Published in final edited form as:

Eur J Protistol. 2011 January ; 47(1): 29–50. doi:10.1016/j.ejop.2010.08.003.

***Cotterillia bromelicola* nov. gen., nov. spec., a gonostomatid ciliate (Ciliophora, Hypotricha) from tank bromeliads (Bromeliaceae) with *de novo* originating dorsal kineties**

Wilhelm Foissner^{a,*} and Thorsten Stoeck^b

^aUniversität Salzburg, FB Organismische Biologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

^bUniversität Kaiserslautern, FB Biologie, Erwin-Schrödinger Strasse, D-67663 Kaiserslautern, Germany

Abstract

Cotterillia bromelicola nov. gen., nov. spec. was discovered in the tanks of the Mexican bromeliad *Tillandsia heterophylla*. Its morphology, ontogenesis, and 18S rDNA were studied with standard methods. *Cotterillia* has many cirral rows on both sides of the body. Uniquely, and thus used to diagnose the new genus *Cotterillia*, it has dorsal kineties originating *de novo*, producing neokinetal waves where the parental dorsal kineties reorganize to “combined rows”, consisting of dorsal bristles anteriorly and of cirri posteriorly. Thus, up to four generations of bristles and cirri occur on the dorsal body surface. *Cotterillia bromelicola* has a gonostomatid body and adoral zone of membranelles, while the dense ciliature and the neokinetal waves resemble kahliellid hypotrichs. However, the *de novo* origin of anlage 1 and the molecular analyses show convincingly that *Cotterillia* belongs to the *Gonostomatidae* Small and Lynn, 1985, for which an improved diagnosis is provided. Thus, neokinetal waves originated several times independently. The molecular differences between *Trachelostyla*, *Gonostomum*, and *Cotterillia* are small (5%) compared to their distinct morphologies and ontogeneses, suggesting that the 18S rDNA underestimates generic diversity. Our study emphasizes the need of combined morphological, ontogenetic, and molecular investigations to unravel the complex phylogeny and evolution of hypotrich ciliates.

Keywords

Biodiversity; Neokinetal wave; Ontogenesis; Stichotrichida; Terminology; Trachelostylidae

Introduction

The past 20 years have seen a bloom in taxonomic and phylogenetic research of hypotrich ciliates, culminating in the invaluable monographs of Berger (1999, 2006, 2008). Hundreds of new species and many new genera have been carefully described or re-described, mostly from soil (e.g. Foissner et al. 2002) and the sea (e.g. Song et al. 2009). These studies showed a high diversity not only of the morphostatic ciliature but also of the ontogenetic processes, emphasizing the view of Foissner et al. (2008) that 80% of the ciliate diversity is still undescribed.

In spite of the detailed data available, including sequence analyses, evolution within the hypotrichs remained obscure, very likely mainly due to many not yet recognized homoplasies. Foissner et al. (2004) and Foissner and Stoeck (2006, 2008) showed that even complex morphological and ontogenetic features have evolved independently in sometimes widely distant groups, for instance, the “urostyliid” midventral complex and the “oxytrichid” 18 cirral pattern. Certainly, the very incomplete knowledge on alpha-diversity strengthens the problems, as mentioned above. The new genus described here is an impressive example, showing that a neokinetal wave (Eigner 1995) can be initiated not only by cirral rows but also by dorsal kineties.

The recognition of homoplasies needs combined morphological, ontogenetic, and molecular investigations (Foissner et al. 2004; Foissner and Stoeck 2006, 2008; Gong et al. 2006; Schmidt et al. 2007; Shao et al. 2007). This applies also to the new ciliate described here. Morphologically and ontogenetically, *Cotterillia* could be classified either in the Gonostomatidae or Kahliellidae, while the 18S rDNA assigns it unambiguously to the *Gonostomum* clade.

Material and Methods, Terminology

Material and cultivation

Cotterillia bromelicola was discovered in tanks of *Tillandsia heterophylla*, a common bromeliad in the cloud mountain forest near the town of Jalapa, Veracruz province, Mexico. Further details, see section “Type locality”.

The species first appeared in tank water and mud slightly enriched with some wheat grains. Pure cultures could be established on Eau de Volvic (French mineral water) enriched with some squashed wheat grains to promote growth of indigenous bacteria. However, the cultures were difficult to maintain, i.e., were fragile and high abundances were rarely reached. Further details, see section “Occurrence and ecology”.

Morphological methods

Living cells were studied using a high-power oil immersion objective and interference contrast. Protargol impregnation and scanning electron microscopy (SEM) were performed as described by Foissner (1991).

Counts and measurements of silvered specimens were performed at a magnification of 1,000×. In vivo measurements were conducted at magnifications of 40–1,000×. Drawings of live specimens were based on free-hand sketches and micrographs; those of impregnated cells were made with a drawing device. In the ontogenetic stages, parental structures are shown by contour, while newly formed structures are shaded black. Each of the stages depicted has been seen in at least two specimens. The description is very detailed because some processes are complex and *C. bromelicola* is a phylogenetically important species.

Molecular methods

To extract genomic DNA for 18S rDNA phylogenies, about 10 specimens of *Cotterillia bromelicola* were picked with a micropipette and transferred into 180 µl ATL buffer (Qiagen) and 20 µl Proteinase K (20 mg/ml). Subsequently, the genomic DNA was extracted using the protocol for cultured animal cells of the DNEasy Tissue Kit (Qiagen, Hildesheim, Germany). 18S rDNA was amplified using the universal eukaryotic primers EukA and EukB (Medlin et al. 1988). The amplification reaction contained 10–20 ng of DNA template, 2.5 U HotStar Taq DNA polymerase (Qiagen) in the manufacturer-provided reaction buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, and 0.5 µM of each oligonucleotide

primer. The final volume was adjusted to 50 µl with sterile distilled water. The PCR protocol for 18S rDNA gene amplification consisted of an initial hot start incubation of 15 min at 95 °C followed by 30 identical amplification cycles (i.e., denaturing at 95 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 2.5 min), and a final extension at 72 °C for 7 min. Negative control reactions included *Escherichia coli* DNA as a template. The resulting PCR products were cleaned with the PCR MinElute Kit (Qiagen) and cloned into a vector using the TA-Cloning kit (Invitrogen, Carlsbad, CA). Plasmids were isolated with Qiaprep Spin Miniprep Kit (Qiagen) from overnight cultures and PCR-reamplified using M13F and M13R primers to screen for inserts of the expected size (ca. 1.8 kb in case of the SSu-rDNA fragment). Three clones were sequenced bidirectionally (M13 sequence primers) with the Big Dye terminator kit (Applied Biosystems, Foster City, CA) on an ABI 3730 automated sequencer.

For assessment of the phylogenetic placement of *Cotterillia bromelicola*, its 18S rDNA sequence was aligned to 18S rDNA sequences of spirotrich ciliates available in GenBank. As outgroup we chose two urostylids (*Urostyla grandis*, *Holosticha multistilata*). Alignments were constructed, using ClustalX (Thompson et al. 1997), and were manually refined in MacClade (Maddison and Maddison 2003). The resulting alignment included 1,533 characters and 38 taxa and is available from the authors upon request. Distance, maximum likelihood and parsimony analyses were conducted for phylogenies. Neighbour joining evolutionary distance (BioNJ) and parsimony analyses were carried out in the seaview program package (vers. 4.2, Galtier et al. 1996). Maximum-likelihood bootstrapping analyses were carried out with 100 replicates using RAXML with the setting as described in Stamatakis et al. (2008). ML analyses were conducted online on the CIPRES Portal V 2.0 (<http://www.phylo.org>). Pairwise sequence similarities were calculated with the module pairalign as implemented in the JAguc software package (<http://www.wagak.informatik.uni-kl.de/JAguc>). The GenBank accession number of *Cotterillia bromelicola* is HM750260.

Based on a suggestion of the Associate Editor, we tried as outgroup also four oligotrichs (*Varistrombidium* sp., *Omegastrombidium elegans*, *Novistrombidium sinicum*, *Strombidium purpureum*). However, this did not change the position of *C. bromelicola*.

Terminology

Terminology of hypotrichs is very complex. Thus, we used a vernacular description style wherever this appeared sufficient. For general and specific terms, see Berger (1999), Corliss (1979), Eigner (1995), Foissner (1996), Foissner and Al-Rasheid (2006), and Lynn (2008). “Undulating membranes” is a common term for paroral plus endoral membrane.

In ciliates, the ventral side is usually that bearing the oral apparatus (Corliss 1979; Lynn 2008). Thus, there are also a dorsal, a right and a left side. In hypotrichs, however, terminology is different (Berger 1999, 2006, 2008; Kahl 1932): irrespective of the oral apparatus, the side with cirri is considered ventral and that with bristles dorsal, possibly because most hypotrichs are flattened and their oral apparatus extends more or less onto the right side.

