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## Research Article

# Diversity and endemism of ciliates inhabiting Neotropical phytotelmata

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While the diversity and distribution of macro-organisms living in phytotelmata (plant-container habitats) is well known, detailed taxonomic work on micro-organisms living in the same environments is limited. As a model clade of microbial eukaryotes, sampling of ciliates in Neotropical bromeliad tanks increased, and Neotropical phytotelmata such as bamboo stumps and tree holes were newly sampled. Thirty-three isolates from Brazil, Costa Rica, Dominican Republic, Jamaica and Mexico were sequenced for small subunit rDNA, and placed into a phylogenetic context using non-phytotelmata GenBank accessions. This and the morphological investigations discovered 45 undescribed, possibly endemic ciliate species. The potential endemics are from throughout most clades of the ciliate tree of life, and there is evidence of speciation within the Neotropical phytotelmata habitat. Our data show the number of potential Neotropical phytotelmata-endemic ciliate species increasing as more phytotelmata are sampled. While the new data show that the supposed endemics are mainly recruited from moss and ephemeral limnetic habitats, the bromeliad ciliate fauna is quite distinct from those of other limnetic habitats, lacking many typical and common freshwater genera, such as *Coleps*, *Colpidium*, *Frontonia*, *Paramecium*, *Glaucoma*, *Nassula*, *Stylonychia* and *Trithigmostoma*. There is no indication that specific ciliates are confined to specific bromeliads.

**Key words:** biogeography, bromeliads, Ciliophora, endemic ciliates, morphology, phylogeny

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## Introduction

There are more than 1500 plants from around the world that can normally form phytotelmata (Fish, 1983), although the number is much higher if tree holes and stumps are included (Pimm, 1982; Frank & Lounibos, 1983; Kitching, 2000). These plant-container habitats are interesting to ecologists as they are discrete systems in which ecological phenomena, such as food webs, can be studied in detail (Kitching, 2000, 2001). A vast amount of literature and knowledge has thus accumulated on the biogeography of macro-organismic species inhabiting phytotelmata; e.g. rotifers, nematodes, arthropods and chordates (reviewed in detail by Kitching, 2000). By contrast, we know very little about the microbial eukaryotes that inhabit these same discrete habitats.

One such group of microbial eukaryotes that can be found in phytotelmata is the Ciliophora. Ciliates are diagnosed by 'germline' micronuclei and 'somatic' macronuclei within each cell (Lynn, 2008). As ciliates are central players in the microbial loop in most ecosystems (Azam *et al.*, 1983;

Finlay & Fenchel, 1996; Corliss, 2002), they are indicators of environmental health (Lynn & Gilron, 1992; Berger & Foissner, 2003). Ciliates are primarily sexual (Dunthorn & Katz, 2010), although most individuals in any given population are the result of asexual cell division. Ciliates have been used extensively as model organisms to develop and test hypotheses about microbial biodiversity and biogeography (e.g. Foissner, 1999; Finlay, 2002; Katz *et al.*, 2005; Fenchel & Finlay, 2006; Doherty *et al.*, 2007; Foissner *et al.*, 2008, 2011; Weisse *et al.*, 2008; Stoeck *et al.*, 2010). They are successful models because compared with amoeboid and flagellated organisms they are morphologically diverse, and GenBank contains numerous accessions from known species (Dunthorn & Katz, 2008; Lynn, 2008).

Ecologists originally observed ciliates inhabiting Neotropical phytotelmata in bromeliads (Picado, 1911; Laessle, 1961; Maguire, 1963; Maguire *et al.*, 1968; Carrias *et al.*, 2001; Vandermeer *et al.*, 1972; Esteves & da Silva Neto, 1996). Detailed taxonomic observations only began with Foissner (2003a, 2003b) and Foissner *et al.* (2003), where new and potentially endemic species from bromeliad tanks were described. Since then, several papers

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have been published describing, and sequencing, additional new and endemic species from bromeliads (Foissner, 2005; Foissner *et al.*, 2009; Foissner & Wolf, 2009; Foissner *et al.*, 2011; Foissner & Stoeck, 2011; Omar & Foissner, 2011).

To deepen our understanding of microbial eukaryotes inhabiting Neotropical phytotelmata, we increased the sampling of ciliates from more bromeliads; we also sampled other Neotropical phytotelmata by isolating ciliates from bamboo stumps and tree holes. All new isolates were sequenced for the nuclear small subunit rDNA (SSU-rDNA). With these data we asked: (1) Does the number of potential Neotropical phytotelmata-endemic species increase the more we sample? (2) Are potential Neotropical phytotelmata-endemic species from throughout the ciliate tree of life? (3) Are there clades of potential Neotropical phytotelmata-endemic species? (4) From what original type of environments do phytotelmata species come?

