Endemic ciliates (Protozoa, Ciliophora) from tank bromeliads (Bromeliaceae): a combined morphological, molecular, and ecological study

Wilhelm Foissner¹*, Michaela Strüder-Kypke², Georg W. M. van der Staay³, Seung-Yeo Moon-van der Staay³, and Johannes H. P. Hackstein³

¹Universität Salzburg, Institut für Zoologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria; ²University of Guelph, Department of Zoology, Ontario N1G 2W1, Canada; ³University of Nijmegen, Department of Evolutionary Microbiology, NL–6525 Ed Nijmegen, The Netherlands

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Bromeliads are mainly epiphytic rosette plants occurring only in Central and South America. They collect rain water and particulate materials in tanks (cisterns) formed by the coalescing leaf axils. These tanks form an extensive, highly compartmentalized water and humus body above the ground and are inhabited by many ordinary and endemic organisms. We discovered at least 10 new ciliate species in the tanks of about 15 bromelian species from the Dominican Republic, Ecuador, and Brazil. Since there are 2000–3000 bromelian species with very different lifestyles, they are likely to contain hundreds of novel ciliate species. Two of the new species described here are real “flagships”, that is, they have such a large size that they would have been found in Europe, if they were there. Consequently, these species must have a restricted geographical distribution, disproving the old hypothesis that microscopic organisms are cosmopolitan. Also the smaller ciliates described here represent new genera and families, arguing for a long-lasting, independent evolution driven by ecological constraints and spatial isolation. Almost half of the new species can switch from bacteriophagous, microstome morphs to a predatory, macrostome lifestyle, likely due to the strong competition in these peculiar habitats. The high morphological and ecological diversity of tank bromeliad ciliates is only partially recovered by small-subunit (18S) ribosomal RNA (rRNA) gene sequences.

Key words: Biodiversity; Biogeography; Brazil; Conventional vs. molecular classification; Dominican Republic; Ecuador; Endemic ciliates.

Introduction

Lakes, ponds, and rivers are rare in many tropical and subtropical rain forests because most rain water is immediately collected by the tree roots. Likewise, soils contain little humus because the organic matter is quickly mineralized due to the high air humidity and temperature. In spite of this, a substantial reservoir of water and raw humus is available in undisturbed rain forests of Central and South America, viz., in the tanks (cisterns) of epiphytic and ground bromeliads, which form an extensive, highly compartmentalized ecosystem (Martinelli 2000; Schönborn 2003). Bromeliads are rosette plants of the subclass Liliidae (lilies), and the most famous species is the...
pineapple, Ananas sativus (Sitte et al. 1991). The tanks are formed by the coalescing leaf axils, which collect the rain water, and the rosettes of the larger species may entrap up to 30 litres. There are 2000–3000 bromelia species, many of which exhibit peculiar lifestyles in a wide variety of environments; some or even most release specific substances into the tank water, whose chemical and physical conditions show great local and temporal differences, providing innumerable niches for protists and small metazoans (Picado 1913; Maguire 1971; Martinelli 2000; Schönborn 2003).

Since Picado (1913), it is well-known that bromelian tanks are inhabited by a more or less specific flora and fauna; about 400 metazoan species have been recorded, but many more can be expected because the tanks are generally poorly explored (Schönborn 2003). Protists, although obviously being common and abundant, were never studied in detail (Picado 1913; Laessle 1961; Maguire 1971; Livezekey and Vareschi 1992; Esteves and da Silva Neto 1996; Little and H ebter 1996; Martinelli 2000; Esteves et al. 2001). Only testate amoebae were investigated to some extent (Van Oye 1923), and recently a new species, representing a new family, was described from Brazilian tank bromeliads (Torres-Stolzenberg 2000). Here, we shall show that also many undescribed ciliate species inhabit this peculiar ecosystem, and that their study significantly contributes to understand protist speciation and diversity.