The weakness of this terminology becomes disturbing in *Cotterillia* and several other groups of hypotrichs, especially most kahliellids: as many other ciliates, they have a ventral and dorsal side (Figs 17, 18, 41) and thus also a right and a left side (Figs 2, 6, 39, 40). The problem is strengthened in that ventral, dorsal and lateral are distinguished in most other groups of the class, for instance, the protocruziids, choreotrichs and oligotrichs.

Certainly, a change in terminology would pose problems in reading the previous literature. Although the common terminology is used in the present paper, specialists should think over a more uniform body terminology in ciliates in general and of hypotrichs in particular.

Results

Cotterillia nov. gen.

Diagnosis—Gonostomatidae with dense ciliature on both sides of body; that of dorsal side composed from several generations and with at least one dorsal kinety developing *de novo*, producing a neokinetal wave where the parental kinety reorganizes to a combined row, consisting of dorsal bristles anteriorly and of cirri posteriorly.

Type species—*Cotterillia bromelicola* nov. spec.

Dedication—We dedicate this new genus to Dr. F. P. D. Cotterill, former Principal Curator of Vertebrates in the Natural History Museum of Zimbabwe, for his supreme effort in reviving natural history as a scientific discipline (Cotterill and Foissner 2010). His writings highlight the tentelic role of preserved specimens in the epistemology of the life and earth sciences, and emphasize the impacts of the Second Alexandrian Tragedy on the state of knowledge (Cotterill 1997, 1999, 2002).

Description of *Cotterillia bromelicola* (Figs 1–43, 81, Tables 1, 2)

Diagnosis—Size about $100\ \mu\text{m} \times 40\ \mu\text{m}$ in vivo. Ellipsoidal to ovate with left margin slightly obliquely truncate in anterior third. Two macronucleus nodules and 5 micronuclei on average. Contractile vacuole slightly above mid-body at level of posterior curve of adoral zone. Cortical granules in stripes within cirral and bristle rows, colourless, ovate, about $2\ \mu\text{m} \times 1\ \mu\text{m}$ in size. On ventral side 7 cirral rows, including 2 marginal ones; on dorsal side an average of 4 cirral rows, all distinctly shortened and with some bristles anteriorly. Three dorsal kineties, rows 1 and 2 develop *de novo*; 2 caudal cirri in body midline. Adoral zone about 64% of body length, strongly curved posteriorly, composed of 39 membranelles on average; third ciliary row of membranelles strongly shortened. Nine paroral kinetids on average. Resting cyst globular, with a 3–10 μm thick, smooth, hyaline external layer and a compact, 1–2 μm thick internal layer.

Type locality—In tanks of *Tillandsia heterophylla* (Bromeliaceae) from a cloud mountain forest (1460 m above sea level) in Mexico, Veracruz province, surroundings of the town of Jalapa (Xalapa), N 19°32'51" W 96°57'36".

Type material—1 holotype and 10 paratype slides with protargol-impregnated morphostatic and dividing specimens have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz (LI). Two further paratype slides have been deposited in the Museum of Zoology at the Faculty of Science, Universidad Nacional Autónoma de México (UNAM). Relevant specimens have been marked by black ink circles on the coverslip.

Etymology—The Latin adjective *bromelicola* refers to the habitat the species was discovered.

Description—Size 80–120 $\mu\text{m} \times 35$ –45 μm in raw cultures, usually about $100\ \mu\text{m} \times 40\ \mu\text{m}$ in vivo; slightly smaller after one month of pure culture (Table 1), while slightly larger in the flourishing culture used for SEM (Table 2). Length:width ratio fairly constant within

individual preparations, while moderately variable ((2.1–3.4):1) when data are combined; slightly decreased after prolonged pure culture (Table 1), while slightly increased in the flourishing culture used for scanning electron microscopy (Table 2), where, however, some width shrinkage cannot be excluded. Shape usually very similar to that of the common *Gonostomum affine*, i.e., ellipsoid to slenderly ellipsoid with left margin more or less obliquely truncate in anterior half, making cells somewhat ovate (Figs 1, 9, 10, 12, 13, 19–22, 39, 40); ovate shapes common in preparations, where cell shrinkage tends to be stronger anteriorly than posteriorly (Figs 2, 16, 33–35). Slightly, to up to 2:1 flattened dorsoventrally and more or less curved when observed from the narrower side (Figs 10, 11, 17, 23). Invariably ($n > 100$) two rather closely spaced macronucleus nodules in middle third of cell, anterior nodule slightly nearer to body midline than posterior; both nodules of similar shape and size, viz., broadly to slenderly ellipsoidal, on average $18\ \mu\text{m} \times 9\ \mu\text{m}$. Nucleoli numerous, globular to irregular, $0.5\text{--}2\ \mu\text{m}$ in size. Two to 10 micronuclei near or attached to macronucleus nodules, some possibly masked by the deeply impregnated nodules; in small macronucleus concavities in about half of specimens; globular to ellipsoidal, on average $2\ \mu\text{m} \times 1.5\ \mu\text{m}$ in protargol preparations (Figs 1, 6, 12, 18, 20, 25, 33–35, 72, Table 1). Contractile vacuole underneath posterior curve of adoral zone of membranelles, a curious location caused by the extraordinary length of the zone; basically, as in many other hypotrichs, i.e., slightly above mid-body and near left margin of cell; with lacunar collecting canals; without permanent pore, opening thus rarely recognizable in SEM micrographs (Figs 1, 9–13, 20, 22, 26, 27, 42). Cortex very flexible, gelatinous and fairly conspicuous due to the rather dense granulation (Figs 5, 26, 27). Cortical granules in narrow stripes within cirral and bristle rows; likely ovate and about $(1.5\text{--}2)\ \mu\text{m} \times 1\ \mu\text{m}$ in size; colourless and of similar refractivity as cytoplasm, thus difficult to recognize in vivo (Figs 5, 7, 8, 14, 28); stain red with methyl green-pyronin, becoming up to $3\ \mu\text{m}$ long structures of various shape (Figs 7, 28); do not impregnate with the protargol method used. Cytoplasm colourless, with moderate numbers of lipid droplets $1\text{--}4\ \mu\text{m}$ across and $4\text{--}10\ \mu\text{m}$ -sized food vacuoles most in posterior half of cell (Figs 1, 5, 21, 24, 25). Feeds on up to $8\ \mu\text{m}$ long, spore-producing bacteria and small starch grains from the squashed kernels added to the cultures (Fig 24). Glides moderately fast on microscope slide and mud particles, showing pronounced flexibility.

Cirri $15\text{--}20\ \mu\text{m}$ long and fine, both in vivo and in SEM preparations, frontal cirri up to $25\ \mu\text{m}$ long; evenly spaced, except in ventral row 3 and left marginal row, where cirral distances increase from anterior to posterior; of similar size, except for distinctly thickened frontal cirri and anterior cirri of row 3 (Figs 1, 2, 6, 19–22, 27, 37–40, Tables 1, 2). Ventral and dorsal cirri as well as marginal cirri each composed of two to four, usually two or three ciliary rows; frontal cirri each composed of about eight rows; buccal cirrus of four rows.

Cirral and bristle pattern complex due to many slightly oblique rows of very different length and two neokinetical waves on dorsal side (see ontogenesis). According to the ontogenetic data, there are five frontoventral and two marginal cirral rows on ventral side; while three to seven, usually four rows on dorsal side, all distinctly shortened and with few to several bristles anteriorly (Figs 1, 2, 16–18, 19, 33–35, 39–41, 49, 50, Table 1). Frontoventral cirral rows slightly obliquely extending from anterior right to posterior left margin of cell; row 1 composed of first frontal cirrus; row 2 composed of second frontal cirrus and a single buccal cirrus in mid-region of adoral zone of membranelles; row 3 composed of third frontal cirrus and an average of eight cirri extending to mid-body, i.e., ending above posterior curve of adoral zone of membranelles; row 4 slightly shortened anteriorly, extends to body end abutting on last cirrus of left marginal row; row 5 only slightly shortened anteriorly and posteriorly. Left marginal row commences underneath curved portion of adoral zone of membranelles and extends to near midline of posterior body end. Right marginal row

extends onto dorsal side in anterior half, commences subapically and ends near midline of posterior body end (Figs 1, 2, 16–18, 19, 33–35, 39–41, 49, 50).

