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ORIGINAL ARTICLE

Functional Ecology of the Ciliate *Glaucomides bromelicola*, and Comparison with the Sympatric Species *Bromeliothrix metopoides*

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Competition; feeding threshold; growth rate; life strategy; macrostome formation; neotropics; pH response; *Polytomella* sp.

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ABSTRACT

We investigated the ecology and life strategy of *Glaucomides bromelicola* (family Bromeliophryidae), a very common ciliate in the reservoirs (tanks) of bromeliads, assessing its response to food quality and quantity and pH. Further, we conducted competition experiments with the frequently coexisting species *Bromeliothrix metopoides* (family Colpodidae). In contrast to *B. metopoides* and many other colpodean ciliates, *G. bromelicola* does not form resting cysts, which jeopardizes this ciliate when its small aquatic habitats dry out. Both species form bactivorous microstomes and flagellate-feeding macrostomes. However, only *G. bromelicola* has a low feeding threshold and is able to adapt to different protist food. The higher affinity to the local bacterial and flagellate food renders it the superior competitor relative to *B. metopoides*. Continuous encystment and excystment of the latter may enable stable coexistence of both species in their natural habitat. Both are tolerant to a wide range of pH (4–9). These ciliates appear to be limited to tank bromeliads because they either lack resting cysts and vectors for long distance dispersal (*G. bromelicola*) and/or have highly specific food requirements (primarily *B. metopoides*).

GLAUCOMIDES BROMELICOLA Foissner, 2013 has been recently described in the context of a comprehensive project on the diversity and ecology of ciliates from tank bromeliads (Foissner 2013). Bromeliads are species-rich epiphytic and terrestrial plants that are wide-spread in Central and South America. Many are able to store water in a reservoir formed by the tightly overlapping bases of their rosette leaves. Such phytotelmata (Varga 1928), also known as tanks or cisterns, may contain up to 30 litres of water; they represent a highly specialized aquatic habitat (Armbruster et al. 2002; Foissner et al. 2003; Kitching 2000). In contrast to larger aquatic habitats, tank bromeliads are disconnected from each other. Thus, phytotelmata represent natural model systems for studying major functional characteristics of food webs and for testing community theories (Armbruster et al. 2002; Kitching 2001).

As yet, ca. 40 new ciliate taxa have been discovered in tank bromeliads and several have already been described (Dunthorn et al. 2012; Foissner 2003a,b, 2010, 2013; Foissner and Stoeck 2013; Foissner and Wolf 2009; Foissner et al. 2003, 2011; Omar and Foissner 2011, 2012). *Glaucomides bromelicola* belongs to the Bromeliophryidae and is distantly related to *Glaucoma* (Foissner 2013; Foissner and Stoeck 2013). While the genus *Glaucoma* radiated in more common freshwater habitats, *G. bromelicola* is widespread in tank bromeliads, commonly occurring in several bromeliad species from southern Chile to Mexico (Foissner 2013). The original description focused on morphological and molecular characteristics while the ecology received little attention. Details of the ecology of ciliates from tank bromeliads are hitherto known only from one species, *Bromeliothrix metopoides* (Weisse et al. 2013b). Accordingly, the goal of this study was to investigate the functional ecology of *G. bromelicola* in comparison to that of *B. metopoides*. In contrast to *B. metopoides* and many other colpodean ciliates, *G. bromelicola* does not form cysts (Foissner 2013), which renders this species vulnerable to extinction following desiccation in the tanks. Water volume (typically ca. 0.01–3 liters), light, pH (usually ranging from 4.0 to 7.0), nutrients, and allochtonous organic input from leaf litter are the most important abiotic factors characterizing phytotelmata of tank bromeliads (Brouard et al. 2012; Janetzky 1997; Laessle 1961; Lopez et al. 2009; Marino et al. 2011).

We hypothesized that *G. bromelicola* should have evolved adaptations different from those of *B. metopoides* to compensate for the lack of cyst formation and promote its survival in astatic aquatic environments. We focused on food (quantity and quality), competition, and pH as major environmental variables that are amenable to experimental manipulation in the laboratory. As *G. bromelicola* often co-occurs with *B. metopoides*, the ciliate species that is second in common in tank bromeliads, the traits that enable coexistence are of special interest. To this end, we performed competition experiments in the laboratory. We did not consider temperature in this study because typical temperature variation in the tanks of bromeliads is in the range of (only) 10 °C (summarized by Weisse et al. 2013b), i.e. presumably of minor importance relative to the other physico-chemical variables.

MATERIALS AND METHODS

Origin and maintenance of stock cultures

Glaucomides bromelicola Foissner, 2013 represents a new tetrahymenid genus and species that is frequent in tank bromeliads of Central and South America (Foissner 2013). Similar to *B. metopoides* Foissner, 2010, *G. bromelicola* forms macrostomes when bacterial food is depleted. The common in vivo cell size of cultivated specimen is ca. 57 × 30 μm (microstomes), respectively, 80 × 50 μm (macrostomes).

The organisms used in this study were collected from tanks of small tree bromeliads either in a subtropical forest of Brazil (*B. metopoides*, *Polytomella* sp., heterotrophic bacteria) or in the botanical garden of Puerto Plata, Dominican Republic (*G. bromelicola*). Details of the origin of our study organisms have been reported by Foissner (2010, 2013). Cultures were established in Eau de Volvic (French table water) enriched with some sterilized, crushed wheat grains to promote growth of indigenous bacteria and bacterivorous flagellates. Monospecific protist cultures were obtained by repeated dilution with Volvic and pipetting of individual target cells, respectively, by removing unwanted predators or competitors. Ciliate stock cultures were maintained in "filter caps" culture flasks (Biomedica) with 50 ml of Eau de Volvic enriched with one to two wheat grains. The stock cultures were kept in an incubator at 22.5 °C under a 14:10 h light–dark cycle; pH was ca. 7.5. New cultures were inoculated once per week by transferring 25 ml of the aged culture to a new flask containing 25 ml Eau de Volvic and one new wheat grain.

