**Are natural microcosms composed of community alternative stable states? Insights from a simple community of ciliates and testate amoebae within the phytotelma of *Tillandsia utriculata* L. (Bromeliaceae) in Guánica State Forest, Puerto Rico**

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## ABSTRACT

Changes in environmental parameters such as abiotic factors, trophic dynamics, and nutrient availability have been suggested as the primary drivers of stable alternate community shifts. Abiotic environmental thresholds, such as conductivity and nutrients can be important factors driving these shifts in bromeliad microcosms. Although primarily described for macrobiota, predation could also be an important factor, driving stable alternate microbial communities through density-mediated indirect interactions and direct predation effects. Furthermore, nutrient availability is also known to generate shifts in the community through bottom-up effects. Both a longitudinal study and a manipulation experiment were performed to determine whether conductivity, pH, nutrient availability, and the presence of a generalist predator were significant factors explaining community shifts of testate amoebae and ciliates in the phytotelma of *Tillandsia utriculata.* If abiotic parameters are the drivers of testate amoebae and ciliate alternate stable communities, then changes in such parameters should be concomitant with shifts in testate amoebae and ciliate species diversity and composition. If predation and nutrient availability are significant factors explaining the alternation of testate amoebae and ciliate communities, then testate amoebae and ciliate communities in the predator and nutrient inputs treatments should be significantly different in comparison with their repective controls. Microcosm experiments were conducted in a controlled environment; one experiment with *Macrostomum tuba* as the generalist predator and the other experiment with manipulation of $NO_3^-$ and $PO_4^{-3}$. The testate amoebae community had low abundances and species richness, thus making their community analyses impossible. For the longitudinal study, a multivariate analysis showed a ciliate community shift between wet and dry seasons and a Repeated Measure ANOVA showed significant differences across time for conductivity and phosphate, moreover a PCA revealed that conductivity explains 96% of the ciliate abundance variation. For the predation experiment, an ANOSIM test revealed two different ciliate communities between treatments. Nutrient availability had no effect on the ciliate community as revealed by an ANOVA. In conclusion, I suggest that conductivity, phosphate, and predation could be important factors causing alternate stable-state ciliates communities of *T. utriculata* in the Guánica Dry Forest.

## RESUMEN

Cambios en parámetros ambientales, tales como factores abióticos, dinámicas tróficas y disponibilidad de nutrimentos, han sido sugeridos como promovedores principales de cambios en comunidades alternas estables. Umbrales ambientales abióticos, tales como la conductividad y los nutrimentos pueden ser factores importantes promoviendo estos cambios en microcosmos bromelícolas. A pesar de ser descrita principalmente para la macrobiota, la depredación puede también ser un factor importante promoviendo comunidades alternas estables microbianas a través de interacciones indirectas mediadas por la densidad y por efectos directos de la depredación. Por otra parte, es también conocido que la disponibilidad de nutrimentos genera cambios en la comunidad a través de efectos ascendentes. Ambos, un estudio longitudinal y un experimento de manipulación fueron realizados para determinar si la conductividad, el pH, la disponibilidad de nutrimentos y la presencia de un depredador generalista son factores significativos explicando cambios comunitarios de amebas testadas y ciliados en el fitotelma de *Tillandsia utriculata*. Si los factores abióticos son los impulsores de comunidades alternas estables de amebas testadas y ciliados, entonces cambios en dichos parámetros deben ser concomitantes con cambios en la riqueza y composición de especie. Además si ambos, la depredación y la disponibilidad de nutrimentos son factores significativos, explican la alternancia en comunidades de amebas testadas y ciliados, entonces la comunidad de amebas testadas y ciliados en los tratamientos con el depredador y con entrada de nutrimentos deberá ser diferente en comparación con sus controles respectivamente. Experimentos en microcosmos fueron conducidos en un ambiente controlado; uno de los experimentos contenía *Macrostomum tuba* como el depredador generalista y en el otro experimento con manipulación de las concentraciones de $NO_3^-$ y $PO_4^{-3}$. La comunidad de amebas testadas tenía bajas abundancias y riqueza de especies por lo que un análisis comunitario fue imposible. Para el estudio longitudinal un análisis multivariado demostró cambios en la comunidad de ciliados entre la temporada seca y lluviosa y un Análisis de Varianza de Medidas Repetidas demostró diferencias significativas a través del tiempo para la conductividad y el fosfato, además un Análisis de Componentes Principales reveló que la conductividad explica el 96% de la variación en al abundancia de ciliados. Para el estudio de depredación, un Análisis de Similitud reveló dos comunidades diferentes de ciliados entre los tratamientos. La disponibilidad de nutrimentos no tiene un efecto en la comunidad de ciliados como lo reveló un análisis de varianza. En conclusión, se sugiere que la conductividad, el fosfato y la depredación pueden ser factores importantes generando estados alternos estables en las comunidades de ciliados de *T. utriculata* en el Bosque Seco de Guánica.

"If Nobel prizes were given to the organisms who made the discovery
rather than only to the researchers,
protists would rank prominently on that list."

**Yana Eglit and Moselio Schaechter**

## DEDICATION

**TO GOD...**

**MY PARENTS...**

**MY SIBLINGS...**

**MY FAMILY...**

**THIS IS FOR YOU!**

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# Are natural microcosms composed of community alternative stable states? Insights from a simple community of ciliates and testate amoebae within the phytotelma of *Tillandsia utriculata* L. (Bromeliaceae) in Guánica State Forest, Puerto Rico

## 1. Introduction

Changes in environmental parameters such as nutrient availability, trophic dynamics and abiotic factors have been suggested as the primary drivers of community shifts. This alternative stable state theory suggests that rather than a single-state community, there are different states in an ecosystem changing from one to another (Beisner et al., 2003; Dent et al., 2002; Scheffer et al., 2001; Schröder et al., 2005). Although alternative stable states have been reported from terrestrial and freshwater ecosystems, there is more evidence from laboratory studies than from field studies (Schröder et al., 2005). Small, controlled laboratory systems are more practical to demonstrate alternative stable states and may be due to the complexity of natural ecosystems, including the difficulty to disentangle large community networks. Thus, ideal experimental approaches to evidence alternative stable states in nature are natural microcosms. These microecosystems, such as tank bromeliads, have small communities in simple habitats and are more sensitive to disturbance (Srivastava et al., 2004), making them an excellent model to test whether the alternative stable state theory applies to less complex and contained ecosystems.

Bromeliads are Neotropical plants that are highly abundant in forests, therefore playing key roles on large-scale processes (i.e., primary productivity, species reservoir, methane generation) (Martinson, et al., 2010; Richardson, 2010). Some species of bromeliads have a peculiar arrangement of their leaves that accumulates rainfall, seeds, other organic matter and thus

represents a nutrient rich phytotelma (Kitching, 2000). This phytotelma is known to store different concentration of nutrients over time due to seasonality-dependent inputs like rainfall (i.e., during wet season), and airborne inputs such as aerial dust (i.e., during dry season) (Benzing et al., 2011; Schlesinger & Marks, 1977). Bromeliad phytotelma supports a diverse aquatic community of detritivores such as insect larvae, annelids, ciliates and testate amoebae (Barberis et al., 2011; Carrias et al., 2001; Dunthorn et al., 2012; Richardson, 1999; Srivastava et al., 2004; Torres, 2001). Therefore, using simple trophic webs as those observed in tank bromeliads will ease the determination of which environmental driver may induce, a shift in the community of ciliates and testate amoebae, two predominant microbial groups within phytotelmata (Carrias et al., 2001; Torres, 2001).

Ciliates from tank bromeliads play a pivotal role in nutrient dynamics within the phytotelmata as evidenced by their abundance (Carrias et al., 2001) and the presence of bromeliad-restricted associated species (Dunthorn et al., 2012; Foissner et al., 2003, 2010; Foissner & Stoeck, 2011; Foissner & Wolf, 2009; Foissner, 2003a-b, 2010). For example, Carrias et al. (2010) reported ciliate abundances ranging from 50 to 200 individuals per mL from tank bromeliads in French Guiana (*Aechmea mertensii* (Meyer) Schultes, *Vriesea splendens* (Brongniart) Lemaire and *Vriesea pleiosticha* (Grisebach) Gouda). There are no reported data about the density of testate amoeba in the phytotelma of bromeliads; however, extrapolating from other microcosms such as the semi-aquatic moss *Hylocomium splendens* (Hedw.) Br. Eur., testate amoeba abound up to 29,500 $\pm$ 3,000 per dry gram of dry moss (Mitchell et al., 2004). Recently, Acosta-Mercado et al. (2012) reported testacean abundances (individuals/g of dry weight) between 2.14 to 93.3 for tropical mosses in Dominican Republic. Ciliates and testate amoebae are abundant species in

aquatic microcosms, therefore key players in nutrient cycling in ecosystems (Sherr & Sherr, 1994; Wilkinson & Mitchell, 2010). Thus any shifts in the diversity and abundance of ciliate and testate amoebae communities could also have potential effects concerning the length and number of links within the bromeliad food webs, therefore affecting nutrient dynamics. This effect could move along the whole forest, therefore affecting the contributions that bromeliad make to large-scale processes in ecosystems.

Numerous biotic and abiotic factors have been suggested as regulators of the alternative stable state theory. Two main hypotheses suggest that top-down control via predation and bottom-up control via nutrient inputs can trigger shifts in communities. For example, in the presence of a generalist predator (*Mecistogaster modesta*), ciliate species richness was significantly reduced in a bromeliad ecosystem in a rain forest in Costa Rica (Srivastava & Bell, 2009). This reduction in species richness was unexpected since generalist predators have the same feeding pressure in the whole food-web since they can switch preys very easily (Faga, 1997), therefore maintaining species richness. It has been suggested by Price & Morin (2004) that colonization history of predators affects the community composition of ciliates. For instance, when *Blepharisma americanum* Suzuki was established prior to *Tetrahymena vorax* Kidder, in controlled microcosm experiments, *T. vorax* could not become established; however when *T. vorax* was established prior to *B. americanum* both species coexisted (Price & Morin, 2004). To my knowledge there are no published data on the effect of predation in testate amoebae communities, even though it is known that testate amoebae are prey of other microorganisms such as ciliates (*Frontonia leucas* Ehrenberg) testate amoebae (*Nebela* sp.) and invertebrates

(oligochaetes [*Enchytraeidae*], nematodes, springtails [*Collembola*] and mites [Acarina]) (Dias & D'agosto, 2006; Gilbert et al., 2003; Schroeter, 2001).

In terms of nutrient availability, nitrogen and phosphate are known to induce changes in species composition, species richness of microbial eukaryotic communities. For example, a slow input of phosphorus and nitrogen in lakes increases ciliate species richness from 14 to 22 (Buosi et al., 2011). Similarly, the composition of ciliates associated with the roots of the water hyacinth, changes in the presence of nitrogen and phosphorus in comparison to the community in the absence of this input (Buosi et al., 2011). Furthermore, ciliate species richness in Jiaozhou Bay in China is negatively correlated to soluble reactive phosphate and $NO_2^-$ concentrations and positively correlated to $NH_3^+$ concentrations (Gong et al., 2005). Species abundance is also affected by nutrient status, for example the abundance of the testate amoebae *Bullinaria indica* Penard in *Sphagnum* sp. increased after nitrogen amendments (Mitchell & Gilbert, 2004).

Abiotic factors, such as pH and conductivity, are known to induce changes in community composition, species richness and abundance of ciliates and testate amoebae communities. At least, for freshwater ecosystems, pH could be a crucial factor determining the distribution of ciliates, despite the fact that few studies emphasize in the effect of pH in ciliates communities (Weisse & Stadler, 2006). Under laboratory conditions, a change in pH from 4 to 10 induced a change in both, cell volumes and the population growth rates of *Urotricha farcta* Claparede & Lachmann, *U. furcata* Schewiakoff and *U. castalia* Muñoz, Tellez & Fernandez-Galiano (Weisse & Stadler, 2006). However, it is well known that pH is a crucial factor explaining community composition of testate amoebae. Based on ordination and partial constrained analysis, the

community structure of testate amoebae from Lake Superior seemed to be controlled by environmental factors (i.e., depth to water table and pH) (Booth, 2001). Moreover, the size of the shell of *Nebela tincta-parvula-collaris* group was positively correlated ($r^2$ = 0.68) to pH (Booth, 2001).

Given the trophic position of ciliates and testate amoeba, predation and nutrient availability could be influential factors explaining changes in community composition, abundance and species richness, and thus alternative stable states in nature under different predation and nutrient regimes. In this study, I used ciliates and testate amoeba communities to test first whether an alternative stable community was present in the phytotelma of a tank bromeliad (*Tillandsia utriculata* L.) within the Guánica Dry Forest. In addition to elucidating which parameters induce community shifts; this study generated baseline knowledge about the magnitude, diversity, and community structure of ciliates and testate amoebae in the phytotelma of *T. utriculata* within the Guánica Dry Forest. This was the first field study done with tank bromeliads in Guánica State Forest focusing on the ecology of ciliates and testate amoebae.

## 2. Literature review

### 2.1 Alternative stable states in ecosystems

Although the theory of alternative stable states was proposed in 1969 by Lewontin, ecologists are still looking for evidence within both, natural and controlled ecosystems (Scheffer et al., 2001). The theory suggests that an ecosystem may have a community that exists as more than one persistent or stable community (Schröder et al., 2005) and that these stable states differ in composition and diversity (Scheffer et al., 2001). Thus the functionality of these communities

within the ecosystem may also change. In nature, alternative stable states have been demonstrated in marine (Dudgeon et al., 2010), river (Dent et al., 2002), and lake communities (Scheffer & van Nes, 2007). Major drivers include environmental parameters such as turbidity, water level, pH, salinity, fires (Dent et al., 2002; Scheffer et al., 2001), predation (Price & Morin, 2004), herbivory (Handa et al., 2002), and nutrient availability such as phosphate and nitrate (Scheffer & van Nes, 2007). For example, in shallow lakes in England two alternate stable states communities predominate; the algal state when the turbidity of the lake is high, whereas the plant state occurs in the absence of turbidity (Scheffer et al., 1993). Similarly, plant communities in coastal marshes shift from a salt tolerant (aquatic angiosperms, alkali grasses, sedges and eudicotyledons) to freshwater-grassland (grasses and shrubs) communities. The shift was geomorphologically induced but only when no geese-grazing pressure occurred (Handa et al., 2002).

Terrestrial ecosystems, like woodlands, also exhibit alternative stable states. Plant communities from the Yellowstone National Park in Idaho changed as a consequence of the reintroduction of the top predator, the gray wolf (*Canis lupus* L.) (Beschta & Ripple, 2010). From 1995-1996 wolves were reintroduced to the park in an effort to restore the wolves' population; however as the population of wolves increased, the elk population (*Cervus elaphus* L.) decreased. This reduced the selective pressure from herbivory caused by the elk generated a plant community shift from grassland to a woody plant community. As a consequence there was an increase in the recruitment and abundance of cottonwood (*Populus angustifolia* James and *Populus trichocarpa* Torr. & Gray) and an increase growth of willow (*Salix boothii* Dorn.) (Beschta & Ripple, 2010).

If the population of elks increases as a consequence of wolves' population reduction, then the plant community will probably shift back to grassland.

Microbial communities also exhibit alternative stable states. The phytoplankton community in shallow lakes can be dominated either by green algae or by cyanobacteria (Scheffer et al., 1997). For instance, in Germany, lakes with less than 3 m of depth were dominated by cyanobacteria species (*Planktothrix* and *Oscillatoria*). This dominance is a consequence of high turbidity and phosphate levels (Scheffer et al., 1997), plus the feedback that maintains this state since cyanobacteria also increase turbidity levels. A community switch to green algae requires a decrease of the phosphorus levels. Consequently, cyanobacteria will start to decrease, as well as the turbidity levels, and the green algae community will dominate the lake until subsequent phosphorus flushes out.

**2.2 Bromeliads as microcosms to test alternative stable states in nature**

Bromeliads (Bromeliaceae) are Neotropical plants that are highly abundant and significant contributors of the net primary productivity in rain forests (Martinson et al., 2010; Richardson, et al., 2000). Among the 2,600 species of bromeliads, only one species (*Pitcairnia feliciana* (A. Cheval.) Harms & Mildbr.) is found in the eastern hemisphere in Africa (Acevedo-Rodríguez & Strong, 2005). According to the morphology of the bromeliads, they can be classified into two major types or ecological types. These are the phytotelma and the non-phytotelma bromeliads. The phytotelma bromeliads are classified into three types: weakly developed phytotelma (type II), well developed phytotelma that carries out CAM photosynthesis (type III), and well developed phytotelma that carries out C3 photosynthesis (type IV). The non-phytotelma

bromeliads are classified into two types: non-absorptive foliar trichomes (type I) and the absorptive foliar trichomes over the entire shoot (type V) (Benzing, 2000). Among the various types, the architecture of bromeliads type III and IV generate simple habitats that are colonized by microbial communities.

The composition of microbial communities has been extensively studied in species of *Aechmea, Billbergia, Guzmania, Tillandsia, Thecophyllum* and *Vriesia* (Kitching, 2000). The diversity of species within bromeliad phytotelma has been comprehensively described and can include Archaea (Methanomicrobiales, Crenarchaeota, Methanosaetaceae and Methanocellales) (Martinson et al., 2010), bacteria (purple non-sulfur and fecal coliforms) (Rivera et al., 1988; Soto-Feliciano et al., 2010), protists (algae, ciliates and testate amoebae) (Carrias et al., 2001; Foissner et al., 2003; Sophia, 1999; Torres, 2001) and invertebrates (Annelida, Arthropoda, Mollusca, Platyhelminthes, Rotifera, Gastrotricha, Nematoda and Tardigrada) (Kitching, 2000; Richardson, 1999; Wagner et al., 2008). Nutrient release from this community interaction (Ngai & Srivastava, 2006) is used by the bromeliad to grow and reproduce in forests.

One new family (Petasidae) and 3 new species of testate amoebae that reside in the phytotelma of *Vriesea* spp. have been described: *Petasus astrum* Torres, *Ascoida modesta* Torres, *Kitotrilocula vriesea* Torres (Torres, 2001). A total of 48 species of ciliates have been identified inhabiting the phytotelma of bromeliads (*Aechma* spp., *Guzmania musaica* (Linden & André) Mez., *G. scherzeriana* Mez., *Tillandsia heterophylla* E. Morren) in the Caribbean, Central and South America. From these, 1 new familiy (Bromeliophryidae) plus 13 new species are new taxonomical records (Foissner, 2003). Four new bacterivorous species have been described:

*Bromeliophrya brasiliensis* Foissner, *Cotterillia bromelicola* Foissner, *Orborhabdostyla bromelicola* Foissner and *Pseudovorticella bromelicola* Foissner. Three new species that feed on bacteria and flagellates include: *Bromeliothrix metopoides* Foissner, *Glaucomides bromelicola* Foissner and *Platyophrya bromelicola* Foissner. Finally a new voracious species was described as *Lambornella trichoglossa* Foissner that feeds on both bacteria and flagellates, as well as on rotifers. The remaining species are still in the process of description (Dunthorn et al., 2012; Foissner, 2003a-b, 2010; Foissner et al., 2010, 2003; Foissner & Stoeck, 2011; Foissner & Wolf, 2009). All of these new records suggest that phytotelmata represent an important microecosystem for microbial food webs and within macro-ecosystems.

**2.3 Ciliates as models to test alternative stable states in nature**

Ciliates are a group of heterotrophic eukaryotic microorganisms characterized by a complex alveolar cytoskeleton, nuclear dimorphism and a sexual process known as conjugation (Lynn, 2008). The cytoskeleton of ciliates is composed of three typical fibrillar associations: kinetodesmal fibrils, transverse and postciliary microtubular ribbons. The cellular membrane of ciliates is characterized by the presence of alveoli, giving the superphylum Alveolata its name. The have two types of nuclei, macronuclei and micronuclei vary in form, number, and arrangement between species. During conjugation, they exchange the gametic nuclei, giving the ciliates a high genetic diversity (Lynn, 2008).

Ciliates comprise a monophyletic group (Ciliophora) distributed across eleven classes that are distinguished by nuclear and ciliary arrangement and patterns. They vary in shape and size and inhabit a wide variety of microhabitats such as the intestine of ruminants, and freshwater, marine

and terrestrial ecosystems (Lynn, 2008). A total of 4,500 free living species have been described, with 83% to 89% species still undescribed (Foissner et al., 2007).

Ciliates play key roles in aquatic ecosystems such as lakes and ponds. Their high feeding and growth rates (Jonsson, 1986**)** make them an excellent model to test alternative stable states, since we can track community changes during small periods of time. Predation, nutrient availability, and abiotic factors such as pH are suggested to triggers shifts in ciliate communities (Buosi et al., 2011; Price & Morin, 2004; Srivastava & Bell, 2009; Weisse & Stadler, 2006; Wickham et al., 2004). In a factorial study with 4 replicates in two freshwater ecosystems (lake and littoral) in Sweden, the abundance and biomass of ciliates was reduced by 40% ($p<0.0001$) and by 38% ($p=0.0125$) respectively when the grazers (larvae of chironomids and trichopterans, ostracods, amphipods and isopods) were removed (Wickham et al., 2004). Similarly, a slow release of nutrients such as nitrogen and phosphate from fertilizers increased ciliate biomass by 68% ($p=0.0176$), but their abundance was not affected (Wickham et al., 2004). This implies that nutrient inputs via fertilizer addition or by nutrient release via grazing pressure can control the biomass contribution of ciliates to freshwater ecosystems.

**2.4 Testate amoebae as models to test alternative stable states in nature**

In contrast to ciliates, testate amoebae constitute a polyphyletic group of heterotrophic ameboidal microorganisms that are enclosed in a shell. From its aperture, the organism extends its pseudopodia to move and to capture its prey. Pseudopodia can be lobose (Amoebosoa) or filifom (Filosea) (Qin et al., 2011). The test can be proteinaceous, siliceous, calcareous, or made from foreign material (xenosomes) (Meisterfeld, 2000). The morphometry of the test is used to

identify the testate amoebae. Amoebosoa includes larger species (> 100 µm) and are represented in four families: Difflugiidae, Centropyxidae, Arcellidae and Hyalospheniidae. Filosea include smaller species (< 100 µm) and are represented by two families Euglyphidae and Trinematidae. A total of 2,000 taxa have been described, leaving about 2,000 other taxa as undescribed (Smith et al., 2008). They have a variety of forms and are distributed in freshwater ecosystems, soils, and mosses. Testate amoebae play key roles in nutrient cycling, especially in soils ecosystems (Wilkinson, 2008).

The community structure of testate amoebae in *Sphagnum* from Poland was explained by the depth of the water table and by pH (21% of the total variation explained). Changes in pH seemed to generate three communities; one of these at low pH during dry conditions dominated by *Assulina muscorum* Greeff, *Euglypha rotunda* Wailes, *E. tuberculata* Dujardin, *Heleopera sylvatica* Penard, *Nebela tincta* (Leidy) Awerintzew and *Corythion* spp.. A second community also under low pH during wet conditions was dominated by *Nebela griseola* Penard, *Hyalosphenia elegans* Leidy, *Amphitrema flavum* Archer, *A. wrighttianum* Archer, *A. stenostoma* Nusslin, *Arcella discoides* Ehrenberg, *Nebela carinata* (Archer) Leidy and *Difflugia leidyi* Wailes. At high pH, the third community was assembled and dominated by *Centropyxis aculeata* (Ehrenberg) Penard, *C. hirsuta* Deflandre, *C. aerophila* Deflandre, *C. ecornis* (Ehrenberg) Leidy and *Nebela bohemica* Taraneck (Lamentowicz & Mitchell, 2005). These results suggest that pH can shape community composition of testate amoebae, therefore altering the nutrient dynamic in ecosystem.

## 3. Objectives

1. Determine whether the community of ciliates and testate amoebae in the phytotelma of *Tillandsia utriculata* L. in Guánica State Forest exhibits alternative stable states.
2. Test the effect of a generalist predator (*Macrostomum tuba*) on ciliate and testate amoebae abundances, species richness, and composition in a simple trophic web derived from the bromeliad *Tillandsia utriculata* L.
3. Determine if resource nutrient availability (phosphorus and nitrate) can trigger a shift in the community of ciliates and testate amoeba.

## 4. Hypotheses

1. Given that phosphate and nitrate inputs in phytotelma are seasonality-dependent, then there should be concomitant changes in species richness, abundance, and composition of the community of ciliates and testate amoebae between seasons.
2. If predation is a significant factor explaining the alternation of ciliate and testate amoebae communities, then the ciliate and testate amoebae community in the predator treatment should be significantly different in comparison with the control.
3. If nutrient availability is the main driver of alternative stable states in the phytotelma of *T. utriculata* in the Guánica Dry Forest, then bromeliads that differ in terms of nitrate and phosphorus will show significant differences in the abundance, richness, and community composition of ciliates and testate amoebae.

# 5. A longitudinal survey of testate amoebae and ciliates communities coupled to environmental parameters: disentangling community shifts in bromeliad phytotelmata

## 5.1 Introduction

Communities can exhibit different stable states that differ in composition and diversity (Scheffer et al., 2001), in contrast to the traditional single persistent state within the same ecosystem (Schröder et al., 2005). This theory was proposed by Lewontin by the 1960's, however in 1956 Alfred J. Lokta also noted that multiple states can exists (Petraitis & Dudgeon, 2004). The shift from one state to another can occur suddenly or gradually, it will depend on the factor triggering such shift (Scheffer et al., 2001). Biotic factors such as predation (Beschta & Ripple, 2010; Price & Morin, 2004) and herbivory (Handa et al., 2002), and abiotic factors such as nutrient availability of phosphate and nitrate (Buosi et al., 2011) are among the suggested factors driving state shifts. Despite its pervasiveness as an ecological theory scientists are still looking for evidence of what can trigger such stable states, if any, in different environments.

Alternative stable states have been demonstrated in both natural and artificial environments (Schröder et al., 2005), spanning terrestrial (Schmitz, 2004), marine (Dudgeon et al., 2010) and freshwater ecosystems (Dent et al., 2002; Scheffer et al., 1997). Their existence has been primarily observed under controlled artificial environments (Chase, 2003; McCauley et al., 1999; Price & Morin, 2004; Schröder et al., 2005) since small controlled laboratory systems are more suitable to test alternative stable states in contrast to the complexity of natural ecosystems. Natural microcosms are small contained systems (Srivastava et al., 2004), similar to laboratory systems, thus making them excellent natural ecosystems to test alternative stables states.

Bromeliads are one particular microcosm present in Puerto Rico. They are Neotropical plants with a particular leaf arrangement that creates a phytotelma (Benzing, 2000; Kitching, 2000; Srivastava et al., 2004). Phytotelmata store different nutrient levels across time due to seasonality-dependent inputs like rainfall, airborne inputs such as aerial dust and organic matter such as detritus, litter and seeds (Barberis et al., 2011; Richardson et al., 2000; Schlesinger & Marks, 1977). For instance, during dry seasons, airborne and aerial dusts are the main nutrient inputs in bromeliads (Benzing, 2000; Schlesinger & Marks, 1977), as opposed to rainfall and litter fall during wet season (Benzing, 2000, Cavalier et al., 1997). Airborne inputs are richer in nutrient during dry seasons due to the lack of rainfall; however the high amounts of rainfall during wet seasons increase the contribution of nutrients from litter decomposition (Benzing, 2000). This organic input holds up a diverse freshwater community that includes: bacteria (Rivera et al., 1988; Soto-Feliciano et al., 2010), Archaea (Martinson et al., 2010), protists (Carrias et al., 2001; Dunthorn et al., 2012; Foissner et al., 2007; Sophia, 1999; Torres, 2001) and animals (Kitching, 2000; Ngai & Srivastava, 2006; Richardson et al., 2000; Richardson, 1999; Srivastava et al., 2005; Srivastava & Bell, 2009; Wagner et al., 2008). Two of the overlooked groups of organisms in this environment are the ciliates (Carrias et al., 2001) and testate amoebae. Despite our lack of data on their community structure, the fact that recent studies revealed new species of ciliates and testate amoebae (Dunthorn et al., 2012; Foissner et al., 2003; Torres, 2001) suggests that phytotelmata of bromeliad represent an important microecosystem for diversity studies.