Material and methods

Ciliates were collected from the water and mud accumulated in the tanks of epiphytic and ground bromelians from the Dominican Republic, Ecuador, and Brazil. Most samples were taken by W. Foissner (Brazil, Dominican Republic), some were kindly supplied by Dr. T. Cordeiro (Cidade University, João Pessoa, Brazil) and Prof. W. Til (Vienna University; samples from Ecuador and a single sample from the Dominican Republic). Since the tank water is acidic (pH 4–6), delaying decomposition and putrefaction of water and organic materials, samples can be transported for several days, perhaps even for one or two weeks, without special precautions. However, larger predators, such as insect larvae and crustaceans, should be sieved off because they consume most protists, especially in small samples stored for a longer time. Of the other hand, many of these small metazoans are colonized by epizoic peritrichs. Accordingly, they should be sampled and transported separately.

All species shown, except for those living on other animals, could be cultivated in natural, centrifuged tank water and/or Eau de Volvic (French table water) enriched with some cracked wheat grains to stimulate growth of food organisms, viz., bacteria, flagellates, and small ciliates. Predatory species were either fed with flagellates or small ciliates isolated from the tanks, or with ordinary laboratory cultures of Colpidium kleini and Paramedium aurelia. For details, see Foissner (2003a, b).

Most morphological and ecological data presented here, are based on cultivated specimens investigated in vivo and with the methods described in Foissner (1991). Soil (humus) samples were investigated with the non-flooded Petri dish method, as described in Foissner et al. (2002). Sequencing of the 18S rRNA genes was performed with standard procedures.

Results

Most of the new species found are documented here by some figures. Only key characteristics are described due to space constraints. For distribution, see Table 1.

Morphology, ontogenesis, and gene sequences

1. Lambornella trichoglossa Foissner, 2003 (Figs 1, 2): This tetrahymenid ciliate, measuring about 200 × 50 µm, described by Foissner (2003b), possibly represents a new genus because it reorganizes the parental oral apparatus during ontogenesis and does not infect mosquito larvae, in contrast to the congeners. Unfortunately, 18S sequence data are not available. Although L. trichoglossa does not form macrostomes, it can feed on large rotifers. Resting cysts are produced.

2. Bromeliophrya brasiliensis Foissner, 2003 (Figs 6, 14, 19): This species, described by Foissner (2003a), is type of the new genus Bromeliophrya and of the new tetrahymenid family Bromeliophryidae, which is characterized by a partially reduced somatic ciliature and migrating kinetofragments during ontogenesis. The family consists of the genus Bromeliophrya and the two new, not yet described genera mentioned above. Morphology, ontogenesis, and the 18S sequence data show that the Bromeliophryidae are closely related to the Glaucomidae (sequence identity with G. scintillans 98.5%, 98.4% and 98.2%, respectively). The Metopus-shaped type species has a size of about 55 × 35 µm. It possesses two large, unciliated areas and does not form macrostomes. Resting cyst pro-
Figs 1–13. Ciliates from bromelian tanks in the SEM (1, 3–5, 7, 8, 10, 12, 13), in vivo (9), and after silver impregnation (2, 6, 11). 1, 2: Lambornella trichoglossa. Arrowheads mark excretory pores. 3–5: New genus 1, ventral and dorsal (4, asterisk marks unciliated patch) view of microstomes (3, 4) and ventrolateral view of a macrostome (5). 6: Bromeliophrya brasiliensis has a large, unciliated area (asterisk) left of the oral apparatus. 7, 8: New, tetrahymenid genus 3. This gigantic, up to 800 µm long ciliate has a functionless, minute primary oral apparatus (arrows) and a large secondary mouth (OA) used to capture large prey, e.g. Paramecium. 9–13: New genus 4 is a minute colpodid ciliate, which divides in freely motile condition forming a chain (9) with pairs connected by a special structure (10, 13, arrows). LP – left oral polykinetid, MA – macronucleus, OA – oral apparatus, RP – right oral polykinetid. Bars 2 µm (Fig. 13), 10 µm (Figs 8, 10–12), 20 µm (Figs 3–6), 50 µm (Figs 1, 2, 7, 9).
3. New genus 1 (Figs 3–5, 14, 17): This ciliate, about 50 µm long, belongs to the Bromeliophryidae described above. It is rather similar to Glaucoma scintillans, but has an unciliated patch dorsally and can transform to macrostomes. Resting cysts could not be produced.