We kept *G. bromelicola* in nonaxenic stock cultures with and without the as-yet-undescribed flagellate *Polytomella* sp.; this flagellate, which was isolated together with *B. metopoides*, is variable in cell size (10–40 μm), has four flagella at the basis of a distinct papilla, four contractile vacuoles, lacks an eyespot, and has the nucleus in the anterior body half (Foissner 2010). The genus *Polytomella* comprises several colorless nutritionally versatile species that thrive on acetate and other organic acids, peptone, and yeast extract (de la Cruz and Gittleson 1981; Pringsheim 1955). We added two wheat grains to 50 ml of Eau de Volvic to provide an organic-rich medium for our *Polytomella* sp. cultures. Ciliate cell numbers in stock cultures usually ranged from 0.5 to 2.0 × 10^4 cells/ml.

Growth experiments with different food organisms

We measured specific growth rates (μ) of *G. bromelicola* in response to different food quality (heterotrophic bacteria and the flagellates *Polytomella* sp. and *Cryptomonas* sp.) and quantity. *Polytomella* sp. coexists with the ciliate in tank bromeliads, *Cryptomonas* sp. is a cryptophyte common in many freshwater bodies and the preferred food of many planktonic ciliates (Skogstad et al. 1987; Weisse and Müller 1998). If not specified, cell numbers of *G. bromelicola* include microstomes and macrostomes in the following. In asexually reproducing ciliates, μ is a direct proxy of their fitness (Weisse 2006). The ciliate was inoculated together with the respective food organism(s) into 50-ml culture flasks. Treatments with food organisms but without ciliates served as controls. Target food levels lower than satiating (< 2 mg C/L; experiments reported in Fig. 1–3) were obtained by diluting strongly growing

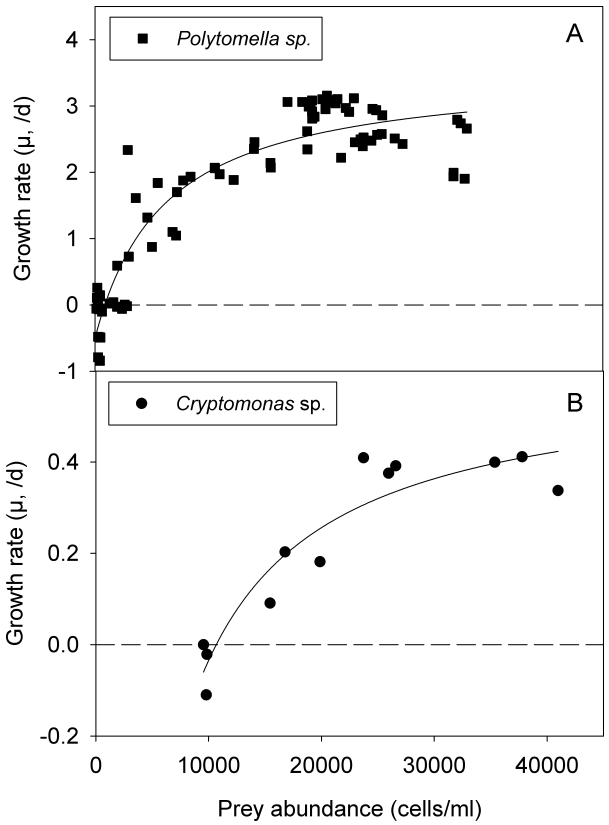

Figure 1 Numerical response of the ciliate *Glaucomides bromelicola*. (**A**) With the auxotrophic flagellate *Polytomella* sp. as food. (**B**) With the phototrophic flagellate *Cryptomonas* sp. as food. The solid lines represent the fit to Eq. 4 (see text).

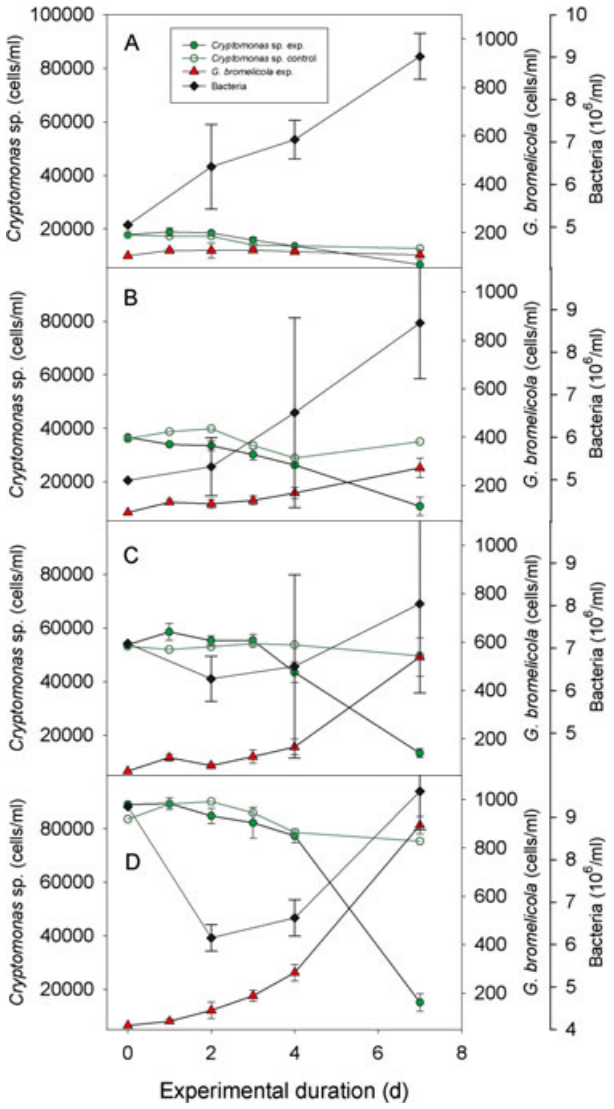

Figure 2 Population dynamics of the ciliate *Glaucomides bromelicola* fed bacteria and the flagellate *Cryptomonas* sp. at four different food levels (**A**–**D**). Symbols represent means of triplicates; error bars denote 1 SD.