## Materials and methods

### Taxon sampling and morphological observations

Most ciliates that were newly isolated were from a variety of tank bromeliads in Jamaica, such as *Achmea* spp., *Guzmania* spp. and *Tillandsia* spp., while others occurred in bamboo stumps and tree holes (Table 1). Previously sequenced phytotelmata sequences are from GenBank (Appendix 1, see supplementary material, which is available on the Supplementary tab of the article's Informaworld page at <http://www.informaworld.com/mpp/uploads/tsab...>). These sequences were then added to three alignments containing non-phytotelmata GenBank sequences: (1) 175 representing all 11 major ciliate clades (Appendix 1, see supplementary material, which is available on the Supplementary tab of the article's Informaworld page at <http://www.informaworld.com/mpp/uploads/tsab...>) (Fig. 1); (2) 41 of just the Spirotrichea (Appendix 2, see supplementary material, which is available on the Supplementary tab of the article's Informaworld page at <http://www.informaworld.com/mpp/uploads/tsab...>) (Fig. 2); and (3) 54 of the Oligohymenophorea, plus *Coleps hirtus* as an outgroup (Appendix 3, see supplementary material, which is available on the Supplementary tab of the article's Informaworld page at <http://www.informaworld.com/mpp/uploads/tsab...>) (Fig. 3). Classification follows Lynn (2008).

Depending on collection circumstances and abundance of the species, specimens were either picked directly from the environment or cultivated, and then were used for the molecular investigations. See Foissner *et al.* (2003) for a brief description of culture methods. The new species were identified by combining live observation with various histological techniques, including silver impregnation meth-

ods and scanning electron microscopy (SEM), following Foissner (1991). New species will be morphologically described elsewhere.

### Sequencing and phylogenetic analyses

To extract genomic DNA, about 50 specimens from each isolate were picked with a micropipette and stored in ATL buffer (Qiagen). Genomic DNA was extracted with the DNEasy Tissue Kit (Qiagen, Hildesheim, Germany) following instructions. PCR and sequencing conditions followed Foissner *et al.* (2011). Sequences were aligned using Hmmer v2.3.2 (Eddy, 1998). Alignments were manually modified and ambiguously aligned positions were conservatively masked in MacClade v4.08 (Maddison & Maddison, 2003). For all three alignments, GTR+I+ $\Gamma$  was the best fitted model selected by AIC as implemented in jModelTest v0.1.1 (Posada, 2008). Maximum likelihood (ML) analyses were run in RaxML-HPC v7.2.5 (Stamatakis, 2006; Stamatakis *et al.*, 2008) with 1000 bootstrap replicates. Bayesian inferences (BI) were run in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003), running two sets of four chains for 20 000 000 generations, sampling every 1000 generations. The first 25% of generations were discarded as burn-in. The remaining trees were used to generate a majority-rule consensus tree to calculate the posterior probabilities. Trees were visualized with FigTree v1.3.1 (Rambaut, 2006).

## Results

### Newly isolated phytotelmata ciliates

From bromeliads, bamboo and tree holes, 33 ciliate species were newly isolated (Table 1). This new sampling greatly increases the number of ciliates sequenced from Neotropical phytotelmata. Of these isolates, the two *Gastrostyla* sp. appear to be the same species morphologically, the three *Vorticella gracilis* are the same species, and the two *Vorticellides* sp. n. 1 are the same species. Of the 45 phytotelmata isolates that have now been sequenced for SSU-rDNA, 28 are from species that are potentially endemic to Neotropical phytotelmata, while the others are also known from other aquatic and terrestrial environments (Table 1).

### Phylogeny of phytotelmata isolates

To place the 45 phytotelmata isolates into a phylogenetic context, we first added them to 175 ciliate SSU-rDNA GenBank sequences that include species from each of the 11 major ciliate clades following the classification of Lynn (2008). The alignment of these 220 sequences contains 1493 included characters, of which 225 are parsimony-informative. The resulting Bayesian inference (BI) tree shows that phytotelmata ciliates nest within

**Table 1.** Taxon sampling of ciliate species that have been isolated from Neotropical phytotelmata. Isolates newly sequenced are in bold.