Phototrophic and heterotrophic protists have been used as model organisms to test alternative stable states in freshwater ecosystems. Colonization history under controlled artificial microcosms is an alternative stable states trigger. Green algae (*Scenedesmus quadricauda* (Turpin) Brébisson, *Selenestrum* sp. and *Ankistrodesmus falcatus* (Corda) Ralfs generated alternative stable states in 250 mL artificial freshwater microcosms. If *S. quadricauda* colonized the microcosms before *Selenestrum* sp. and *A. falcatus,* the latest two became rare or even extinct (Drake, 1991). However, the three species could coexist in the microcosms during 50 days when the colonization order was the following: first *A. falcatus*, second *Selenestrum* sp. and third *S. quadricauda* (Drake, 1991). Similarly, after 48 days, when the ciliate *Blepharisma americanum* Suzuki was established before the introduction of *Tetrahymena vorax* Kidder, in controlled microcosm experiments *T. vorax* could not become established; in contrast when, *T. vorax* was established before *B. americanum* both species could be established (Price & Morin, 2004). The fact that alternative stable states have been detected in protist populations under controlled experimentation suggests that protists are ideal model organisms to test the alternative stable state theory under natural conditions.

Nutrient concentrations may be crucial for shaping protist communities as shown in freshwater ecosystems where both, ciliates and testate amoebae are related to nutrient concentrations (Beaver & Crisman, 1989; Rublee & Partusch-Talley, 1995); therefore any change in nutrient inputs could trigger changes in ciliate and testate amoebae communities. For instance, ciliate species richness associated with the roots of *Eichhornia crassipes* increased from 14 to 22 when nitrate and phosphate were added to the lake (Buosi et al., 2011). Similarly the community composition experienced concomitant changes in species composition, where 25% of the 85

species identified during the study were present only under eutrophic conditions (Buosi et al., 2011). Planktonic ciliates from tropical and temperate lakes followed this trend, changing from being dominated by the suborder Oligotrichida under oligotrophic conditions to being dominated by the subclass Scuticociliatia under eutrophic conditions (Beaver & Crisman, 1982, 1989). High abundance levels of ciliates were also achieved under eutrophic conditions (i.e. 200 cells/mL), while low abundances under oligotrophic conditions (i.e. 2-11 cells/mL) (Beaver & Crisman, 1989). Similarly increases in abundance (1,040 individuals to 2,690 individuals) and biomass levels (0.12 mg C to 0.33 mg C) were recorded for testate amoebae in lotic ecosystems in Alaska, associated withan increase of phosphate and nitrate (Partusch-Talley, 1995). Moreover, nutrients seem to be a crucial factor explaining the abundance and community composition of testate amoebae, at least for bryophyte microcosms. In *Sphagnum* sp. the relative abundance of *Bullinularia indica* Penard increased concomitantly with increases in the nitrogen inputs (Mitchell & Gilbert, 2004) and total phosphorus explained 21% of the variation in testate amoebae communities (Mieczan, 2009a).

Given the sensibility of protist to environmental disturbance (Acosta-Mercado & Lynn, 2006; Yang et al., 2011), ciliates and testate amoebae could be excellent models to test whether alternative stables states are present in these communities. Thus, understanding the primary driver of a community shift within bromeliad phytotelmata is pivotal since it is now well established that bromeliads have a significant effect on large scale processes such as forest methane emissions (Martinson, et al., 2010), primary productivity (Richardson, et al., 2000), and the magnitude of species reservoir (Richardson, et al., 2000). The focus of this part of the study was to assess if the community of ciliates and testate amoebae in the phytotelma of *Tillandsia*

*utriculata* L. in Guánica State Forest exhibits alternative stable states. The Guánica Dry Forest is one of the most preserved subtropical dry forests in the world (Murphy and Lugo, 1990), moreover it was designated a Biosphere Reserve by the UNESCO in 1981. It is important to disentangle the ecological mechanisms sustained within the Guánica Dry Forest, from the microecosystem to macro ecosystem levels. Given that nutrient inputs in the phytotelma microecosystems are seasonality-dependent, then there should be concomitant changes in species richness, abundance, and composition of the community of ciliates and testate amoebae.

## 5.2 Methodology

### 5.2.1 Study site

The Guánica State Forest is located in the south region of Puerto Rico within the municipalities of Guánica, Guayanilla, Peñuelas, Ponce, and Yauco, and has an approximate area of 4,400 ha. The mean annual precipitation is 762 mm. The wet season starts in May and ends in November; whereas the dry season spans the months from December to April. Precipitation peaks have been reported between the months of August and November. Air temperatures in the forest range from 26.6 ˚C to 37.7 ˚C (DRNA, 2006).

A total of six species of *Tillandsia* were confirmed for the Guánica Dry Forest: *T. fasciculata* Sw., *T. flexuosa* Sw., *T. recurvata* (L.)L., *T. setacea* Sw., *T. utriculata* L. and *T. variabilis* Schltdl. (Monsegur-Rivera, 2009). The bromeliads in the forest are scattered along forest trails, where the understory is partially open. They are vertically distributed 1-2 m above the ground. For this study, five randomly selected bromeliads, within the Ballena trail, were longitudinally

sampled to analyze community structure parameters for ciliates and testate amoebae present in their phytotelmata (Fig. 5.1a).

### 5.2.2 Sampling for ciliate and testate amoeba communities

A longitudinal study was made to determine whether the community structure of ciliates and testate amoebae shifted in a stable manner across time period of 12 months. Five randomly selected bromeliads were sampled in 2011 across the wet and dry seasons. In order to study the phytotelma during the dry season, approximately just 1 g of litter from inside the bromeliad's phytotelma were collected, in order to maintiant enough litter for subsequent samplings, and activated by adding 100 mL of filtered river water from the Toro Negro River (TNR) to 1 g of the collected litter in plastic microcosms. This amount of water used (100 mL) was the minimum volume requeried in order to analyze nutrient concentrations and community structure. This stream was selected because it is a non-polluted and non-eutrophicated river in Puerto Rico; water from this stream has been used successfully by colleagues to establish ciliate cultures (Garzón, 2008). To ensure that ciliates and testate amoebae are from the litter within the bromeliad and not from cysts from the Toro Negro River, the water was sterilized by filtration through a 0.20 µm nylon membrane (Whatman™, 7402-004). To resemble the natural architecture of bromeliads and to keep the spatial heterogeneity constant, plastic microcosms were constructed with one plastic cup and six green plastic "leaves", two large, two medium and two small "leaves" (Srivastava, 2006) (Appendix A). After two days of litter reactivation, a subsample of 2 mL was collected and fixed in 8 mL of Bouin's fixative for a Edaphic Quantitative Protargol Staining (EQPS) procedure (Acosta-Mercado & Lynn, 2003). Fixing the ciliates and testate community after two days of activation allowed me to capture a significant

proportion of the diversity of ciliates and testate amoebae (Foissner, 1987; 1992). During wet season, at least 22 mL of suspension were collected from each bromeliad (Fig. 5.1b) and pipetted in a non-destructive manner to avoid destruction of the bromeliad. Admittedly the best method to extract community data is through bromeliad dissection (Jocque et al., 2010); however, Jocque et al. (2010) showed that for studying phytotelmata communities, pipetting was a method that captured representative data. From the collected sample, a subsample of 2 mL was fixed as explained above. Species were identified to the lowest taxonomic level possible using: Berger (1999, 2011); Foissner et al. (2002); Foissner & Xu (2007); Foissner (1993); Ogden & Hedley (1980); Mazei & Tsyganov (2006). Environmental parameters, such as pH and conductivity were measured during sampling. The remaining water from the sample was filtered through a 0.20 µm nylon membrane (Whatman, 7402-004) for a nutrient extraction of phosphate, nitrate, and ammonium. The samples were analyzed by the Water Quality Analysis Laboratory at the University of New Hampshire

### 5.2.3 Statistical analysis

To test whether there were significantly different communities of ciliates and testate amoebae in the phytotelma of *T. utriculata* between seasons, an Analysis of Similarity (ANOSIM) test was performed (Hammer, et al., 2012). A *t*-test was performed to test if there were significant differences in the abundance and species richness of ciliate and testate amoebae between seasons (Di-Rienzo et al., 2012). An appropriate test to analyze the abundances and species richness between seasons is a Repeated Measures ANOVA due to the interaction of time; however some samples were lost during the EQPS procedure, therefore a *t*-test was performed. A Repeated Measures ANOVA, with autoregressive model was performed to determine whether there were

statistically significant differences among the environmental parameters across time (SAS® Institute Inc, v 9.3, 2013). An autoregressive model assumes correlation structures within the data, so is an effective analysis for this experiment. The community composition, defined as species dominance, of ciliates and testate amoebae were analyzed with a non-Metric Multidimensional Scaling analysis (nMDS) and a principal component analysis (PCA) to determine if there is an ordination pattern between the community and the environmental parameters (Hammer et al., 2012). A Stepwise Multiple Regression was calculated to determine which environmental factor is determining the community structure (Di-Rienzo et al., Infostat 2012).

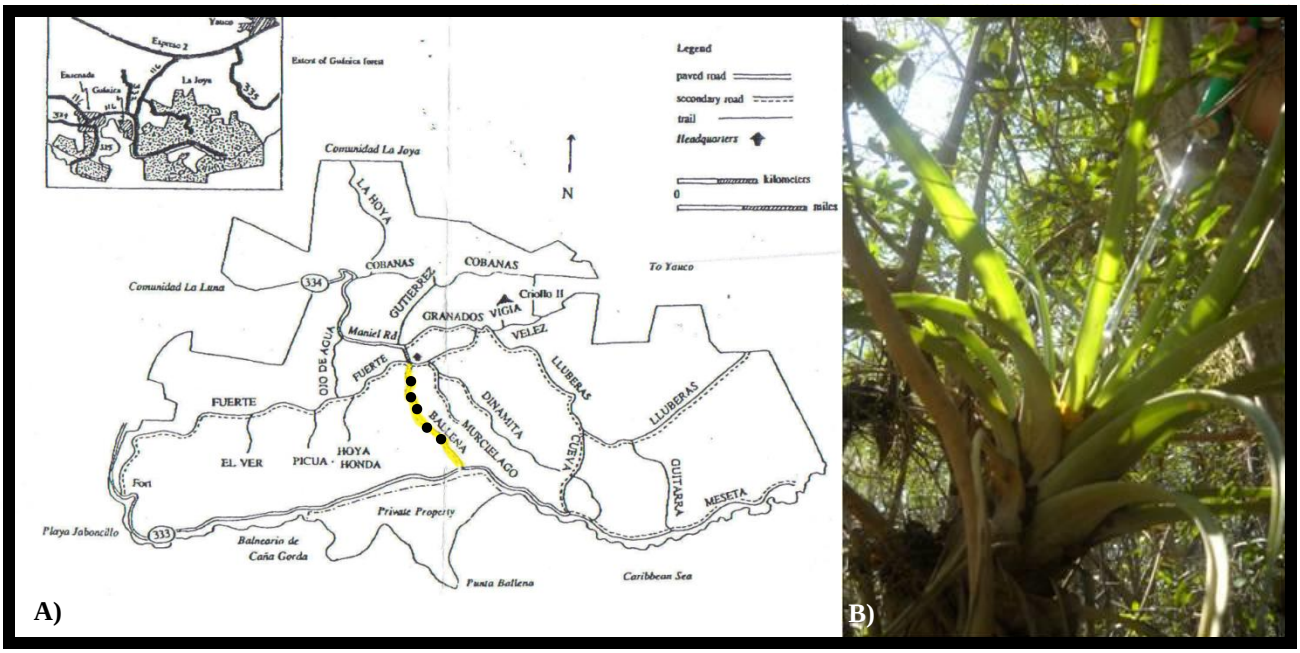


Figure 5.1 A) Map of the trails in the Guánica Dry Forest (DNER). The community of ciliates and testate amoebae in the analyzed phytotelma of *Tillandsia utriculata* was collected from five randomly selected bromeliads (black dots) scattered along the Ballena Trail. B) A non-destructive sampling technique with pipettes was performed to ensure the preservation of the bromeliad.

## 5.3 Results

### 5.3.1 Environmental variables dynamics

Overall, the water accumulated in the bromeliad was slightly acid for both seasons (Table 5.1). The pH for the water during the dry season ranged from 5.5 to 7.2 whereas for the wet season ranged from 4.20 to 7.30. A repeated measures ANOVA, showed that pH differs significantly between seasons ($p$= 0.006) but not over time within season ($p$= 0.34) (Table 5.1). Figure 5.2 shows the temporal dynamics of precipitation recorded Guánica during 2011. Figure 5.3 shows the temporal dynamics of pH in the phytotelma of *T. utriculata* throughout year 2011 in the Guánica State Forest.

Conductivity levels, ranged from 22 µS/cm to 1,116 µS/cm. Higher conductivity levels were recorded for dry season (486.40 ± 243.46 µS/cm) in contrast to wet season (210.50 ± 186.27). A Repeated Measures ANOVA, demonstrated significant differences for both, between seasons ($p$= 0.005) and over time within season ($p$= 0.0009) (Table 5.1). Figure 5.4 shows the temporal dynamic of conductivity in the phytotelma of *T. utriculata* through year 2011 in the Guánica State forest.

Nutrient concentration analyses showed no significant differences for ammonium and nitrate for neither of both, between seasons and over time within season (Table 5.1). The mean (± SD) ammonium concentration was 484.4 ± 603.3 µg/L, while the mean nitrate concentration was 0.67 ± 2.51 mg/L. However, the Repeated Measures ANOVA showed significant differences in the phosphate concentration for both, between season ($p$= 0.02) and over time within season ($p$=

0.01) (Table 5.1). Lower phosphate levels were recorded in dry season (59.70 ± 95.98 µg/L) than the wet season (257.10 ± 413.06 µg/L). Figures 5.5, 5.6 and 5.7 show respectively the temporal changes of ammonium, nitrate and phosphate in the phytotelma of *T. utriculata* throughout year 2011 in the Guánica State forest.

Table 5.1 Mean ± SD of the environmental variables measured between the wet and dry seasons. The *p* values are the result of repeated measures ANOVA with an autoregressive model.

| **Environmental variable** | **Season** | | ***p* value** | |
|---|---|---|---|---|
| | **Dry** | **Wet** | **Between seasons** | **Over time within season** |
| pH | 6.8 ± 0.10 | 5.9 ± 0.1 | **0.006** | 0.344 |
| Conductivity (µS/cm) | 486.4 ± 243.46 | 210.5 ± 186.3 | **0.005** | **0.0009** |
| Ammonium (µg/L) | 442 ± 728.6 | 527 ±460.8 | 0.120 | 0.730 |
| Nitrate (mg/L) | 0.2 ± 0.35 | 1.2 ± 3.51 | 0.060 | 0.500 |
| Phosphate (µg/L) | 59.7 ± 95.89 | 257.1 ± 413.1 | **0.020** | **0.010** |

### 5.3.2 Ciliates community

There was a shift in the community composition of ciliates in the phytotelma of *T. utriculata* in the Guánica Dry Forest concomitant to the seasonality regime of the forest as demonstrated by an ANOSIM test ($p$= 0.01) and an nMDS (Stress= 0.16) (Fig. 5.7). According to a SIMPER test the communities are 94% dissimilar. A total of 37 ciliates morphospecies were identified through the longituinal study (Table 5.2). Significant differences were recorded for abundance and species richness, as showed by a *t*-test (Table 5.3). Higher abundances (224.5 ± 118.61 individuals/mL) and species richness (12.75 ± 2.16 number/mL) were recorded for the dry season in contrast to the abundances (63.6 ± 43.52 individuals/mL) and species richness (6.4 ± 2.77 number/mL) in the wet season (Table 5.3). Throughout the dry season, the most abundant

ciliates were represented by the orders Colpodida, Hymenostomatida and Scuticociliatida. On the other hand, through the wet season there were higher abundances for the class Spirotrichea and the orders Peniculida, Prorodontida and Sessilida. The dominat species in both season was *Colpoda maupasi* Enriques, followed by *Platyophrya bromelicoa* Foissner & Wolf. These two species compromised the 66% of the community. Colpodea was the most represented class with eleven species identified, followed by Spirotrichea and Litostomea, both with five species, and the subclasses Peritrichia and Scuticociliatia both with four species.

### 5.3.3 Ordination between ciliate community and environmental variables

Conductivity was the environmental variable that explained the ciliate community abundances, as demonstrated by a principal component analysis (PCA). Conductivity explained 94% of the community abundance between seasons (Fig. 5.8). The multiple regression supported this finding, demonstrating that conductivity had a significant effect on community abundance across time ($p$= 0.006, $R^2$=0.45). In addition, the multiple regression depicted that phosphate is also accounting for the community abundance as follows:

$$[\boldsymbol{Total\ abundance} = \mathbf{0.83}\left(\boldsymbol{Conductivity}\ \frac{\mu\mathbf{S}}{\mathbf{cm}}\right) - \mathbf{0.26}\left(\boldsymbol{Phosphate\ concentration}\ \frac{\mu\mathbf{g}}{\mathbf{L}}\right) + \mathbf{0.61}] \quad \textbf{(5.1)}$$

### 5.3.4 Testate amoebae community

A community shift in the testate amoebae community was not observed since a comprehensive analysis of this community was not possible due to low levels of abundances and species richness. However, 14 species were observed (Table 5.2), of which 9 had the ameboidal organism inside the shell, whereas 5 were always observed as empty shells. The live testate amoeba belonged to the orders: Euglyphida (*Euglypha cristata* Leidy, *E. hyalina* Couteaux, *E.*

*rotunda* Ehrenberg, *E. tuberculata* Dujardin, *Trinema lineare* Penard and *Sphenoderia lenta* Schlumberger) and Arcellinida (*Cyclopixis khali* Deflandre, *Hyalosphenia* sp. and *Nebela tubulata* Brown). The empty shells belonged to the orders: Euglyphida (*Assulina muscorum* Greef, *Corythion dubium f. minima* Chardez, *Euglypha laevis* (Ehrenberg) Perty, *Placocista glabra v. minima* Decloitre) and Arcellinida (*Arcella arenaria Greeff*). A total abundance of 22 individuals/mL were recorded for the wet season whereas 8.5 individuals/mL for dry season. *Euglypha rotunda* compromises the 70% of the community during wet season and 41% during dry season.

Table 5.2 Ciliates and testate amoebae identified in the bromeliad *Tillandsia utriculata* in the Guánica Dry Forest.

| **Species** | **Season** | |
|---|---|---|
| | **Dry** | **Wet** |
| **Ciliates** | | |
| *Colpoda cucullus* | ‡ | + |
| *Colpoda inflata* | ‡ | + |
| *Colpoda maupasi* | ‡ | + |
| *Colpoda steinii* | ‡ | + |
| *Grossglockneria* sp. | ‡ | + |
| *Oxytricha ottowii* | ‡ | + |
| Scuticociliatida 1 | ‡ | + |
| Scuticociliatida 2 | ‡ | + |
| Scuticociliatida 3 | ‡ | + |
| Scuticociliatida 4 | ‡ | + |
| *Spathidium* sp.2 | ‡ | + |
| *Telotrochidium* sp. | ‡ | + |
| *Bresslaua vorax* | ‡ | - |
| *Bresslauides discoideus* | ‡ | - |
| Bryophirid like | ‡ | - |
| *Chillodonella unicinata* | ‡ | - |
| *Colpoda aspera* | ‡ | - |
| *Colpoda lucida* | ‡ | - |
| Enchelydid sp. | ‡ | - |
| *Enchelys* sp. | ‡ | - |
| *Glaucoma reniformis* | ‡ | - |

| | | |
|---|---|---|
| *Kreyella minuta* | ‡ | - |

Table 5.2 Cont.

| Species | Season | |
|---|---|---|
| | **Dry** | **Wet** |
| Litonotidae sp. | ‡ | - |
| *Pattersoniella vitiphila* | ‡ | - |
| *Spathidium* sp. | ‡ | - |
| *Balanion planctonicum* | + | ‡ |
| *Coleps hirtus* | + | ‡ |
| *Gonostomum affine* | + | ‡ |
| *Oxytricha longigranulosa* | + | ‡ |
| *Oxytricha* sp. | + | ‡ |
| *Platyophrya bromelicola* | + | ‡ |
| Vorticellid 1 | + | ‡ |
| Vorticellid 2 | + | ‡ |
| Vorticellid 3 | + | ‡ |
| *Cyrtolophosis major* | - | ‡ |
| *Frontonia depressa* | - | ‡ |
| *Tetrahymena piriformis* complex | - | ‡ |
| **Testate amoebae** | | |
| *Assulina muscorum* | ‡ | + |
| *Cyclopixis khali* | ‡ | + |
| *Euglypha hyalina* | ‡ | + |
| *Euglypha rotunda* | ‡ | + |
| *Arcella arenaria* | ‡ | - |
| *Hyalosphenia* sp | ‡ | - |
| *Nebela tubulata* | ‡ | - |
| *Sphenoderia lenta* | ‡ | - |
| *Euglypha cristata* | + | ‡ |
| *Trinema lineare* | + | ‡ |
| *Euglypha laevis* | - | ‡ |
| *Corythion dubium f. minima* | - | ‡ |
| *Euglypha tuberculata* | - | ‡ |
| *Placocista glabra v. minima* | - | ‡ |

*Note:*

Double dagger symbol (‡)= Highest mean abundance

Minus symbol (-)= Organism not observed

Plus symbol (+)= Organism observed

Table 5.3 Mean ± SD of the ciliate community parameters in the dry and wet seasons. The *t*-test showed significant differences between seasons.

| Community parameter | Season | | |
|---|---|---|---|
| | Dry | Wet | *p* value |
| Abundance (individuals/mL) | 224.5 ± 237.21 | 63.6 ± 43.52 | 0.020 |
| Species richness (number/mL) | 12.6 ± 2.16 | 6.4 ± 2.77 | 0.004 |

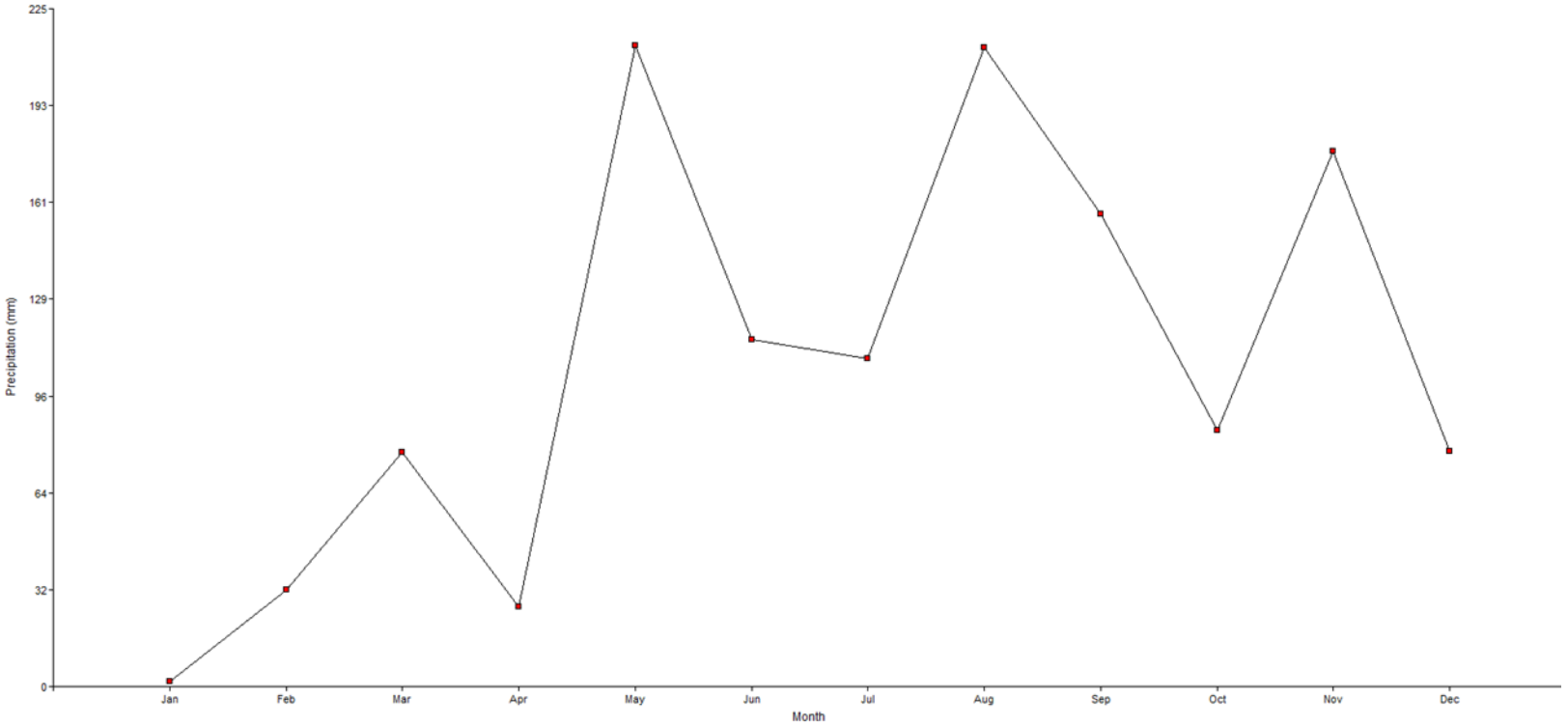


Figure 5.2 Temporal changes in rainfall precipitation (mm) recorded for the Ensenada meteorological station in 2011 (The Southeast Regional Climate Center).