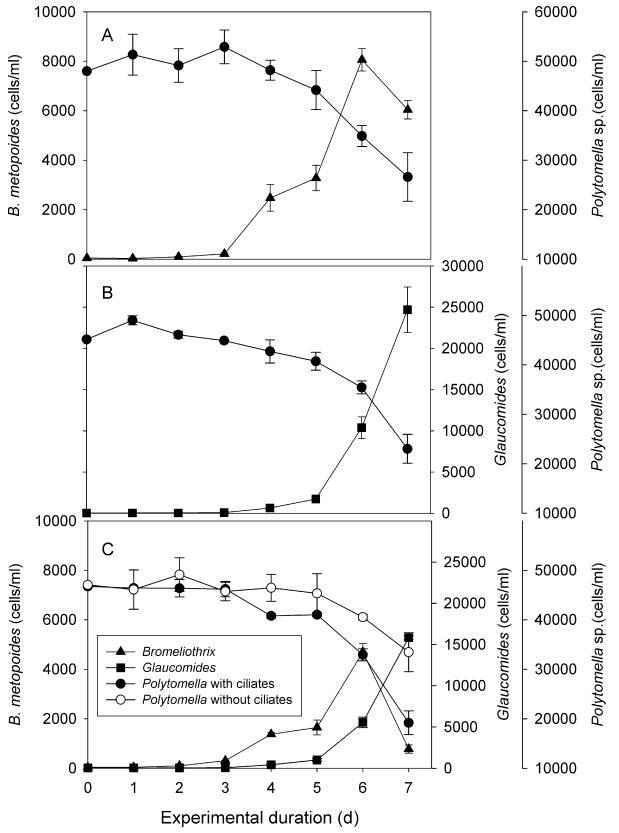

Figure 3 Population dynamics of the ciliates *Bromeliothrix metopoides* and *Glaucomides bromelicola* fed bacteria and the flagellate *Polytomella* sp. (A**)** *B. metopoides* in single growth experiments. (**B)** *G. bromelicola* in single growth experiments. (**C)** Both ciliates in pairwise growth (= competition) experiments. Symbols represent means of triplicates; error bars denote 1 SD.

cultures with Eau de Volvic. Flagellate prey concentration ranged from a few hundred cells/ml to > 80,000 cells/ml (equivalent to < 0.05–> 2 mg C/L), depending on the experimental conditions. Bacterial levels ranged from 10^6 to 10^8 cells/ml. Except for the experiments with *Cryptomonas* sp., the bacterial flora used in this study was enriched from the original habitat. The bacteria present in the *Cryptomonas* cultures remained unidentified but were, most likely, qualitatively different from those used in the other experiments. The initial experimental ciliate abundance was 20–110 cells/ml. All growth experiments were performed in the dark to prevent photoautotrophic food (the flagellate *Cryptomonas* sp.) from growing. Similarly, we removed remnants from wheat grains from the experimental containers to limit bacterial growth. The experiments lasted from several days to several weeks.

Samples (5 ml) were taken from the experimental containers at 24 h intervals and fixed with acid Lugol's iodine (final concentration 2% v/v). Ciliate cell numbers were determined microscopically either in counting chambers of 3 ml volume or in Sedgewick Rafter chambers of 1 ml volume. Flagellate cell numbers were also counted microscopically, either together with the ciliates or separately in a 1-ml Sedgewick chamber. At higher abundance (> 10^4 cells/ml), flagellates were also counted and sized electronically by means of an automatic particle counter (CASY 1-model TTC; Schärfe System, Reutlingen, Germany; Weisse and Kirchhoff 1997). Bacterial levels were measured in Formalin-fixed (2% v/v) samples (2 ml) taken together with the ciliate samples. Bacterial cell numbers were assessed by flow cytometry after staining with the green fluorescent nucleic acid stain SYTO-13 (Molecular Probes, Invitrogen™, Carlsbad, CA, USA), using a FACSCalibur flow cytometer (Becton Dickinson BD Biosciences, San Jose, CA) equipped with an argon ion laser emitting light at 488 nm.

Cell size of ciliates and flagellates was measured with Lugol's fixed material using an inverted microscope and a

semi-automatic image analysis system (LUCIA version 4.51, Laboratory Imaging, Prague, Czech Republic). Flagellates were also measured in unfixed material. The automatic particle counter yielded an independent estimate of flagellate cell size, which was used mainly to determine the cell volume of living cells. Both methods yielded similar results.

The cell volume of the prey flagellates used in this study (*Polytomella* sp. and *Cryptomonas* sp.) was converted to carbon units assuming the allometric equation provided by Menden-Deuer and Lessard (2000), i.e. pg C/cell = 0.216 × cell volume$^{0.939}$. To calculate carbon biomass of bacteria, we assumed a conservative estimate of 26 fg C/cell for our cultivated bacteria (Troussellier et al. 1997).

Cell volume (V) of the ciliates was determined from length (l) and width (w) measurements, assuming a prolate spheroid shape:

$$V(\mu m^3) = \pi/6 \times 1 \times w \times b \quad (1)$$

where b is cell breadth (in μm, as l and w). Measurements were made on 50 ciliates each at the end of several experiments. As we could not measure the third dimension, we assumed that b is equal to 0.6 × w (Foissner 2013).

Competition experiments

The experiments described above assumed that *G. bromelicola* is the only ciliate feeding on bacterial and flagellate food. However, in the tank bromeliads, this ciliate is often encountered together with *B. metopoides*, a small colpodean ciliate (Dunthorn et al. 2012; Foissner 2010). Accordingly, we compared the growth response of both ciliates when kept alone to that of pairwise experiments, i.e. with the potential competitor present. In each of four different competition experiments, we offered a combination of bacteria and *Polytomella* sp. as food; initial food levels were saturating for both ciliate species (> 2 mg C/L, Weisse et al. 2013b; this study) but declined in the course of the experiments (Fig. 3, 5).