On dorsal side a mixture of cirral and bristle rows (see above and Figs 6, 16–18, 35, 38, 39, 41), caused by the neokinetal waves produced by dorsal bristle rows 1 and 2 (see ontogenesis). Invariably three ordinary (without cirri) rows of dorsal bristles: row 1 on left margin of cell and shortened anteriorly by an average of 32%; rows 2 and 3 bipolar, i.e., only slightly shortened anteriorly and extending to the caudal cirri (Figs 6, 16–18, 35, 39, 41, Tables 1, 2). Bristles 3–4 μm long in vivo, of ordinary structure, both in “complete” rows and in “compound” rows with bristles anteriorly and cirri posteriorly (Figs 24, 27, 38, 39). Invariably two fine, narrowly spaced caudal cirri in midline of posterior body end, i.e., between the marginal rows (Figs 1, 4, 15, 24, 31, Table 1).

Oral apparatus gonostomatid (Berger 1999) and conspicuous because adoral zone of membranelles extends over 64% of body length on average (Figs 1, 2, 12, 17, 19, 25, 33, 34, 40, Tables 1, 2). Adoral zone narrowly curved anteriorly, followed by a long and slightly sigmoidal portion extending to mid-body and curving abruptly rightwards and slightly posteriorly to end underneath mid-body and near body midline; composed of four to six frontal membranelles with about 20 μm long cilia partially covered by a 5 μm high scutum (Figs 1, 25, 37–40) and about 35 ventral membranelles with cilia decreasing in length from about 15 μm anteriorly to 5 μm posteriorly. Largest membranelles 6–7 μm wide both in vivo and in protargol preparations, of usual structure, except for row 3 which is shortened to an about 2 μm long stump (Figs 4, 36).

Buccal cavity narrow and flat both in vivo and SEM preparations, commences near anterior body end and extends posteriorly along adoral zone of membranelles, forming a flat furrow widening triangularly when reaching bend of adoral zone. Buccal lip inconspicuous because only about 3 μm wide, commences at right margin of posterior widening of buccal cavity and merges into buccal vertex covering proximal fifth of adoral zone (Figs 1, 2, 17, 19, 25, 33, 34, 40, 41, 43). Paroral membrane at right margin of buccal cavity at level of posterior bend of adoral zone, inserts in a minute furrow on buccal lip short, consisting of an average of nine comparatively widely spaced kinetids with 5–7 μm long cilia. Endoral membrane commences slightly posterior of paroral, extends diagonally to proximal end of adoral zone, composed of very narrowly spaced dikinetids with rather long cilia extending into the cytopharynx. Pharyngeal fibres without peculiarities, extend almost perpendicularly to right margin of cell (Figs 1, 2, 17, 25, 34, 36, 43, Tables 1, 2).

Resting cysts—These were obtained by isolating about 50 specimens from the raw culture in a large drop of Eau de Volvic and storing the microscope slide in a wet chamber. After 12 h, most specimens were encysted. Mature cysts were studied 10 d after isolation. Specimens kept in pure cultures for some weeks hardly encysted; most died within a week, and few made cysts after two to three weeks.

Size on average 38 μm ($\bar{x}$ 38.0, M 37, SD 4.6, SE 0.8, CV 15.2, Min 30, Max 50, n 30). Usually globular, rarely very broadly ovate; colourless (Figs 15, 29–32). Wall composed of two distinct layers: internal layer compact, 1–2 μm thick, possibly composed of two sublayers (Fig 31); stains reddish with methyl green-pyronin. External layer possibly consisting of compact slime, 3–10 μm thick, very hyaline becoming recognizable due to adhering mud and bacteria (Figs 15, 29, 30); frequently lacking or thin in immature cysts from pure cultures. Cortex distinct, granules absent and thus possibly used to build the external cyst layer, contains a highly characteristic membrane composed of bi- and trifurcated, fibre-like structures (Figs 3, 32). Underneath cortex a layer of narrowly spaced, pale mitochondria. Cyst contents dominated by the nuclear apparatus; macronucleus nodules

in cyst centre, not fused, with distinct nucleoli. Cyst plasm granular and with many globular inclusions 2–3 μm across, possibly autophagous vacuoles.

Occurrence and ecology—As yet found only in two samples from the type locality. Although being a bacteria feeder, *C. bromelicola* needs oligosaprobic culture conditions because it is very sensitive to oxygen depletion. Usually glides on bottom of Petri dish and between small mud accumulations. Resting cysts are formed when food is depleted.

Ontogenesis of *C. bromelicola* (Figs 44–80)

The temporal sequence of the ontogenetic processes is rather instable in *C. bromelicola*. For instance, is the stage shown in Fig 44 (buccal cirrus disintegrated, oral primordium very small) really earlier than that shown in Fig 48 (buccal cirrus still present, but oral primordium distinctly advanced, i.e., with protomembranelles anteriorly)? The same problem occurs in the specimens shown in Figs 56, 59.

Division of nuclear apparatus and cell fission—Both occur in the way typical for the Hypotricha, i.e., the daughters are complete when they separate, except for the adoral membranelles, where the minute fourth ciliary row is added only in post-dividers (Figs 71, 77, 78). The micronuclei become rather distinctly inflated, and thus the chromosomes (possibly 6) can be recognized (Figs 57, 58, 64, 65, 69).

Oral apparatus, including anlage 1—The oral primordium, i.e., an anarchic field of basal bodies, develops postorally between the left marginal row and frontoventral cirral row 4 (Figs 44, 72). It originates apokinetally, i.e., without any contact to parental structures, and can be seen in the SEM because the basal bodies become ciliated very early (Fig 49). Concomitantly, a long streak of basal bodies, i.e., proter's anlage 1 develops *de novo* right of the middle portion of the adoral zone of membranelles (Figs 44, 45, 72). This anlage, which will generate proter's undulating membranes and frontal cirrus 1, cannot be seen in the SEM because it is not yet ciliated (Figs 49, 50). When the oral primordium has grown to the appropriate size, protomembranelles each composed of two rows of basal bodies assemble at its anterior end (Figs 47, 48, 50).

Next, anlage 1 develops in the opisthe, i.e., a streak of basal bodies separates from the right margin of the oral primordium. This streak, which is the anlage for the undulating membranes and frontal cirrus 1, possibly contributes to the anlagen for cirral rows 2 and 3. Concomitantly, protomembranelles develop along the right margin of the oral primordium (Figs 51, 55, 56, 60, 61, 73).

In early middle dividers, the oral primordium has formed protomembranelles throughout, those which will become frontal membranelles have added a short third row (Fig 61). Proter and opisthe anlage 1 have arranged to dikinetal files that divide into two anlagen (Figs 61, 62, 74): the short anterior portion becomes frontal cirrus 1, while the long posterior portion gives rise to the undulating membranes (Fig 66). Interestingly, the anlage for the first frontal cirrus is distinctly curved to the right (Figs 61, 62; seen in several specimens). Further, the parental buccal cavity flattens, causing the paroral to become located right of the endoral.

In middle dividers, the macronucleus nodules have fused (Figs 65, 75), and the opisthe's adoral zone consists of three types of protomembranelles (Fig 66): the prospective frontal membranelles are composed of three ciliary rows of equal length; they are followed by some protomembranelles composed of two long and a short third row; and the posterior half of the zone consists of two-rowed protomembranelles. In the proter, the adoral zone of membranelles has lost the gonostomatid shape, and the minute fourth ciliary row has been resorbed in the individual membranelles. The buccal cavity has flattened, and the undulating

membranes are resorbing (Fig 67). Further, the anterior portion of anlage 1 assembled to the first frontal cirrus, while the posterior portion spreads in a Y-like shape: the right row, which is slightly shorter and consists of comparatively widely spaced monokinetids, will become the paroral membrane, while the left row, which is composed of very narrowly spaced dikinetids, will become the endoral (Figs 66–68).

In late dividers, when cell furrowing commences, the adoral zone comprises two types of protomembranelles (Figs 68, 76): four to six frontal ones, each consisting of three ciliary rows of equal length, and many ventral protomembranelles each consisting of two long rows and a third short row. The new undulating membranes shortened slightly, and the invaginating buccal area pushes the paroral membrane left of the endoral (Fig 68).

In very late dividers, the adoral zone of proter and opisthe obtains the gonostomatid shape; the paroral membrane is reduced to the species-specific number of cilia; and the scutum develops (Figs 71, 77). Still, the ventral adoral membranelles consist of only three rows of cilia, both in proter and opisthe; the fourth, minute row is added in early post-dividers, where the membranelar zone becomes distinctly curved posteriorly, extending to body midline (Fig 78).

Ventral cirral rows (anlagen 2–5)—The special origin of anlage 1 has been described above. The other cirral rows originate as usual, possibly except for proter's row 2. Its anlage originates in early dividers when basal bodies migrate out of the oral primordium and meet those from the disintegrated buccal cirrus, forming a long, slightly disordered streak (Figs 44, 45, 47, 48), which will produce the second frontal cirrus and the buccal cirrus. However, we cannot exclude that proter's anlage 2 originates entirely from the oral primordium because a distinct streak evolves even then when the buccal cirrus has not yet disintegrated (Figs 47, 48). Unfortunately, the process cannot be followed by SEM because the basal bodies of the anlage are not yet ciliated (Figs 49, 50). Proter's anlage 3 originates in late early dividers by intrakinetal reorganization of the parental row (Figs 51, 56). All cirri are transformed, except two at the anterior end and frontal cirrus 3. The opisthe anlagen 1–3 are produced as usual, i.e., by the oral primordium, together with the undulating membranes, as described in the previous section (Figs 48, 51, 55, 56).