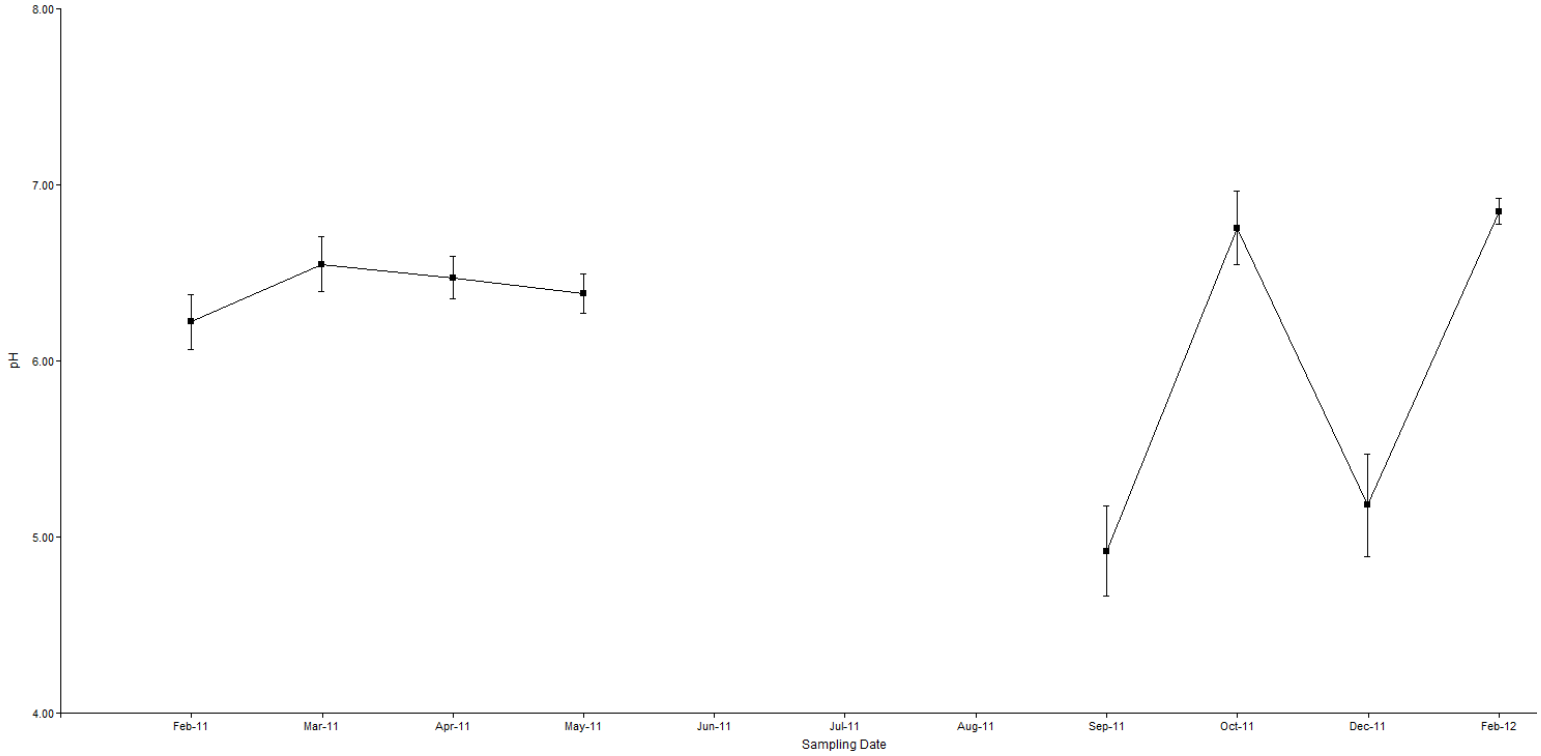


Figure 5.3 Temporal changes in pH ± SEM within the phytotelma of *T. utriculata* through year 2011. Dry season spans from February to April whereas wet season spans from May to December.

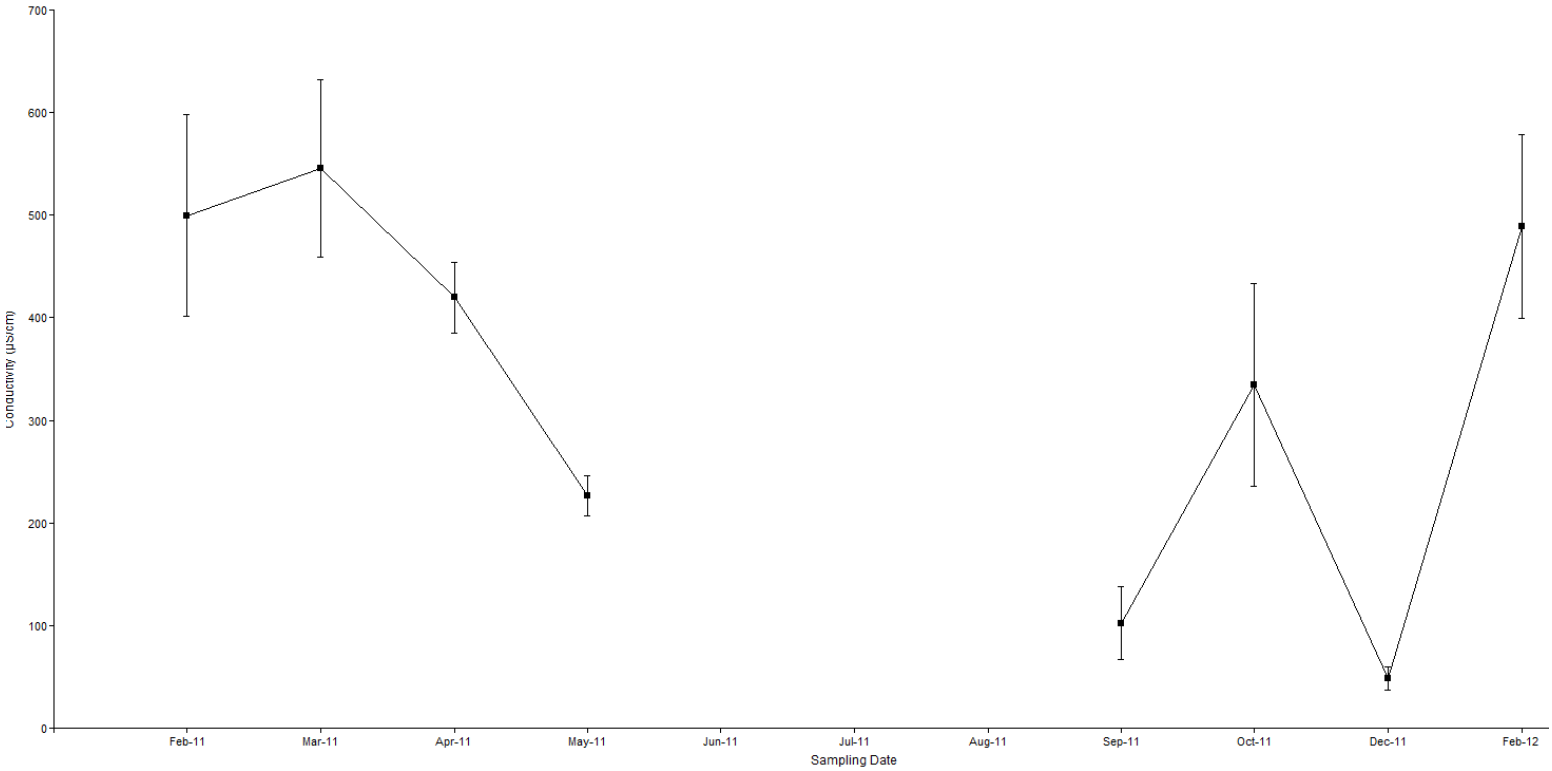


Figure 5.4 Temporal changes in conductivity ± SEM within the phytotelma of *T. utriculata* through year 2011. Dry season spans from February to April whereas wet season spans from May to December.

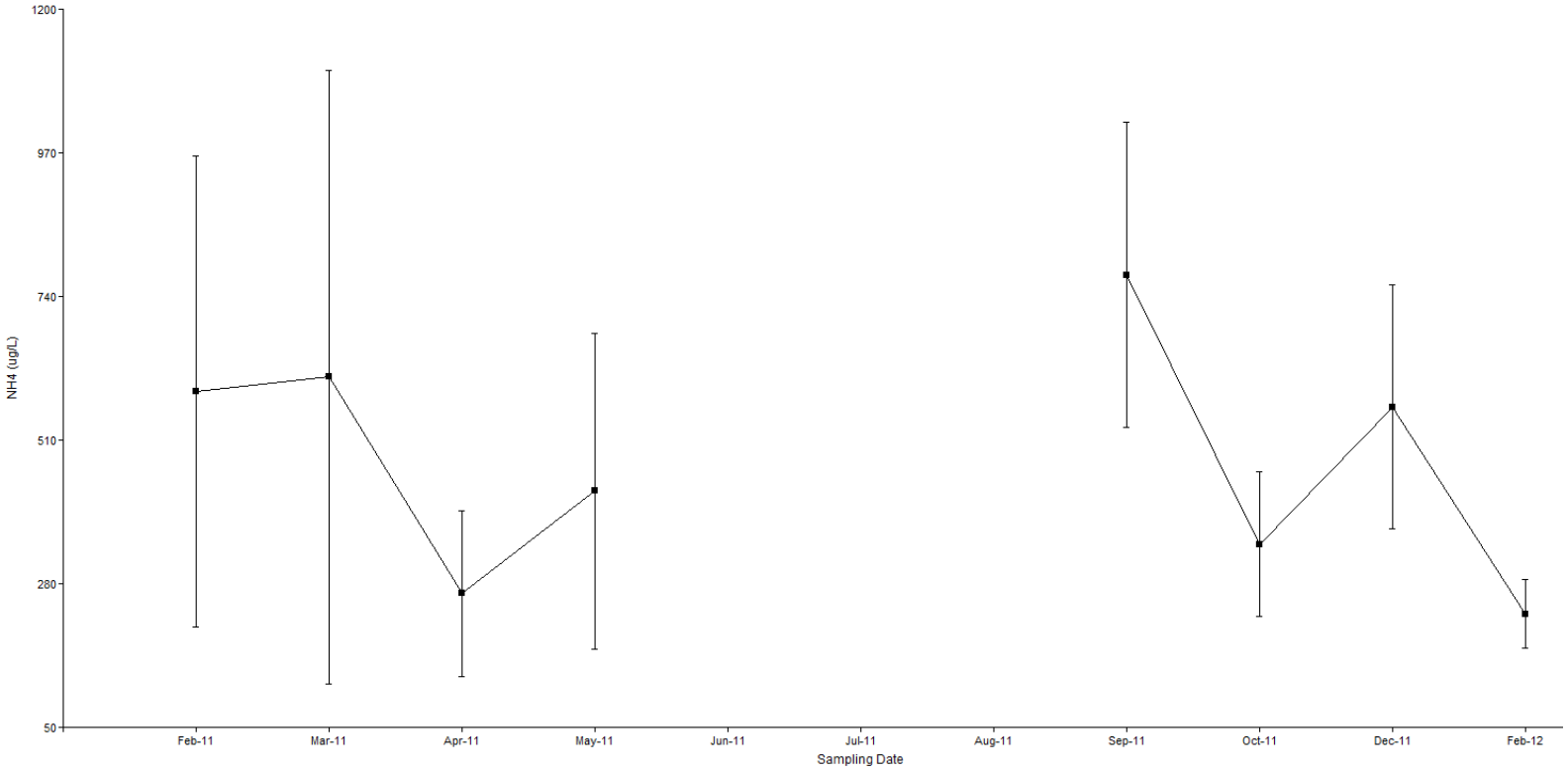


Figure 5.5 Temporal changes in ammonium ± SEM within the phytotelma of *T. utriculata* through year 2011. Dry season spans from February to April whereas wet season spans from May to December.

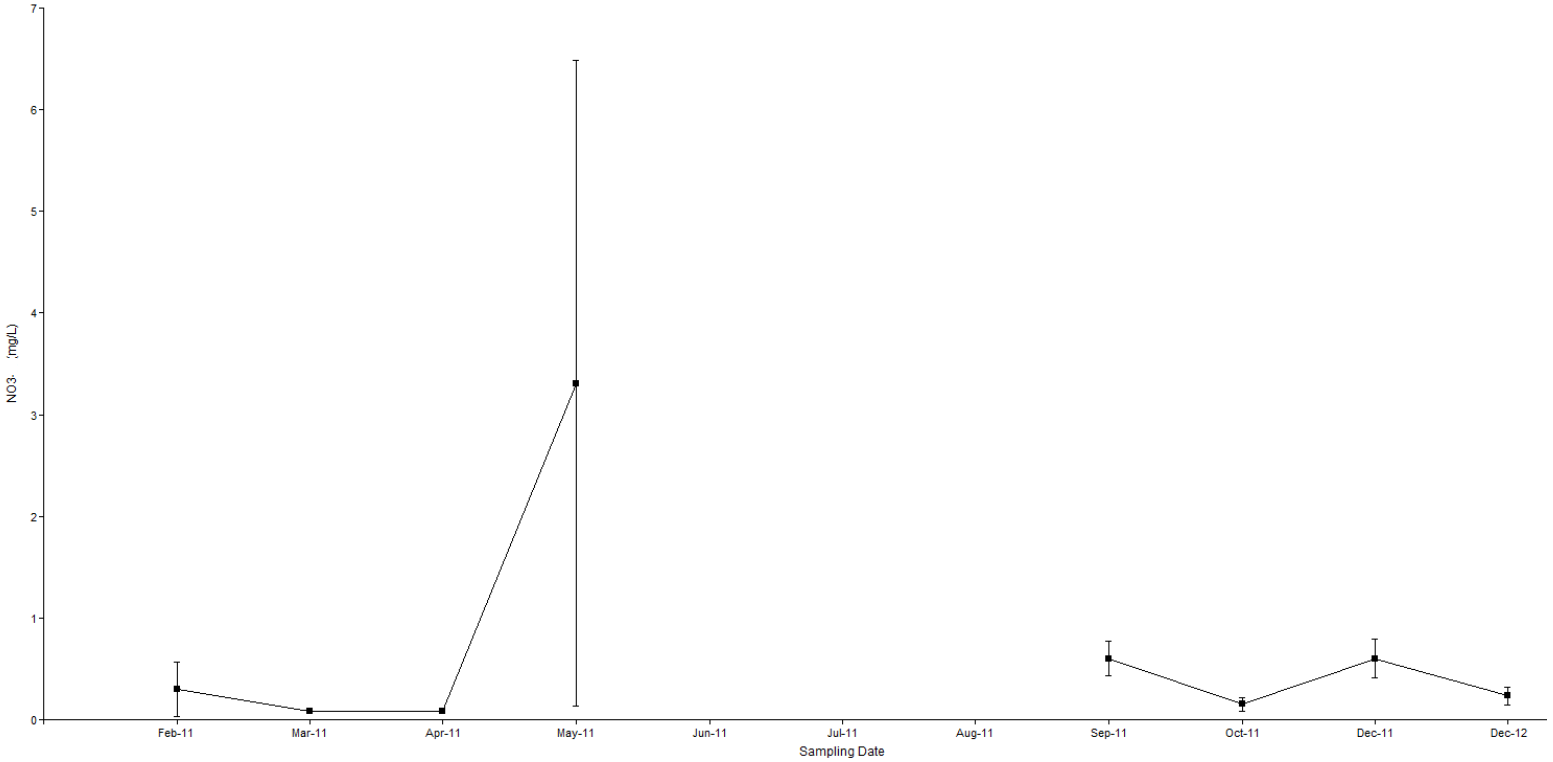


Figure 5.6 Temporal changes in nitrate ± SEM within the phytotelma of *T. utriculata* through year 2011. Dry season spans from February to April whereas wet season spans from May to December.

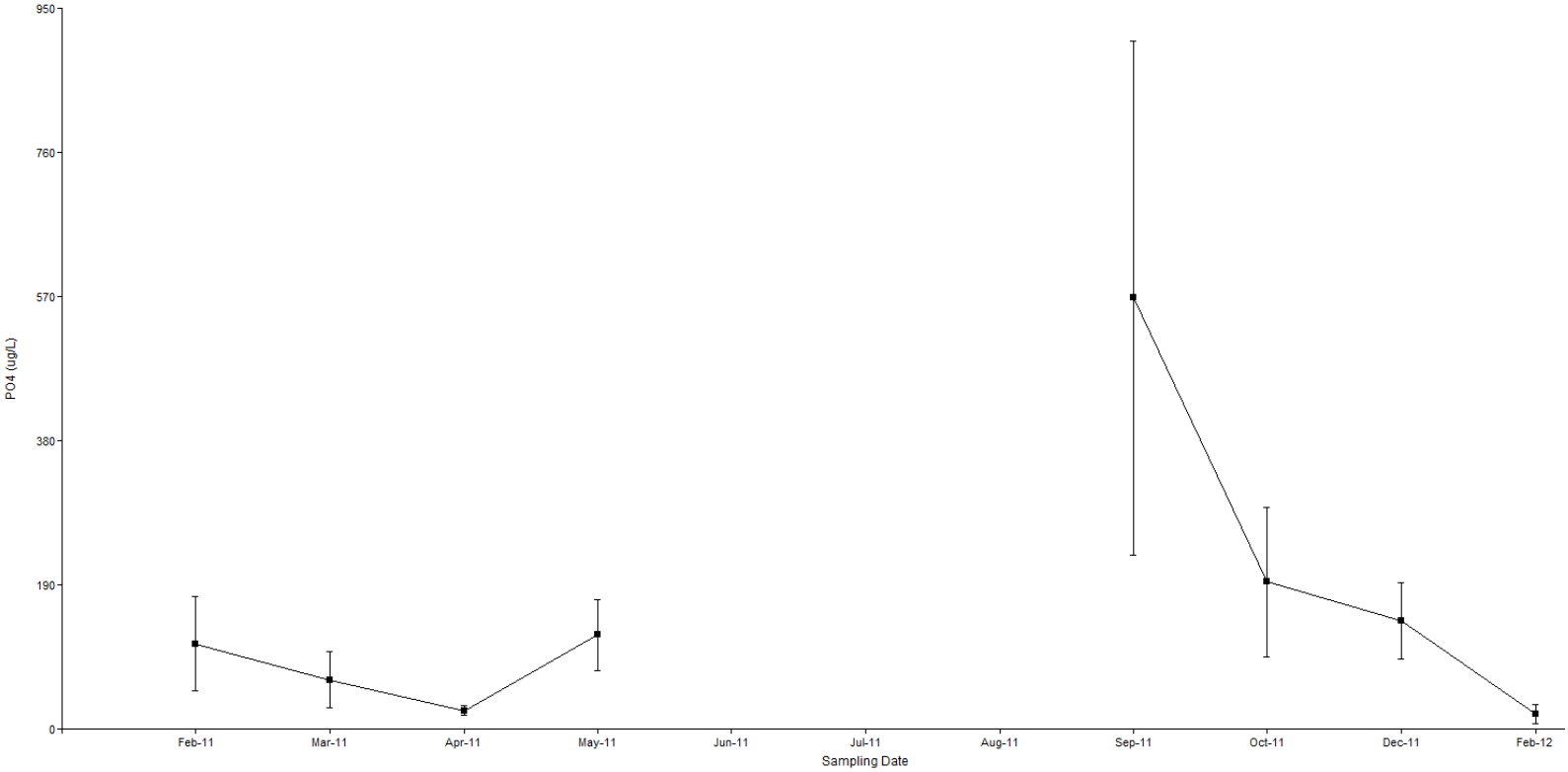


Figure 5.7 Temporal changes in phosphate ± SEM within the phytotelma of *T. utriculata* through year 2011. Dry season spans from February to April whereas wet season spans from May to December.

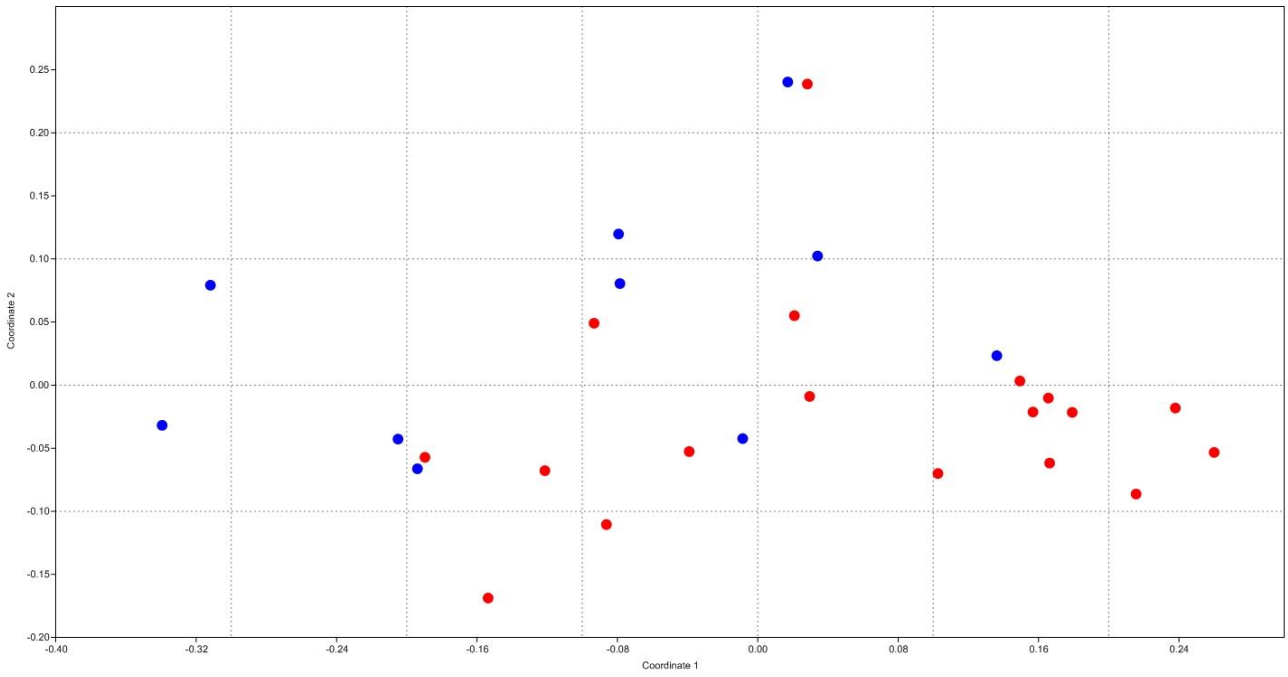


Figure 5.8 The nMDS revealed two different communities ($p$= 0.01, stress=0.16) between seasons: Dry season (red dots) and Wet season (blue dots)

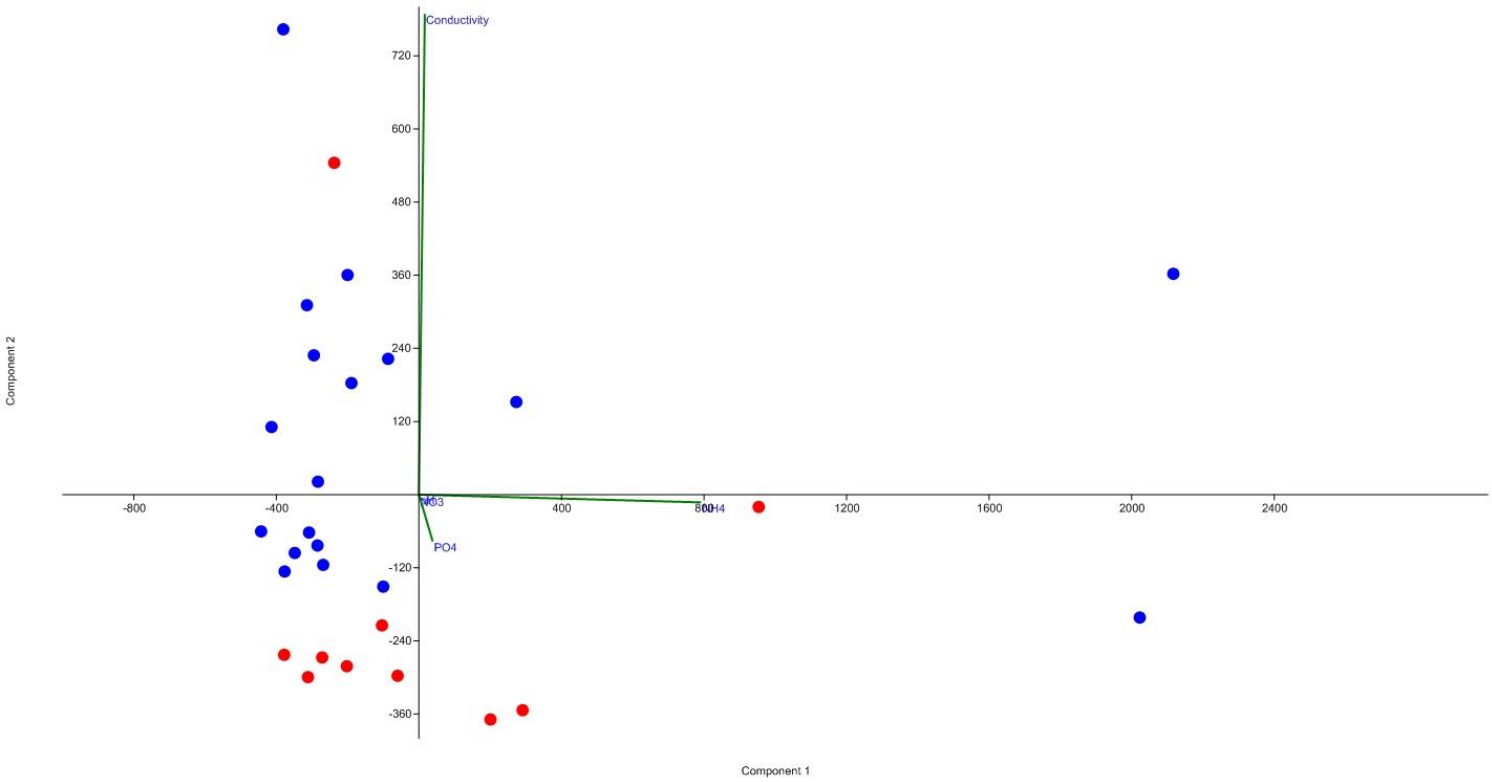


Figure 5.9 A Principal Component Analysis shows that conductivity explained 96% of the variance in the ciliate abundance in the phytotelma of *T. utriculata*. Dry season (red dots) and wet season (blue dots)

## 5.4 Discussion

### 5.4.1 Environmental variables dynamics

This study aimed to know whether the community of ciliates and testate amoebae undergoes community shifts and if so, then elucidate what environmental variables could be triggering the change in the community. The pH values of the accumulated water in the phytotelma of *T. utriculata* are similar to those in published in other phytotelmata through the tropics (Table 5.4). Bromeliad phytotelmata gather water mainly, if not exclusively, from rainfall, therefore pH could be directly affected by the pH of rainfall. It is known that pH of rainfall is slightly acid (5 –

7) (Charlson & Rodhe, 1982; Möller & Zierath, 1986; Sanhueza et al., 1987), explaining the acidity of the water accumulated in phytotelmata.

On the other hand, conductivity levels reported for the bromeliad *T. utriculata* (359.6 ± 257.91 µS/cm) are higher (Table 5.4) than those reported for different bromeliad species phytotelma, like *Neoregelia cruenta* (Graham) L.B. Smith (40 ± 30 µS/cm) and *Aechmea nudicaulis* Griseb. (40 ± 20 µS/cm) (Guimarães-Souza et al., 2006) and *Guzmania brasiliensis* Ule (10 to 50 µS/cm) (Torreias & Ferreira-Keppler, 2011) in Brazilian forests. However, conductivity levels from other phytotelmata, like tree holes in the midstory of a lowland moist forest in Barro Colorado Island (462 ± 194 µS/cm) (Yanoviak, 1999) and rainforest in Perú (398 ± 261 µS/cm) (Yanoviak et al., 2006) are similar to our values. The higher values of conductivity in *T. utriculata* could be due to salt spray depositions from the Caribbean Sea. It is well established that salt spray depositions are affected by: wind speed, wave amplitude and surface area of plants, and thus substantial amounts of salt spray depositions have been recorded up to 6.3 km from the sea (Maun, 2009). I suggest that salt spray is causing an accumulation of salts, higher in the dry season due to the lack of rainfall, which cause an increase in the conductivity levels in the phytotelma of *T. utriculata* in Guánica Dry Forest (Fig. 5.9).

Regarding nutrient levels, the phytotelma of *T. utriculata* accumulates higher amounts of nitrate and ammonium than *Guzmania* and *Vriessa* spp. in El Yunque Rainforest (Table 5.4) (Richardson, et al. 2000). While phosphate levels in *T. utriculata* are higher than those reported from *Guzmania* and *Vriessa* spp. in in El Yunque Rainforest (Table 5.4). The Guánica Dry Forest is known to have high nutrient (nitrates and phosphate) storages, even higher than other

tropical forests (Lugo & Murphy, 1986). For instance, soils in the Guánica Dry Forest have higher storage of nitrogen (9,100 kg/ha) than those reported from a tropical wet forest in Venezuela (3,507 kg/ha), tropical premontane wet forest in Costa Rica (3,310 kg/ha) and from a dry forest in Belize (7,500 kg/ha) (Lugo & Murphy, 1986). Likewise, phosphate storages in Guánica Dry Forest (1,820 k/ha) are higher than those reported for subtropical lower rainforests in Puerto Rico (705 kg/ha) and tropical moist forests in Panamá (11-33 kg/ha) (Lugo & Murphy, 1986). Therefore, higher amounts of nutrients were expected from the phytotelma of *T. utriculata*.