To investigate the effect of competition on growth and survival of *B. metopoides,* we added *G. bromelicola* as competitor to the experimental containers (50-ml culture flasks) with *B. metopoides* and vice versa. To ensure that ciliate growth rates with and without competitor were directly comparable, we inoculated, in the first competition experiment, from a vigorously growing *B. metopoides* culture, one treatment without competitor (Fig. 3A) and another one with its competitor, *G. bromelicola* (Fig. 3C); similarly, we inoculated simultaneously from a *G. bromelicola* culture two different treatments without (Fig. 3B) and with its competitor, *B. metopoides* (Fig. 3C). Each treatment was then split into three replicates. The flagellate *Polytomella* sp. (initial abundance ca. 50,000 cells/ml, equivalent to ca. 2.7 mg C/L) served as food for the macrostomes; bacteria (initial abundance ca. 10^8 cells/ml, equivalent to ca. 2.6 mg C/L) were present and served as food for the microstomes, but were unimportant as food for the macrostomes. This first competition experiment, therefore, consisted of three different predator–prey treatments: (A) *B. metopoides* plus *Polytomella* sp. and bacteria; (B) *G. bromelicola* plus *Polytomella* sp. and bacteria; (C) *B. metopoides* and *G. bromelicola* plus *Polytomella* sp. and bacteria. The duration of this experiment (1 wk) turned out to be too short to reveal whether the ciliates may outcompete each other. Further, we did not monitor bacterial levels. To this end, we repeated this competition experiment with several modifications and extended the duration up to 4 wk; we also measured bacterial concentration together with flagellate and ciliate abundance. In the second competition experiment (Fig. 5A, B), we provided *Polytomella* sp. and bacteria at similar initial satiating amounts as in the previous experiment (ca. 50,000 *Polytomella*/ml, ca. 10^8 bacteria/ml) but did not add additional food in the course of the experiment. Due to ciliate grazing, food limitation was likely after several days. To reduce food limitation, we added flagellate food 5 d after the beginning of the third competition experiment (Fig. 5C, D). Finally, in a fourth competition experiment (Fig. 5E, F), we added food upon each sampling occasion, beginning on experimental day 5. Initial ciliate abundance ranged from 30 to 40 cells/ml in all competition experiments. The general experimental conditions, the analyses of protist and bacterial cell numbers, and the calculation of protist growth rates were identical to the growth experiments reported above.

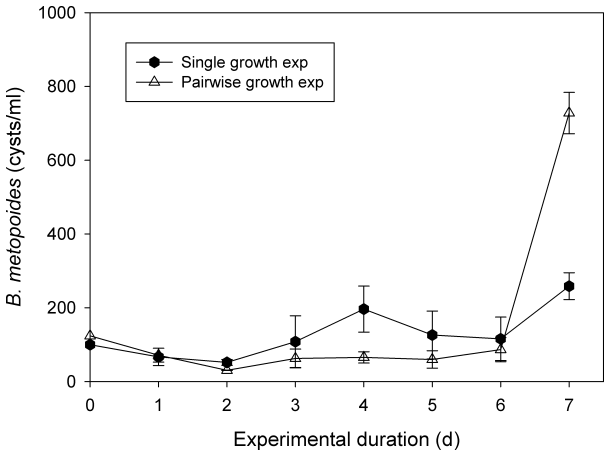

Figure 4 Cyst formation of the ciliate *Bromeliothrix metopoides* in the experiments shown in Fig. 3A and C.

Response to pH

We investigated the growth and survival of *G. bromelicola* over pH ranging from 4.0 to 9.0; pH was measured using a microprocessor pH-mV meter (model pH 526; WTW, Weilheim, Germany) to the nearest 0.01 unit. The pH sensor was 2-point calibrated with standard buffer solutions of pH = 6.87 and pH = 9.18 before each series of measurement. Ciliates and their prey (provided at satiating amounts) were acclimated to the experimental conditions in steps of

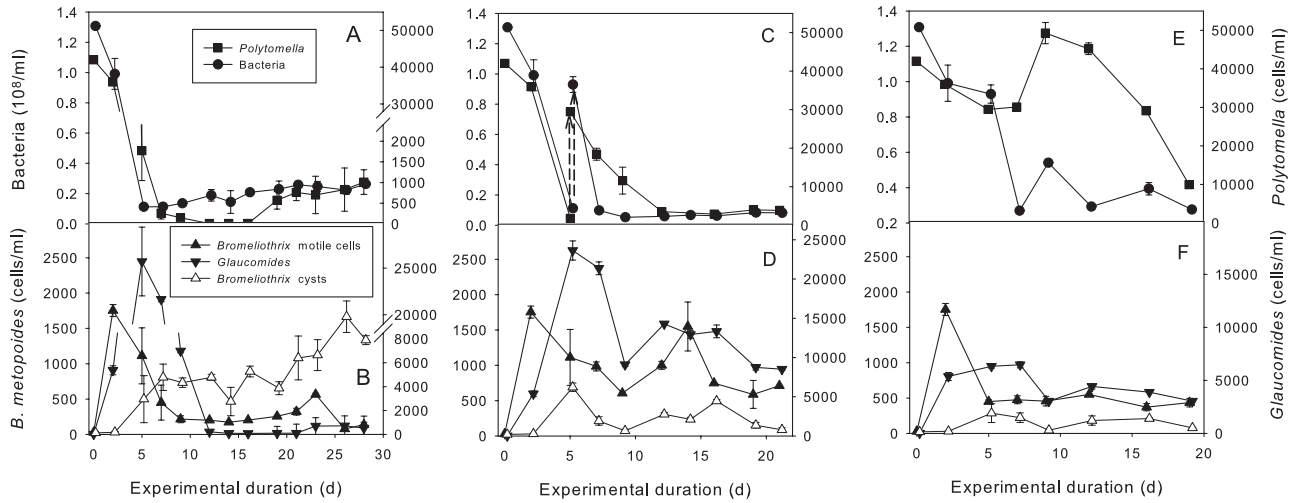

Figure 5 Competition experiment with the ciliates *Bromeliothrix metopoides* and *Glaucomides bromelicola* (bottom panels) fed bacteria and the flagellate *Polytomella* sp. (top panels). (**A**, **B**) No additional food was added in the course of the experiment. (**C**, **D**) Additional food was added on day 5 of the experiment (indicated by dashed lines and arrows in Fig. 5C). (**E**, **F**) Additional food was added on each sampling occasion, beginning on day 5 of the experiment. Bacterial abundance is related to the left *y*-axis in Fig. 5A, C, E; flagellate abundance is related to the right *y*-axis in Fig. 5A, C, E. Symbols represent means of duplicates, error bars denote 1 SD.