4. New genus 2 (Figs 14, 18): This ciliate, about 60 µm long, also belongs to the Bromeliophryidae and is rather similar to B. brasiliensis, but the unciliated areas are less distinct and the right side preoral ciliary rows are less conspicuously elongated. Resting cysts could not be produced.

5. New genus 3 (Figs 7, 8, 14): This gigantic ciliate, up to 800 µm long, is also a tetrahymenine hymenostome, according to both the morphological and the 18S sequence data. It is outstanding by its large size, in being able to feed on Paramecium, and in having two entirely different mouths, both completely reorganized during ontogenesis. The original (primary), tetrahymenine oral apparatus is located subapically at the left margin of the cell and is likely functionless because it is minute and lacks a paroral membrane; it possesses, however, 4–8 (X6) adoral membranelles, while all other tetrahymenids have only three. The functional (secondary) mouth is a large funnel produced by the invaginated anterior body end and lacks any specific oral structures. This species also shows some sort of microstome-macrostome transformation and produces large resting cysts. Morphologically, the ciliate is so different from all the known tetrahymenids that it could be a representative of at least a new family. However, its 18S rRNA sequence is almost identical (99.3%) to that of Tetrahymena corlissi (see Discussion).

6. Peritrichs: Several, likely undescribed peritrichs were found, most attached to small metazoans, such as oligochaetes and mosquito larvae. Some are rather conspicuous because they form large colonies and have zooids up to 150 µm long. Although difficult to quantify, contractility appears more pronounced than in many related species from ordinary limnetic habitats. All feed on bacteria. No macrostomes were observed. Most likely they produce resting cysts.

7. New genus 4 (Figs 9–13, 15): This ciliate, only 20–50 µm long, briefly described by Foissner and Cordeiro (2000), represents not only a new genus, but likely also a new family. Morphologically, it belongs to the order Colpodida, while gene sequences place it somewhere between the Colpodida.
Ecological data are still very incomplete because only a few bromelian species and sites were investigated. Nonetheless, some interesting observations were made, which will be briefly described.

(1) Two main ciliate habitats can be distinguished in the tanks, viz., the water and mud, and the body surface of small metazoans, such as da and Grossglockneriida. This species is outstanding by dividing in freely motile condition (most other colpodids of this type reproduce in division cysts), whereby chains of four individuals are produced with the individual pairs connected by a special structure highly reminiscent of an ordinary patent fastener. Further, it forms bacteriophagous microstomes and flagellate-feeding macrostomes, and produces two types of resting cysts.

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Figs 16–19.

The three genera (Figs 17–19) of the new family Bromeliophryidae Foissner, 2003a evolved from a Glaucoma scintillans-like ancestor (Fig. 16), according to both morphologic data and ribosomal gene sequences (Fig. 14). The new genus 1 (Figs 3–5, 17), which looks like G. scintillans ventrally (Figs 3, 16, 17), has an unciliated patch dorsally (Fig. 4) and forms macrostomes (Fig. 5). The ciliary rows of the new genus 2 (Fig. 18), which also has an unciliated patch dorsally, are elongated preorally (arrow) and interrupted left laterally (asterisk). Bromeliophrya brasiliensis (Figs 6, 19), which has a large, unciliated patch each dorsally and left laterally (asterisk), possesses strongly elongated preoral ciliary rows (arrow) and two short kinetofragments (arrowhead) which migrate from left to right during ontogenesis, an outstanding feature previously not known in tetrahymenid ciliates (Foissner 2003a). Evolution within the family Bromeliophryidae, composed of the genus Bromeliophrya and the new genera 1 and 2, is still obscure.