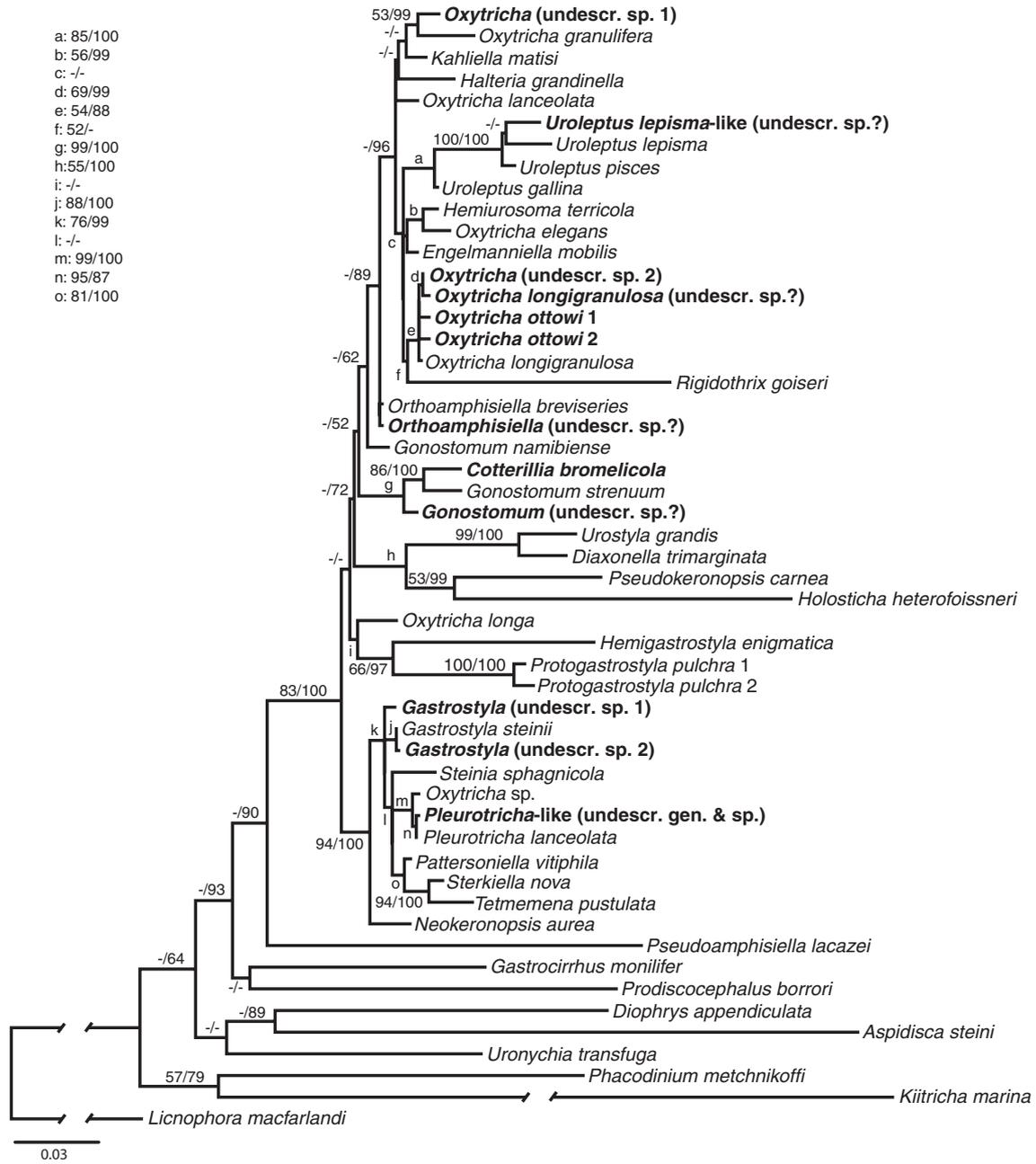
Taxon	Place of collection	GenBank number	Found only in phytotelmata	Found elsewhere
<i>Bromeliophrya brasiliensis</i>	bromeliad, Brazil	AJ810075	x	
<b><i>Bromeliophrya minor</i><sup>1</sup></b>	<b>bromeliad, Jamaica</b>	<b>JQ723961</b>	<b>x</b>	
<i>Bromeliotrix metopoides</i>	bromeliad, Brazil	AY398684	x	
<i>Cotterillia bromelicola</i>	bromeliad, Mexico	HM750260	x	
<b><i>Cyrtolophosis minor</i></b>	<b>bromeliad, Jamaica</b>	<b>JQ723962</b>		<b>x</b>
<b><i>Dexiotricha</i> sp.</b>	<b>bromeliad, Jamaica</b>	<b>JQ723963</b>		<b>x</b>
<b><i>Epistylis</i> sp.</b>	<b>bromeliad, Jamaica</b>	<b>JQ723964</b>	<b>x<sup>3</sup></b>	
<b><i>Fuscheria terricola</i></b>	<b>bromeliad, Rio de Janeiro, Braz</b>	<b>JQ723965</b>		<b>x</b>
<b><i>Gastrostyla</i> (undescr. sp. 1)<sup>2</sup></b>	<b>bromeliad, Jamaica</b>	<b>JQ723966</b>	<b>x</b>	
<b><i>Gastrostyla</i> (undescr. sp. 2)<sup>2</sup></b>	<b>bromeliad, Mexico</b>	<b>JQ723967</b>	<b>x</b>	
Gigantic tetrahymenid (undescr. gen. & sp.)	bromeliad, Dominican Republic	AJ810076	x	
<i>Glaucomides bromelicola</i> <sup>1</sup>	bromeliad, Dominican Republic	AJ810077	x	
<b><i>Glaucomides</i> (undescr. sp. 1)</b>	<b>bromeliad, Jamaica</b>	<b>JQ723968</b>	<b>x</b>	
<b><i>Glaucomides</i> (undescr. sp. 2)</b>	<b>bromeliad, Jamaica</b>	<b>JQ723969</b>	<b>x</b>	
<b><i>Gonostomum</i> (undescr. sp.)</b>	<b>bromeliad, Jamaica</b>	<b>JQ723970</b>	<b>x</b>	
<b><i>Kreyella</i> -like (undescr. gen. &amp; sp.)</b>	<b>bamboo stump, Jamaica</b>	<b>JQ723971</b>	<b>x</b>	
<b><i>Lagynophrya acuminata</i> (?)</b>	<b>bamboo stump, Jamaica</b>	<b>JQ723972</b>		<b>x</b>
<b><i>Lambornella</i> (undescr. sp.)</b>	<b>bromeliad, Jamaica</b>	<b>JQ723973</b>	<b>x</b>	
<i>Lambornella trichoglossa</i>	bromeliad, Brazil	AJ810078	x	
<i>Leptopharynx bromelicola</i>	bromeliad, Jamaica	HQ668466	x	
<i>Maryna</i> sp. n.	bromeliad, Jamaica	JF747218		x
<i>Orborhabdostyla bromelicola</i>	bromeliad, Jamaica	GQ872428	x	
<b><i>Orthoamphisiella</i> (undescr. sp.?)</b>	<b>bromeliad, Jamaica</b>	<b>JQ723974</b>	<b>x</b>	
<b><i>Oxytricha longigranulosa</i> (undescr. sp.?)</b>	<b>bromeliad, Jamaica</b>	<b>JQ723975</b>	<b>x<sup>3</sup></b>	