Cirral rows 4 and 5 reproduce by intrakinetal anlagen becoming recognizable in late early dividers (Figs 51, 56). An anlage each is produced in anterior third and subequatorially, leaving some parental cirri anteriorly, in mid-body, and posteriorly (Figs 59, 61, 73, 74). All anlagen segregate cirri concomitantly in middle and late middle dividers in the usual way, i.e., from anterior to posterior (Figs 66, 68, 76). The species-specific cirral pattern is obtained in late and very late dividers, when most of the parental cirri have been resorbed (Figs 68, 71, 76, 77).

Marginal cirral rows—These originate as usual, that is, a primordium each develops in proter and opisthe of late early dividers (right row) or early middle dividers (left row). The primordia are produced by several cirri each, those not involved will be resorbed in late and very late dividers (Figs 56, 61, 66, 68, 71, 74, 76, 77). Dorsomarginal kineties absent.

Dorsal ciliature—Dorsal ontogenesis commences comparatively early, i.e., when the oral primordium and frontoventral anlagen 1 and 2 appear. First, a new dorsal kinety 1 develops *de novo* between the adoral zone of membranelles and parental dorsal kinety 1 (Figs 44, 48, 72). This process is not visible in the scanning electron microscope because the basal bodies are not yet ciliated. The parental dorsal kinety is unchanged. Slightly later, dorsal kinety 2 develops *de novo* left of the parental kinety, while dorsal kinety 3 develops intrakinetally by proliferation of basal bodies in mid-body (Fig 54). The parental kinety 2 is unchanged, while

parental kinety 1 develops an anlage subequatorially (Fig 54), and minute bristles become visible in the new dorsal kinety 1 (Fig 51).

When anlagen formation commences on the ventral side, the new dorsal kineties have produced the morphostatic number of bristles (dikinetics) and divide in mid-body, while kinety 3 still produces basal bodies. Concomitantly, anlagen formation commences in parental dorsal kineties 1 and 2 (Figs 56, 58, 73, 74). The production of basal bodies is more intense in the opisthe than the proter anlagen (Fig 63). At this stage, i.e., in early middle dividers and in middle dividers anlagen formation commences also in the compound rows, but only in the bristle portion, while the cirral portion remains silent (Figs 63, 65). These anlagen will produce cirri for the proter (Fig 69).

In late and very late dividers, caudal cirri develop at the posterior end of the newly formed dorsal kineties 1 and 2; cirri assemble in the posterior portion of parental dorsal kineties 1 and 2; and cirri develop in the anlagen of the compound kineties of the proter. Further, bristles and cirri are resorbed partially or completely in the grand-parental and great-grandparental rows (Figs 63, 66, 69, 70, 79, 80).

Molecular analysis

The 18S rDNA sequence of *C. bromelicola* is 1,773 bp long and available under GenBank accession number HM 750 260. We show only the maximum likelihood (ML) tree; the other trees are available from the authors upon request.

Comparing the sequence of *C. bromelicola* with sequences from representative oxytrichid and amphisiellid hypotrichs recovers the *Gonostomum* clade with high bootstrap support as the closest relative in all phylogenetic analyses (Fig 81). Note the often low bootstrap values for other clades, showing that the tree is far from being robust.

Sequence similarities for the *Gonostomum* clade are as follows: *G. strenuum* – *G. namibiense*: 97.4%; *G. namibiense* – *Cotterillia bromelicola*: 96.4%; *G. strenuum* – *C. bromelicola*: 97.9%.

The position of *C. bromelicola* within the *Gonostomum* clade offers three possibilities (Fig 81): *Cotterillia* is possibly a subgenus of *Gonostomum*; *Gonostomum* is non-monophyletic; or the phylogenetic tree is biased. Based on the morphological data, the first option seems unlikely, while the latter two are possible scenarios with the non-monophyly of *Gonostomum* being a result of a biased phylogenetic tree. In the phylogenetic 18S rDNA gene analysis, we are facing two challenges: the first one regards the low taxon sampling in *Gonostomum* and *Cotterillia* and the second one concerns the highly conserved nature of the taxonomic marker gene, which often fails to resolve deep nodes. Yet, the fact remains that the closest related sequence to *Cotterillia* known to date is *G. strenuum*, which finds full support (100/ 100/ 100) in all three analyses conducted (ML/ P/ NJ).

While *G. strenuum* is rather similar to *G. affine*, type of the genus (for a review, see Berger 1999), *G. namibiense* Foissner et al., 2002 is a tailed species with frontoventral cirral pairs. Increased taxon sampling and analysing additional genes, for instance the mitochondrial 18S rDNA, might shed light on the phylogenetic position of the *Gonostomum*/ *Cotterillia* clade as well as its topology.

Discussion

The new genus *Cotterillia*

Cotterillia is unique by producing dorsal kineties 1 and 2 *de novo*. This is associated (caused?) with (i) neokinetal waves, (ii) compound kineties composed of dorsal dikinetids anteriorly and of cirri posteriorly, and (iii) a fully ciliated left body side. This combination of features is unique within the Hypotricha, justifying at least a distinct genus. Of special interest is the *de novo* origin of two full dorsal kineties because this is extremely rare (Berger 1999, 2006, 2008), occurring, for instance, in *Engelmanniella* which produces the very short kinety 2 *de novo* (Wirnsberger-Aeschl et al. 1989).

The new species *Cotterillia bromelicola*

We could not find a species in the literature that is identical with *C. bromelicola*. Thus, we classified it as a new species. Whether or not it is a specific inhabitant of tank bromeliads is not known. However, we did not find *C. bromelicola* in any other of about 200 samples from bromeliads of Central and South America. Likewise, we did not find it in over 1000 soil samples collected globally (Foissner 1998; Foissner et al. 2002).

In vivo, *C. bromelicola* is easily confused with quite a lot of genera and species, especially the common, cosmopolitan *Gonostomum affine*, which has the same size and overall appearance but lacks cirral rows on both sides of the body (for a review, see Berger 1999). Even more similar are species of the genus *Wallackia* because they have cirral rows on the ventral side (Foissner et al. 2002); fortunately, the dorsal side bears only bristle rows, i.e., lacks the compound cirral rows so typical for *Cotterillia* (Figs 6, 16, 35, 39). *Cotterillia bromelicola* resembles also various kahliellids, for instance, *Kahliella simplex* (adoral zone of membranelles distinctly shorter; Eigner 1995), *Parastrongylidium* spp. (adoral zone distinctly shorter and not gonostomatid; Aeschl and Foissner 1992) and *Cladotricha* ssp. (no cirri on dorsal side; Blatterer and Foissner 1988).

Ontogenetic comparison

Besides the *de novo* originating dorsal kineties, *C. bromelicola* has another very characteristic ontogenetic feature: frontoventral anlage 1, which produces the first frontal cirrus and the proter's undulating membranes, also originates *de novo*. There are only two other genera, which have the same mode: *Gonostomum* (Eigner 1999; Foissner et al. 2002) and *Wallackia* (Foissner et al. 2002). Thus, these three genera form a natural group that can be united in the family Gonostomatidae Small and Lynn, 1985. Very likely, *Paragonostomum* Foissner, Agatha and Berger, 2002 also belongs to that family because it differs from *Gonostomum* only by the lack of transverse cirri (Foissner et al., 2002, 2005). Whether or not the other genera assigned to the Gonostomatidae by Small and Lynn (1985) and to the Trachelostylidae by Lynn (2008) belong to this family, needs detailed ontogenetic investigations.

Generally, the (*de novo*) origin of proter's anlage 1 is often difficult to prove because of the close association with the undulating membranes. Thus, it is possible that it has been overlooked or misinterpreted in some genera and species.

There are also pronounced ontogenetic differences between the genera *Gonostomum*, *Wallackia*, and *Cotterillia*. *Gonostomum* produces long primary primordia and six frontoventral cirralanlagen, while *Wallackia* and *Cotterillia* have only a single primary primordium (anlage 2) and possess only five frontoventralanlagen. And, of course, *Cotterillia* has a unique ontogenesis of the dorsal side ciliature, but the morphostatic state is

the same as in *Gonostomum* and *Wallackia*: all have three dorsal kineties, supporting our claim that these genera form a natural assemblage.