Figs 14, 15. 18S rRNA sequence trees from some new bromelian tank ciliates (neighbour-joining analysis). 14: The new genera 1 and 2 and Bromeliophrya brasiliensis form a new family related to the Glaucomidae, represented by Glaucoma scintillans. The new, gigantic (up to 800 µm) tetrahymenid ciliate, though having a unique morphology, clusters close to Tetrahymena corlissi. The numbers at nodes represent bootstrap percentages from 100 replicates. The scale bar corresponds to 5 substitutions per 100 nucleotide sequences. 15: Tamura-Nei evolutionary distances show that the new colpodid ciliate belongs to the order Colpodida.

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mosquito larvae and oligochaetes, where epizoic peritrichs are frequent. (2) Distribution of the new species appears rather scattered, that is, only *Lambornella trichoglossa* was found at all sites investigated (Table 1). (3) The tank bromeliad ciliate community consists of several endemic, undescribed taxa and ordinary, cosmopolitan freshwater (e.g., *Halteria*, *Cinetochilum*) and moss (e.g., *Colpoda*, *Drepanomonas*, *Leptopharynx*) species. True soil and euplanktonic species are rare (Table 1). (4) None of the new species was found in any of about 150 soil samples from Central and South America. They also could not be recovered in two dried and re-wetted tank mud samples from the Dominican Republic, although most of them produce resting cysts. On the other hand, the new colpodid (genus 4) grows well when added to various ordinary soil samples. (5) Garden bromeliads from two sites of the Dominican Republic had lost the original ciliate community and were inhabited by pond and waste-water ciliates, indicating that sometimes cleared waste-water was used for irrigation. (6) Most tank ciliates are bacteriophagous or feed, after transformation to macrostomes, on flagellates (new genera 1, 2, 4). Trials to adapt the new colpodid to feed on small ciliates (*Colpoda steinii*, *Tetrahymena*) failed. Large species, such as *Lambornella trichoglossa* and the new genus 3 feed on a variety of protists and small metazoans; genus 3 is even cannibalistic.

**Discussion**

**Contrasting morphological and molecular classifications**

We shall discuss here mainly the gigantic tetrahymenid ciliate (= new genus 3) because it is the most informative example. Morphological and molecular data both support the assignment to the tetrahymenine hymenostomes (Fig. 14). However, a comparison of the 18S sequence with those available in the public data bank shows that this species is very closely related to *Tetrahymena corlissi*, a small, possibly ubiquitous ciliate. This contrasts sharply with the morphological and ecological data, which suggest at least a distinct family for this peculiar ciliate, especially if the differences are compared with those found in other tetrahymenid families, for instance, the Tetrahymenidae and Glaucomidae (Lynn 1994). Potentially, other molecular markers than the 18S might represent the morphological divergence more adequately. However, even identical 18S sequences of two species a priori do not allow detailed predictions about the morphological similarity, as exemplified by the minute 18S divergence of man and chimpanzee. With respect to the 18S sequences analysed here, all tetrahymenids could be united in a single genus, notwithstanding their high morphological and ecological diversity. A similar, but less striking situation is obvious with the new colpodid, which appears as a typical member of the order Colpodi da, according to its morphology (Figs 9–12) and evolutionary distances (Fig. 15), while 18S sequence trees (not shown) classify it as a member of the Grossglockneriida.

**Endemic ciliates in bromelian tanks**

When one of us (W. Foissner) collected some water and mud from a ground bromelia in Brazil, this was done only for fun because the mainstream ideology fostered the expectation that such small biota should be inhabited by common, cosmopolitan ciliate species. However, the sample contained two new taxa (Foissner 2003a, b), and further samples provided many other new species, disproving the widespread, old hypothesis that, in the microbial world, “everything is everywhere, the environment selects”. Some of these new species, viz., the large, *Paramecium*-feeding tetrahymenid presented here and the 200 µm long *Lambornella trichoglossa* Foissner (2003b) are too conspicuous to assume that they were overlooked in Europe (Figs 1, 2, 7). Thus, the little ponds in the cisterns of bromelian plants add significant evidence for the occurrence of free-living protists with restricted, geographic distribution (for reviews, see Foissner 1999 and Foissner et al. 2002).