0.5 pH unit change per day for 2–5 d. We measured and adjusted the pH in each experimental container twice per day; when pH differed by more than 0.2 from the target pH, it was adjusted by addition of small amounts (15–35 µl) of 0.1 mol/l NaOH or HCl (Weisse and Stadler 2006; Weisse et al. 2007, 2013c). The general experimental design followed that of the growth experiments.

We used an incubator with a constant temperature of 22.5 °C without illumination for all experiments. Except for the competition experiments 2–4 (results reported in Fig. 5), which were performed in duplicate, all experiments were run in triplicate. Results reported are mean values ± 1 standard deviation (SD).

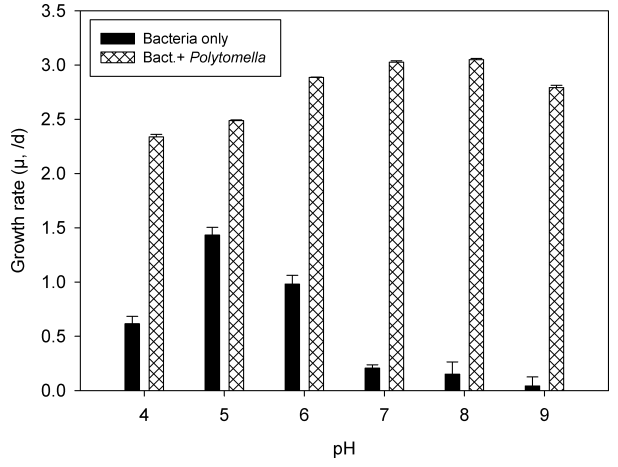

Figure 6 pH response of the ciliate *Glaucomides bromelicola* fed bacteria only, respectively bacteria plus the flagellate *Polytomella* sp. Bars represent means of triplicates, error bars denote 1 SD.

Data analysis and statistics

Ciliate growth rate (µ) was calculated from changes in cell numbers, assuming exponential growth over the experimental period according to

$$\mu = \ln(N_t/N_0)/t \quad (2)$$

where N_0 and N_t are ciliate numbers at the beginning and at the end of the experimental period, respectively. Please note that 'experimental period' does not denote the total duration of an experiment, but specific periods of 1–4 d each during which the ciliate population increased exponentially. Details are reported in the Results section.

Ciliate growth rates were related to the geometric mean prey concentration (*P*) during the experimental period (Frost 1972; Heinbokel 1978) according to Eq. 3:

$$P = \frac{P_t - P_0}{\ln(P_t/P_0)} \quad (3)$$

where P_0 and P_t are the initial and final prey concentrations (cells/ml) during incubations.

Ciliate growth rates were fit to Eq. 4, which includes a positive *x*-axis intercept, using the Marquardt-Levenberg algorithm (SigmaPlot; SPSS Inc., Chicago, IL).

$$\mu = \mu_{max}(P - X')/(k + P - X') \quad (4)$$

where µ is the growth rate (/d), μ_{max} is the maximum growth rate (/d), *P* is the geometric mean prey concentration (Eq. 3), *k* is a constant (cells/ml), and *x*′ is the *x*-axis intercept (i.e. threshold concentration, cells/ml, where µ = 0). This equation is similar to the Michaelis–Menten model and Holling's type II functional response (Holling 1959), but assumes a positive *x*-axis intercept where

population growth equals mortality (Weisse et al. 2002). Accordingly, the constant k of Eq. 4 is similar, but not identical to the half saturation constant known from Michaelis–Menten kinetics. The curve resulting from Eq. 4 that describes the change in predator density as a function of change in prey density is known as numerical response (Solomon 1949).

One-way ANOVA and Student's t-test were used to test for significant differences between several pairwise treatments. Results were considered statistically significant if $p < 0.05$.

RESULTS

Response to food supply

With *Polytomella* sp. and bacteria as food, *G. bromelicola* showed a typical numerical response curve (Fig. 1A). The nonlinear curve fit (Eq. 4) yielded a maximum growth rate (μ_{max}) of 3.55/d, a constant k = 7,035 *Polytomella* cells/ml, and an x-axis intercept (x' in Eq. 4, where $\mu = 0$) of 868 *Polytomella* cells/ml. All parameter estimates and the curve fit were statistically significant ($p < 0.001$, $R^2 = 0.882$). When converted to carbon units, food levels at near-to-maximum growth rates ($\mu = 3.0$) were 1.92 mg C/L, k was reached at 0.38 mg C/L, and x' amounted to 0.05 mg C/L. The percentage of flagellate-feeding macrostomes ranged from 1.0% to 8.5% of the total *G. bromelicola* cell number. The calculations above ignore the presence of bacteria that were, most likely, primarily used as food by the microstomes. Bacterial levels ranged from 1.1 to 10.5 $\times$ 10^6 cells/ml in the numerical response experiment, equivalent to 0.03–0.28 mg C/L. As bacterial abundance was positively correlated with *Polytomella* abundance, the threshold food concentration of *G. bromelicola* was likely close to 0.1 mg C/L, and μ_{max} was recorded when total food concentration (i.e. *Polytomella* sp. plus bacteria) reached ca. 2.2 mg C/L.

We did not assess the numerical response of *G. bromelicola* to bacteria as sole food, because we had observed in our routine cultures that ciliate growth rates are lower in the absence of the flagellate (Weisse, unpubl. data, but see below, Response to pH).