<b><i>Oxytricha ottowi</i> 1<sup>2</sup></b>	<b>bromeliad, Jamaica</b>	<b>JQ723976</b>		<b>x</b>
<b><i>Oxytricha ottowi</i> 2<sup>2</sup></b>	<b>bromeliad, Rio de Janeiro, Braz</b>	<b>JQ723977</b>		<b>x</b>
<b><i>Oxytricha</i> (undescr. sp. 1)</b>	<b>bromeliad, Mexico</b>	<b>JQ723978</b>	<b>x</b>	
<b><i>Oxytricha</i> (undescr. sp. 2)</b>	<b>bromeliad, Mexico</b>	<b>JQ723979</b>		<b>x</b>
<i>Platyophrya bromelicola</i>	bromeliad, Jamaica	EU039905	x	
<b><i>Pleurotricha</i> -like (undescr. gen. &amp; sp.)</b>	<b>bamboo stump, Jamaica</b>	<b>JQ723980</b>	<b>x</b>	
<b><i>Spirostomum ambiguum</i> 1</b>	<b>bromeliad, Jamaica</b>	<b>JQ723981</b>		<b>x</b>
<b><i>Telotrochidium</i> -like (undescr. gen. &amp; sp.)</b>	<b>tree hole, Jamaica</b>	<b>JQ723982</b>		<b>x</b>
<b><i>Tetrahymenid</i> (undescr. gen. &amp; sp.)</b>	<b>tree hole, Jamaica</b>	<b>JQ723983</b>	<b>x</b>	
<b><i>Tokophrya infusionum</i></b>	<b>bromeliad, Mexico</b>	<b>JQ723984</b>		<b>x</b>
<b><i>Uroleptus lepisma</i> -like (undescr. sp.?)</b>	<b>bromeliad, Rio de Janeiro, Braz</b>	<b>JQ723985</b>	<b>x</b>	
<b><i>Usconophrys</i> (undescr. sp.?)</b>	<b>bromeliad, Jamaica</b>	<b>JQ723986</b>	<b>x</b>	
<b><i>Vorticella convallaria</i></b>	<b>bromeliad, Jamaica</b>	<b>JQ723987</b>		<b>x</b>
<b><i>Vorticella gracilis</i> 1<sup>2</sup></b>	<b>bromeliad, Jamaica</b>	<b>JQ723988</b>		<b>x</b>
<b><i>Vorticella gracilis</i> 2<sup>2</sup></b>	<b>bromeliad, Jamaica</b>	<b>JQ723989</b>		<b>x</b>
<b><i>Vorticella gracilis</i> 3<sup>2</sup></b>	<b>bromeliad, Costa Rica</b>	<b>GQ872429</b>		<b>x</b>
<b><i>Vorticellides aquadulcis</i></b>	<b>bromeliad, Mexico</b>	<b>JQ723990</b>		<b>x</b>
<b><i>Vorticellides astyliformis</i></b>	<b>bromeliad, Costa Rica</b>	<b>GQ872427</b>		<b>x</b>
<b><i>Vorticellides</i> (undescr. sp. 1a)<sup>2</sup></b>	<b>bamboo stump, Jamaica</b>	<b>JQ723991</b>	<b>x<sup>3</sup></b>	
<b><i>Vorticellides</i> (undescr. sp. 1b)<sup>2</sup></b>	<b>bamboo stump, Jamaica</b>	<b>JQ723992</b>	<b>x<sup>3</sup></b>	
<b><i>Vorticellides</i> sp. n. 2</b>	<b>bamboo stump, Jamaica</b>	<b>JQ723993</b>	<b>x</b>	