There are 2000–3000 bromelia species, many of which have special lifestyles and grow in extreme environments, such as the tepuis in Venezuela and the Andean semi-deserts (Martinelli 2000). We investigated less than 20 bromelia species from only eight sites, but found at least 10 new species (Table 1). Since samples were taken just once and from a few plants only, it is likely that merely a tiny fraction of the species actually present was encountered. Common sense suggests that the highly compartmentalized and diverse bromelian tank ecosystem will host hundreds, perhaps thousands of novel ciliate species, as do soils globally (Foissner et al. 2002).
Ecology and selective forces

Although the bromelian tank ciliate community has an original character due to those species not found elsewhere, it likely evolved from cosmopolitan pond and moss ciliate biota, as indicated by the frequent occurrence of bacteriophagous tetrahymenids, epizoic peritrichs, and euryoecious species of the genus Colpoda. Typical soil inhabitants are rare. This matches the observation that we could not find any of the new bromelian ciliates in about 150 soil samples from Central and South America. Interestingly, the bromelian tank ciliate community is also highly different from that found in astatic road and meadow puddles (Gleis 1954; Dingfelder 1962), mainly due to the rarity or lack of nassulids (likely because filamentous cyanobacteria are rare), colpodid marynids, rapacious haptorids, and euplanktonic stichotrich hypotrichs.

The new ciliates occurring in bromelian tanks are basically similar to those found in ordinary limnetic habitats, but frequently represent new genera and families; these could suggest a long, independent evolution, which matches the age of the bromelian plant family documented to date from at least 65 million years ago (Sitte et al. 1991). Morphology suggests at least two selective forces. The ability to switch between bacteriophagous microstomes and predaceous macrostomes (Figs 3, 5) and the overall changes in the oral structures, even those found in the large tetrahymenid (Figs 7, 8), become understandable when the habitat is considered. Desiccates during dry periods (thus, many of the species can form resting cysts), whereby intra- and interspecific competition must become extremely high due to the decreasing space and resources. More difficult to understand are the rather pronounced changes in body shape and somatic ciliary pattern because they have no obvious ecological advantage and the somatic ciliary pattern is usually much more stable than the oral one. Probably, these changes are caused by long spatial isolation and/or the substances secreted by the bromelian leaves secreted into the tank water.

The Bromeliophryidae, an impressive example of ciliate evolution

When looking at the three known species of the family Bromeliophryidae and the sequence data, it becomes obvious that they evolved from a holotrichously ciliated, Glaucoma-like ancestor (Fig. 14), possibly from an ancestor shared with Glaucoma scintillans, a very frequent, euryoecious cosmopolitan (Foissner et al. 1994). Commencing with G. scintillans, the following features gradually increase/change, reaching a maximum in Bromeliophrya brasiliensis (Figs 16–19): the length and curvature of the right side preoral ciliary rows, the reduction of the somatic ciliature, the body asymmetry, and the length of adoral membranelle 3. Unfortunately, the sequence data (Fig. 14) do not follow this "straight" line, and a cladistic analysis of the morphological features (not shown) also gives doubtful results. Further, the proposed stem-species, G. scintillans, is rather speculative because the silverline pattern and the ability to transform the oral apparatus match G. ferox better, a species whose geographic origin is, unfortunately, unknown (Puytorac et al. 1973). There is also conspicuous parallelism: the preoral ciliary patterns of the Bromeliophryidae are highly similar to those found in the genera Dexistoma, Paracolpidium, and Colpidium of the tetrahymenid family Turaniellidae (for reviews, see Ganner and Foissner 1989; Lynn 1994).

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