The accumulation of nutrients in bromeliad phytotelmata will also be influenced by the bromeliad metabolism. Several bromeliad species are known to be evolutionarily adapted for both, efficient phosphate uptakes via luxury consumption (e.g., higher phosphate absorption than needed for the plant's metabolism and growth due to high levels of phosphate availability) (Chapin, 1980) and efficient absorption rates (Winkler & Zotz, 2009; Zotz, 2004). Moreover, the fact that phosphate precipitation is higher at higher pH values (Otsuki & Wetzel, 1972) and that higher levels of pH (6.8 ± 0.1) were recorded for *T. utriculata* during dry season, could explain the lower levels of phosphate during dry season. Lower pH values increases the phosphate solubility (Brown, 1973) therefore the higher amounts of phosphate during the wet season in *T. utriculata.*

Table 5.4. Limnological parameters measured in this study and data from diverse phytotelmata ecosystems in the tropics [Modified from Jocque & Kolby (2012)].

| Parameter | Study | Phytotelma | Mean | SD | Country | Note |
|---|---|---|---|---|---|---|
| **pH** | Eterovick* | *Aechmea saxicola* | 4.8 | 0.3 | Brazil | |
| | | *Aechmea victoriana* | 5.2 | 0.6 | | |
| | | *Vriesea neoglutinosa* | 5.3 | 1.3 | | |
| | De Oliveira & Nava | *Vriesea* sp. and *Nidularium* sp. | 4.6 | 0.4 | | |
| | Souza et al.* | *Nidularium cruenta* | 4.6 | 0.8 | | |
| | | *Nidularium nidicaulis* | 5.6 | 0.9 | | |
| | Torreias et al.* | *Guzmania brasiliensis* | 4 | 0.1 | | |
| | Lopez et al.* | *Aechmea nudicaulis* | 5 | 0.8 | | |
| | Torreias & Ferreira-Keppler (2011) | *Guzmania brasiliensis* | 4.6 | | | Lowest value |
| | Guimarães-Souza et al. (2006) | *Neoregelia cruenta* | 4.6 | 0.8 | | |
| | | *Aechmea nudicaulis* | 5.6 | 0.9 | | |
| | Benzing* | *Aechmea bracteata* | 4.6 | | Jamaica | |
| | Janetzky* | *Aechmea paniculigera* | 6 | 0.4 | | |
| | | *Hohenbergia* sp. | 5.8 | 0.3 | | |
| | Epler & Janetzky | *Aechmea paniculigera* | 5.6 | | | |
| | Richardson (1999) | *Guzmania* and *Vriesia* spp. | 5.9 | | Puerto Rico | |
| | **This study** | *Tillandsia utriculata* | 6.3 | 0.7 | | |
| | Yanoviak (1999) | Tree Holes | 6 | 0.1 | Panamá | Understory |
| | | | 6.3 | 0.2 | | Midstory |
| | | | 6.1 | 0.1 | | Canopy |
| **Conductivity (µS/cm)** | Guimarães-Souza et al. (2006) | *Neoregelia cruenta* | 0.04 | 0.03 | Brazil | |
| | | *Aechmea nudicaulis* | 0.04 | 0.02 | | |
| | De Oliveira & Nava | *Vriesea* sp. and *Nidularium* sp. | 17.5 | 2 | | |
| | Torreias & Ferreira-Keppler (2011) | *Guzmania brasiliensis* | 10 | | | Lowest value |
| | Epler & Janetzky | *Aechmea paniculigera* | 27.2 | | Jamaica | |
| | **This study** | *Tillandsia utriculata* | 359.6 | 257.9 | Puerto Rico | |
| | Yanoviak (1999) | Tree Holes | 286 | 23.5 | Panamá | Understory |
| | | | 462 | 194 | | Midstory |
| | | | 324 | 34.4 | | Canopy |
| **Nutrients** | | | | | | |
| **Ammonium (mg/L)** | Richardson et al. (2000) | *Guzmania* and *Vriesia* spp. | 0.57 | | Puerto Rico | T site |
| | | | 0.12 | | | PC site |
| | | | 0.11 | | | DF site |
| | Richardson et al. (2001) | *Heliconia caribaea* | 0.89 | | | U bracts |

Table 5.4 Cont.

| Parameter | Study | Phytotelma | Mean | SD | Country | Note |
|---|---|---|---|---|---|---|
| | | | 1.78 | | | M bracts |
| | | | 3.26 | | | L bracts |
| | | *Guzmania berteroniana* | 0.57 | | | |
| | **This study** | *Tillandsia utriculata* | 0.46 | 0.6 | | |
| **Nitrate (mg/L)** | Richardson et al. (2000) | *Guzmania* and *Vriesia* spp. | 0.03 | | | T site |
| | | | 0.03 | | | PC site |
| | | | 0.02 | | | DF site |
| | **This study** | *Tillandsia utriculata* | 0.64 | 2.3 | | |
| **Phosphate (mg/L)** | Richardson et al. (2000) | *Guzmania* and *Vriesia* spp. | 0.43 | | | T site |
| | | | 0.18 | | | PC site |
| | | | 0.03 | | | DF site |
| | Richardson et al. (2001) | *Heliconia caribaea* | 0.32 | | | U bracts |
| | | | 2.37 | | | M bracts |
| | | | 5.63 | | | L bracts |
| | | *Guzmania berteroniana* | 0.43 | | | |
| | **This study** | *Tillandsia utriculata* | 0.15 | 0.29 | | |

Note: * Data in Jocque & Kolby (2012)
T site: "Tabonuco" site; PC site: "Palo Colorao" site; DF site: Dwarf Forest site, all sites are from El Yunque Rainforest
U bracts: Upper bracts; M bracts: Middle bracts; L bracts: Lower bracts

### 5.4.2 Ciliate Community

Although ciliates have been observed by ecologists in bromeliad phytotelma (Carrias et al., 2001; Srivastava & Bell, 2009), only two publications have a detailed taxonomic check list (Dunthorn et al., 2012; Foissner et al., 2003). Foissner et al. (2003) suggested that there could be hundreds of new species in bromeliads, due to the fact that bromeliads exhibit a high variety of morphological and lifestyle traits (Benzing, 2000). Up to 70% of the ciliates in the community of *T. utriculata* are new reports for a bromeliad phytotelmata, probably because this is the first report of ciliates communities for a dry forest bromeliad. Moreover, among the unidentified species, novel species and even new genera could be found.

Total ciliates richness from *T. utriculata* (38 species) was higher than that reported for bromeliads from Dominican Republic (24 species), Ecuador (24 species) and Brazil (19 species) (Foissner, et al. 2003). In other microcosms such as *Sphagnum* spp. in Poland the ciliate richness have similar values from *T. utriculata*, reaching a peak of 37 species (Mieczan, 2009b). On the other hand ciliate richness from soils is higher in dry forests (Foissner, 1995) and tropical rain forests (Bamforth, 2007), both with 80 species reported, whereas Li et al. (2010) reported a total of 114 species in soils from evergreen forests in Baiyun Mountain, China.

Regarding abundance, the levels reported for ciliates in *T. utriculata* (144 ± 119.5 individuals/mL) were between the ranges reported from bromeliads in French Guiana (50 - 200 individuals/mL). Nevertheless, ciliate abundance in bromeliads is still in comparison to other ecosystems such as, lakes (Jürgens et al. 1999), litter (Bamforth, 2007) and soils (Bamforth, 2007; Li et al., 2010) that fluctuate in the order of hundreds to thousands. For instance, Jürgens et al. (1999) reported total ciliates abundances in the range of 100-600 in Denmark, while Bamforth (2007) reported abundances ranging 1,000–25,000 per gram of litter and 1,000-8,000 per gram of underground soil in Puerto Rico. Similarly Li et al. (2010) reported ciliate abundance ranging from of 9,200-38,700 per gram of soils from China. The lower abundances for ciliates in bromeliads could be due to the fact that bromeliads are semi-closed ecosystems, therefore there is less probability for ciliate cysts to arrive to the phytotelma.

### 5.4.3 Ordination between ciliate community and environmental variables

The longitudinal study revealed that the community of ciliates exhibits a community shift between dry and wet seasons, concomitant to shifts in conductivity and phosphate levels.

Conductivity seems to be a critical factor generating community shifts in this bromeliad community, but mostly during the dry season. A conductivity effect on community composition has been reported for ciliates in German lakes (Pfister et al. 2002). In the Pfister's et al. (2002) case, conductivity was correlated to salinity which might explain the presence of marine ciliate species (e.g. *Euplotes patella* Ehrenberg, *Glaucoma scintillans* Ehrenberg and *Strombidium conicum* Lohmann) in lakes with high conductivity levels (1,180-1348 µS/cm). In our case mostly during the dry season, ciliate species reported are salinity tolerant or have been found in brackish and saline environments (e.g. *Colpoda. steinii, C. inflata*, *C. cucullus*) (Foissner, 1993).

On the other hand, during the wet season, phosphate may be the triggering factor for a community shift. Contrary to published data (Hwang & Heath, 1997), were there is a positive relationship between ciliate abundance and total phosphorus (Pearson's correlation=0.742, $p<0.01$), our ciliates abundances were reduced as phosphate increased. Mathematical models predictions also suggest that an increase in phosphate should be concomitant to increases in ciliates abundance (Thingstad, 2005).

### 5.4.4 Testate amoebae community

A comprehensive analysis of the testate amoebae community was not possible due to low abundances. There was a total of 22 species with mean abundance lower than 1 individual/2 mL. In fact, bromeliads ecosystems should be added to the list of tropical microcosm with low abundances of testate amoebae such as: mosses from Dominican Republic were the lowest mean abundance (individuals/ g of dry weight) was 2.14 for *Acroporia pungens* and the highest was 93.3 for *Thuidium urceolatum* (Acosta-Mercado et al., 2012), freshwater permanent and

ephemeral lakes and ponds in Barbados (<100 individuals per site) (Roe & Patterson, 2006) and anchialine holes in Mexico, were the highest abundance recorded was less than 200 individuals/mL (Van Hengstum et al., 2008a). A similar trend is observed for species richness. Testate amoebae species richness recorded for *T. utriculata* was lower than that reported in tropical bryophytes (83 species) (Acosta-Mercado et al., 2012). However the total species richness from *T. utriculata* are higher than those reported for permanent and ephemeral ponds in Barbados (14 species) (Roe & Patterson, 2006).

The dominant species of testate amoebae in *T. utriculata* is a spineless morphotype of *Euglypha rotunda,* contributing with 45% of the community abundance during the dry season. The dominance of spineless testate amoebae, at least for anchialine holes in Mexico, is associated to salinity increases. Despite the fact that spineless testate amoebae are also found in freshwater ecosystems, salinity is probably one of the environmental variables that together with others variables such as predation and pH, could influence the presence of spines. Additionally, it is believed that spines are involved in buoyancy, and if this statement is true, then it is not surprising what Van Hengstum et al. (2008) suggested since there is a direct relationship between salinity and buoyancy. Moreover none of the morphospecies of testate amoebae observed in *T. utriculata* had spines, even though some morphospecies usually bears spines (e.g. *Euglypha cristata*, *Euglypha rotunda*).

### 5.4.5. A model for ciliates community shifts in *T. utriculata* from Guánica Dry Forest

A possible alternate stable state is occurring in the community of ciliates in the bromeliad *T. utriculata* in the Guánica Dry Forest, as shown in the figure 5.9. This model takes in

consideration the linear regression and the principal coordinate analysis, as well as a Similarty Percentage test. Due to the lack of rain during the dry season, bromeliads in the Guánica Dry Forest start to accumulate salts as a result of salt spray generations from the sea, and the phosphate in the phytotelma starts to precipitate (due to higher pH values). A small amount of humidity in the phytotelma, gathered from dew, still maintains a wet environment and possibly small ciliates species in the phytotelma such as *Colpoa steinii, C. aspera, C. maupasi, Platyoprya bromelicola* and the order Scuticociliatida. Larger species such as *Frontonia depressa* and the subclass Stichotrichia will probably encyst, hence remaining in the phytotelma. Encysted ciliates that tolerate small amount of salts will be reactivated during wet periods. The order Colpoda dominates the ciliate community during the dry season. The orders Hymenostomatida and Scuticociliatida, while not dominant through the dry season, can increase in abundance during this period. When the rain season spawns, the salts are quickly washed from the bromeliads, and phosphate is quickly dissolved as with other temporary aquatic ecosystems (Williams, 2006). The increase in phosphate seems to be an important variable during wet season, causing a shift in the ciliate community of *T. utriculata*. For instance, the abundances of species of the class Spirotrichea and the orders Peniculida, Prorodontida and Sessilida were increased, whereas the abundance of the order Colpoda was reduced. However, the dominant ciliate species during the both seasons is *Colpoda maupasi*. *Colpoda maupasi* is known to have a wide range of optimal temperatures (29-35 ºC), can survive anaerobic conditions and is ubiquitous in a wide variety of environments (e.g. soils, aquatic ecosystems, mosses, caves) (Foissner, 1993); all these could be the reasons of the dominance of this species in T. *utriculata.* The fact that the genus Colpoda, whose species are known to have high population growth rates (Foissner, 1993) and a rapidly response to environmental change (Acosta-Mercado & Lynn,

2006), dominate in this ecosystem during dry season, indicate that *T. utriculata* turns into an extreme environment. This is corroborated by the drastically changes in conductivity and phosphate levels between seasons. In fact, r-selected species {*Colpoda* species [generation time (GT) between 3-4 hours]} dominates during dry season, enabling a successful exploit of resources in *T. utriculata* during extreme conditions (e.g. higher salinity, and lower resources like phosphate), while k-selected species dominates during wet season [e.g. *Vorticella* species (GT between 7-9 hours), *Coleps hirtus* (GT between 24 – 90 hours), *Gonostomum affine* (GT 11 hours)] possibly due to less extreme conditions (e.g. lower salinity, higher resources like phosphate). I suggest that salinity and phosphate could be important environmental variables explaining alternate stable state in the ciliate community *of T. utriculata* in the Guánica Dry Forest, Puerto Rico.

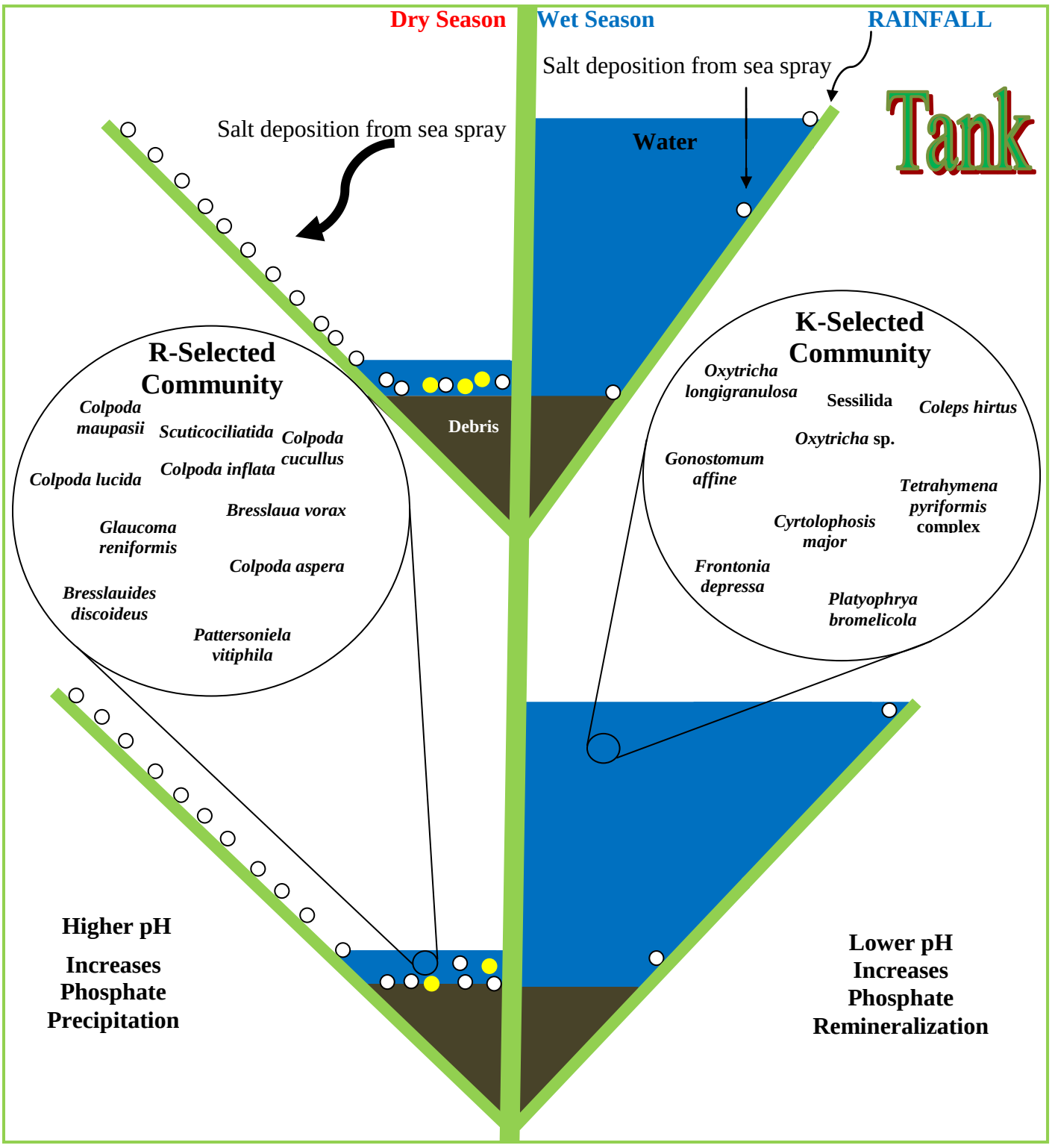


Figure 5.10. Model for the possible alternative stable states of ciliate community in *Tillandsia utriculata* from the Guánica Dry Forest, Puerto Rico. Non-dashed arrows represent inputs (salts, rainfall), the thickness of the arrows represents the magnitude; white circles represent salts from sea and yellow circles represent ciliate cysts.

# 6. Ciliates, testate amoebae and predators: a microcosm study to assess top-down effects on community structure

## 6.1 Introduction

The alternative stable state theory suggests that rather than a single state community, there are different community states in an ecosystem, changing from one to another (Schröder et al., 2005) across time and spatial scales. Species interactions are among the suggested biotic factors that can trigger such shifts (Beschta & Ripple, 2010; Handa et al., 2002; Price & Morin, 2004). For instance, generalist and specialist predators can generate shifts through selective feeding or through indirect interactions such as behaviorally or density-mediated interactions (Heithaus et al., 2008). Behavior interactions take place when changes in the abundance of a species trigger a change in the behavior of a second species that in turn influences a third species (Heithaus et al., 2008). On the other hand, a density-mediated interaction is when the abundance of a species affects the density of a second species and so on (Heithaus et al., 2008). Thus, predators cause a top-down effect that moves along the community food web through a trophic cascade effect ensued by either selective feeding or indirect interactions (Heithaus et al., 2008). The latter was observe after the reintroduction of gray wolves in the mid-1990 in the Yellowstone park, were the predators caused a decline in the herbivore population and thus a community composition shift from a grassland to a woody plant community (Beschta & Ripple, 2010). These changes in the community could trigger concomitant changes in the number, and the length of the links within the food web of the ecosystem, therefore changes in the nutrient cycling dynamics**.**

In fact, changes in the food web were recorded for Flathead Lake in Montana, USA, after the invasion in 1985 of *Mysis diluviana* Audzijonyte & Väinölä, 2005 (formerly *M. relicta* Lovén, 1862), a plankton predatory shrimp (Ellis et al., 2011). The shrimp triggered a density-mediated response that moved along the whole ecosystem stimulating a change in the migration route of the top-predator, the bald eagle, thus reducing its abundance in the zone from nearly 639 eagles before the invasion of *M. diluviana* (1981) to 25 eagles after the invasion (1987) (Spencer et al., 1991). When the shrimp became established, the kokanee salmon abundance was also reduced due to feeding competition, since both species fed on the same prey (large cladocerans). However since *M. diluviana* is an omnivorous feeder (phytoplankton and zooplankton), it survives the competition against the kokanee salmon which is a specialist feeder (zooplankton) (Ellis et al., 2011). Ultimately, the invasion of *M. diluviana*, also generated a shift in the composition and diversity of the microfaunal community. From larger and slower zooplankton (*Bosmina longirostris* O. F. Müller, 1776, *Epischura nevadensis* Lilljeborg, 1889 and *Diacyclops thomasi* S. A. Forbes, 1882) to smaller and faster zooplankton (*Daphnia thorata* Forbes, 1893, *Leptodiaptomus ashlandi* Marsh, 1893). After the invasion the abundances of the previous zooplankton community were reduced in more than a 90% and some species even became extinct in the lake after the invasion (e.g. *Daphnia rosea* G. O. Sars, 1862 and *Scapholeberis kingi* Sars, 1903) (Ellis et al., 2011).

According to published models, the community shifts could be either un-stabilized (Pimm & Lawton, 1978; Trzcinski et al., 2005) or stabilized (Fagan, 1997) by predators. Omnivores can quickly respond to community disturbances by switching to different preys within the food web

according to fluctuations of prey abundances, therefore stabilizing communities by preventing species blooms and by consuming from different trophic levels (Fagan, 1997).

Given that trophic cascade effects can move along whole communities from macroecosystem to microecosystem levels and vice versa, it is important to understand if predation can drive community shifts within bromeliad phytotelmata since the latter can have a significant effect on large scale processes such as forest methane emissions (Martinson et al., 2010), primary productivity (Richardson et al., 2000), magnitude of species reservoir (Barberis et al., 2011; Richardson et al., 2000) and nutrient dynamics (Ngai & Srivastava, 2006). Bromeliads serve as reservoir for a wide variety of organism like: bacteria (Rivera et al., 1988; Soto-Feliciano et al., 2010), Archaea (Martinson et al., 2010), protists (Carrias et al., 2001; Dunthorn et al., 2012; Foissner et al., 2003; Sophia, 1999; Torres, 2001) and animals (Kitching, 2000; Ngai & Srivastava, 2006; Richardson, 1999; Srivastava et al., 2005; Wagner et al., 2008). The dominant protist community within bromeliads includes algae, ciliates and testate amoebae. The last two are known to be key heterotrophic microorganisms in microbial food webs (Sherr & Sherr, 1994; Wilkinson & Mitchell, 2010).

With high feeding and population growth rates, ciliates (Jonsson, 1986) and testate amoebae (Laybourn & Whymant, 1980) could serve as model organisms to study the effect of predation on alternative stable states. Changes in species composition and abundance in these communities can be tracked in short time scales making it possible to disentangle predation effects on communities. In less than a month, Srivastava and Bell (2009) tracked a decrease in ciliate richness from artificial microcosms resembling Costa Rican bromeliads, suggesting an indirect

predation effect from predatory damselflies larvae (*Mecistogaster modesta* Selys). In contrast to ciliates, predation effects on testate amoeba have not been published. Nonetheless, they are known to serve as prey for ciliates, other testate amoebae and invertebrates (Dias & D'agosto, 2006; Gilbert et al, 2003; Schroeter, 2001).

The aim of this part of the study was to test whether predation from a generalist predator (*Macrostomum tuba*) triggers a shift in the ciliate and testate amoebae communities within the phytotelma of *Tillandsia utriculata* L. from the Guánica Dry Forest in Puerto Rico. If predation is a significant factor explaining the alternation of ciliate and testate amoebae communities, then, in the presence of the generalist predator the ciliate and testate amoebae communities should be significantly different when compared with the community of ciliates and testate amoebae in absence of the generalist predator.

## 6.2 Methodology

### 6.2.1 Sampling of ciliate and testate amoebae communities

A total of 20 g of litter from inside the phytotelma of *T. utriculata* were collected from ten randomly selected bromeliads during the dry season (December-May) in the Guánica Dry Forest. Ciliates and testate amoeba were activated in the litter by adding 2000 mL of filtered river water from the Toro Negro River (TNR). This water source was selected because it is a non-polluted and non-eutrophicated river in Puerto Rico and a previous study from colleagues in the laboratory were able to maintain successful cultures (Garzón, 2008). To ensure that ciliates and testate amoeba were from the litter within the bromeliad and not from cysts from the Toro Negro

River, the water was sterilized by filtration through a 0.20 µm nylon membrane (Whatman, 7402-004). After two days of wetting the litter, a subsample of 50 mL was added to the Predator (P) and Control (C) treatments.

### 6.2.2 Predator sampling

The invertebrate community within *T. utriculata* from Guánica Dry Forest included: oligochaetes, tipulids, chironomids, culicids, and Platyhelminthes (flatworms). Given that flatworms have been reported as generalist predators for ciliates (Altwegg et al., 2004) and zooplankton communities (Blaustein, 1990) I collected a flatworms as our generalist predator. In fact, Altwegg et al. (2004) found that *Euplotes* sp. populations induced morphological defenses after being in the presence of a *Stenostomum* sp., a predatory platyhelminth. Moreover, the degree of morphological defense was affected by the abundance of the predators. Based on biogeographical data and morphology of the habitus and the penis, the flatworm in *T. utriculata* was identified as *Macrostomum tuba* Graff (Delp, 2002; Gamo & Leal-Zanchet, 2004; Schockaert et al., 2007). Furthermore *M. tuba* has been used successfully on predation studies with freshwater mussels (Delp, 2002). Delp calculated the predation rate of *M. tuba* under controlled laboratory conditions and found that at 20º C the predation rate on mussel larvae was 0.26 mussels·worms·$h^{-1}$, a similar rate of predation was found for other preys (*Ceriodaphnia reticulata* Jurine, 1820) when the mussels were present. Because the focus of the study was to test if predation can trigger shift in the ciliate and testate amoebae communities, I proceeded to collect the species (*M. tuba*) from bromeliads phytotelmata in order to attain significant abundances for the replicates. To ensure that the predators used for the treatment were free of ciliates and testate amoebae, each predator was washed in three changes of TNR filtered water.

Predators were used for the Predator treatment (P) and for the Predator Control treatment (PC). The PC treatment was made in order to verify that the predator washing was successful; similarly the water used to wash the predator for the third time was used for the Wash Control Treatment (WC).

### 6.2.3 Microcosm experimental set up

All microcosms were assembled with plastic cups and green plastic “leaves”, to resemble the natural architecture of bromeliads. To maintain the spatial heterogeneity across all replicates, microcosms consisted of one plastic cup and six green plastic “leaves”: two large, two medium and two small "leaves" (Srivastava, 2006). The microcosms were covered with a piece of fabric to avoid any external interference, but allowing gas exchange. The P and C consisted of 50 mL of the ciliate and testate amoebae community, and 50 mL of TNR water; the P treatment also included 5 *M. tuba*. This number of predators was used since we were limited by the abundance of *M. tuba* collected.