<sup>1</sup>Description in preparation<sup>2</sup>Same species<sup>3</sup>Possibly found in non-phytotelmata habitats

seven of these 11 clades (Fig. 1): Colpodea, Heterotrichea, Litostomatea, Nassophorea, Oligohymenophorea, Phyllopharyngea and Spirotrichea. Species from the anoxic Arthroporeia and Plagiopylea, the mostly marine intertidal Karyorelictea, and marine and freshwater Prostomatea, have yet to be sequenced (or observed) from Neotropical phytotelmata.

To more thoroughly analyse the phylogenetic placement of some of these phytotelmata isolates, we first looked at those that nest just within the Spirotrichea by adding them to 41 non-phytotelmata spirotrich sequences. The alignment of these 52 sequences contains 1624 included characters, of which 222 are parsimony-informative. Here we present the most likely maximum likelihood (ML) tree with node



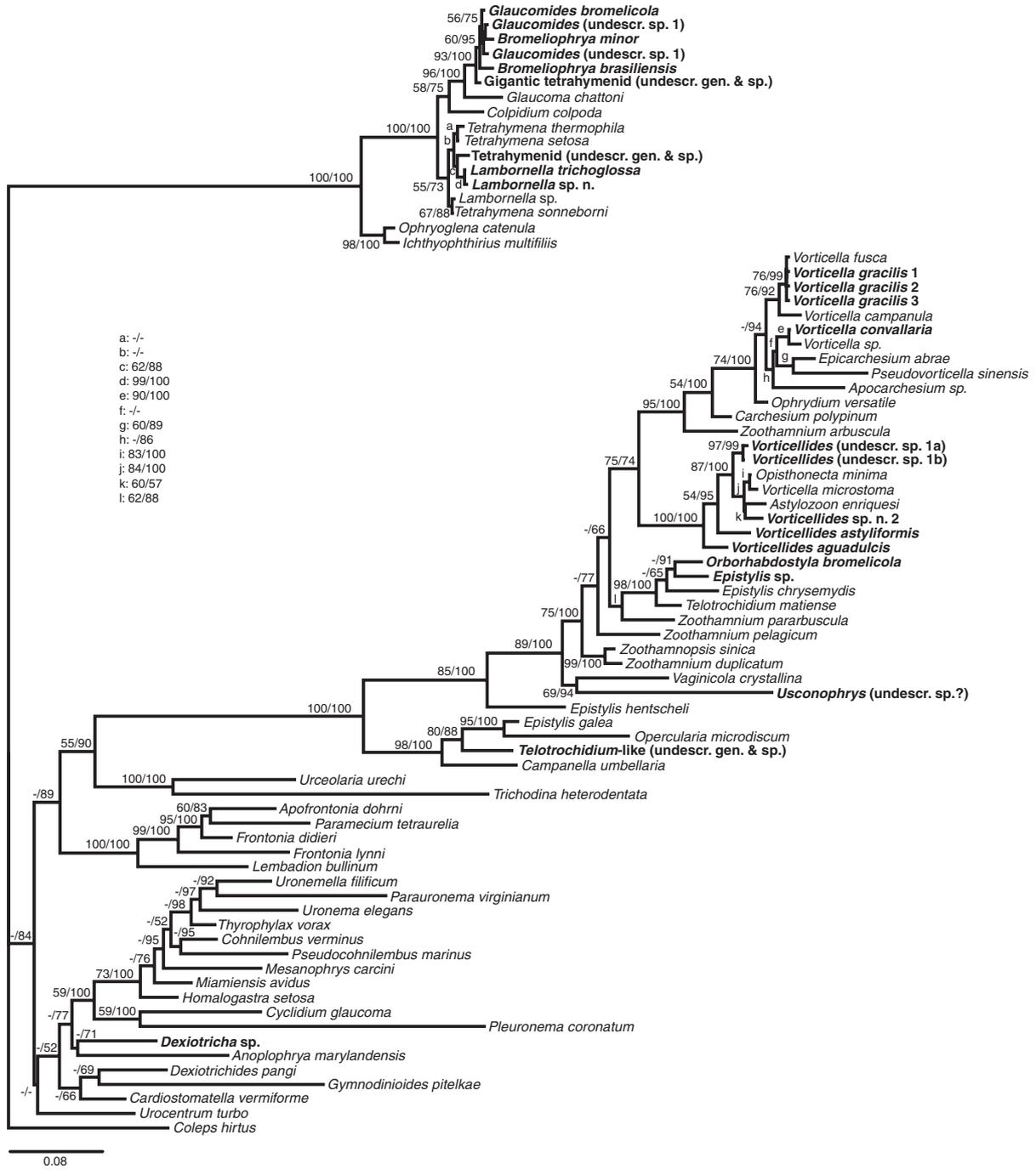


**Fig. 2.** Phylogenetic tree of just the Spirotrichea. Most likely ML tree is shown, with node support as: ML bootstraps/BI posterior probability. Values  $\leq 50$  are shown as '-'. Phytotelmata species are in bold.