When we replaced *Polytomella* sp. by *Cryptomonas* sp., *G. bromelicola* needed approximately 3 d to adapt to the new food (Fig. 2). Irrespective of the food level, ciliate growth rates were low to moderate during the first 2–3 d of the experiment and then increased. Differences between *Cryptomonas* abundance in the experimental containers with ciliates and controls without ciliates were small and mostly statistically not significant during this period, suggesting that *G. bromelicola* did not at all or only sparsely feed upon the flagellates. However, at day 7, *Cryptomonas* levels were significantly lower (pairwise t-tests, $p < 0.001$ in each case) in each experimental container than in the respective controls, and ciliate numbers had increased, relative to the first 4 d of the experiment (Fig. 2A–D). Only at the lowest flagellate abundance (ca. 15,000 cells/ml), the ciliates did not grow during the period of observation, although bacteria were present in moderate abundance at the beginning of the experiment and continuously increased up to 9 $\times$ 10^6 cells/ml to the end of the observation period (Fig. 2A). The decline in bacterial levels recorded in Fig. 2C, D demonstrates that *G. bromelicola* microstomes fed on bacteria, because bacterial levels did not decline in the controls without ciliates. Similarly, the initial bacterial growth in treatments A and B was lower than in the respective controls (data not shown). The difference between *Cryptomonas* sp. abundance in the experimental containers and controls indicates that the flagellate was ingested by *G. bromelicola* at the lowest flagellate abundance tested. However, the cryptophyte biomass of ca. 0. 4 mg C/L did not support positive ciliate growth. Including bacteria, the total concentration of potential food was ca. 0. 6 mg C/L at the end of this experiment.

When we plotted the ciliate growth rate measured between experimental days 4–7 vs. the mean *Cryptomonas* abundance during this period, we obtained a numerical response curve with a statistically significant ($R^2 = 0.896$) curve fit (Fig. 1B). However, comparing Fig. 1B to A demonstrates that μ_{max} of *G. bromelicola* was significantly lower (0.60/d) with *Cryptomonas* sp. as food and the cellular threshold prey concentration was approximately tenfold higher for *Cryptomonas* sp. than for *Polytomella* sp. In terms of biomass, the difference was lower, ca. 0. 30 mg C/L (*Cryptomonas* sp.), respectively, 0.05 mg C/L (*Polytomella* sp.). Including bacteria, we estimated a total threshold concentration of ca. 0.5 mg C/L for *G. bromelicola* fed *Cryptomonas* sp. and bacteria. Tables 1, 2 summarize the results obtained in both numerical response experiments.

Competition for food

When kept alone with their prey, both ciliates started growing exponentially after an initial lag phase (Fig. 3A, B). In contrast to *B. metopoides* (Fig. 3A), exponential growth of *G. bromelicola* continued to the final day of observation (Fig. 3B); μ calculated from linear regression of ln cell numbers vs. time was 1.19 $\pm$ 0.12/d (days 1–6, $n = 6$, $R^2 = 0.960$) for *B. metopoides*, respectively, 1.37 $\pm$ 0.07/d (days 2–7, $n = 6$, $R^2 = 0.989$) for *G. bromelicola*. Highest growth rates from day to day were reached for both ciliates during days 3–4, with μ_{max} = 2.45 $\pm$ 0.16/d (*B. metopoides*) and μ_{max} = 2.01 $\pm$ 0.20/d (*G. bromelicola*). In the latter case, average ciliate cell number increased from 84.3 to 632.0 cells/ml during days 3–4; due to the large scale of the y-axis, this is difficult to deduce from Fig. 3B. In the pairwise experiment (Fig. 3C), *B. metopoides* reached the same continued growth rate, 1.20 $\pm$ 0.09/d, as in the single growth experiment, but for a shorter period (days 1–4, $n = 4$, $R^2 = 0.989$); during days 4–6, μ of *B. metopoides* was $\leq$ 1.0/d. Similarly, highest day to day growth rate was reached during days 3–4; however, μ_{max} = 1.51 $\pm$ 0.02/d in the competition experiment was significantly reduced ($p < 0.001$) compared to the single growth experiment. Maximum cell number in the former

Table 1. Parameter estimates of the numerical response curves (see text, Eq. 4) of *Glaucomides bromelicola* fed the flagellates *Polytomella* sp., respectively, *Cryptomonas* sp.

Variable	Coefficient	SE	Coefficient	SE
	With *Polytomella* sp. as food		With *Cryptomonas* sp. as food	
μ_{max}	3.55	0.23***	0.60	0.14**
x'	868	170***	10,678	670***
k	7,035	1,470***	12,364	6,782*
R^2	0.88	0.44***	0.90	0.07***

*$p < 0.102$.
**$p < 0.01$.
***$p < 0.0001$.
μ_{max}, maximum growth rate (per d), x', threshold food concentration (cells/ml), k, a constant (cells/ml), R, coefficient of determination, SE, standard error.

was 4,700 cells/ml, compared to 8,060 cells/ml in the single growth experiment. Final abundance of *B. metopoides* was 6,041 ± 374 cells/ml (single growth), respectively, 777 ± 166 cells/ml (pairwise growth). These differences in the duration of exponential growth and final cell numbers were affected by a switch to mass encystment observed in the competition experiment during days 6–7 (Fig. 4). On day 7, cyst abundance in the competition experiment was significantly higher ($p < 0.001$) than in the single growth experiment. The opposite was true for days 2 and 4; we recorded no difference in cyst abundance between the two treatments during days 0, 1, 5, and 6.

Continued exponential growth rate of *G. bromelicola* in the pairwise experiment (Fig. 3C) was identical to that measured in the single growth experiment (1.36 ± 0.08/d; days 3–7, $n = 5$, $R^2 = 0.988$); similarly, μ_{max} = 1.88 ± 0.10/d reached during days 3–4 and was not different ($p = 0.369$) from the single growth experiment. Considering that *G. bromelicola* grew exponentially, differences in its final abundance reported in Fig. 3B, C were minor.