In contrast to these treatments, the PC and WC treatments included 100 mL of TRN water, to ensure that microcosm lasted for 1 week, due to evaporation rates. The PC treatment also included 5 *M. tuba*, and the WC treatment included the last water change used to wash the predators. To ensure that we could track community changes, a subsample of 2 mL of the P and C treatments were fixed in Bouin's fixative at Day 2 and 7, followed by staining using the Edaphic Quantitative Protargol Staining procedure was performed (Acosta-Mercado & Lynn, 2003). Species were identified to the lowest taxonomic level possible using Berger (1999, 2011); Foissner (1993); Foissner & Xu (2007); Foissner et al. (2002); Ogden & Hedley (1980); Mazei &

Tsyganov (2006). Live observations were done for the PC and WC treatments (1 mL) under light microscopy at 10X, to verify the effectiveness of the washing technique. The treatments were arranged in a complete randomized block design (Figure 6.1) under controlled environmental conditions, therefore any significant differences in the ciliate and testate amoebae abundance, species richness and composition could be attributed to the predator presence. Environmental parameters (pH and conductivity) were simultaneously measured at each sampling date. Ciliates and testate amoebae communities were analyzed in terms of species richness, abundance, and community composition. At Day 7, the remaining water in the P, C and PC treatment were filtered through a 0.20 µm Nylon Millipore™ membrane to perform a nutrient analysis of phosphate, ammonium, and nitrate in the University of New Hampshire at the Water Quality Analysis Laboratory.

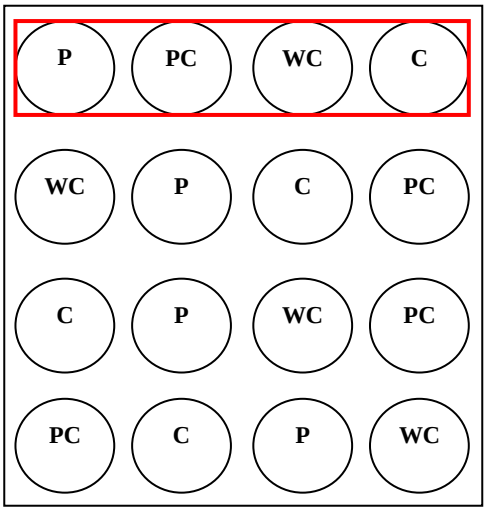


Figure 6.1 Complete randomized block design of the microcosms. The red rectangle represents a block, for a total of 4 blocks assembled, each with four treatments: P, C, PC and WC.

### 6.2.4 Statistical analysis

The community structure of ciliates and testate amoebae was analyzed with an Analysis of Similarity (ANOSIM) test to determine whether there were significant differences in the

community structure between the P and C treatments (Clarke & Gorley, PRIMER-E 2006). A Similarity Percentage (SIMPER) test was performed to identify the species composition between the P and C treatments (Clarke & Gorley, PRIMER-E 2006). A non-metric multidimensional scaling analysis was performed to observe if ciliates and testate amoebae were different in terms of species composition under predator and control treatments (Clarke & Gorley, PRIMER-E 2006). A *t*-test was performed to determine whether there were significant differences in the environmental parameters between P and C treatments (Di-Rienzo et al., Infostat 2012). An ANOVA test was performed to test whether there were significant differences in the phosphate, nitrate, and ammonium concentrations among treatments (Di-Rienzo et al., Infostat 2012). When significant differences were found, a Tukey test was performed. The coefficients of variation of ciliate and testate amoebae community parameters were calculated to determine whether predation had a stabilizing effect on ciliate and testate amoebae abundances and richness. Data were examined to meet the requirements of all models, if the data did not fit the model, then non-parametric equivalent tests were performed (e.g., Wilcoxon-Man-Whitney and Kruskal Wallis).

## 6.3 Results

### 6.3.1 Predator controls (PC) and washes control (WC) treatments

The predator control was established in order to observe if the Platyhelminthes could survive without the protist community and to observed if the predators were free from ciliates and testate amoebae. From the 20 Platyhelminthes used in the PC treatment, 14 flatworms were recovered. Based on personal observations, the sizes of the recovered flatworms were basically the same sizes as the size of the day the flatworms were added to the treatments. No ciliates or testate

amoebae were observed in the PC treatments. The phosphate concentration for the PC treatment was higher than that for the P and C treatments (ANOVA, $R^2$= 0.78 *p*= 0.0005) as shown by the Tukey test (Table 6.1). A multiple comparison in the Kruskal Wallis test also showed significant differences in the nitrate concentration among treatments (*p*= 0.0001) (Table 6.1) and no significant differences among treatments for the ammonium (*p*=0.8107).

The wash control was made in order to verify the effectiveness of the washing procedure. A total of 3 ciliates were observed but only on the second day of sampling. The ciliates observed belong to the subclass Stichotrichia (Class Spirotrichea). No testate amoebae were observed in the wash controls neither of the sampling dates.

Table 6.1. Mean nutrient concentrations ± SD in the Predator (*Macrostomum tuba* + ciliate and testate amoebae community), Control (Ciliate and testate amoebae community) and Predator Control (*M. tuba*) treatments. Only phosphate (*p*= 0.0005) and nitrate (*p*=0.002) varied among treatments.

| **Treatment** | **Nutrient concentrations (mean ± SD (µg/L))** | | | | | |
|---|---|---|---|---|---|---|
| | **$PO_4$** | | **$NH_4$** | **$NO_3$** | | |
| Predator (P) | 4.20 ± 7.759 | A | 174.60 ± 77.999 | 68.00 ± 10.000 | B | |
| Control (C) | 3.25 ± 2.217 | A | 158.50 ± 57.047 | 12.00 ± 10.000 | A | |
| Predator control (PC) | 23.75 ± 4.500 | B | 171.00 ± 32.404 | 14.00 ± 20.000 | A | |

Different letters represent significant differences at *p*= 0.01

### 6.3.2 Predator (P) and control (C) treatments

#### 6.3.2.1 Environmental parameters between treatments

Environmental parameters were concomitantly measured in order to identify potential sources of variation among all treatments. Environmental variables had comparable effects on the

community across treatments as shown in Table 6.2, since pH ($p$= 0.895) and conductivity ($p$= 0.724) were not significantly different ($t$-test). The pH levels were neutral for all treatments ranging from 7.6 to 8.3. Conductivity ranged from 326 µS/cm to 401 µS/cm, suggesting that nutrients such as phosphate, ammonium and nitrate were also comparable across treatments. In fact no significant differences were observed in the phosphate and ammonium levels between treatments P and C (Table 6.1). On the other hand, there were significant differences in nitrate concentrations found for the nitrate concentrations among treatments (Table 6.1).

Table 6.2. Mean ± SD of two environmental parameters in the Predator and Control treatments.

| **Environmental parameters** | **Mean ± SD** | | ***p* value** |
|---|---|---|---|
| | **P** | **C** | |
| pH | 7.9 ± 0.25 | 7.9 ± 0.12 | 0.895 |
| Conductivity (µS/cm) | 359.17 ± 31.29 | 353.6 ± 14.50 | 0.724 |

**6.3.2.2 Testate amoebae community**

After one week of microcosms assemblage, only live cells were used to analyze the effect of *M. tuba* predation on the community of testate amoebae, thus empty or non-stained shells were discarded for the purpose of this study. However, the effect of predation by generalist predator on the community composition of testate amoebae could not be assessed due to their low abundances in the microcosms (Table 6.5). In general, 7 species of testate amoebae were recorded in the predator treatment, while 11 species in the control treatment (Table 6.5). Total abundances followed a similar pattern, i.e. 27 individuals/mL (P) and 44.5 individuals/mL (C). The most abundant species in all treatments was *Euglypha rotunda* Ehrenberg with a dominance of 67% in predator treatment, and 62%)\ in the control treatment. Although there were no

significant differences in abundance ($p$= 0.366) and species richness ($p$= 0.260) between treatments, the presence the generalist predator reduced the CV% (variation) of both, abundance and species richness (Table 6.4).

Table 6.3 Mean ± SD of community parameters of testate amoebae in the P and C treatments.

| Treatment | Community parameter | |
|---|---|---|
| | **Abundance (individuals/mL)** | **Species richness (number/mL)** |
| Predation (P) | 7.71 ± 10.673 | 2.14 ± 0.900 |
| Control (C) | 17.8 ± 37.043 | 2.80 ± 4.658 |

Table 6.4 Coefficient of variation of testate amoebae community parameters in the P and C treatments.

| Treatment | Coefficient of Variation (%) | |
|---|---|---|
| | **Abundance** | **Species richness** |
| Predation (P) | 138.35 | 41.99 |
| Control (C) | 208.11 | 166.37 |

**6.3.2.3 Predation effect on the ciliate community structure**

A total of 26 ciliate species were identified, from which 23 were present in the predator treatment (P) and 17 in the control treatment (C) (Table 6.5). Based on an nMDS, predation had an effect only on ciliate species composition (Fig. 6.2). A SIMPER test revealed that the community of ciliates was dominated by 4 species (*P. bromelicola*, *G. affine*, *Thylakidium* like and *C. maupasi*) in the predator treatment (Table 6.5), while 6 species dominated the community of ciliates (*P. bromelicola*, *Thylakidium* like, *C. maupasi*, *G. affine*, Scuticociliatidia sp. and

*Chilodonella uncinata*) in the control treatment (Table 6.5). These differences were not significant for abundance ($p$= 0.458) and species richness ($p$= 0.880) (Table 6.7). Similar to that observed with testate amoebae, the generalist predator reduced the CV% (variation) (Table 6.6).

Table 6.5. Mean abundance ± SD (individuals/mL) of testate amoebae and ciliates communities in the Predator and Control treatments.

| Species | Treatment | |
|---|---|---|
| | **P** | **C** |
| **Testate amoebae** | | |
| *Euglypha rotunda* | 2.6 ± 4.49 | 5.5 ± 11.48 |
| *Euglypha laevis* | 0.5 ± 0.61 | 0.8 ± 1.79 |
| *Assulina muscorum* | 0.5 ± 0.61 | 0.2 ± 0.45 |
| *Nebela sp* | 0.2 ± 0.57 | 0.4 ± 0.90 |
| *Centropyxis constricta* | 0.1 ± 0.19 | 0 |
| *Centropyxis kahli* | 0.1 ± 0.19 | 0 |
| *Hyalosphenia sp* | 0.1 ± 0.19 | 0 |
| *Cryptodifflugia compressa* | 0 | 0.6 ± 1.34 |
| *Trinema lineare* | 0 | 0.6 ± 0.82 |
| *Cryptodifflugia oviformis* | 0 | 0.2 ± 0.45 |
| *Euglypha cristata* | 0 | 0.2 ± 0.45 |
| *Euglypha hyalina* | 0 | 0.2 ± 0.45 |
| *Centropyxis elongata* | 0 | 0.1 ± 0.23 |
| *Nebela carinata* | 0 | 0.1 ± 0.23 |
| **Ciliates** | | |
| *Platyophrya bromelicola* | **212.8 ± 143.94** | **136.8 ± 50.36** |
| *Thylakidium* like sp | **27.7 ± 50.12** | **7.5 ± 2.58** |
| *Colpoda maupasi* | **17.0 ± 30.44** | **7.8 ± 10.67** |
| *Gonostomum affine* | **10.7 ± 10.67** | **13.2 ± 13.22** |
| Scuticociliatidia 1 | 6.5 ± 10.53 | **6.1 ± 5.61** |
| *Oxytricha longigranulosa* | 1.4 ± 2.81 | 1.2 ± 1.6 |
| *Frontonia depressa* | 1.2 ± 1.32 | 0.4 ± 0.48 |
| Vorticellidae sp. 2 | 0.6 ± 1.09 | 1.2 ± 1.55 |
| Stichotrichia 1 | 0.5 ± 1.14 | 0 |
| *Oxytricha ottowi* | 0.3 ± 0.57 | 0.3 ± 1.07 |
| *Spathidium* sp1 | 0.3 ± 0.57 | 0.3 ± 0.50 |
| *Chilodonella caudata* | 0.2 ± 0.39 | **1.9 ± 3.75** |
| *Chilodonella uncinata* | 0.2 ± 0.25 | 0.4 ± 0.48 |
| *Colpoda steinii* | 0.2 ± 0.25 | 0.2 ± 0.25 |
| *Tetrahymena pyriformis* complex | 0.1 ± 0.19 | 0.7 ± 0.85 |
| *Colpoda aspera* | 0.1 ± 0.19 | 0 |
| *Colpoda inflata* | 0.1 ± 0.19 | 0 |

Table 6.5 Continuation

| Species | Treatment | |
|---|---|---|
| | **P** | **C** |
| Litostomatea 2 | 0.1 ± 0.19 | 0 |
| *Tachysoma* sp | 0.1 ± 0.19 | 0 |
| *Halteria* sp. | 0 | 0.6 ± 1.25 |
| *Pattersoniella vitiphila* | 0 | 0.6 ± 1.25 |

Table 6.6. Coefficient of variation of ciliate community parameters in the predator (P) and control (C) treatments.

| Community parameters | Coefficient of variation % | |
|---|---|---|
| | **P** | **C** |
| Abundance (individuals/mL) | 42.8 | 61.4 |
| Species richness ( number/mL) | 40.6 | 37.4 |

Table 6.7. Mean ± SD of the ciliate community parameters in the predator (P) and control (C) treatments

| Community parameters | Mean ± SD | | *p* value |
|---|---|---|---|
| | **P** | **C** | |
| Abundance (individuals/mL) | 607.7± 260.32 | 351.6 ± 215.80 | 0.458 |
| Species richness (number/mL) | 8.3 ± 3.39 | 10.0 ± 3.74 | 0.880 |

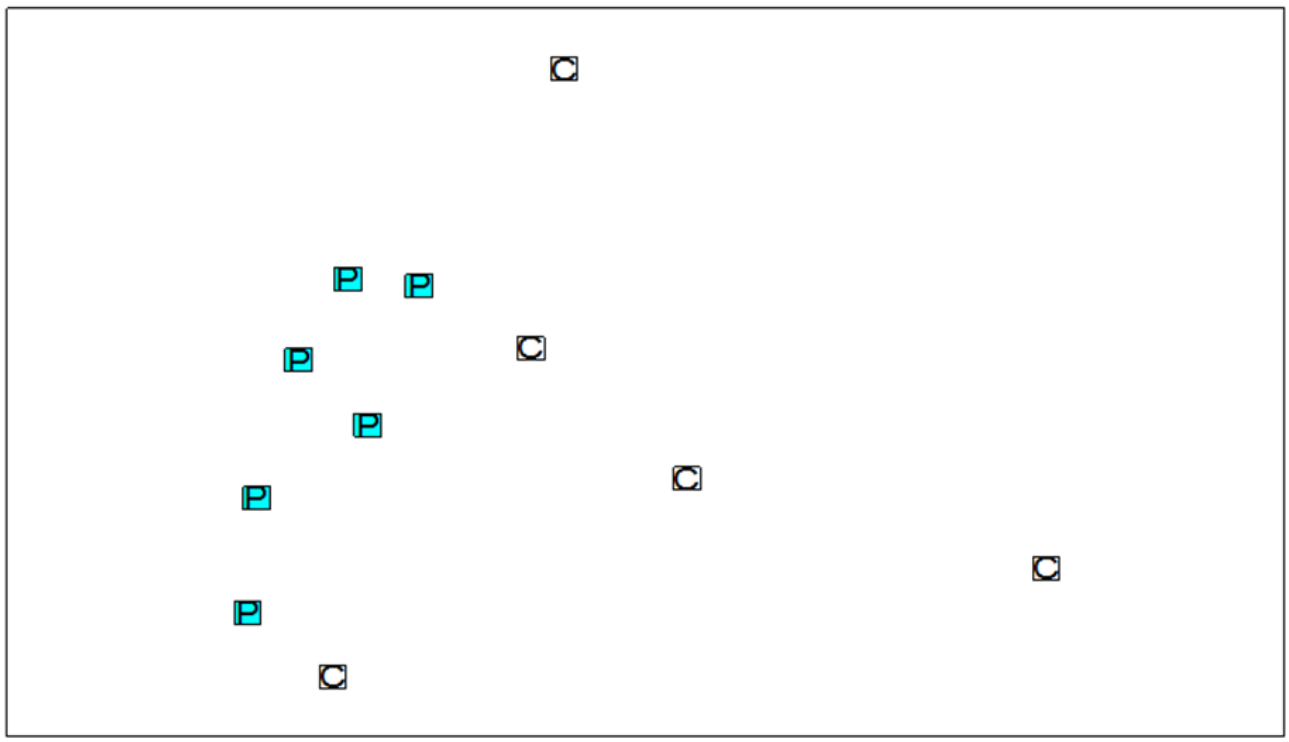


Figure 6.2 The nMS revealed two different communities (ANOSIM, R=0.4 p=0.03, stress=0.1) between the treatments P (predator, in blue box) and C (control, in the white box).

## 6.4 Discussion

### 6.4.1 Testate amoebae community

This manipulation microcosm study was made in order to test whether the presence of a generalist predator had an effect on community parameters of both, ciliates and testate amoebae within the phytotelma of *T. utriculata*. The effect of predation on testate amoebae community could not be fully analyzed due to low abundances and species richness (Table 6.5). However, after the dissection of *M. tuba* to observe the penis stylet, the content of the intestine was expelled, and empty silica shells, mainly of *T. lineare*, were observed. Therefore, as suggested by previous studies for edaphic ecosystems (Wilkinson & Mitchell, 2010; Wilkinson, 2008), testate amoebae could also have a key role in aquatic microecosystems, especially regarding the silica cycle. Moreover testate amoebae must be added to the list of prey organisms of predatory flatworms, especially to the diet of *M. tuba* in bromeliad microecosytems.

### 6.4.2 Ciliate community

Recently, the role of omnivory in food-webs has been the main focus of food-web ecologists. Omnivory is known to have a stabilizing effect on food-webs, particularly when the prey exhibits an inducible defense (Kratina et al., 2012). Previous studies with ciliates in the presence of flatworms have found that *Euplotes* species can exhibit two types of inducible defenses: behavioral (i.e., higher maximum movement rates) and morphological defenses (i.e. increase in width) (Hammill et al., 2008). The low coefficients of variation for abundance suggest that the generalist predator could have a stabilizing effect. It is very likely that ciliates species in the bromeliad ecosystem exhibit predatory induced defenses such as, behavioral defenses and thus lower coefficient of variation in the presence of *M. tuba*. *Macrostomum tuba* is forced to change from prey to prey in a non-selective manner and thus maintaining abundances more homogeneous.

The fact that no significant differences were observed for ciliate abundances between treatments could be linked to the nature of our selected predator. It is known that strong links such as specialist predators with their prey are responsible for changes in the community even extinctions (McCann et al., 1998). Specialist predators have a restricted ability to switch to alternate prey, consequently reducing the abundance of the main prey (Andersson & Erlinge, 1977). In contrast, generalist predators can switch prey rather easy; therefore, any decline in abundance is more evenly distributed along the food-web (Fagan, 1997). Moreover, in contrast to that seen in other phytotelmata (Srivastava & Bell, 2009), the predator had no effect on ciliate species richness. This could be explained by the fact that the food web in the artificial

microcosms was both, simplified and dominated by ciliates of the class Colpodea, which are known to have high population growth rates (Foissner, 1993) and to rapidly respond to environmental change (Acosta-Mercado & Lynn, 2006). Although there was no evidence of prey selectivity, the high population growth rates of Colpodea could overshadow the functional response of the generalist predator on species richness. In fact generalist predators, in theory, feed upon the dominant taxa, when a prey is too low in abundance they shift to another prey (Fagan, 1997).

Predation had a top-down effect on ciliate community composition in the bromeliad *T. utriculata*, since conductivity, an environmental factor triggering shifts (**See chapter 5)**, was constant between the predator and control treatments. Four ciliate species dominated the predator treatment and six species in the control treatment (Table 6.5), probably due to the nature of the generalist predator, shifting from dominant prey to the next dominant. A top-down effect in ciliate community composition occurred in a Danish lake, were small ciliates (i.e., Scuticociliatidia) had higher abundances in the presence of cyclopoid copepods and *Daphnia* sp. whereas in their absence larger ciliates (i.e., Haptorida) were most abundant (Jürgens et al. 1999).

### 6.4.3 Nutrient cycling in the food-web of *T. utriculata*

As suggested by published models (Ngai & Srivastava, 2006), the nitrate released in this simple trophic web could be enhanced by predation, suggesting that predators could facilitate the nitrate uptake by bromeliads However, published models for *Vriesea gigantea* Gaudichaud, an epiphytic bromeliad, suggests a preference towards ammonium absorption rather than nitrates

(Inselsbacher et al., 2007). Furthermore, most aquatic organisms are ammonotelics (Goyal & Sastry, 2010), therefore higher amounts of ammonium could be available in the phytotelma. Since this study was made in plastic microcosms the absorption of ammonium should not have occured, suggesting that the higher levels of nitrate reported in our microcosms could be due to the oxidation of ammonia excreted from *M. tuba*, ciliates and testate amoebae. This oxidation step was very likely present in the microcosms since there is evidence of high copies of ammonia monooxygenase genes (responsible for ammonium oxidation) by archeas reported in other natural microecosystems (roots of the freshwater macrophyte *Littorella uniflora* (L.) Asch.) (Herrmann et al., 2008). This adds to the monumental evidence that tank bromeliads are sources and sinks of critical ecosystem function.

Food-webs in bromeliad phytotelmata have been classified as detritus based (Brouard et al., 2012), suggesting that the main sources for predators are mainly metazoans and detritivorous or bacterivorous heterotrophic protists. However, recent studies suggest that producers (i.e. algae) are a relevant group within phytotelmata food-webs (Brouard et al., 2011). Despite the fact that algae have been observed in bromeliad phytotelma since earliest phytotelma studies (Laessle, 1961; Maguire, 1971; Sophia, 1999; Sophia et al., 2004), no evidence was found to support their relevance in the food-web of *T. utriculata*. Only 4 morphospecies of diatoms: one centric and three pennate, were observed while only two algivorous ciliates were detected: *Chilodonella uncinata* and *C. caudata*. The lack of producers and herbivore protists suggests that the food-web within the phytotelma of *T. utriculata* is detritus based, at least for the Guánica Dry Forest.

### 6.4.4 Suggested model for the top-down effect in the phytotelma of *T. utriculata*

Predation by *M. tuba* is triggering a top-down effect on community composition of ciliates from the bromeliad *T. utriculata* in the Guánica Dry Forest, as shown in the figure 6.3. The apparent lack of prey selectivity and the fact that generalist predators can target prey abundances evenly in the food-web (Fagan, 1997) seems to stabilize the ciliate abundances. The ciliate composition is shifted by the presence of *M. tuba*. For instance, 4 species dominated in the presence of *M. tuba* whereas 6 species dominated in its absence (Table 6.5). Additionally, predation by *M. tuba* seemed to increase the levels of nitrogen, possibly through excretions of ammonium. The ammonium is then absorbed by the plant and/or transformed to nitrate through oxidation mechanisms by prokaryotes. The possible increase in ammonium excretions is beneficial to epiphytic bromeliads, as suggested by higher activities of urease in Tillandsioideae species (i.e. *Vriesea gigantea*) (Inselsbacher et al., 2007). Omnivorous or generalist predators could be added to the list of generators of possible alternative stable states in the ciliate community of *Tillandsia utriculata* (Fig. 6.3).

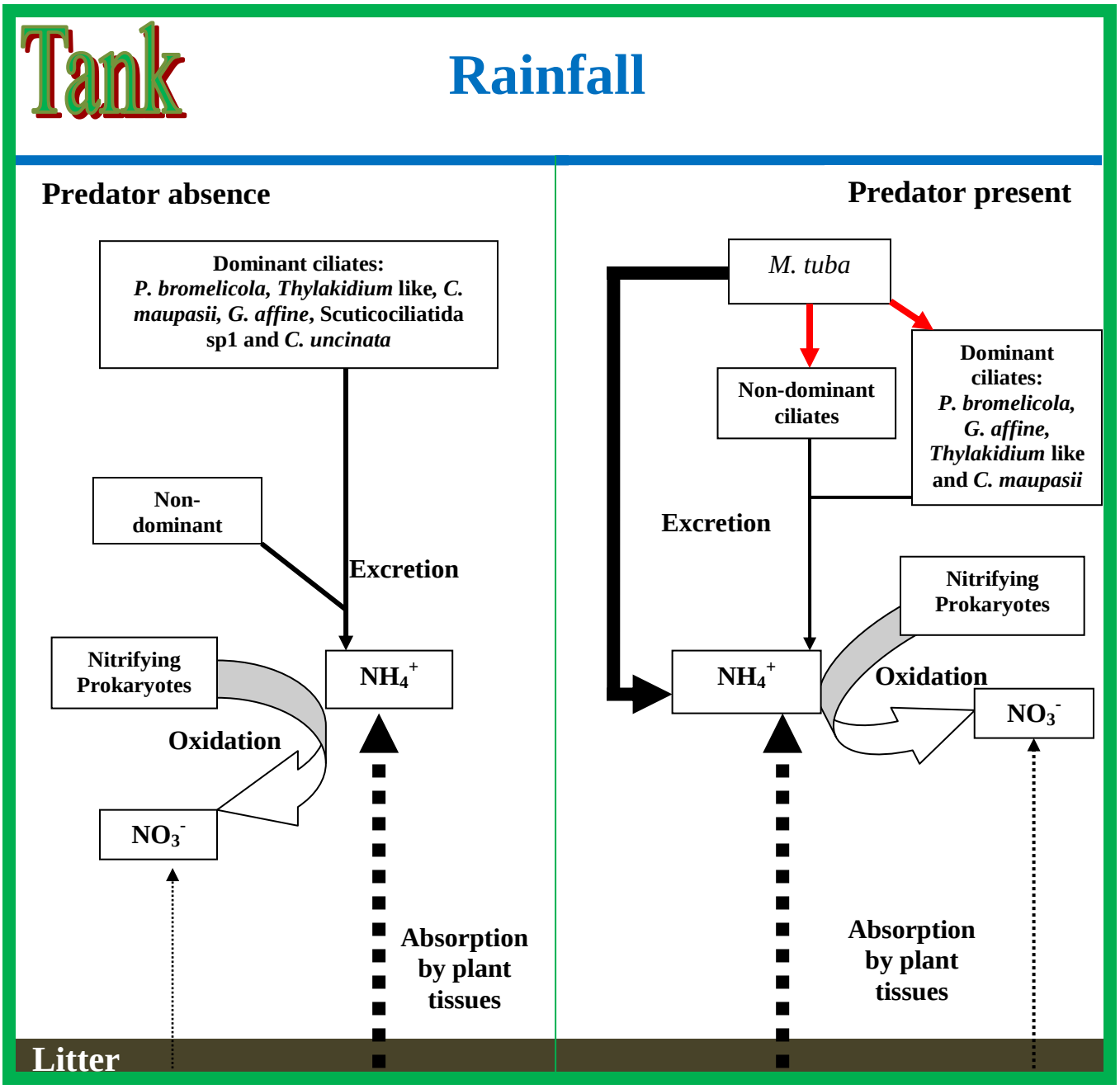


Figure 6.3. Schematic model of the possible alternate stable state triggered by top-down effects in the bromeliad *T. utriculata* from the Guánica Dry Forest. Based on nitrogen model from Inselsbacher et al., 2007. Red arrows represent predation interaction, dashed arrows represent absorption by plant tissues, non-dashed arrows represent nitrogen excretion and the thickness of the arrows represents the magnitude. Non-dominant ciliates are listed Table 6.5.

# 7. Is the ciliate and testate amoebae community in bromeliad phytotelmata bottom-up regulated? A microcosm study to assess bottom-up effects.

## 7.1 Introduction

Ecological communities can shift from one state to another as suggested by the alternative stable state theory (Beisner et al., 2003). The theory suggests that community shifts can be observed if the community overcome certain environmental thresholds (Beisner et al., 2003). These thresholds range from biotic environmental variables like predator-prey interactions (Price & Morin, 2004) and top-down effects (Beschta & Ripple, 2010) to abiotic environmental variables such as turbidity level (Scheffer & Nes, 2007), dissolved organic matter (Crump et al., 2003) and bottom-up effects generated by nutrient concentrations (Buosi et al., 2011; Wickham et al., 2004). Alternate nutrients flushes could stimulate specific ciliate species that ultimately generate a community shift. Ciliates and testate amoebae have faster growing and feeding rates than plants and animals (Jonsson, 1986; Laybourn & Whymant, 1980), making them excellent model organisms to test nutrient availability effects in community structure in short scales of time.

