, 1837		X
<i>D. gramen</i> Penard, 1902		X
<i>D. helvetica</i> var. <i>multilobata</i> G.L. & Th., 1958		X
<i>D. kempny</i> Stepánek, 1953		X
<i>D. lanceolata</i> Penard, 1902		X
<i>D. limnetica</i> (Levander, 1900)		X
<i>D. lingula</i> Penard, 1911		X
<i>D. litophila</i> Penard, 1902		X
<i>D. lobostoma</i> Leidy, 1879		X
<i>D. lobostoma</i> var. <i>cornuta</i> G.L. & Th., 1958		X
<i>D. lobostoma</i> var. <i>multilobata</i> G.L. & Th., 1958		X
<i>D. lobostoma</i> var. <i>tuberosa</i> G.L. & Th., 1958		X
<i>D. microclaviformis</i> (Kourov, 1925)		X
<i>D. multidentata</i> G.L. & Th., 1958		X
<i>D. muriculada</i> G.L. & Th., 1958		X
<i>D. muriformis</i> G.L. & Th., 1958		X
<i>D. muriformis</i> f. <i>crucilobata</i> G.L. & Th., 1958		X
<i>D. nebeloides</i> G.L. & Th., 1958		X
<i>D. oblonga</i> Ehrenberg, 1838		X
<i>D. parva</i> (Thomas, 1954)		X
<i>D. pleustonica</i> Dioni, 1970		X

	Endogenous	Exogenous
<i>D. pseudogramen</i> G.L. & Th., 1958		X
<i>D. schuurmani</i> Van Oye, 1931		X
<i>D. stellastoma</i> Vucetich, 1989		X
<i>D. urceolata</i> Carter, 1864		X
<i>D. ventricosa</i> Deflandre, 1926		X
<i>Pontigulasia compressa</i> (Carter, 1864)		X
<i>Protocucurbitella coroniformis</i> var. <i>ecornis</i> G.L. & Th., 1960		X
<i>Suiadifflugia multipora</i> Green, 1975		X
HELEOPERIDAE		
<i>Heleopera petricola</i> Leidy, 1879		X
HYALOSPHEIIDAE		
<i>Nebela penardiana</i> Deflandre, 1936		X
LESQUEREUSIIDAE		
<i>Lesquereusia mimetica</i> Penard, 1902		X
<i>L. modesta</i> Rhumbler, 1896		X
<i>L. modesta</i> var. <i>caudata</i> (Playfair, 1917)		X
<i>L. ovalis</i> G.L. & Th., 1960		X
<i>L. spiralis</i> (Ehrenberg, 1840)	X	
<i>L. spiralis</i> var. <i>hirsuta</i> (G.L. & Th., 1958)	X	
<i>L. spiralis</i> var. <i>caudata</i> Playfair, 1917	X	
<i>Netzelia oviformis</i> (Cash, 1909)		X
<i>N. tuberculata</i> (Wallich, 1864)		X
<i>N. wailesi</i> (Ogden, 1980)		X
PHRYGANELLIDAE		
<i>Phryganella hemisphaerica</i> Penard, 1902		X
PLAGIOPYXIDAE		
<i>Hoogenraadia criptostoma</i> G.L. & Th., 1958	X	
<i>Plagiopyxis callida</i> Penard, 1910	X	
TRIGONOPYXIDAE		
<i>Cyclopyxis kahli</i> (Deflandre, 1929)		X
EUGLYPHIDA		
EUGLYPHIDAE		
<i>Euglypha acanthophora</i> (Ehrenberg, 1841)	X	
TRINEMATIDAE		
<i>Trinema lineare</i> Penard, 1890	X	