Classification of *Cotterillia*

We agree with Lynn (2008) that “the taxonomy of stichotrichians is one of the most confused in the phylum”. Thus, we follow pragmatically the monographs of Berger (1999, 2006, 2008). Then, *Cotterillia* belongs to the Hypotricha (~ stichotrichs), which comprise all non-euplotid hypotrichs (Berger 2006). Within the Hypotricha, *Cotterillia* belongs to the unranked taxon “non-dorsomarginalian Hypotricha” because it lacks dorsomarginal kineties and includes taxa like *Gonostomum* and the Amphiseliidae. However, *Cotterillia* shows also pronounced similarities with the Kahliellidae, as defined by Eigner (1995): “Euhypotrichina with more than one longitudinal cirral row on right side of body. Neokinetal anlagen develop during morphogenesis”.

Obviously, *Cotterillia* has important features from both, the “non-dorsomarginalian Hypotricha” and the Kahliellidae. Within the “non-dorsomarginalian Hypotricha”, *Cotterillia* strongly resembles genera like *Wallackia* and *Gonostomum* in body shape and size, in the oral apparatus (a long, gonostomatid adoral zone of membranelles; a flat, inconspicuous buccal cavity; and a paroral membrane composed of few, comparatively widely spaced cilia), and the ontogenesis (see above). Typical kahliellid features are many cirri in longitudinal rows on both sides of the body and neokinetal waves, which produce compound kineties (Eigner 1995).

Fortunately, the 18S rDNA sequence assigns *Cotterillia* unambiguously to the *Gonostomum* clade (Fig 81). Accordingly, the kahliellid features of *Cotterillia* are either plesiomorphies (possibly the many cirral rows) or homoplasies (very likely the neokinetal waves on dorsal side).

In some molecular trees, the *Gonostomum* clade appears rather close to *Trachelostyla pediculiformis* (Gong et al. 2006) or *Trachelostyla* appears at the base of a larger clade including the Gonostomatidae and Amphiseliidae (Shao et al. 2007), similar to our tree (Fig 81). This is not surprising because sequence similarity is ~96% between *Gonostomum* and *Trachelostyla*. However, bootstrap values are low in all trees, indicating that this highly curious genus has not yet settled. We assume that genes with higher resolution will provide better insights into the deeper nodes of hypotrich families. See Shao et al. (2007) and Berger (2008) for morphological descriptions of *Trachelostyla* and a critical discussion of its specific features.

Improved diagnosis of the family Gonostomatidae Small and Lynn, 1985

Oxytrichid (?) Hypotricha with gonostomatid oral apparatus. Cirri may occur on ventral side only, forming a kind of *Oxytricha* pattern or on both sides of the cell, forming distinct rows, i.e., a *Kahliella* pattern. Frontoventral anlage 1 originates *de novo*, the other anlagen develop ordinarily or as primary primordia. The dorsal kineties reproduce intrakinetally or *de novo*, initiating neokinetal waves.

Type genus—*Gonostomum* Sterki, 1878.

Genera assignable—*Gonostomum* Sterki, 1878; *Paragonostomum* Foissner, Agatha and Berger, 2002; *Cotterillia* nov. gen.

Remarks—Small and Lynn (1985) provided a rather meagre diagnosis: “With two or more frontoventral cirral files parallel to long axis of oral region along its right border; at least two

frontoventral files extend almost length of body". The last feature would exclude the type species, *G. affine*, which does not have such rows (see also Berger 1999). Lynn (2008) synonymized the Gonostomatidae with the Trachelostylidae. This is neither supported by the molecular data (see above) or by the morphological and ontogenetic investigations (Shao et al. 2007), which suggest maintaining both families.

Acknowledgments

This study was supported by the Austrian Science Foundation (FWF, project P 20360-B17) and the German Science Foundation (DFG, project STO 414/3-1). The technical assistance of Mag. Barbara Harl, Robert Schörghofer, and Andreas Zankl is greatly acknowledged. Special thanks to Carlos Durán Ramírez for collecting the samples.

References

- Aescht E, Foissner W. Biology of a high-rate activated sludge plant of a pharmaceutical company. Arch. Hydrobiol. 1992; (Suppl. 90):207–251.
- Berger H. Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Monogr. Biol. 1999; 78:1–1080.
- Berger H. Monograph of the Urostyloidea (Ciliophora, Hypotricha). Monogr. Biol. 2006; 85:1–1304.
- Berger H. Monograph of the Amphiseliidae and Trachelostylidae (Ciliophora, Hypotricha). Monogr. Biol. 2008; 88:1–737.
- Blatterer H, Foissner W. Beitrag zur terricolen Ciliatenfauna (Protozoa: Ciliophora) Australiens. Stapfia, Linz. 1988; 17:1–84.
- Corliss, JO. The Ciliated Protozoa. Characterization, Classification and Guide to the Literature. 2nd ed. Pergamon Press; Oxford–New York–Toronto–Sydney–Paris–Frankfurt: 1979.
- Cotterill, FPD. The Second Alexandrian Tragedy, and the fundamental relationship between biological collections and scientific knowledge. In: Nudds, JR.; Pettitt, CW., editors. The Value and Valuation of Natural Science Collections. Geological Society; London: 1997. p. 227–241.
- Cotterill FPD. Toward exorcism of the ghost of W.T. Thistleton-Dyer: a comment on “overduplication” and the scientific properties, uses and values of natural science specimens. Taxon. 1999; 48:35–39.
- Cotterill, FPD. The future of natural science collections into the 21st century. Conferencia de Clausura. Actas del I Simposio sobre el Patrimonio Natural en las Colecciones Públicas en España; Vitoria. 25–27 Septiembre 2001; Vitoria: Departamento de Cultura, Diputación Foral de Alava; 2002. p. 237–282. ISBN:84-7821r-r497-6
- Cotterill FPD, Foissner W. A pervasive denigration of natural history misconstrues how biodiversity inventories and taxonomy underpin scientific knowledge. Biodivers. Conserv. 2010; 19:291–303. [PubMed: 21472034]
- Eigner P. Divisional morphogenesis in *Deviata abbrevescens* nov. gen., nov. spec., *Neogeneia hortualis* nov. gen., nov. spec., and *Kahliella simplex* (Horváth) Corliss and redefinition of the Kahliellidae (Ciliophora, Hypotrichida). Eur. J. Protistol. 1995; 31:341–366.
- Eigner P. Comparison of divisional morphogenesis in four morphologically different clones of the genus *Gonostomum* and update of the natural hypotrich system (Ciliophora, Hypotrichida). Eur. J. Protistol. 1999; 35:34–48.
- Foissner W. Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. Eur. J. Protistol. 1991; 27:313–330. [PubMed: 23194842]
- Foissner, W. Ontogenesis in ciliated protozoa, with emphasis on stomatogenesis. In: Hausmann, K.; Bradbury, PC., editors. Ciliates: Cells as Organisms. Fischer; Stuttgart–Jena–Lübeck–Ulm: 1996. p. 95–177.
- Foissner W. An updated compilation of world soil ciliates (Protozoa, Ciliophora), with ecological notes, new records, and descriptions of new species. Eur. J. Protistol. 1998; 34:195–235.
- Foissner W, Al-Rasheid K. A unified organization of the stichotrichine oral apparatus, including a description of the buccal seal (Ciliophora: Spirotrichea). Acta Protozool. 2006; 45:1–16.