Small freshwater ecosystems, such as bromeliad phytotelmata, can suffer changes in the nutrient dynamics through airborne inputs, rainfall and litter fall (Barberis et al., 2011; Richardson et al., 2000; Schlesinger & Marks, 1977). For instance, nutrient inputs in bromeliads during dry seasons are mainly from airborne inputs such as aerial dusts, in contrast suring wet seasons the main sources of nutrients are rainfall (Benzing, 2000; Cavalier et al., 1997; Schlesinger & Marks, 1977). Therefore changes in the community that inhabit the bromeliads could be triggered.

The community of organisms that inhabit bromeliads includes bacteria (Rivera et al., 1988; Soto-Feliciano et al., 2010), Archaea (Martinson et al., 2010), algae (Laessle, 1961; Maguire, 1971; Sophia, 1999; Sophia et al., 2004) ciliates (Dunthorn, 2012 and Foissner et al., 2003), testate amoebae (Torres, 2001) and animals (Kitching, 2000). Ciliates and testate amoebae are two overlooked group of organisms in this environment (Carrias et al., 2001). Despite the lack of data on the community structure, recent studies revealed new species of both ciliates and testate amoebae (Dunthorn et al., 2012; Foissner et al., 2003; Torres, 2001), suggesting that phytotelmata of bromeliad represent an important microecosystem for diversity studies.

Community shifts have been reported for ciliates in other ecosystems such as lakes and streams. For instance, a slow input of phosphorus and nitrogen in Garças Lake, Brazil, was known to increase ciliate species richness from 14 to 22, and also to change the community composition of ciliates associated with the roots of the water hyacinth *Eichhornia crassipes* (Buosi et al., 2011). Domènech et al. (2006), found that for the Furisos stream in Spain, the abundance of ciliates increased from $0.21 \pm 0.03 \times 10^3$ individuals/$cm^2$ to $0.46 \pm 0.09 \times 10^3$ individuals/$cm^2$ after nitrate and phosphate loadings. Moreover, the abundance of the orders Pleurostomatida and Peniculida showed a positive response to nutrient inputs (Domènech et al., 2006). Although there is no clear association between community shifts and nutrient inputs for testate amoebae, it is well known that abundance and biomass of testate amoebae changes across a nutrient gradient (Rublee & Partusch-Talley, 1995). For instance, in Kuparuk River, Alaska, testate amoebae biomass and abundance was $0.12 \pm 0.02$ mg C/ PFU (polyfoam units) and $1.04 \pm 0.11 \times 10^3$ individuals/PFU in the control treatment whereas in the phosphorus and nitrogen treatments the biomass levels increased to $0.33 \pm 0.04$ mg C/ PFU and the abundances to $2.69 \pm 0.31 \times 10^3$

individuals/PFU. These changes could move along the food-web of the forest; therefore, possibly changing trophic forest dynamics through allometric (biomass-density) relationships.

This part of the study aims to determine if resource (phosphate and nitrate) availability can trigger a shift in the community of ciliates and testate amoeba. If nutrient availability is the main driver of alternative stable states in the phytotelma of *T. utriculata* in the Guánica Dry Forest, then bromeliads that differ in terms of nitrate and phosphorus will show significant differences in the abundance, richness, and community composition of ciliates and testate amoebae.

## 7.2 Methodology

### 7.2.1 Microcosms experimental set up

Samples were taken from 11 randomly selected bromeliads (*T. utriculata*) during the dry season in the Guánica Dry Forest. A total of 30 grams of litter was collected from inside the phytotelma of the bromeliads. In order to maintain the same levels of carbon between treatments, the litter was cut in pieces of approximately 1 $cm^2$. Carbon content of seven independent litter samples were analyzed in the Water Quality Analysis Laboratory at the University of New Hampshire. The shredded litter was homogenized in a plastic container and 1 g was added to the microcosms. To resemble the natural architecture of bromeliads and to keep the spatial heterogeneity constant, all the microcosms were constructed with plastic cups and six green plastic “leaves”: two large, two medium, and two small "leaves" following the method of

Srivastava (2006). A total of 21 microcosms were constructed and organized in a complete randomized block design (Figure 7.1).

Each microcosm was filled with 100 mL with either one of the following treatments: phosphate solution (P), nitrogen solution (N), or Toro Negro River (TNR) sterilized water as a control solution (C). The P treatment concentration was 0.639 mg/L and the N treatment concentration was 0.052 mg/L. These solutions were based on published nutrient levels from bromeliads in El Yunque Rainforest (Richardson et al., 2000) since no data were available regarding nutrient concentrations from the Guánica Dry Forest prior to the realization of this experiment. Each solution was prepared with TNR sterilized water and the chemical compound, $KH_2PO_4$ for P and $KNO_3$ for N treatments. This river was selected because it is a non-polluted and non-eutrophicated river in Puerto Rico and previous studies from colleagues of our laboratory used it for bryophytes microcosms with successful results (Garzón, 2008). The TRN water was sterilized through filtration with a 0.20 µm nylon membrane (Whatman, 7402-004) in order to guarantee that the community of ciliates and testate amoebae activated was from the litter and not from the TRN water. After 4 d of the experimental assemblage, a subsample of 2 mL was collected and fixed in 8 mL of Bouin's fixative for the Edaphic Quantitative Protargol Staining (QPS) procedure (Acosta-Mercado & Lynn, 2003). During each sampling day, pH and conductivity were measured. Ciliates and testate amoebae species were identified to the lowest taxonomic level possible using: Berger (1999, 2011); Foissner (1993); Foissner & Xu (2007); Foissner et al. (2002); Ogden & Hedley (1980); Mazei & Tsyganov (2006).

Figure 7.1 Complete randomized block design arrangement of the microcosms. A total of seven blocks were assembled with three treatments: P, N and C

### 7.2.2 Statistical analysis

An ANOSIM test was performed to determine whether there were significant differences in the community composition among treatments (Hammer et al., PAST 2012). An ANOVA test was used to corroborate whether the abundance and richness of ciliate and testate amoebae differed among treatments (Di-Rienzo et al., Infostat, 2012). For testate amoebae, only tests containing its ameboidal organism were considered for the analysis; empty tests were discarded from the analysis. An ANOVA test was also performed to test whether there were significant differences in the pH and conductivity among treatments (Di-Rienzo et al., Infostat 2012). The data were examined to determine whether it met all the assumption of the models. If the data didn't fit the model, non-parametric equivalent test were performed (e.g., Kruskal Wallis).

## 7.3 Results

### 7.3.1 Environmental parameters among treatments

No significant differences were detected for pH and conductivity among treatments as shown by the ANOVA test ($p$=0.632, and $p$=0.304 respectively) (Table 7.1). The mean pH remained neutral (6.8 ± 0.1) ranging from 6.6 to 7.0 and conductivity (429.9 ± 63.1 µS/cm) ranged from 294 µS/cm to 561 µS/cm (Table 7.1). The carbon input among microcosms is assumed to be relatively similar as demonstrated by the low standard deviation of the samples analyzed (0.450 ± 0.0032 g).

Table 7.1 Mean ± SD of environmental parameters in artificial microcosms with litter from *T. utriculata*. The ANOVA test showed no significant differences among treatments.

| **Environmental parameter** | **Treatment** | | | |
|---|---|---|---|---|
| | **Phosphate** | **Nitrate** | **Control** | ***p* value** |
| pH | 6.8 ± 0.095 | 6.8 ± 0.09 | 6.82 ± 0.13 | 0.632 |
| Conductivity (µS/cm) | 460.3 ± 49.92 | 407.4 ± 79.74 | 421.9 ± 51.59 | 0.304 |

### 7.3.2 Testate amoebae community

A comprehensive analysis of the testate amoebae community could not be performed due to low abundances and species richness across all treatments (Table 7.2). However, three species of testate amoebae were identified, one belonging to the order Arcellinida (*Arcella catinus* Penard) and two belonging to the order Euglyphida (*Assulina muscorum* Greef and *Euglypha rotunda* Ehrenberg). No testate amoebae were recorded from the nitrogen treatment (Table 7.2), two

species were identified in the phosphorus treatment while only one species was recorded in the control treatment (Table 7.2).

### 7.3.3 Ciliate community

Under this experimental set-up, the community of ciliates was not affected by nitrogen, nor phosphate availability. A total of 30 species of ciliates were identified with 8 orders represented: Colpodida, Cyrtolophosidida, Cyrtophorida, Haptorida, Peniculida, Sessilida, Scuticociliatida, and Stichotrichida. No significant differences in community composition were detected among treatments (ANOSIM, R=0.01 $p$=0.331). There were no significant differences in abundance or species richness among treatments (Table 7.3). The mean for ciliate abundances mean was 260 ± 370.9 individuals/2 mL and for species richness was 7.3 ± 3.1 species/2 mL. The colpodeans *Colpoda cucullus* O. F. Muller and *Colpoda maupasii* Enriques were the most abundant species in each treatment (Table 7.2).

## 7.4 Discussion

Phosphate and nitrate concentration were manipulated in artificial microcosms in order to analyze and understand the effect of nutrient availability in ciliate and testate amoebae communities in the natural microcosms of *T. utriculata*. As expected, because of previous results (i.e., low abundance and species richness) the testate amoebae community could not be analyzed.

However, the abundance of testate amoebae was expected to increase with nutrient inputs. In fact, the arcellinid *Bullinularia indica* was observed to increase its abundance in the moss *Sphagnum* concomitant to nitrogen inputs (Mitchell & Gilbert, 2004).

In contrast to the trends in literature, phosphate and nitrate inputs did not trigger changes in the ciliate community (abundance, species richness, composition) and this may be due to methodological artifacts. For example, I added high amounts of nutrients in a short scale of time (1 d), whereas Bousis's et al. (2011) method included adding the high amounts of nutrients distributed during 33 d. My high nutrient loading could have caused a ciliatostasis effect, or encystment of the organisms within the community. However, *Colpoda cucullus* and *Colpoda maupasi* are known to exploit resources in extreme environments and thus it was expected for them to become dominant in each treatment (Foissner, 1993). A nutrient effect has also been detected on marine ciliates in Norway, where the abundance of ciliates increased after adding nitrogen and phosphate (Gismervik et al., 2002). Similarly to Bosui et al. (2011), Gismervik et al. (2002) added nutrients in an exponential manner over 2 weeks. To fully understand the nutrient and population dynamics in the phytotelma of *T. utriculata*, manipulation studies should add nutrients (i.e. phosphate and nitrate) overtime and not in a single load.

Table 7.2 Mean abundance ± SD (individuals/mL) of the ciliates and testate amoebae in the phosphate (P), nitrate (N) and control (C) treatments in artificial bromeliad microcosms with litter from *T. utriculata*

| Species | Treatment | | |
|---|---|---|---|
| | **P** | **N** | **C** |
| **Ciliates** | | | |
| *Colpoda cucullus* | 103.2 ± 99.63 | 73.2 ± 85.5 | 29.3 ± 58.9 |
| *Colpoda maupasi* | 21.65 ± 25.79 | 111.2 ± 191.64 | 24.3 ± 38.17 |
| *Colpoda steinii* | 1.15 ± 0.88 | 0.7 ± 0.54 | 0.8 ± 1.47 |
| *Oxytricha ottowii* | 0.9 ± 1.03 | 1.4 ± 1.09 | 1.6 ± 1.09 |
| *Spathidium* sp. | 0.7 ± 1.77 | 14.4 ± 14.00 | 3.4 ± 1.33 |
| *Colpoda inflata* | 1.5 ± 2.10 | 1.88 ± 2.44 | 0.9 ± 1.21 |
| Vorticellid 2 | 0.8 ± 0.89 | 0.2 ± 0.25 | 0.6 ± 1.51 |
| *Platyophrya bromelicola* | 0.4 ± 0.70 | 0.9 ± 1.46 | 0.36 ± 0.56 |
| *Frontonia depressa* | 0.3 ± 0.37 | 0.2 ± 0.40 | 0.3 ± 0.48 |
| *Pattersoniela vitiphila* | 0.1 ± 0.23 | 0.4 ± 0.50 | 0.2 ± 0.40 |
| *Frontonia* sp | 0.1 ± 0.26 | 0.2 ± 0.40 | 0 |
| *Colpoda aspera* | 0.3 ± 0.53 | 0 | 0 |
| Litonotidae 1 | 0 | 1.1± 2.12 | 0.4 ± 0.45 |
| *Gonostomum affine* | 0 | 0.3 ± 0.40 | 0.5 ± 0.77 |
| *Chilodonella uncinata* | 0 | 0.4 ± 0.57 | 0.1 ± 0.19 |
| Vorticellid 1 | 0 | 0.4 ± 0.57 | 0.1 ± .019 |
| *Trachelophyllum* sp. | 0 | 0.1 ± 0.25 | 0.1 ± 0.19 |
| Scuticociliatida 1 | 0 | 0.1 ± 0.19 | 0.2 ± 0.57 |
| Scuticociliatida 4 | 0 | 0.1 ± 0.19 | 0.2 ± 0.57 |
| *Enchelys* sp. | 0 | 0.1 ± 0.19 | 0.1 ± 0.19 |
| *Oxytricha longrigranulosa* | 0 | 0.1 ± 0.40 | 0 |
| *Colpoda lucida* | 0 | 0.1 ± 0.19 | 0 |
| *Glaucoma reniformis* | 0 | 0 | 0.1 ± 0.19 |
| *Telotrochidium* sp. | 0 | 0 | 0.1 ± 0.19 |
| **Testate amoebae** | | | |
| *Arcella catinus* | 0.1 ± 0.19 | 0 | 0 |
| *Assulina muscorum* | 0.1 ± 0.19 | 0 | 0 |
| *Euglypha rotunda* | 0 | 0 | 0.1 ± 0.19 |

Table 7.3. Mean ± SD of the ciliate community parameters in the phosphate (P), nitrate (N) and control (C) treatments. The Kruskal Wallis test for abundance and ANOVA test for species richness showed no significant differences between treatments.

| | **Mean ± SD** | | | |
|---|---|---|---|---|
| **Community parameters** | **P** | **N** | **C** | ***p* value** |
| Abundance (individuals/2 mL) | 210.3 ± 256.54 | 440.9 ± 540.87 | 120.9 ± 158.53 | 0.195 |
| Species richness (number/2 mL) | 5.7 ± 1.97 | 9.3 ± 3.50 | 6.7 ± 2.69 | 0.084 |

## 8. Conclusions

- Recent studies have included ciliates in the analysis of bromeliad phytotelmata communities, moreover new species and families have been described from these microcosms. Although ciliate community abundances report are scarce, *Tillandsia utriculata* should be added to the list of tropical ecosystems with low ciliates abundance. However, the ciliates richness in *T. utriculata* is higher than those reported from other bromeliads, but lower than soil and freshwater ecosystems.

- Although testate amoebae are undersampled in bromeliad phytotelmata, *Tillandsia utriculata* should be added to the list of tropical ecosystems with low species richness and abundances for testate amoebae.

- A bottom-up effect through conductivity seems to be a triggering factor in the ciliate community of *T. utriculata* in the Guánica Dry Forest. This may be because salinity levels increased after ocean spray traveling through the forest. Similarly phosphate is also an important factor structuring ciliate abundances in *T. utriculata* from the Guánica Dry Forest.

- A top-down effect through predation by a generalist predator can also trigger ciliate community composition shifts. Moreover, a generalist predator reduced the CV% therefore stabilizing the abundance of the ciliate community.

- Species list serve to elucidate biogeographical trends. This study generated a species list for ciliates and testate amoebae inhabiting the phytotelma of *T. utriculata*. Moreover, we possibly have one new species (*Thylakidium* like).

## 9. Recommendations

- Species inventories should include ciliate and testate amoebae from different bromeliad species and from *T. utriculata* across different forests types. It could be useful to determine whether testate amoebae follow the same trend observed in this study. The new data generated from these studies will elucidate the general pattern describing the testate amoebae communities in bromeliad phytotelmata and will serve to make comparisons with other natural microcosms such as mosses, tree holes and bracts of Heliconiaceae.

- Studies that intend to disentangle which environmental factors are crucial for structuring ciliate communities should include phosphate and salinity (conductivity) as a measured variable.

- Silverline and protargol stains, together with molecular studies, should be performed on the possible new specie (*Thylakidium* like) inhabiting *T. utriculata*.

- Use rainwater collected from the forest to modify the longitudinal study to activate the ciliate and testate amoebae communities from the litter to validate the results.

- Modify the nutrient experiment so that the addition of nutrients is progressive, to test nutrient addition in ciliate and testate amoebae communities from bromeliad phytotelma.

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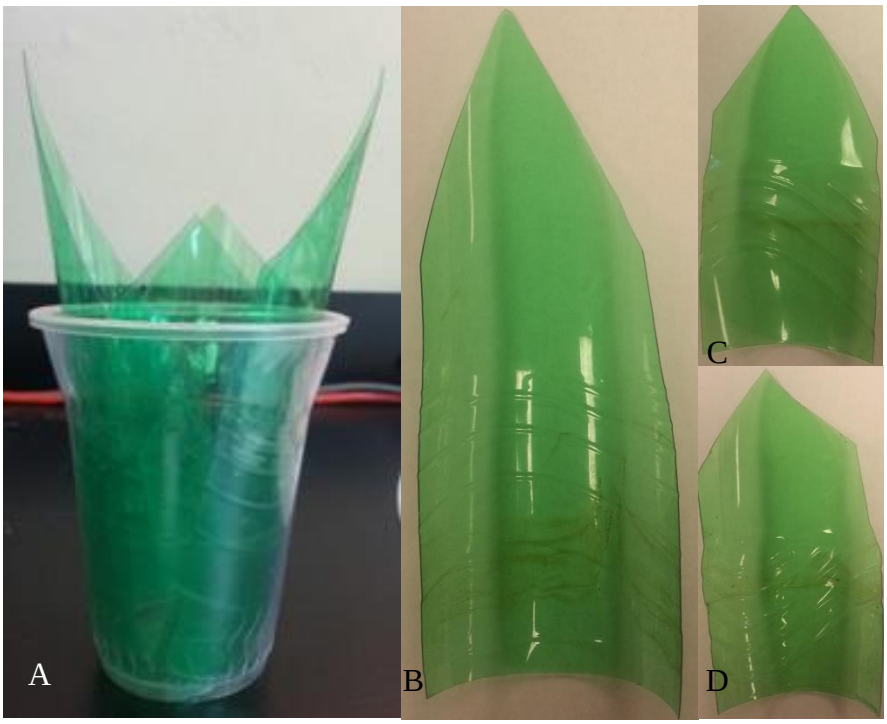
# APPENDICES




Appendix A: A) Plastic microcosm set-up, B-D) Leaves, B) Large (170 mm x 75 mm), C) Small (110 mm x 60 mm) and D) Medium (130 mm x 75 mm)