support from ML bootstraps and BI posterior probabilities (Fig. 2). The one clade of just phytotelmata ciliate sequences is comprised of *Oxytricha longigranulosa* (undescribed?) and *Oxytricha* (undescr. sp. 2), with

moderate to high node support (69 ML bootstrap/99 BI posterior probability).

We also looked at isolates that nested with the Oligohymenophorea by adding them to 55 non-phytotelmata



**Fig. 3.** Phylogenetic tree of the Oligohymenophorea, rooted with *Coleps*. Most likely ML tree is shown, with node support as: ML bootstraps/BI posterior probability. Values  $\leq 50$  are shown as '-'. Phytotelmata species are in bold.

sequences. The alignment of these 78 sequences contains 1510 included characters, of which 191 are parsimony-informative. As above, we present the most likely ML

tree with node support from ML bootstraps and BI posterior probabilities (Fig. 3). There are three clades containing just phytotelmata sequences: *Orborhabdostyla*

*bromelicola/Epistylis* sp., with low node support (< 50/91); *Lambornella* (undescribed sp.)/*Lambornella tri-choglossa/Tetrahymenid* (undescribed gen. & sp.), with low node support (62/88); and *Bromeliophrya brasiliensis/Bromeliophrya minor/Gigantic tetrahymenid* (undescribed gen. & sp.)/*Glaucomides* (undescribed sp. 1 and sp. 2), with high to full node support (93/100).

### Distribution of potential endemic bromeliad ciliates

We were able to identify some of the bromeliads sampled during the 2008 fieldwork (Table 2). These data, in combination with the identified ciliate species, reveal two aspects. First, only some of the ciliates collected in Neotropical phytotelmata between the years 2004 and 2011 were collected in 2008. Second, those ciliate species found more than once in the Neotropics occurred in more than one bromeliad genus and/or species.

## Discussion

### Phytotelmata diversity: a black box for microbial eukaryotes

After centuries of morphological investigations, and decades of molecular sequencing, we now know much about which ciliate species are located in many environments, such as in ponds and streams (Foissner, 1994), intertidal zones (Carey, 1992), offshore in the oceans (Agatha, 2011; Doherty *et al.*, 2007), anoxic basins (Behnke *et al.*, 2010; Stoeck *et al.*, 2010) and in soils (Foissner, 1995, 1998; Foissner *et al.*, 2002). For example, *Halteria grandinella* (Katz *et al.*, 2005), *Cyrtolophosis mucicola* (Foissner, 1993) and *Urocentrum turbo* (Stoeck *et al.*, 2007) are common in freshwater environments, while *Gonostomum affine* (Foissner *et al.*, 2001) and *Colpoda* spp. (Foissner, 1993) are often found in soils.

On the other hand, we are just beginning to know the ciliates in specific, and the microbial eukaryotes in general, that inhabit Neotropical phytotelmata (Picado, 1911; van Oye, 1923; Laessle, 1961; Maguire, 1963, 1971; Maguire *et al.*, 1968; Lyra, 1971, 1976; Vandermeer *et al.*, 1972; Addicott, 1974; Esteves & da Silva Neto, 1996; Torres-Stolzenberg, 2000; Carrias *et al.*, 2001; Foissner, 2003a, 2003b, 2005; Foissner *et al.*, 2003, 2011; Foissner *et al.*, 2009; Foissner & Wolf, 2009; terHorst, 2010; Foissner & Stoeck, 2011; Omar & Foissner, 2011). If we are to have a complete understanding of the ecology of these plant-container habitats, including the microbial loop of nutrients (e.g. Azam *et al.*, 1983), then we also need to have a detailed knowledge of which microbial eukaryotic species can be found there – not only previously known ones, but also any new species that potentially may be endemic. The work needed for such knowledge is not a job for ecologists; rather, morphological and molecular taxonomists are required, such as done here.