- Foissner W, Stoeck T. *Rigidothrix goiseri* nov. gen., nov. spec. (Rigidotrichidae nov. fam.), a new “flagship” ciliate from the Niger floodplain breaks the flexibility-dogma in the classification of stichotrichine spirotrichs (Ciliophora, Spirotrichea). *Eur. J. Protistol.* 2006; 42:249–267. [PubMed: 17113471]
- Foissner W, Stoeck T. Morphology, ontogenesis and molecular phylogeny of *Neokeronopsis (Afrokeronopsis) aurea* nov. subgen., nov. spec. (Ciliophora: Hypotricha), a new African flagship ciliate confirms the CEUU hypothesis. *Acta Protozool.* 2008; 47:1–33. [PubMed: 20700468]
- Foissner W, Agatha S, Berger H. Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert. *Denisia.* 2002; 5:1–1459.
- Foissner W, Moon-van der Staay SY, van der Staay GWM, Hackstein JHP, Krautgartner W-D, Berger H. Reconciling classical and molecular phylogenies in the stichotrichines (Ciliophora, Spirotrichea), including new sequences from some rare species. *Eur. J. Protistol.* 2004; 40:265–281.
- Foissner W, Berger H, Xu K, Zechmeister-Boltenstern S. A huge, undescribed soil ciliate (Protozoa: Ciliophora) diversity in natural forest stands of Central Europe. *Biodivers. Conserv.* 2005; 14:617–701.
- Foissner W, Chao A, Katz LA. Diversity and geographic distribution of ciliates (Protista: Ciliophora). *Biodivers. Conserv.* 2008; 17:345–363.
- Galtier N, Gouy M, Gautier C. SEAVIEW and PHYLO WIN: two graphic tools for sequence alignment and molecular phylogeny. *Comput. Appl. Biosci.* 1996; 12:543–548. [PubMed: 9021275]
- Gong J, Song W, Li L, Shao C, Chen Z. A new investigation of the marine ciliate, *Trachelostyla pediculiformis* (Cohn, 1866) Borror, 1972 (Ciliophora, Hypotrichida), with establishment of a new genus, *Spirotrachelostyla* nov. gen. *Eur. J. Protistol.* 2006; 42:63–73. [PubMed: 17070752]
- Kahl A. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Tierwelt Dtl.* 1932; 25:399–650.
- Lynn, DH. The Ciliated Protozoa. Characterization, Classification, and Guide to the Literature. 3rd ed. Springer; Dordrecht: 2008.
- Maddison, DR.; Maddison, WP. McClade. Version 4.0. Sinauer Associates; Sunderland, MA: 2003.
- Medlin L, Elrod HJ, Stickel S, Sogin ML. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene.* 1988; 71:491–499. [PubMed: 3224833]
- Schmidt SL, Bernhard D, Schlegel M, Foissner W. Phylogeny of the Stichotrichia (Ciliophora; Spirotrichea) reconstructed with nuclear small subunit rRNA gene sequences: discrepancies and accordances with morphological data. *J. Eukaryot. Microbiol.* 2007; 54:201–209. [PubMed: 17403161]
- Shao C, Song W, Yi Z, Gong J, Li J, Lin X. Morphogenesis of the marine spirotrichous ciliate, *Trachelostyla pediculiformis* (Cohn, 1866) Borror, 1972 (Ciliophora, Stichotrichia), with consideration of its phylogenetic position. *Eur. J. Protistol.* 2007; 43:255–264. [PubMed: 17870427]
- Small, EB.; Lynn, DH. Phylum Ciliophora Doflein, 1901. In: Lee, JJ.; Hutner, SH.; Bovee, EC., editors. *An Illustrated Guide to the Protozoa.* Society of Protozoologists; Lawrence: 1985. p. 393–575.
- Song, W.; Warren, A.; Hu, X. Free-living Ciliates in the Bohai and Yellow Seas, China. Science Press; Beijing: 2009.
- Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web-servers. *Syst. Biol.* 2008; 75:758–771. [PubMed: 18853362]
- Sterki V. Beiträge zur Morphologie der Oxytrichinen. *Z. Wiss. Zool.* 1878; 31:29–58.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 1997; 25:4876–4882. [PubMed: 9396791]
- Wirnsberger-Aesch E, Foissner W, Foissner I. Morphogenesis and ultrastructure of the soil ciliate *Engelmanniella mobilis* (Ciliophora, Hypotrichida). *Eur. J. Protistol.* 1989; 24:354–368. [PubMed: 23195727]

Figures 1–15.

Cotterillia bromelicola from life (1, 3, 5, 8, 9–15), after protargol impregnation (2, 4, 6), and in a methyl green-pyronin stain (7). **1.** Ventral view of a representative specimen, length 100 μm . Arrow marks contractile vacuole underneath buccal cavity and adoral zone. **2, 6.** Ventral and dorsal view of holotype specimen, length 87 μm . Arrowhead marks paroral membrane; numerals in (6) denote compound rows. **3.** Cortex pattern in resting cyst (cp. Fig 32). **4.** Fine structure of adoral membranelles. Note the unusually short third ciliary row. **5, 8, 14.** Optical section (5) and surface view (14), showing the gelatinous cortex studded with hyaline granules (8) with a size of (1.5–2) $\mu\text{m} \times 1 \mu\text{m}$. **7.** Cortical granules after staining with methyl green-pyronin, length up to 3 μm . **9–13.** Shape variability. Figs 10, 11 show the same specimen in dorsal and lateral view. **15.** Resting cyst, diameter 40 μm . AZM, adoral zone of membranelles; B, bacteria; C, cirri; CC, caudal cirri; CV, contractile vacuole; DB, dorsal bristle; DK1–3, dorsal kineties; EL, external cyst layer; FV, food vacuole; G, cortical granules; IL, internal cyst layer; LM, left marginal row; MA, macronucleus nodule; MI, micronucleus; P, pharyngeal fibres; RM, right marginal row; R1–5, cirral rows. Scale bars 30 μm .

Figures 16–18.

Cotterillia bromelicola, cirral and bristle pattern of dorsal (16), oral (17), and aboral (18) side after protargol impregnation. This species has three ordinary rows of dorsal bristles (DK1–3) and six compound rows (numerals 1–6) composed of bristles anteriorly and of cirri posteriorly. Thus, the body is comparatively densely ciliated. Arrowhead (16) marks caudal cirri. Note that dorsal kinety 1 is close to the left margin of the adoral zone of membranelles. AZM, adoral zone of membranelles; DK1–3, dorsal kineties; EM, endoral membrane; MA, macronucleus nodule; MI, micronucleus; PM, paroral membrane; RM, right marginal row; R5, cirral row 5. Scale bars 30 μ m.

Figures 19–32.

Cotterillia bromelicola, trophic (19–28) and cystic (29–32) specimens from life (19–27, 29–32) and in a methyl green-pyronin stain (28). **19–23.** Overview of freely motile specimens showing, *inter alia*, body shape, the long cirri, and the contractile vacuole in the dorsal side of the cell (23, arrow) and at level of curved posterior portion of adoral zone (20, 22). **24.** The cortical granules (arrowheads) are inconspicuous. The food vacuoles contain bacteria and their spores. **25–28.** Slightly squashed cells, showing some main organelles (25, 26) and rows of cortical granules (27, arrowheads) becoming distinct when stained (28). Arrows in Figs 26, 27 mark the canals of the contractile vacuole. **29.** Resting cyst covered with a thick layer of bacteria (bright field). **30.** Resting cyst with contents pressed out. **31.** The internal cyst layer is possibly composed of two sublayers (opposed arrowheads). **32.** Surface view of the cortex of an encysted specimen. AZM, adoral zone of membranelles; B, bacteria; C, cirri; CV, contractile vacuole; DB, dorsal bristles; EL, external cyst layer; FV, food vacuoles; IL, internal cyst layer; MA, macronucleus nodules; S, scutum. Scale bars 5 μm (24, 27, 28, 30–32), 30 μm (23, 29), and 50 μm (19–22, 25, 26).

Figures 33–38.

Cotterillia bromelicola after protargol impregnation (33–36) and in the scanning electron microscope (37, 38). **33–36.** Ventral and dorsal views, showing the cirral and bristle pattern as well as the oral and nuclear apparatus. On the dorsal side occur compound parental and grand-parental rows, consisting of dorsal bristles anteriorly and of cirri posteriorly (35, transition sites marked by asterisks). The third ciliary row of the adoral membranelles is unusually short (36, arrow). Note the short “gonostomatid” paroral membrane consisting of comparatively widely spaced basal bodies (33, 34, 36). **37.** Ventral view of anterior body portion, showing the narrow scutum and the long, thin cirri. **38.** Dorsal view of anterior body portion, showing five elongated frontal membranelles (FM) and some thin cirri (arrowheads). AM, adoral membranelles; AZM, adoral zone of membranelles; CC, caudal cirri; DB, dorsal bristles; DK1–3, dorsal kineties; EM, endoral membrane; FC1–3, frontal cirri; FM, frontal membranelles; LM, left marginal row; MA, macronucleus nodules; MI, micronuclei; PM, paroral membrane; RM, right marginal row; R1–5, cirral rows; S, scutum. Scale bars 10 μ m (36–38) and 30 μ m (33–35).

Figures 39–43.

Cotterillia bromelicola in the scanning electron microscope. **39.** Dorsal view, showing dorsal kineties 1 and 2 as well as several compound cirral rows with an anterior tail of dorsal bristles (transition sites marked by asterisks). Note body shape, the elongated frontal adoral membranelles, and the thin caudal cirri. **40.** Ventral overview, showing the arrangement of the cirral rows; row 1 is composed of frontal cirrus 1 (FC1); row 2 consists of frontal cirrus 2 and the buccal cirrus (arrow). **41.** Oral overview showing, *inter alia*, dorsal kinety 1 and the slightly sigmoidal adoral zone of membranelles. **42.** The opening of the contractile vacuole is not strengthened by specific cortical differentiations, and thus recognizable only when emptying. **43.** Details of oral apparatus showing, *inter alia*, the long and comparatively wide-spaced cilia of the paroral membrane, which inserts into the convex buccal lip. Arrow marks buccal cirrus. AZM, adoral zone of membranelles; BC, buccal cavity; CC, caudal cirri; DK1–3, dorsal kineties; EP, opening (“excretory pore”) of contractile vacuole; FC1, frontal cirrus 1; LM, left marginal row; PM, paroral membrane; R3–5, cirral rows; RM, right marginal row. Scale bars 2 μm (42), 10 μm (43), and 30 μm (39–41).