**Appendix B: Testate amoebae observed**

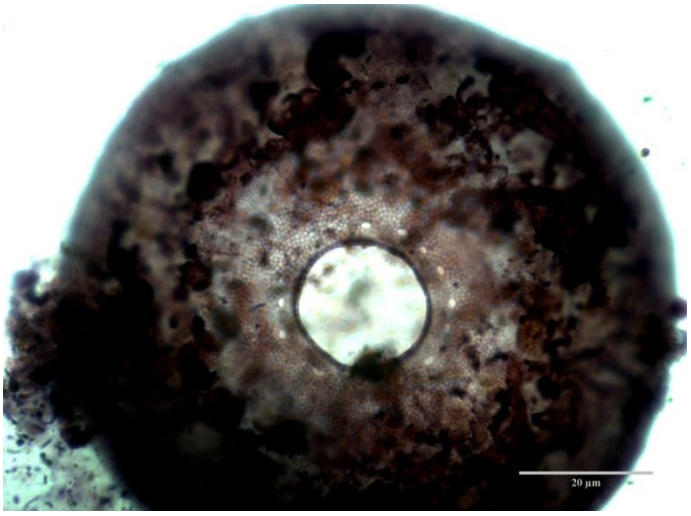
**Amoebozoa**

**Lobosea**

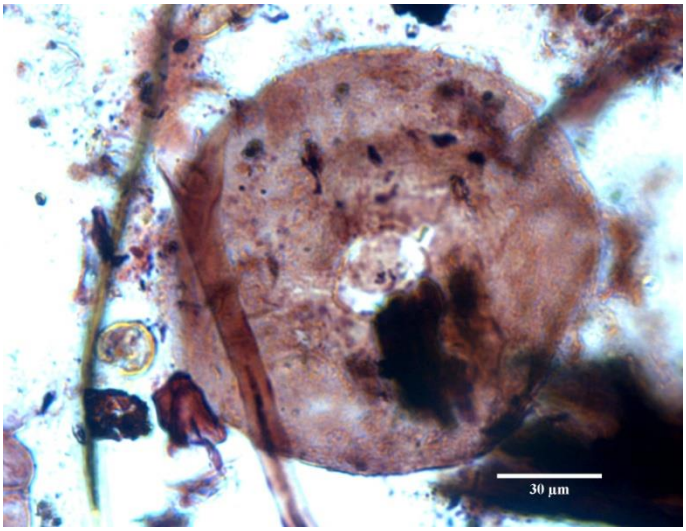
**Arcellinida**

***Arcella***

***A. arenaria*** Greeff



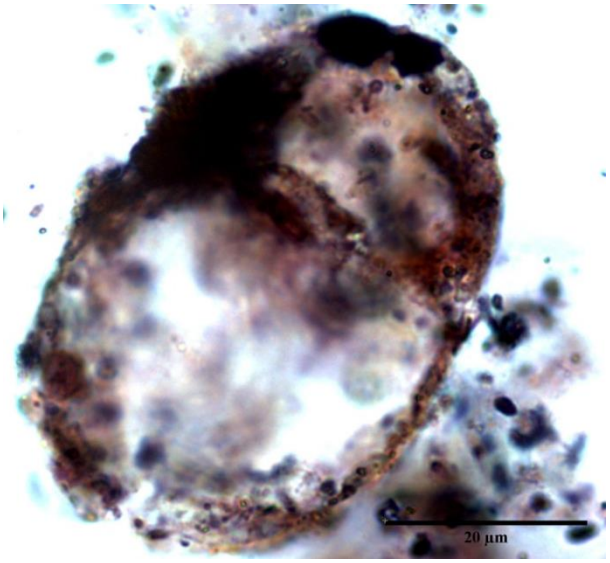

***A. catinus*** Penard



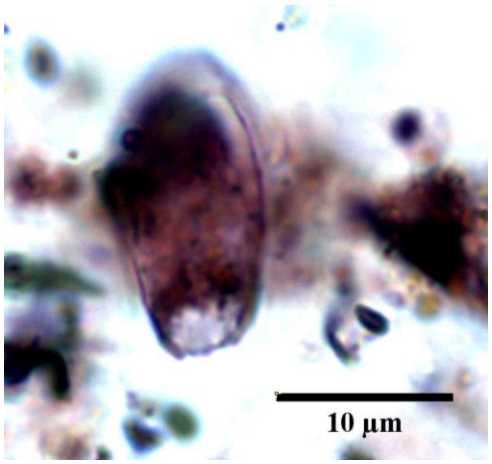

**Centropyxidae**

**Centropyxis**

***C. constricta*** Ehrenberg



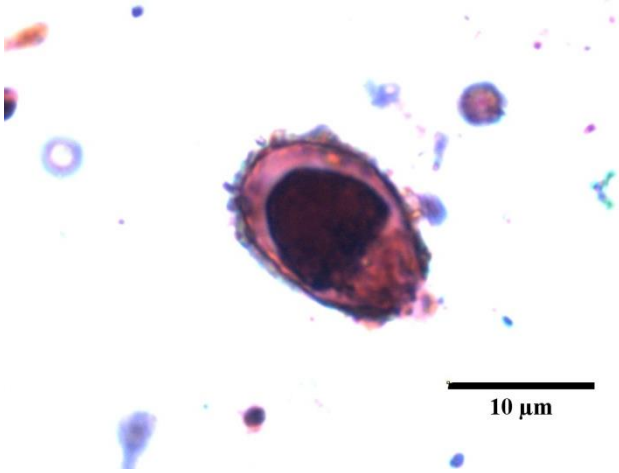
***C. elongata*** (Penard) Thomas



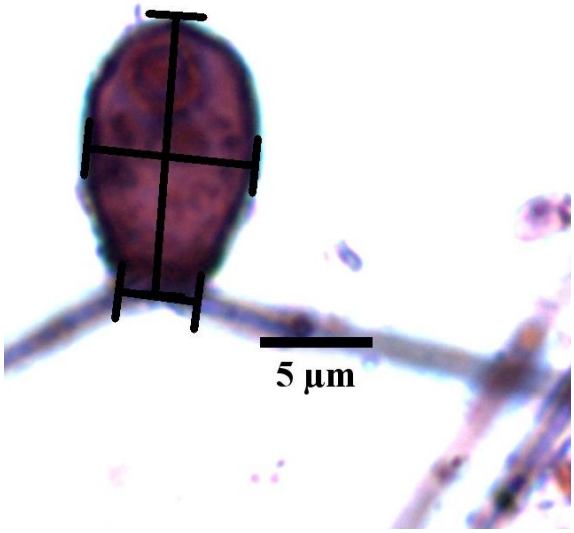

**Cryptodifflugiidae**

**Cryptodifflugia**

***C. compressa*** Penard



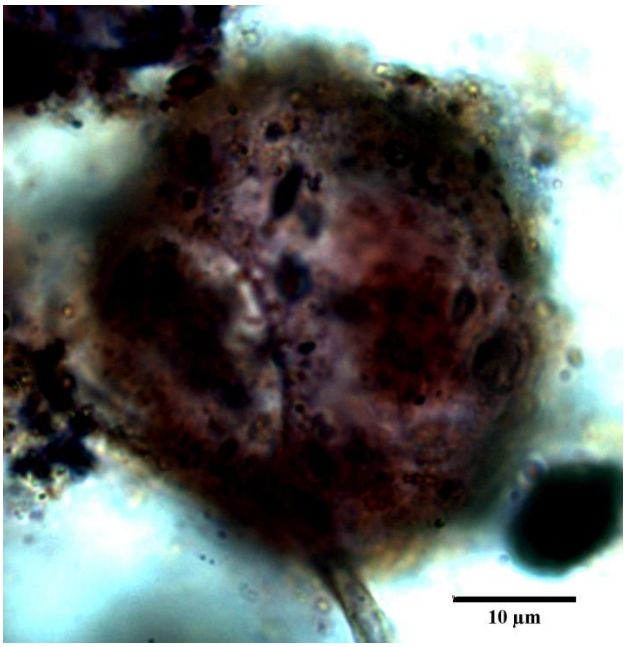

***C. oviformis*** Penard



**Trigonopyxidae**

***Cyclopyxis***

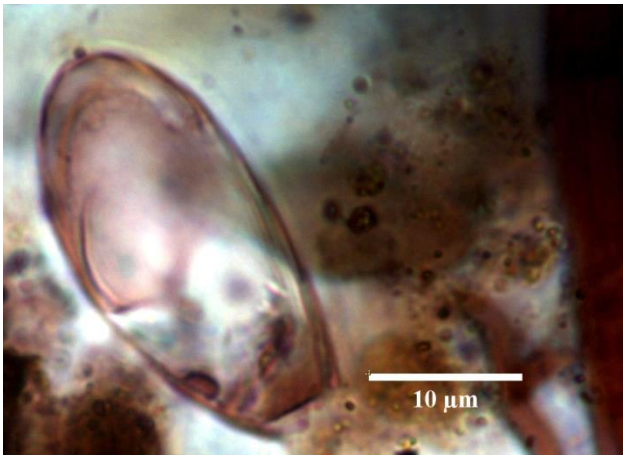
***C. kahli*** Deflandre




**Hyalospheniidae**

***Hyalosphenia***

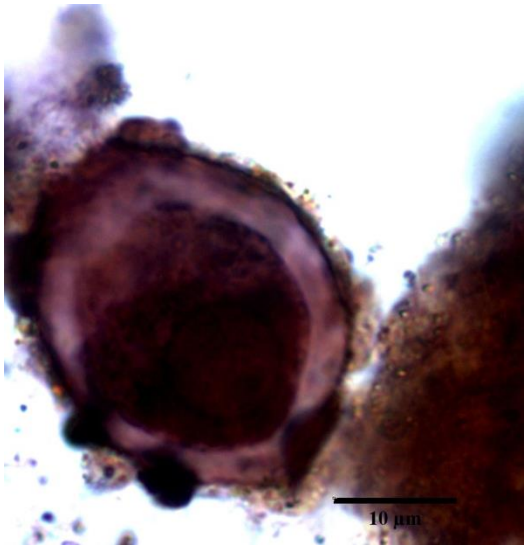
***Hyalosphenia* sp.**



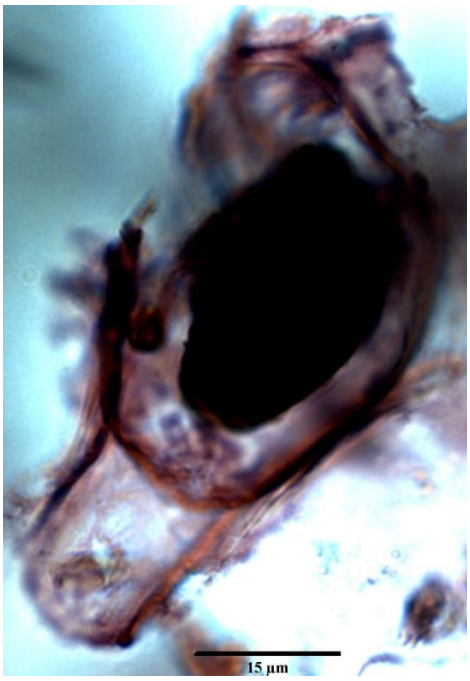

**Nebelidae**

***Nebela***

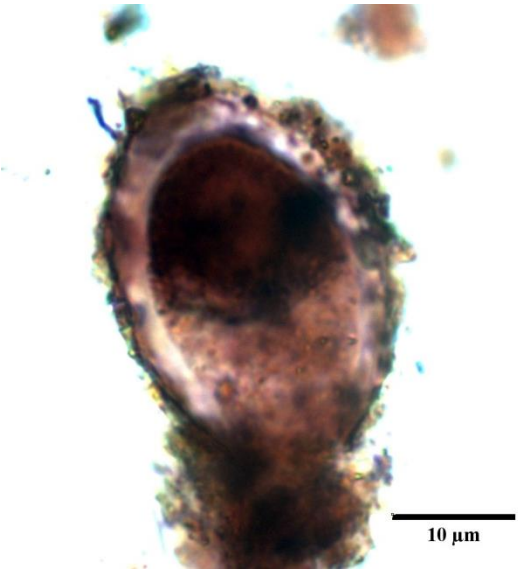
***N. carinata*** (Archer) Leidy



***N. tubulata*** Brown




***Nebela sp.***




**Cercozoa**

**Imbricatea**

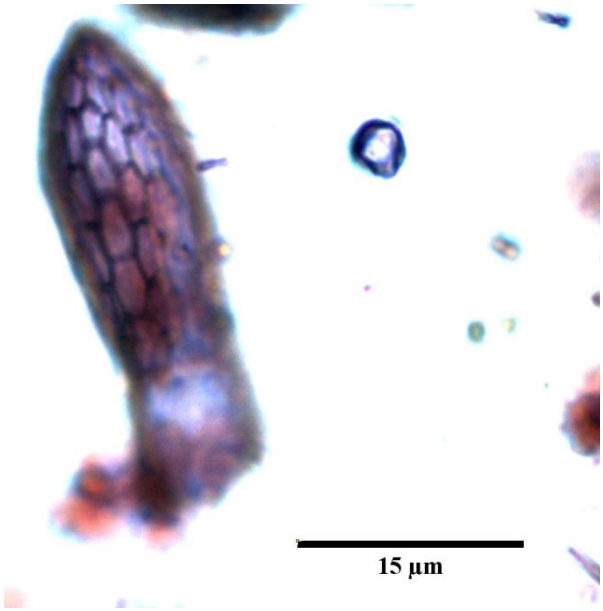
**Euglyphidae**

***Assulina***

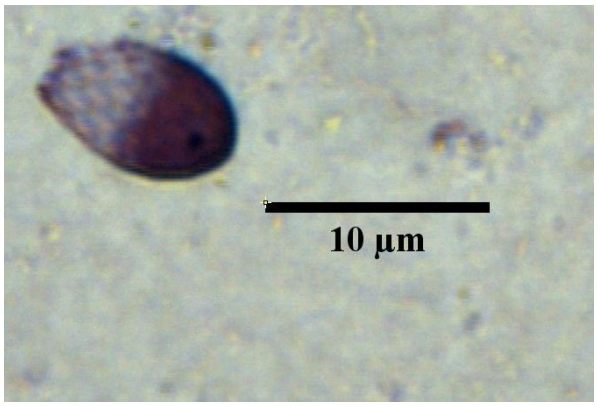
***A. muscorum*** Greeff

***Euglypha***

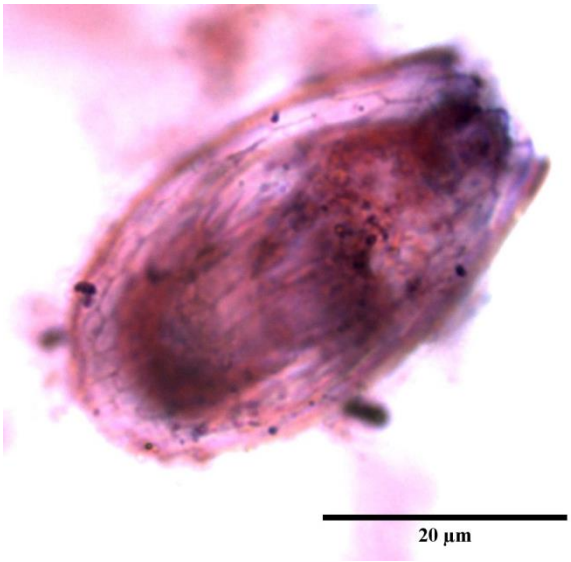
***E. cristata*** Leidy



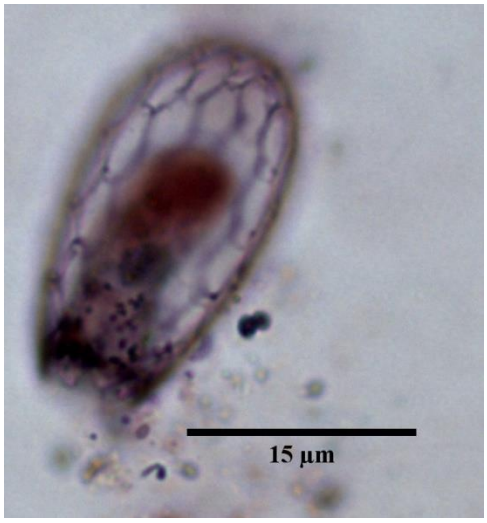

***E. hyalina*** Coûteaux



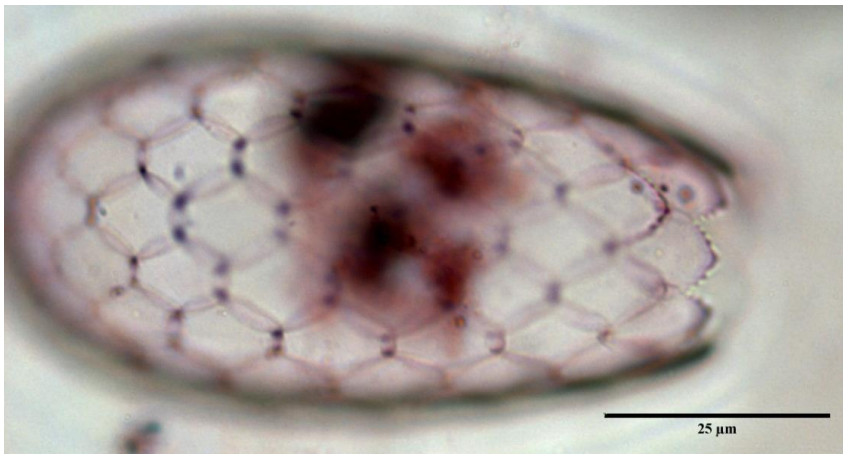

***E. laevis*** Perty



***E. rotunda*** Wailes

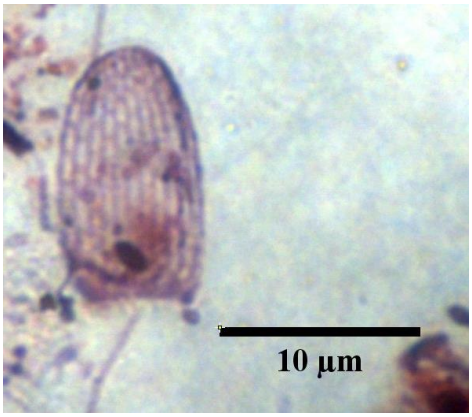



***E. tuberculata*** Dujardin



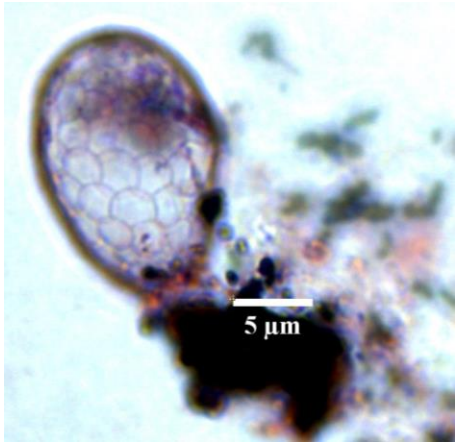

***Placocista***

***P. glabra v. minima*** Decloitre



***Sphenoderia***

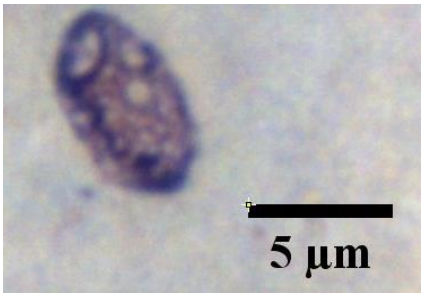
***S. lenta*** Schlumberger




**Trinematidae**

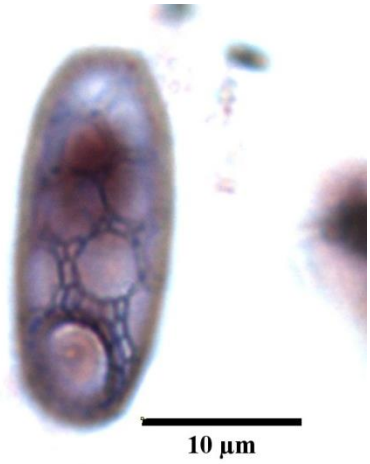
***Corythion***

***C. dubium f. minima*** Chardez




***Trinema***

***T. lineare*** Penard



**Appendix C: Ciliates observed**

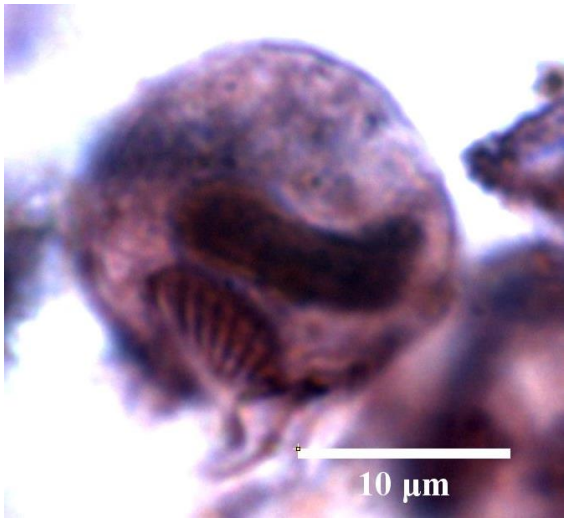
**Ciliophora**

**Spirotrichea**

**Halteriidae**

***Halteria***

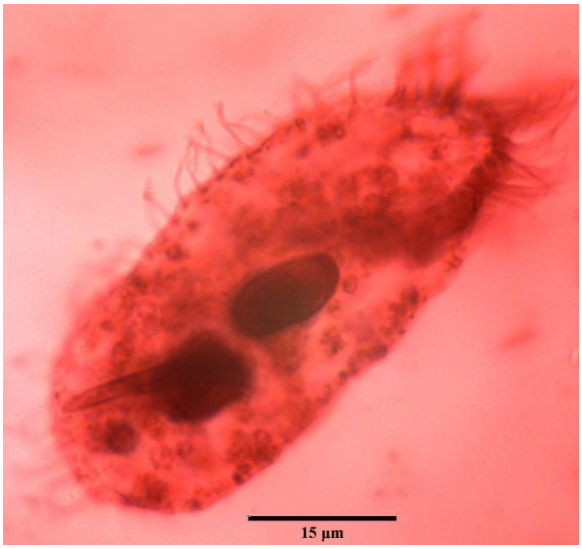
***Halteria sp.***




**Oxytrichidae**

***Oxytricha***

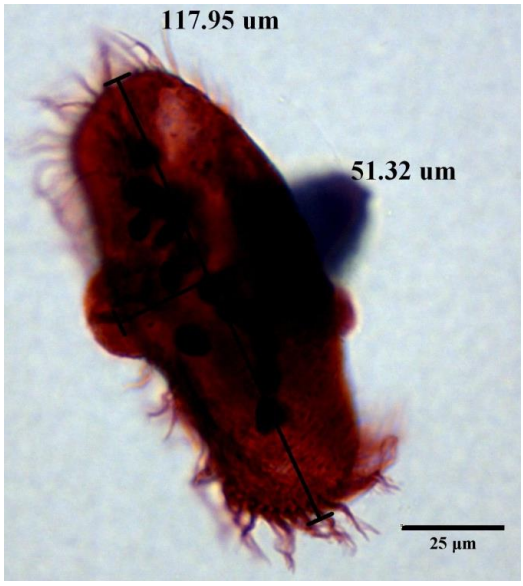
***O. longigranulosa*** Shin




***O. ottowi*** Foissner

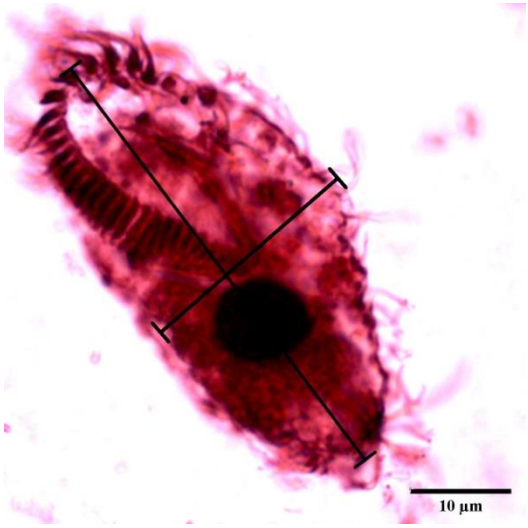
***Pattersoniella***

***P. vitiphila*** Foissner




***Thachysoma***

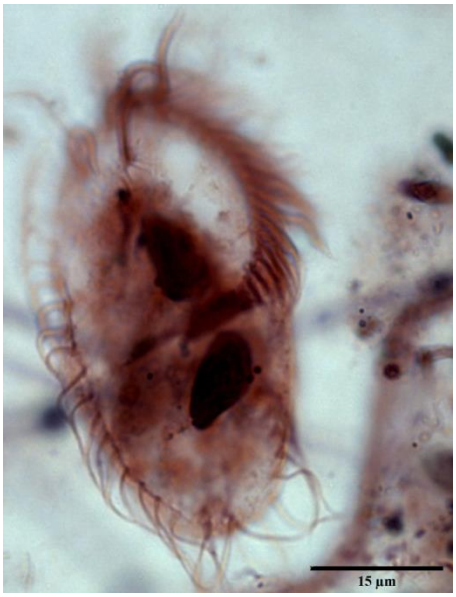
***Tachysoma* sp.**




**Trachelostylidae**

***Gonostomum***

***G. affine*** Stein



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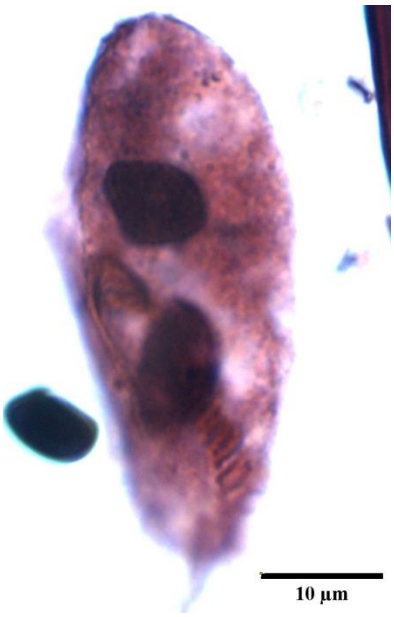