Both ciliate species formed macrostomes in the competition experiments, with typical percentages ranging from 2% to 7% of the total cell number.

In summary, the first competition experiment demonstrated some effect of *G. bromelicola* on *B. metopoides*, but virtually no effect of the latter on the former. The experimental duration was too short to reveal if *B. metopoides* may be outcompeted by *G. bromelicola*. In the following three competition experiments, we extended the duration up to 4 wk; we also measured bacterial concentration together with flagellate and ciliate abundance. Results shown in Fig. 5 demonstrate that both ciliates may coexist over several weeks. In the second competition experiment, without feeding (Fig. 5A, B), both ciliates coexisted with small, but relatively stable populations (*B. metopoides*: 242 ± 150 cells/ml, *G. bromelicola* 313 ± 285 cells/ml) after 2 wk, when the abundance of the food flagellate *Polytomella* sp. remained < 1,000 cells/ml, and bacterial levels were close to 0.2×10^8 cells/ml (Fig. 5A). Starting 1 wk after the beginning of the experiment, the abundance of encysted *B. metopoides* exceeded that of motile cells and continuously increased until the end of the experiment (Fig. 5B). Maximum abundance of *G. bromelicola* (25,715 ± 3,692 cells/ml) was more than 10-fold higher than that of *B. metopoides* (1,753 ± 85 cells/ml). Both ciliates were, most likely, food limited in this experiment after the initial week.

In the third competition experiment, we added flagellate food 5 d after the beginning of the experiment (Fig. 5C). The ciliates coexisted in this experiment for the following 2 wk with higher cell numbers than in the previous experiment (Fig. 5D). The average abundance of *G. bromelicola* (12,608 ± 4,553 cells/ml) was ca. 14× higher than that of *B. metopoides* (884 ± 337 cells/ml) during the second and

Table 2. Key variables characterizing the life strategies of the ciliates *Bromeliothrix metopoides* and *Glaucomides bromelicola* from tank bromeliads

Variable	*B. metopoides*	*G. bromelicola*	Source
Taxonomic affiliation (class, order)	Colpodea, Colpodida	Oligohymenophorea, Tetrahymenida	Foissner (2010, 2013)
Cell length	20–55 μm	25–80 μm	Foissner (2010, 2013), Weisse et al. (2013b)
Average cell volume in cultures	8,570 μm^3	8,250 μm^3	Weisse et al. (2013b), This study
Macrostomes	Yes	Yes	Foissner (2010, 2013)
Cysts	Yes	No	Foissner (2010, 2013)
Division chains	Yes	No	Foissner (2010, 2013)
Food organisms	Bacteria; *Polytomella* sp.	Bacteria; *Polytomella* sp.; other flagellates	Weisse et al. (2013b), This study
Sensitivity to competition	Moderate	Low	This study
Growth rate threshold	1.4[a]–2.3[b]mg C/L	0.05[c]–0.1[a]mg C/L ~0.5[d]mg C/L	Weisse et al. (2013b), This study
μ_{max} (/d)	3.55	4.71	Weisse et al. (2013b), This study
pH tolerance	< 4–> 9	< 4–> 9	Weisse et al. (2013b), This study
pH optimum	~8	7–8	Weisse et al. (2013b), This study

[a]With bacteria and *Polytomella* sp. as food, i.e. microstome and macrostome feeding.
[b]With bacteria as sole food, i.e. only microstome feeding.
[c]With *Polytomella* sp. as food, ignoring the bacterial background.
[d]With bacteria and *Cryptomonas* sp. as food, i.e. microstome and macrostome feeding.

third week of the experiment. Different from the second competition experiment without additional feeding, motile cells of *B. metopoides* prevailed over encysted cells throughout this experiment.

In the fourth competition experiment, with food added upon each sampling occasion from experimental day 5 onwards, food levels of bacteria and *Polytomella* sp. were higher than in the previous experiments (Fig. 5E). Ciliate growth rates cannot be calculated directly from the results shown in Fig. 5F because the experimental containers were diluted 1:3 upon each addition of food. However, the nearly constant ciliate cell numbers recorded for both species during experimental days 5–19 suggest that the ciliate populations increased with a relatively constant rate close to 0.55/d. Similar to the previous experiment, (i) the average abundance of *G. bromelicola* (4,534 ± 1,539 cells/ml) was ca. 10-fold higher than that of *B. metopoides* (456 ± 58 cells/ml), and (ii) motile cells of *B. metopoides* prevailed over encysted cells.

Individual and average ciliate cell size was variable in the different growth and competition experiments, mainly depending on food and the percentage of macrostomes in the population (data not shown). Assuming typical average dimensions of $l = 42$ μm and $w = 25$ μm and accounting for 10% shrinkage due to fixation, we calculated an average cell volume of *G. bromelicola* of 8,250 μm^3 under food replete conditions. The average cell size of *G. bromelicola* measured in the present investigation was thus smaller than the in vivo cell size reported by Foissner (2013) for well-fed laboratory specimens.

Response to pH

We investigated the pH response of *G. bromelicola* over pH ranging from 4 to 9 with combined bacterial and flagellate (*Polytomella* sp.) food, respectively bacteria without any other food organism (Fig. 6). To avoid food limitation, prey organisms were provided in satiating amounts (> 2 mg C/L). With the combined food, *G. bromelicola* was relatively insensitive to pH, reaching high growth rates (> 2.4/d) over the entire pH range tested; growth rates peaked at pH 7–8, where μ was not different (*t*-test, $p = 0.073$). Highest growth rates, $\mu_{max} = 3.05$/d, obtained in the pH experiment with *Polytomella* sp. plus bacterial food at pH 7–8 were close to μ_{max} measured in the numerical response experiment with the same food organisms (cf. Fig. 1A). If bacteria were the sole food, the ciliates (i) reached lower maximum growth rates, (ii) the pH optimum was shifted to acidic conditions, and (iii) the highest pH barely supported positive growth rates (Fig. 6).