With our morphological and molecular methods we were able to get a deeper view of the diversity of ciliates inhabiting Neotropical phytotelmata. First, with the increased sampling of bromeliads, and the expanded sampling of other types of phytotelmata (Table 1), the data support the hypothesis of Foissner *et al.* (2003) that these habitats harbour potentially many novel ciliates, with unique morphologies, unknown from anywhere else. The number of potential Neotropical phytotelmata-endemic ciliate species does indeed increase the more we sample. As more phytotelmata are sampled throughout the Neotropics, more potential endemics will assuredly be discovered and described. Additional phytotelmata from other tropical areas, as well as temperate environments, need similar morphological and molecular sampling to determine if these novel species are endemic to Neotropical phytotelmata, or if they are found in worldwide phytotelmata.

Second, SSU-rDNA sequences from these Neotropical phytotelmata isolates show that they are from throughout most of the major clades of the ciliate tree of life (Fig. 1). The clades these isolates are found in are for the most part species rich, and often found in non-extreme (from a human's point of view) environments: Colpodea, Heterotrichea, Litostomatea, Nassophorea, Oligohymenophorea, Phyllopharyngea and Spirotrichea. Those clades that are not represented by new species in the phytotelmata are primarily composed of species that are found in extreme environments; e.g. in anoxic habitats.

Third, for the first time the new sampling here points to potential clades of Neotropical phytotelmata-endemic species in the groups Spirotrichea and Oligohymenophorea (Figs 1–3), although for most of these clades node support is low. That is, once a ciliate species entered the phytotelmata habitat, there were one or more speciation events or radiations. The exact conditions that led to these radiations are unknown, but spatial isolation and strong competition are very likely main factors (Foissner *et al.*, 2003). Such strong competition has been shown for *Paramecium* by Vandermeer *et al.* (1972), who found experimentally that this clade is competitively excluded from the bromeliad habitat, confirming earlier observations by Laessle (1961) and Maguire & Belk (1967). Likewise, we never found a *Paramecium* in over 200 bromeliad samples.

There is still much to be learned about the biogeography of ciliates inhabiting phytotelmata. In the Neotropics, bromeliads alone – living on a variety of substrates from soil to upper tree canopy – can hold up to 50 000 litres of water per hectare (Williams, 2006). Thus phytotelmata represent a significant habitat for many different lineages of ciliates to live, and to experience a variety of micro- and macro-evolutionary pressures. Just how unique this habitat is in relation to phytotelmata from other parts of the world (especially given that bromeliads are largely restricted to the Neotropics; Benzig, 2000; Givnish *et al.*, 2004), and

**Table 2.** Distribution of proposed bromeliad-specific ciliates in Jamaica from several sampling campaigns. The bromeliads were identified only during the 2008 campaign.

Ciliate species	Bromeliad species												
	<i>Achmea paniculigera</i>	<i>Catopsis berteroniana</i>	<i>Guzmania monostachia</i>	<i>Hohenbergia abbreviata</i>	<i>Hohenbergia inermis</i>	<i>Hohenbergia penduliflora</i>	<i>Hohenbergia polycephala</i>	<i>Hohenbergia proctorii</i>	<i>Hohenbergia spinulosa</i>	<i>Hohenbergia urbaniana</i>	<i>Hohenbergia</i> sp.	<i>Tillandsia</i> sp.	<i>Vriesea sanguinolenta</i>
<i>Bromeliophrya brasiliensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bromeliophrya minor</i>	-	+	+	-	-	-	-	+	-	-	+	-	+
<i>Bromeliothrix metopoides</i>	-	+	+	-	-	+	-	-	-	-	-	-	-
<i>Coriplites proctori</i>	+	-	-	-	-	-	-	-	-	+	-	-	-
<i>Cultellothrix</i> (undescr.)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Epistylis</i> (undescr.?)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Epistylis</i> cf. <i>plicatilis</i> (undescr.?)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Gastrostyla</i> (undescr. 1)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Gastrostyla</i> (undescr. 2)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Glaucomides</i> (undescr.?)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Glaucomides bromelicola</i>	+	+	+	-	+	+	-	+	+	+	+	+	+
<i>Gonostomum</i> (undescr.)	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>Lambornella</i> (undescr.)	+	+	+	-	-	+	-	+	-	+	+	-	-
<i>Leptopharynx bromelicola</i>	-	-	-	-	-	+	-	-	-	-	-	-	-
<i>Leptopharynx bromeliophilus</i>	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>Maryna</i> (undescr.)	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Orborhabdostyla bromelicola</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Orthoamphisiella</i> (undescr.)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oxytricha</i> (undescr. 1)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oxytricha</i> (undescr. 2)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Oxytricha longigranulosa</i> (?)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Platyophrya bromelicola</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Protospathidium</i> (undescr.)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhabdostyla</i> (undescr.)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spathidium</i> (undescr. 1)	-	-	-	-	-	-	-	-	-	+	-	-	-
<i>Spathidium</i> (undescr. 2)	+	-	-	-	+	-	-	-	-	-	-	-	-
<i>Sterkiella</i> (undescr.)	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tetrahymena</i> (undescr.)	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>Uroleptus</i> cf. <i>lepisma</i> (undescr.?)	+	-	+	-	-	-	-	-	+	+	-	-	-
<i>Usconophrys</i> (undescr.)	-	-	+	-	-	-	-	-	-	-	-	-	+

from other freshwater and terrestrial Neotropical habitats, will require additional sampling using both morphological and molecular approaches. Nevertheless, the Neotropical phytotelmata ciliate fauna is already well recognizable by the lack of typical and common freshwater genera, such as *Coleps*, *Colpidium*, *Frontonia*, *Glaucoma*, *Nassula*, *Paramecium*, *Stylonychia* and *Trithigmotoma*, which one of us (W. Foissner) did not observe in over 200 bromeliad samples.