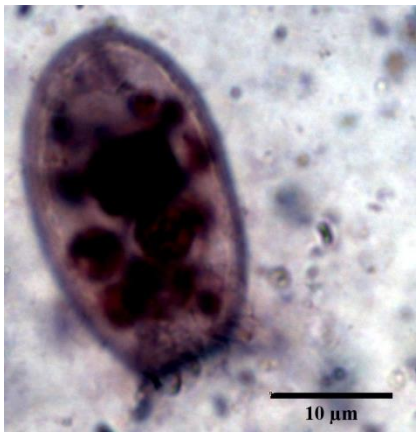

**Litostomatea**

**Haptorida**

**Enchelyidae**

***Enchelys***

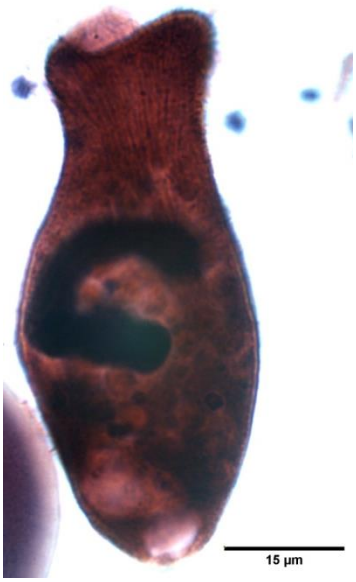
***Enchelys* sp.**




**Spathidiidae**

***Spathidium***

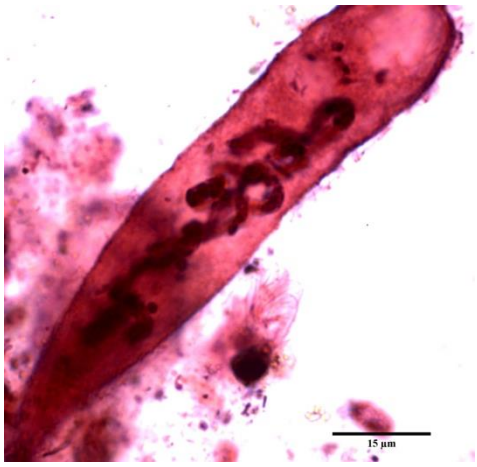
***Spathidium* sp.**



**Trachelophyllidae**

***Trachelophyllum***

***Trachelophyllum* sp.**



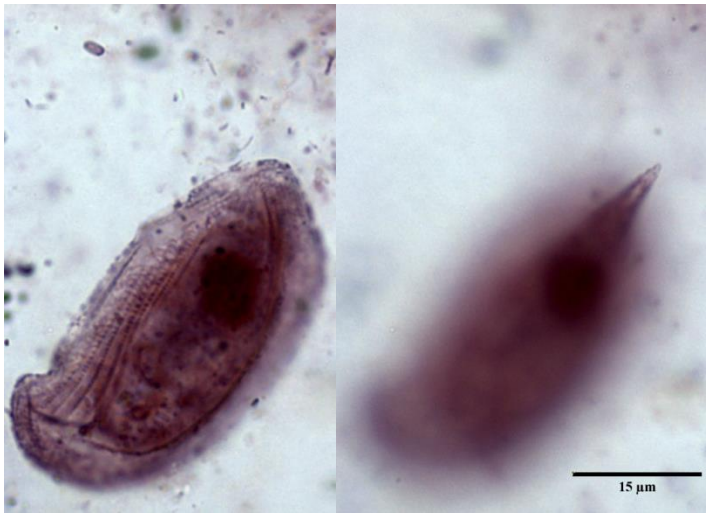

**Phyllopharyngea**

**Chlamydodontida**

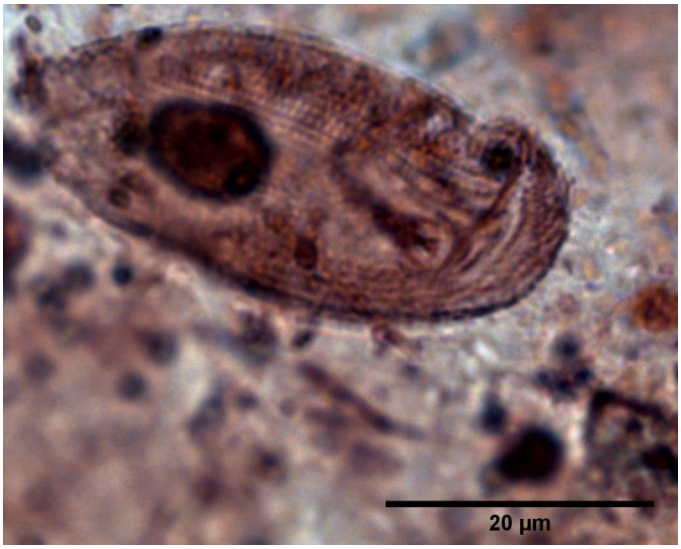
**Chilodonellidae**

***Chilodonella***

***C. caudata*** Stokes




***C. uncinata*** Ehrenberg

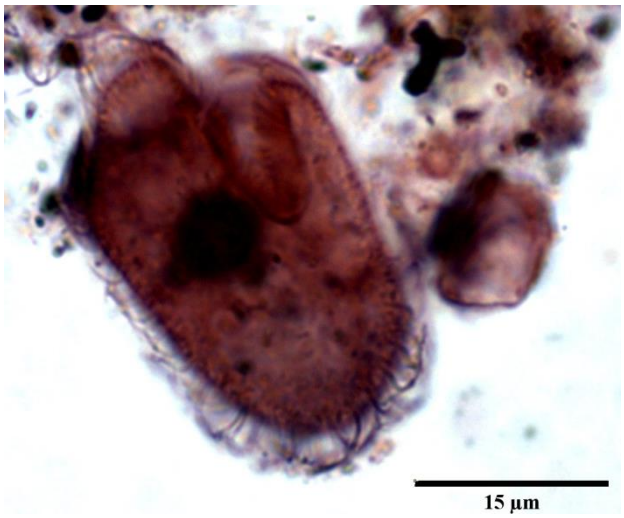


**Colpodea**

**Bryometopida**

**Bryometopidae**

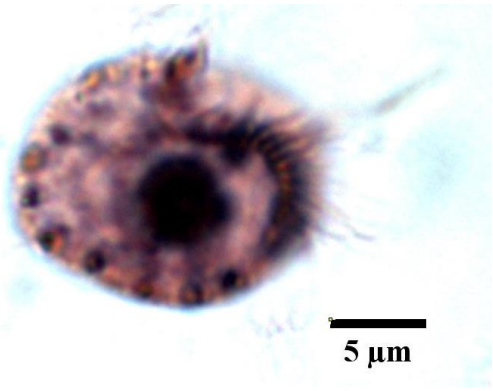
***Thylakidium* like**




**Kreyellidae**

***Kreyella***

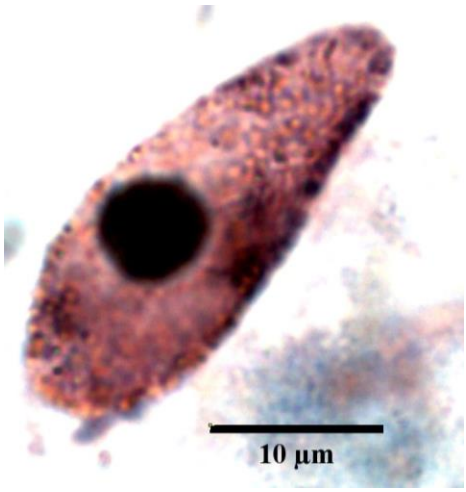
***K. minuta*** Foissner




**Bryophryida**

**Bryophryidae**

**Bryophrydid like**

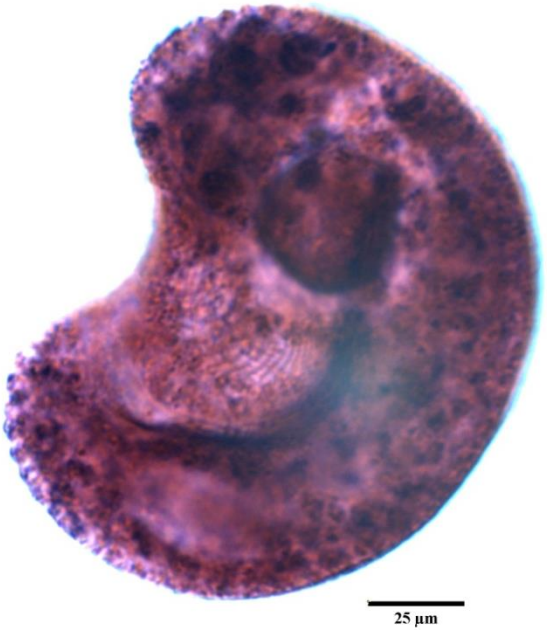


**Colpodida**

**Colpodidae**

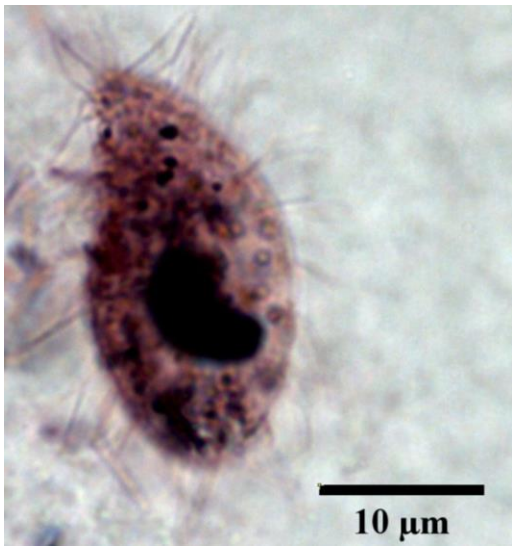
***Bresslaua***

***B. vorax*** Kahl

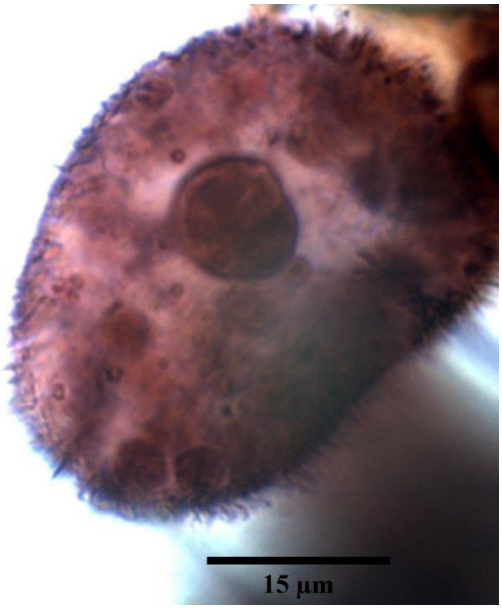



***Colpoda***

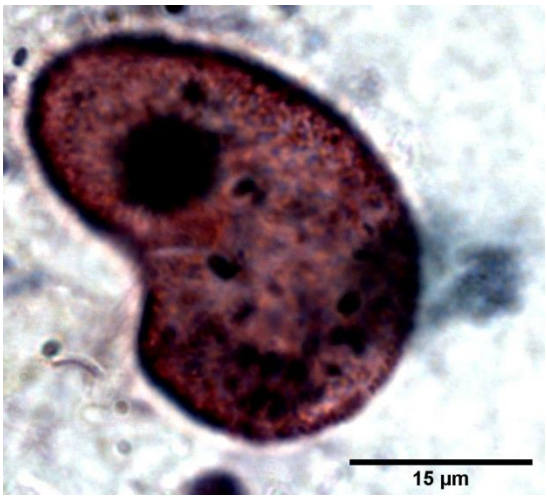
***C. aspera*** Kahl




***C. cucullus*** Muller

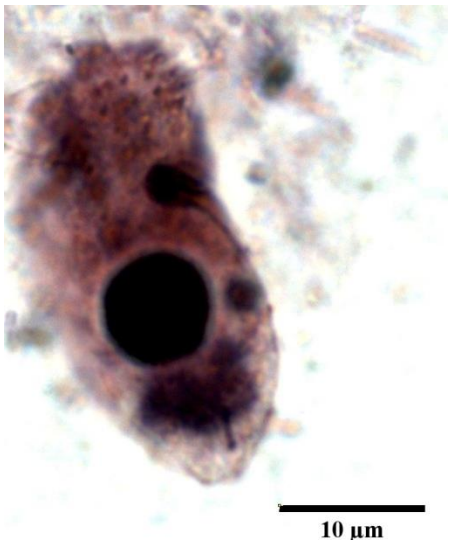


***C. inflata*** Stokes

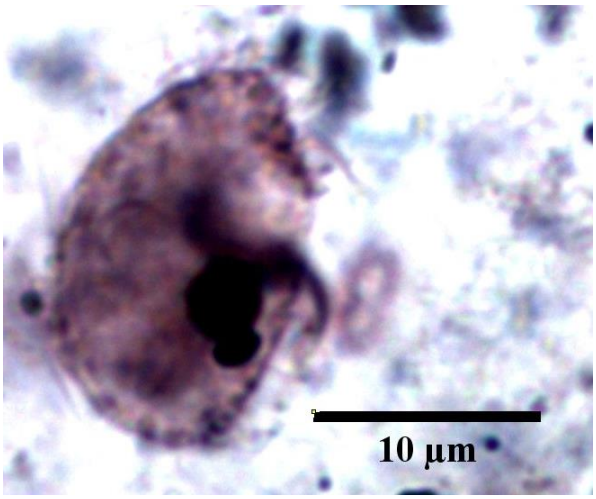



***C. lucida*** Greeff


***C. maupasi*** Enriquez



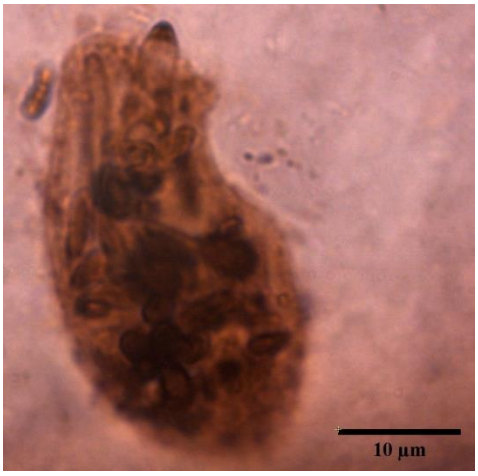
***C. steinii*** Maupas




**Grossglockneriidae**

***Grossglockneria***

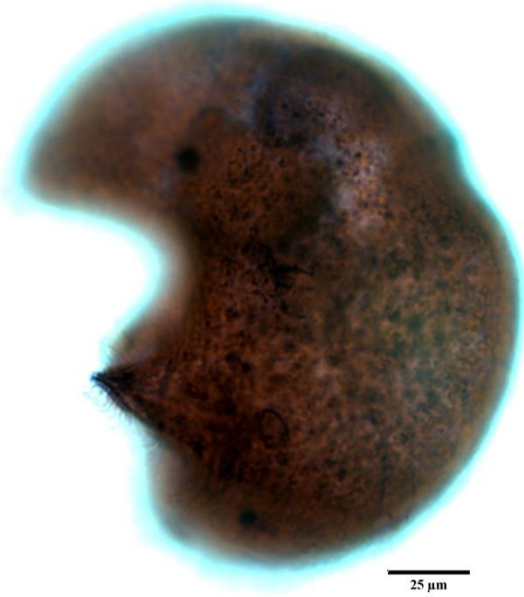
***Grossglockneria* sp**




**Hausmanniellidae**

***Bresslauides***

***B. discoideus*** Kahl

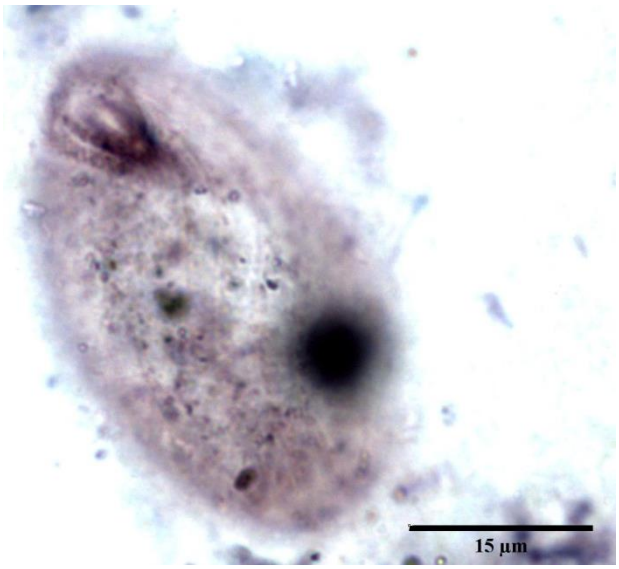


**Cyrtolophosidida**

**Cyrtolophosididae**

***Cyrtolophosis***

***C. major*** Kahl




**Platyophryidae**

***Platyophrya***

***P. bromelicola*** Foissner

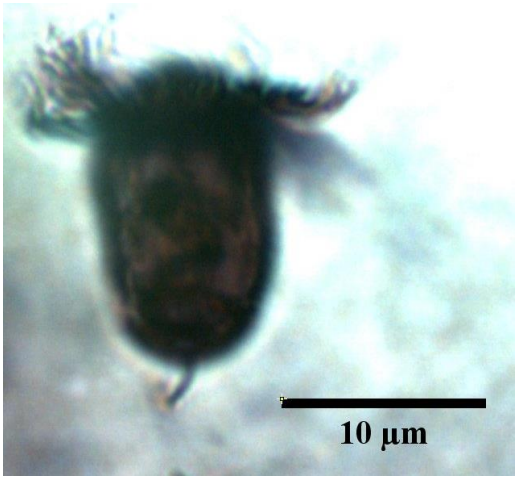

**Prostomatea**

**Prorodontida**

**Balanionidae**

***Balanion***

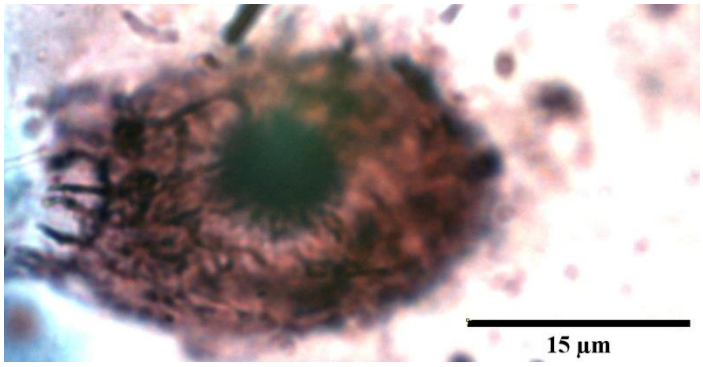
***B. planctonicum*** Foissner, Oleksiv and Müller



**Colepidae**

***Coleps***

***C. hirtus*** Nitzsch



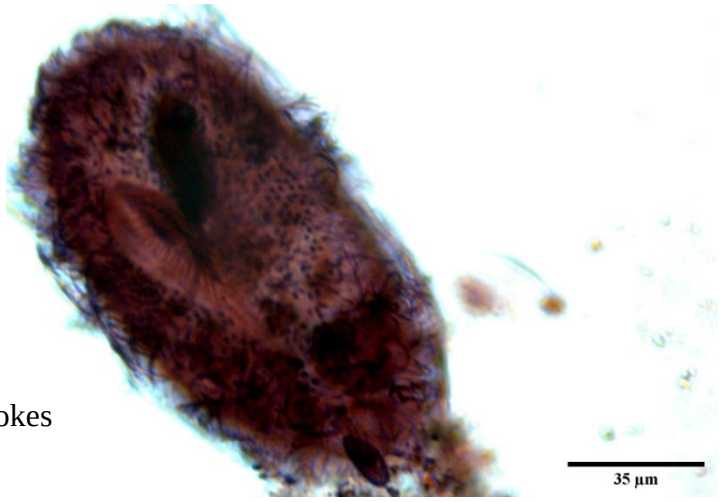

**Oligohymenophorea**

**Peniculida**

**Frontoniidae**

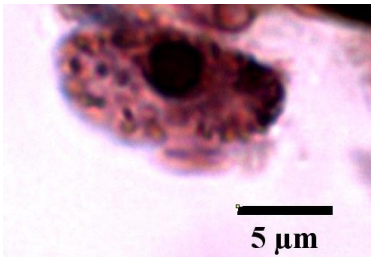
***Frontonia***

***F. depressa*** Stokes

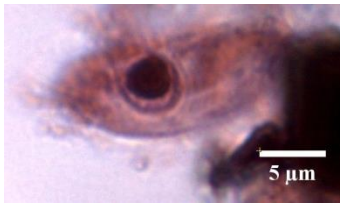



**Scuticociliatia**

**Scuticociliatidia 1**




**Scuticociliatidia 2**

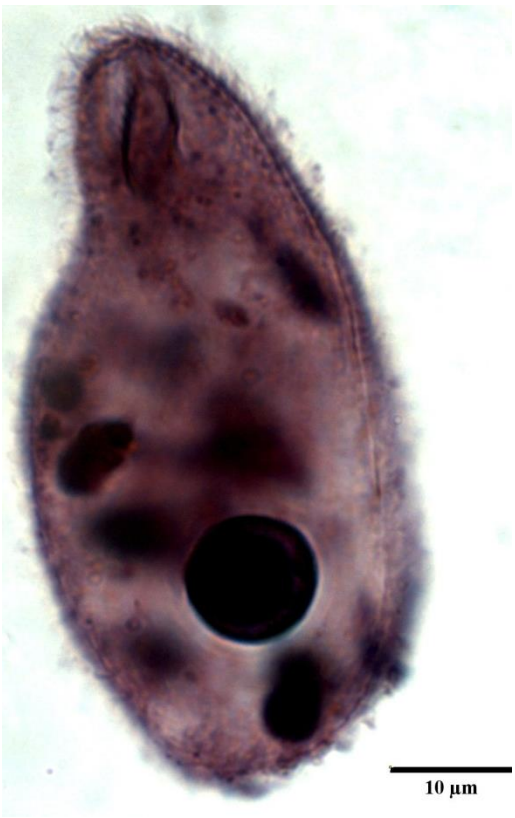


**Tetrahymenida**

**Glaucomidae**

***Glaucoma***

***G. reniformis*** Schewiakoff




**Tetrahymenidae**

***Tetrahymena***

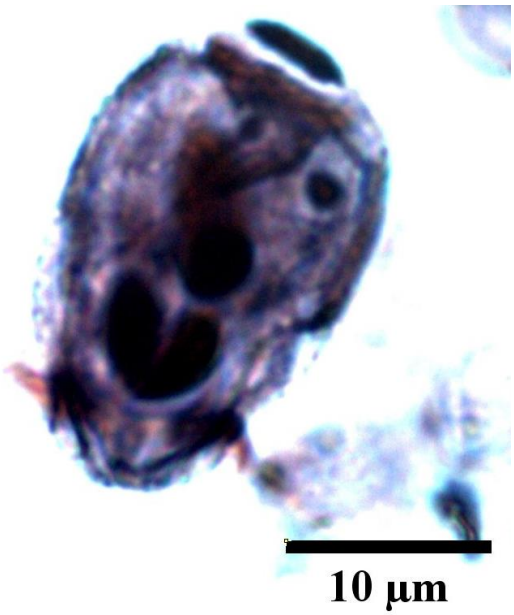
***T. pyriformis*** **complex**

**Sessilida**

**Opisthonectidae**

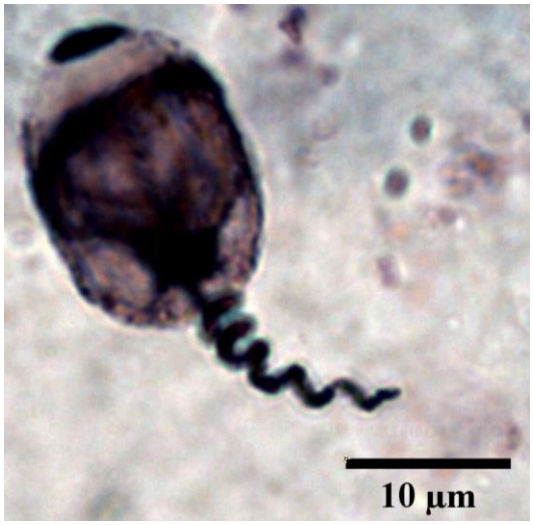
***Telotrochidium***

***Telotrochidium* sp**

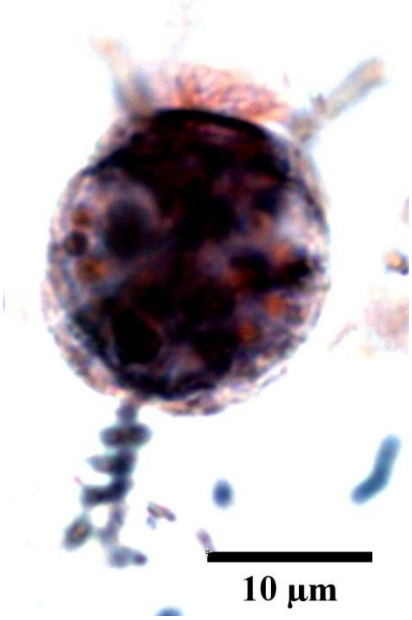



**Vorticellidae**

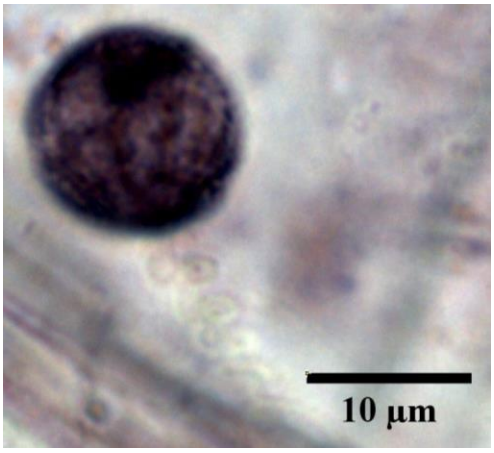
**Vorticellid 1**




**Vorticellid 2**



**Vorticellid 3**



Appendix D: Mean ± SD (individuals/2 mL) ciliate and testate amoebae abundance (individuals/2 mL) observed in *T. utriculata* in Guánica Dry Forest, Puerto Rico.

| Species | Study | | |
|---|---|---|---|
| | **Longitudinal** | **Predation** | **Nutrients** |
| **Testate amoebae** | | | |
| *Arcella arenaria* | 0.02 ± 0.22 | - | - |
| *Arcella catinus* | - | - | 0.1 ± 0.22 |
| *Assulina muscorum* | 0.02 ± 0.22 | 1.0 ± 1.20 | 0.1 ± 0.22 |
| *Centropyxis constricta* | - | 0.1 ± 0.35 | - |
| *Centropyxis elongata* | - | 0.1 ± 0.35 | - |
| *Corythion dubium f. minima* | 0.1 ± 0.43 | - | - |
| *Cryptodifflugia compressa* | - | 0.6 ± 0.90 | - |
| *Cryptodifflugia oviformis* | - | 0.2 ± 0.6 | - |
| *Cyclopixis khali* | 0.07 ± 0.57 | 0.1 ± 0.32 | - |
| *Euglypha cristata* | 0.02 ± 0.27 | 0.4 ± 1.06 | - |
| *Euglypha hyalina* | 0.2 ± 1.41 | 0.3 ± 0.71 | - |
| *Euglypha laevis* | 0.02 ± 0.22 | 0.6 ± 1.88 | - |
| *Euglypha rotunda* | 0.9 ± 6.47 | 5.5 ± 8.33 | 0.15 ± 0.22 |
| *Euglypha tuberculata* | 0.1 ± 0.77 | - | - |
| *Hyalosphenia* sp.1 | 0.02 ± 0.22 | - | - |
| *Hyalosphenia* sp.2 | - | 0.1 ± 0.35 | - |
| N*ebela carinata* | - | 0.1 ± 0.35 | - |
| *Nebela* sp. | - | 0.7 ± 1.49 | - |
| *Nebela tubulata* | 0.02 ± 0.22 | - | - |
| *Placocista glabra v. minima* | 0.1 ± 0.65 | - | - |
| *Sphenoderia lenta* | 0.1 ± 0.86 | - | - |
| *Trinema lineare* | 0.1 ± 0.43 | 1.0 ± 1.85 | - |
| **Ciliates** | | | |
| *Balanion planctonicum* | 0.1 ± 0.37 | - | - |
| *Bresslaua vorax* | 0.2 ± 0.80 | - | - |
| *Bresslauides discoideus* | 0.02 ± 0.16 | - | - |
| Bryophirid like | 6.5 ± 19.92 | - | - |
| *Chilodonella unicinata* | 0.2 ± 0.57 | 0.5 ± 0.71 | 0.2 ± 0.70 |
| *Chilodonella caudata* | - | 1.8 ± 4.67 | - |
| *Coleps hirtus* | 0.1 ± 0.22 | - | - |
| *Colpoda aspera* | 0.6 ± 1.89 | 0.1 ± 0.32 | 0.2 ± 0.67 |
| *Colpoda cucullus* | 27.1 ± 63.57 | - | 139.9 ± 170.10 |
| *Colpoda inflata* | 24.8 ± 84.08 | 0.20 ± 0.42 | 1.3 ± 1.98 |
| *Colpoda lucida* | 2.8 ± 8.92 | - | 0.1 ± 0.22 |
| *Colpoda maupasi* | 139.7 ± 259.00 | 11.5 ± 47.48 | 114.7 ± 241.36 |
| *Colpoda steini* | 50.7 ± 104.34 | 0.3 ± 0.49 | 1.7 ± 2.01 |

Appendix D: Continuation

| Species | Study | | |
|---|---|---|---|
| | **Longitudinal** | **Predation** | **Nutrients** |
| *Cyrtolophosis major* | 0.02 ± 0.16 | - | - |
| Enchelydid sp. | 0.02 ± 0.19 | - | - |
| *Enchelys* sp. | 0.2 ± 0.67 | - | - |
| *Frontonia depressa* | 0.2 ± 0.65 | 1.9 ± 2.28 | 0.6 ± 0.83 |
| *Glaucoma reniformis* | 1.6 ± 4.35 | - | 0.1 ± 0.22 |
| *Gonostomum affine* | 1.1 ± 4.01 | 23.20 ± 23.31 | 0.50 ± 1.10 |
| *Grossglockneria* sp. | 0.4 ± 2.34 | - | - |
| *Halteria* sp. | - | 0.50 ± 1.581 | - |
| *Kreyella minuta* | 0.02 ± 0.156 | - | - |
| Litonotidae sp. | 0.12 ± 0.557 | - | 0.25 ± 1.12 |
| Litostome 2 | - | 0.1 ± 0.32 | - |
| *Oxytricha longrigranulosa* | 1.5 ± 4.85 | 2.8 ± 4.87 | 0.2 ± 0.49 |
| *Oxytricha ottowii* | 1.8 ± 5.21 | 0.40 ± 0.97 | 1.9 ± 2.06 |
| *Oxytricha* sp. | 0.02 ± 0.16 | - | - |
| *Pattersoniella vitiphila* | 0.2 ± 0.99 | 0.8 ± 1.62 | 0.6 ± 0.83 |
| *Platyophrya bromelicola* | 23.8 ± 47.70 | 469.8 ± 511.12 | 1.1 ± 2.05 |
| Scuticociliatidia 1 | 4.6 ± 12.37 | 11.7 ± 18.25 | 0.2 ± 0.70 |
| Scuticociliatidia 2 | 1.6 ± 4.79 | - | - |
| Scuticociliatidia 3 | 0.02 ± 0.16 | - | - |
| Scuticociliatidia 4 | 1.2 ± 5.99 | - | 0.3 ± 0.92 |
| *Spathidium* sp. | 0.2 ± 0.98 | 0.60 ± 1.1 | 7.3 ± 10.92 |
| *Spathidium* sp.2 | 0.1 ± 0.49 | - | - |
| Stichotrich 1 | - | 0.6 ± 1.90 | - |
| *Tachysoma* sp. | - | 0.1 ± 0.32 | - |
| *Telotrochidium* sp. | 0.5 ± 1.78 | - | 0.1 ± 0.22 |
| *Tetrahymena piriformis* complex | 0.02 ± 0.16 | 0.6 ± 1.27 | - |
| *Thylakidium* like sp. | - | 54.7 ± 92.01 | - |
| *Trachelophyllum* sp. | 0.1 ± 0.37 | 0.10 ± 0.32 | 0.20 ± 0.70 |
| Vorticellid 1 | 6.1 ± 12.52 | - | 0.20 ± 0.70 |
| Vorticellid 2 | 5.6 ± 18.46 | 1.20 ± 2.10 | 0.75 ± 2.05 |
| Vorticellid 3 | 0.7 ± 2.31 | - | - |

Appendix E: Carbon concentration of seven randomly selected litter samples in the nutrient experiment to corroborate the assumption of a homogeneous distribution of carbon among the treatments phosphate, nitrate and control.

| Sample | %C | Grams of C |
|---|---|---|
| 1 | 44.7 | 0.45 |
| 2 | 45.2 | 0.45 |
| 3 | 45.2 | 0.45 |
| 4 | 44.8 | 0.45 |
| 5 | 44.7 | 0.45 |
| 6 | 45.0 | 0.45 |
| 7 | 45.5 | 0.45 |

Appendix F. Total abundance ± SD (individuals/2 mL) of the ciliates and testate amoebae in the phosphate (P), nitrate (N) and control (C) treatments in artificial bromeliad microcosms with litter from *T. utriculata.*

| Species | Treatment | |
|---|---|---|
| | **P** | **C** |
| **Testate amoebae** | | |
| *Euglypha rotunda* | 36 | 55 |
| *Euglypha laevis* | 6 | 8 |
| *Assulina muscorum* | 6 | 2 |
| *Nebela sp* | 3 | 4 |
| *Centropixis constricta* | 1 | 0 |
| *Cyclopyxis kahli* | 1 | 0 |
| *Hyalosphenia sp* | 1 | 0 |
| *Cryptodifflugia compressa* | 0 | 6 |
| *Trinema lineare* | 0 | 6 |
| *Cryptodifflugia oviformis* | 0 | 2 |
| *Euglypha cristata* | 0 | 2 |
| *Euglypha hyalina* | 0 | 2 |
| *Centropixis elongata* | 0 | 1 |
| *Nebela carinata* | 0 | 1 |
| **Ciliates** | | |
| *Platyophrya bromelicola* | 3113 | 960 |
| *Gonostomum affine* | 211 | 68 |
| *Thylakidium* like sp | 145 | 302 |
| *Colpoda steinii* | 60 | 75 |
| Scuticociliatidia 1 | 45 | 80 |
| *Frontonia depressa* | 13 | 7 |
| *Oxytricha longigranulosa* | 11 | 17 |
| *Colpoda maupasii* | 9 | 25 |
| *Spathidium* sp1 | 6 | 0 |
| *Stichotrich* 1 | 6 | 0 |
| *Halteria* sp. | 5 | 0 |
| *Tetrahymena pyriformis* complex | 4 | 2 |
| *Tachysoma* sp | 2 | 0 |
| *Chilodonella caudata* | 1 | 17 |
| Vorticellid sp 2 | 1 | 7 |
| *Chilodonella uncinata* | 1 | 4 |
| *Oxytricha ottowi* | 1 | 3 |

Appendix F. Continuation

| Species | Treatment | |
|---|---|---|
| | P | C |
| *Colpoda aspera* | 1 | 0 |
| *Colpoda inflata* | 1 | 0 |
| *Litostome* 2 | 1 | 0 |
| *Pattersoniella vitiphila* | 0 | 7 |

Appendix G. Total abundance ± SD (individuals/2 mL) of the ciliates and testate amoebae in the phosphate (P), nitrate (N) and control (C) treatments in artificial bromeliad microcosms with litter from *T. utriculata.*

| Species | Treatment | | |
|---|---|---|---|
| | **P** | **N** | **C** |
| **Ciliates** | | | |
| *Colpoda cucullus* | 1438 | 1150 | 410 |
| *Colpoda maupasii* | 328 | 1694 | 340 |
| *Colpoda steinii* | 15 | 8 | 11 |
| *Oxytricha ottowii* | 10 | 20 | 7 |
| *Colpoda inflata* | 10 | 11 | 6 |
| *Spathidium* sp1 | 8 | 114 | 24 |
| Vorticellid 2 | 5 | 2 | 8 |
| *Platyophrya bromelicola* | 4 | 13 | 5 |
| *Frontonia depressa* | 3 | 3 | 5 |
| *Colpoda aspera* | 3 | 0 | 0 |
| *Pattersoniela vitiphila* | 2 | 6 | 3 |
| *Frontonia* depressa | 1 | 3 | 0 |
| Litonotidae 1 | 0 | 17 | 5 |
| Scuticociliatida 4 | 0 | 5 | 1 |
| *Gonostomum affine* | 0 | 3 | 7 |
| *Chilodonella uncinata* | 0 | 3 | 1 |
| *Trachelophyllum* sp. | 0 | 3 | 1 |
| Vorticellid 1 | 0 | 3 | 1 |
| *Oxytricha longrigranulosa* | 0 | 3 | 0 |
| *Enchelys* sp | 0 | 2 | 1 |
| Scuticociliatida 1 | 0 | 1 | 3 |
| *Colpoda lucida* | 0 | 1 | 0 |
| *Glaucoma reniformis* | 0 | 0 | 1 |
| *Telotrochidium* sp. | 0 | 0 | 1 |
| **Testate amoebae** | | | |
| *Arcella catinus* | 1 | 0 | 0 |
| *Assulina muscorum* | 1 | 0 | 0 |
| *Euglypha rotunda* | 0 | 0 | 1 |