Figures 44–48.

Cotterillia bromelicola, early dividers after protargol impregnation. The oral primordium originates postorally between the left marginal and the fourth frontoventral cirral row and develops a long basal body streak extending anteriorly. The buccal cirrus (48, arrow) is incorporated into this streak (44), which is a primary primordium producing the second frontal cirrus both in proter and opisthe. Left of the long streak develops a primordium *de novo* (arrowheads), producing the first frontal cirrus and the undulating membranes of the proter (Figs 56, 59, 61, 66). Concomitantly, a new dorsal kinety 1 (N1) develops *de novo*. AZM, adoral zone of membranelles; DK1–3, parental dorsal kineties; EM, endoral membrane; N1, new dorsal kinety 1; OP, oral primordium; PM, paroral membrane. Scale bars 30 μ m.

Figures 49–52.

Cotterillia bromelicola, scanning electron micrographs of dividers. **49.** Very early divider, comparable to those shown in Figures 44 and 48, with intact buccal cirrus (arrow). Only the oral primordium is recognizable because the developing proter anlagen 1 and 2 are not yet ciliated (cp. Figs 44, 48). This applies also to the new dorsal kinety 1 (cp. Fig 51). **50.** An early divider with a cirral anlage in row 3 (arrow). The buccal cirrus has been resorbed. The developing proter anlagen 1 and 2 are not recognizable because they are not yet ciliated (cp. Figs 48, 56). **51.** A late early divider with cirral anlagen in rows 2–5 (arrowed numerals). Still, proter anlage 1 is not ciliated and thus invisible. The undulating membranes separate from the oral primordium and a cirral anlage (likely for row 3) becomes recognizable at right (arrowhead). **52.** Dorsal view of a very late divider already having the morphostatic cirral and bristle pattern. A new dorsal kinety 2 has been generated, while the parental row reorganized to cirri in the posterior half (asterisks). Note the five conspicuous frontal adoral membranelles. AZM, adoral zone of membranelles; DK2, 3, parental dorsal kineties; FC3, frontal cirrus 3; LM, left marginal row; N1, *de novo* dorsal kinety 1; OP, oral primordium; PM, paroral membrane; R2–5, anlagen in cirral rows; UM, anlage for the undulating membranes. Scale bars 40 μ m.

Figures 54–58.

Cotterillia bromelicola, late early dividers after protargol impregnation. **54.** Dorsal view of a stage between those shown in Figures 48 and 56. The new dorsal kinety 2 originates *de novo* (N2), and parental dorsal kinety 1 develops anlagen intrakinetally (arrowhead). The caudal cirri were resorbed or are not recognizable. **55–58.** Ventral and dorsal view, showing the formation of membranelles in the oral primordium and of anlagen (numerals 1–5) in all frontoventral cirral rows and the right marginal row (56, asterisks). Two dorsal kineties (N1, N2) developed *de novo* and divided equatorially. The parental bristles of dorsal kineties 1 and 2 (DK1, 2) transform into cirri in the posterior half of the rows (56, 58, arrows). Dorsal kinety 3 develops intrakinetical anlagen (arrowheads). The macronucleus is still unchanged, while the micronuclei are slightly inflated showing six chromosomes each. AZM, adoral zone of membranelles; DK1–3, parental dorsal kineties; EM, parental endoral membrane; MA, macronucleus nodules; MI, micronucleus; N1, 2, dorsal kineties generated *de novo*; OP, oral primordium; PM, parental paroral membrane; R5, frontoventral cirral row 5; UM, newly forming undulating membranes. Scale bars 30 μm .

Figures 59–68.

Cotterillia bromelicola, dividers after protargol impregnation. **59, 60.** Late early divider similar to that shown in Figure 56, but without anlagen (numerals) in cirral row 4 and with a very small anlage for the undulating membranes of the opisthe. Thus, this could be a slightly malformed specimen. **61–64.** Ventral and dorsal view of an early middle divider, showing forming adoral membranelles and disintegration of the parental oral apparatus, i.e., flattening of the buccal cavity and resorption of the paroral membrane. New cirri are developing in the anlagen (numerals 1–5) of the frontoventral and marginal (asterisks) rows. On the dorsal side, the reorganizing parental (DK1–3) and the *de novo* (N1, 2) generated dorsal kineties are recognizable. Further, anlagen develop in grand-parental dorsal kineties 1 and 2 (arrowheads). The macronucleus nodules became globular and the micronuclei commence division. **65–67.** Ventral view of a middle divider with fused macronucleus nodules. A short third row of basal bodies is developing in the opisthe adoral membranelles, while the minute fourth row is resorbed in the parental ones. The parental endoral membrane is resorbed (Figs 66, 67), and new undulating membranes originate from the posterior portion of anlage 1, forming a Y-like pattern. New cirri have been formed in all anlagen and in the posterior portion of the parental dorsal kineties 1 and 2 (arrow; Fig 69). **68.** Ventral view of a late divider (dorsal side shown on next plate). A third row of basal bodies has been added to the ventral opisthe membranelles, and the developing buccal cavity pushes the paroral (asterisks) left of the endoral. Parental cirri not used in anlagen formation are resorbing. Scale bars 30 μm . Explanation of abbreviations, see Figs. 69–71.

Figures 69–71.

Cotterillia bromelicola, dividers after protargol impregnation. **69.** Dorsal view of the late divider shown in Figure 68. The macronucleus nodules are dividing a second time and the micronuclei are finishing division. Both, in proter and opisthe cirri have developed in the posterior portion of parental dorsal kineties 1 and 2 (DK1, 2), while caudal cirri developed in the *de novo* kineties. **70, 71.** Dorsal and ventral view of a very late divider. The daughters are complete, except for ciliary row 4 of the adoral membranelles, which is produced in post-dividers (Fig 78). Arrowheads mark the new buccal cirri. The arrow denotes some not yet resorbed marginal cirri. AZM, adoral zone of membranelles; B, buccal cirrus; CC, new caudal cirri; DK1–3, parental dorsal kineties; EM, parental endoral membrane; FC1–3, frontal cirri; LM, left marginal row; MA, macronucleus nodules; MI, micronuclei; N1, 2, *de novo* dorsal kineties; NE, new endoral membrane; NP, new paroral membrane; OP, oral primordium; PM, paroral membrane; RM, right marginal row; UM, undulating membranes. Scale bars 30 μm.

Figures 72–77.

Cotterillia bromelicola, protargol-impregnated dividers. **72.** Ventral view of a late early divider with three anlagen streaks (numerals 1–3) and a small oral primordium hidden almost entirely by the posterior macronucleus nodule. Note the early *de novo* origin of dorsal kinety 1 (N1). **73, 74.** Ventral view of a late early divider and an early middle divider. The specimen shown in (74) lost the anterior macronucleus nodule during preparation. In the oral primordium, adoral membranelles and the undulating membranes are forming. Cirral anlagen occur in all frontoventral rows (numerals 1–5) and in the marginal rows (74, asterisks). Both, parental dorsal kinety 1 (DK1) and the *de novo* generated dorsal kinety 1 (N1) are recognizable. **75.** Dorsal view of a middle divider, showing the fusing macronucleus nodules, the *de novo* generated dorsal kinety 2 (N2), and the reorganizing parental kineties 1 and 2 (DK1, 2). Arrowheads mark anlagen in grand-parental dorsal kineties 1 and 2; they will produce cirri in the proter. **76.** Ventral view of a late divider. The somatic and oral structures have been largely completed both in proter and opisthe. Arrows mark some of not yet resorbed parental cirri. **77.** Ventral view of a very late divider. The daughters are complete, already having a gonostomatid body shape and oral apparatus. Scale bars 30 μ m. Explanation of abbreviations, see Figs 78–80.

Figures 78–80.

Cotterillia bromelicola after protargol impregnation. **78, 79.** Ventral and dorsal view of a post-divider. A minute fourth row of cilia has been added to the adoral membranelles (cp. Fig 4). The daughters, which are considerably smaller than morphostatic cells, have completely reorganized the ventral cirral pattern, while parental, grand-parental, and great-grandparental (arrows) bristles and cirri occur on the dorsal side (see following scheme). **80.** The dorsal ciliature of *C. bromelicola* is composed of dikinetal “bristles” and of cirri. Basically, there are three rows (1–3) of bristles (dorsal kineties, Figs 78, 79): rows 1 and 2 originate *de novo* and produce a neokinetal wave each, while kinety 3 reproduces ordinarily: 1+2= kineties 1 and 2, which originate *de novo*; 3 = kinety 3, which reproduces by intrakinetal proliferation of basal bodies; P+P= parental kineties 1 and 2, each composed of bristles anteriorly and of cirri posteriorly; GP + GP = grand-parental kineties 1 and 2, part of the bristles and cirri has been resorbed. GGP + GGP = great-grandparental bristles and cirri. The remaining bristles and cirri which will be resorbed in the next generation. AZM, adoral zone of membranelles; DK1–3, parental dorsal kineties; FC1, frontal cirrus 1; GP, grand-parental compound row; LM, left marginal row; MA, macronucleus nodules; MI, micronuclei; N1, 2, *de novo* dorsal kineties; OP, oral primordium; P, parental compound row; RM, right marginal row; UM, undulating membranes. Scale bar 30 μm .