## Distribution

Distribution studies of Neotropical ciliates are difficult because the presence of species in specific phytotelmata de-

pends on the size of the water tank, water and organic matter contents, and the age of the water (sample taken at beginning or end of wet season). The present and unpublished data from Costa Rica, the Dominican Republic, Venezuela, Peru and Chile indicate that spatial distance is probably more important than the bromeliad species (Table 2).

## Endemism of microbial eukaryotes: a testable hypothesis

There are many issues that need to be dealt with when evaluating different views of eukaryotic microbial biogeography and biodiversity. One of these is establishing hypotheses that can be tested in natural environments. For example,

the 'ubiquity model', where everything is everywhere and the environment selects (Finlay *et al.*, 1996; Finlay, 2002; Fenchel & Finlay, 2004, 2006) is near impossible to accurately falsify. Under this model, a claimed absence of a ciliate species from a normally welcoming environment can be merely dismissed as poor or inappropriate sampling. While a million passerines or ungulates are not easily missed when sampling a community, a million *Colpoda* ciliates will go unnoticed if the sampled soil was a millimetre off. A claimed absence of a species can also be dismissed by stating that the actual microenvironment that was sampled was entirely different from the larger environment of the community.

The 'moderate endemism model', where there are many ubiquitous microbial eukaryotic species but also biogeographically restricted ones (Foissner, 1999, 2006, 2011; Foissner *et al.*, 2008, 2011), on the other hand does allow for testable hypotheses of endemism (Foissner *et al.*, 2008). If the claimed endemic is found elsewhere, then the hypothesis is falsified. Such that, the Neotropical phytotelmata-endemic ciliates proposed here are indeed legitimate hypotheses that are at least explicitly testable and falsifiable. There are now 45 SSU-rDNA sequences that can be potentially detected in environmental sequencing projects that are designed to sample other Neotropical habitats such as soils and the numerous rivers and swamps that can be found in those ecosystems, as well as phytotelmata in different parts of the world. Most of these phytotelmata-endemic ciliate species are also morphologically described in great detail or will be in future papers. Well-trained morphologists can sample Neotropical soils, rivers and swamps looking for these same species.

### Whence Neotropical phytotelmata ciliates came?

Foissner *et al.* (2003) hypothesize that the ciliates found in Neotropical phytotelmata – both non-endemics and the progenitors of endemics – are from communities that are commonly found in ponds and mosses. This view echoes a similar observation by Picado (1911), who describes the bromeliad habitat as like a fragmented swamp. These swamps, then, could have various gradations of wetness among the fragments. Other authors suggest that phytotelmata should be viewed more as islands (Siefert, 1975; Frank & Lounibos, 1987); however, this is just playing with metaphors, as bromeliads and other phytotelmata could be islands of swamps.

Foissner *et al.* (2003) originally did not find Neotropical phytotelmata ciliates that were from communities normally found in ephemeral (astatic) habitats, e.g. puddles formed on roads and in meadows after it has rained, such as colpodean marnyids and members of the family Nassulidae. However, Foissner *et al.* (2011) sequenced a *Maryna*

species isolated from a Jamaican bromeliad. The original type of environments that phytotelmata species therefore came from should be expanded from the earlier hypothesis of Foissner *et al.* (2003) to include not just the more stable aquatic and moss environments, but also to those that are often subject to variable periods of near to complete dryness.

### Conclusions

Although bromeliads and other phytotelmata represent a significant and extensive habitat for Neotropical microbial eukaryotes, we are far from understanding the ecological process that structure ciliate communities, and the spatial and temporal scales of these ecological processes. What can be said of these communities is that they are composed of both ubiquitous and endemic species, some of these may be the result of speciation within the phytotelmata habitat, and the number of supposed endemics increases the more these habitats are sampled.

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**Appendix 1.** GenBank accessions of non-phytotelmata ciliates from each of the 11 major clades used in Fig. 1.

**Appendix 2.** GenBank accessions of non-phytotelmata ciliates from the Spirotrichea used in Fig. 2.

**Appendix 3.** GenBank accessions of non-phytotelmata ciliates from the Oligohymenophorea, plus *Coleps hirtus* as an outgroup, used in Fig. 3.