DISCUSSION

Different life strategies of *Glaucomides bromelicola* and *Bromeliothrix metopoides* enable their stable coexistence in tank bromeliads

Results of this study and a related recent study (Foissner 2010, 2013; Weisse et al. 2013b) demonstrate contrasting life strategies of the two common ciliates from tank bromeliads (summarized in Table 2). As *G. bromelicola* cannot form cysts, this species is threatened by desiccation that may be common in many small bromeliads (Kitching 2001; Marino et al. 2011). Accordingly, a *G. bromelicola* population can escape extinction only via dispersal into new suitable habitats. The preferred flagellate prey, *Polytomella* sp., is common in tank bromeliads but it was not found in every reservoir (Foissner, unpubl. data). The ability to adapt to different prey such as *Cryptomonas* sp. may enable survival of the ciliate in less suitable habitats. The adaptation period of ca. 3 d that we observed (Fig. 2) appears to be short enough to prevent extinction of the population. Although μ_{max} was significantly reduced with *Cryptomonas* sp. as food ($\mu_{max} = 0.60$/d), it is still in a range typical for many aquatic ciliates known to feed on *Cryptomonas* sp. (Skogstad et al. 1987; Weisse 2006; Weisse and Müller 1998). Secondly, the food threshold of *G. bromelicola*, i.e. the prey level that is needed to sustain the population, is relatively low, compared to most planktonic ciliates (Weisse 2006), and orders of magnitudes lower than that of *B. metopoides* (Weisse et al. 2013b). This nutritional versatility may compensate for the lack of cyst formation in *G. bromelicola*.

Bromeliothrix metopoides is unable to use *Cryptomonas* sp. and other flagellates and small ciliates as food (Weisse et al. 2013b). With its preferred food, *Polytomella* sp., the threshold concentration is close to 1.4 mg C/L (Table 2), i.e. higher than that of any other aquatic ciliate investigated thus far under comparable experimental conditions (Weisse et al. 2013b). The microstomes of *B. metopoides*, which feed on bacteria, require an even higher food threshold (2.3 mg C/L) to proliferate. In contrast, microstomes of *G. bromelicola* can ingest bacteria at concentrations < 10^7/ml, equivalent to ca. 0.2 mg C/L (Fig. 2C, D). The lower affinity to its preferred bacterial and flagellate food is likely the cause why *B. metopoides* is the inferior competitor when paired with *G. bromelicola*. However, encystment and excystment enable *B. metopoides* to coexist with *G. bromelicola* over weeks (Fig. 5). During short periods of time, the former may even reach higher growth rates than the latter (Table 2 and Fig. 3). We repeatedly observed greater than five cell divisions per day of *B. metopoides* in our laboratory cultures (Foissner 2010; Weisse et al. 2013b), similar to the shortest generation times known from small terrestrial colpodeans (reviewed by Weisse et al. 2013b). If the ciliates reach such high growth rates in situ remains at present unknown.

Why are both ciliates restricted to tank bromeliads?

A central issue of this research was to explain why the most common ciliates from tank bromeliads appear to be restricted to this peculiar habitat. Of the four environmental variables studied (pH, food quantity and quality, competition), pH was seemingly of minor importance, as both ciliate species were widely tolerant to changing pH (Weisse et al. 2013b; this study). However, recent

experimental evidence with freshwater ciliates, flagellates, and microeukaryotes revealed that the realized pH niche may be confined to a small range, relative to the fundamental pH assessed in the laboratory under optimized conditions. This is because there is a significant interaction of pH with food and temperature (Moser and Weisse 2011; Weisse 2006; Weisse et al. 2002, 2013a, b). Interaction of pH with food quality was obvious for both ciliate species of this study; the pH niche was narrower and, in the case of *G. bromelicola*, restricted to the pH range typical of bromeliad reservoirs if bacteria were the only food (Fig. 6; Weisse et al. 2013b). It appears that (metabolism of) the flagellate *Polytomella* sp. conditioned the medium in a favorable way for *G. bromelicola*, promoting high growth rates even under alkaline conditions.

The unusually high food threshold and its demand for specific food items (*Polytomella* sp. and some bacteria) are the main reasons why *B. metopoides* cannot thrive in other environments (Weisse et al. 2013b), in spite of its ability to form cysts and endure desiccation of its natural habitat. Summarizing the sparse literature on the abundance of bacteria and flagellates in tank bromeliads, Weisse et al. (2013b) concluded that, while it is likely that the high food levels needed by *B. metopoides* are occasionally met in situ, it remains at present unknown if such favorable conditions are persistent in tank bromeliads.

The results of this study further suggest that motile cells of this species are inferior competitors, relative to other ciliates with higher affinity to prey common in bromeliads and elsewhere. Weisse et al. (2013b) concluded that *B. metopoides* has reached a narrow peak along its fitness landscape and any deviation from the optimum conditions will reduce its fitness. This conclusion does not apply to *G. bromelicola*; based upon its feeding and growth characteristics reported in this study, this species should be able to live in a wider array of aquatic habitats than *B. metopoides*. Indeed, *G. bromelicola* was found in samples from 11 different bromeliad species, while *B. metopoides* occurred in only three bromeliad species (Dunthorn et al. 2012).

The lack of cyst formation severely limits dispersal of *G. bromelicola*. Long range dispersal of ciliates and other microbes is only possible in the form of resting stages resistant to desiccation (Foissner 2006, 2007, 2011; Weisse 2008). We hypothesize that dispersal of *G. bromelicola* over narrow ranges is primarily mediated via insects specifically attracted to bromeliads, explaining why this species is endemic in tank bromeliads. A corollary of this conclusion is that it supports the earlier notion that the high degree of endemicity observed in the bromeliad tanks points to speciation within this habitat (Dunthorn et al. 2012; Foissner 2013; Foissner et al. 2003). Both species used in the present investigation possess a micronucleus (Foissner 2010, 2013). However, conjugation was observed only in *G. bromelicola* (Foissner, unpubl. data), while most colpodids very likely lack sex at all (Foissner 1993). Thus far, no attempts have been made at mating in the laboratory. The genetic population structure of tank bromeliads is an area that awaits future research.

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