Figure 81.

18S rDNA maximum likelihood tree showing the phylogenetic placement of *Cotterillia bromelicola* nov. gen., nov. spec. (in bold). Bootstrap values above 50 for the neighbour-joining evolutionary distance (BioNJ, 1000 replicates), parsimony (P, 1000 replicates), and maximum-likelihood (ML, 100 replicates) analyses are given at the individual nodes. For details about tree construction, see “Methods” section.

Table 1

Morphometric data on *Cotterillia bromelicola*.

Characteristics ^a	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length (first raw culture)	86.1	85.0	5.2	1.2	6.0	78.0	98.0	19
Body, width (first raw culture)	35.0	35.0	3.8	0.9	10.8	30.0	44.0	19
Body, length:width ratio (first raw culture)	2.5	2.5	0.2	0.1	7.3	2.2	2.7	19
Body, length (after 1 month of pure culture)	80.0	80.0	5.9	1.3	7.3	66.0	93.0	19
Body, width (after 1 month of pure culture)	34.6	35.0	3.6	0.8	10.4	25.0	39.0	19
Body, length:width ratio (after 1 month of pure culture)	2.3	2.3	0.2	0.1	8.6	2.1	2.7	19
Anterior end to end of AZM, distance	54.8	55.0	2.2	0.5	4.0	50.0	59.0	19
Left body margin to end of AZM, distance	19.2	20.0	2.1	0.5	11.2	15.2	23.0	19
Longest base of adoral membranelles, length	6.4	6.0	–	–	–	6.0	7.0	19
Adoral membranelles, number	38.5	39.0	1.6	0.4	4.3	36.0	41.0	19
Anterior end to begin of paroral membrane, distance	38.0	39.0	2.1	0.5	5.5	34.0	41.0	19
Anterior end to begin of endoral membrane, distance	38.3	39.0	2.1	0.5	5.4	34.0	41.0	19
Paroral membrane, length	5.2	5.0	0.8	0.2	15.1	4.0	7.0	19
Paroral membrane, number of cilia	9.3	9.0	1.5	0.3	15.6	7.0	13.0	19
Endoral membrane, length	17.7	18.0	1.3	0.1	7.5	15.0	20.0	19
Anterior end to first MA-nodule, distance	23.4	23.0	1.4	0.3	5.9	21.0	26.0	19
Anterior end to second MA-nodule, distance	50.6	50.0	2.7	0.6	5.3	46.0	55.0	19
Macronucleus nodules, distance in between	10.3	11.0	2.0	0.5	19.4	6.0	13.0	19
Anterior macronucleus nodule, length	17.3	18.0	1.7	0.4	10.0	14.0	22.0	19
Anterior macronucleus nodule, width	9.5	10.0	1.1	0.2	11.3	7.0	11.0	19
Posterior macronucleus nodule, length	17.3	18.0	1.5	0.4	8.8	14.0	19.0	19
Posterior macronucleus nodule, width	8.8	9.0	1.2	0.3	13.5	7.0	10.0	19
Macronucleus nodules, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	19
Micronuclei, length	2.0	2.0	–	–	–	1.6	2.5	19
Micronuclei, width	1.5	1.5	–	–	–	1.2	1.8	19
Micronuclei, number	5.3	5.0	2.0	0.5	38.2	2.0	10.0	19
Anterior end to first frontal cirrus, distance	6.4	6.0	0.8	0.2	11.9	5.0	8.0	19
Frontal cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	19

Characteristics ^a	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Anterior end to buccal cirrus, distance	29.1	29.0	1.8	0.4	6.3	26.0	33.0	19
Buccal cirri, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	19
Ventral cirral rows, number ^b	5.0	5.0	0.0	0.0	0.0	5.0	5.0	19
Dorsal cirral rows (with bristles), number	4.1	4.0	0.5	0.1	11.2	3.0	5.0	19
Dorsal cirral rows (without or few bristles), number	1.4	1.0	–	–	–	0.0	2.0	19
Ventral row 3, AE to begin, distance	8.5	9.0	0.6	0.1	7.2	7.0	9.0	19
Ventral row 3, AE to end, distance	45.7	45.0	2.6	0.6	5.7	42.0	52.0	19
Ventral row 3, number of cirri	8.1	8.0	0.8	0.2	10.5	7.0	10.0	19
Ventral row 4, AE to begin, distance	13.2	13.0	1.4	0.3	10.5	11.0	15.0	19
Ventral row 4, AE to end, distance	82.7	83.0	4.7	1.1	5.7	75.0	91.0	19
Ventral row 4, number of cirri	14.1	14.0	1.3	0.3	9.0	12.0	16.0	19
Ventral row 5, AE to begin, distance	6.9	7.0	0.9	0.2	13.6	6.0	9.0	19
Ventral row 5, AE to end, distance	81.3	81.0	5.9	1.4	7.3	72.0	95.0	19
Ventral row 5, number of cirri	14.4	14.0	1.3	0.3	9.3	12.0	17.0	19
Right marginal row, AE to begin, distance	13.5	13.0	2.4	0.5	17.6	10.0	20.0	19
Right marginal row, AE to end, distance	83.8	83.0	6.2	1.4	7.3	72.0	97.0	19
Right marginal row, number of cirri	14.3	15.0	1.9	0.4	13.2	11.0	17.0	19
Left marginal row, AE to begin, distance	53.4	53.0	2.0	0.5	3.7	50.0	57.0	19
Left marginal row, AE to end, distance	83.6	83.0	4.8	1.1	5.7	75.0	91.0	19
Left marginal row, number of cirri	8.7	9.0	0.9	0.2	10.0	7.0	10.0	19
Dorsal kineties, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	19
Dorsal kinety 1, AE to begin, distance	27.6	27.0	2.7	0.6	9.8	24.0	32.0	19
Dorsal kinety 1, number of bristles	20.7	21.0	2.0	0.5	9.6	17.0	25.0	19
Dorsal kinety 2, AE to begin, distance	12.1	12.0	3.5	0.8	29.3	6.0	21.0	19
Dorsal kinety 2, number of bristles	22.3	22.0	2.4	0.6	10.9	18.0	27.0	19
Dorsal kinety 3, number of bristles	23.0	23.0	2.9	0.7	12.5	19.0	31.0	19
Caudal cirri, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	19

^aData based on mounted, protargol-impregnated, and randomly selected specimens from a raw culture, except of characters 4–6, which are from a pure culture. Measurements in μm . AE, anterior end of body; AZM, adoral zone of membranelles; CV, coefficient of variation in %; M , median; MA, macronucleus; Max, maximum; Min, minimum; n , number of specimens investigated; SD, standard deviation; SE, standard error of arithmetic mean; $\bar{x}$, arithmetic mean.

^bWith marginal rows, but without first and second cirral row consisting only of the first frontal cirrus and the second frontal cirrus plus the buccal cirrus, respectively.

Table 2
Scanning electron microscopic morphometric data on *Cotterillia bromelicola*.

Characteristics ^a	$\bar{x}$	<i>M</i>	<i>SD</i>	<i>SE</i>	<i>CV</i>	<i>Min</i>	<i>Max</i>	<i>n</i>
Body, length	92.1	90.0	8.7	2.0	9.5	81.0	110.0	19
Body, width	33.1	32.0	3.9	0.9	11.7	28.0	43.0	19
Body, length:width ratio	2.8	2.7	0.2	0.1	6.9	2.6	3.4	19
Cirri, length (marginal and ventral)	16.2	16.0	2.4	0.5	14.8	12.0	21.0	19
First frontal cirrus, length	18.7	18.0	3.1	0.8	16.8	14.0	25.0	19
Dorsal bristles, length	2.8	2.6	0.7	0.2	24.8	2.0	5.0	19
Frontal membranelles, cilia length	16.1	16.0	1.5	0.4	9.6	13.0	19.0	19
Ventral membranelles, cilia length	10.1	10.0	1.4	0.3	13.5	7.0	12.0	19
Paroral membrane, cilia length	6.8	7.0	1.1	0.3	16.3	5.0	8.0	12

^aData based on randomly selected specimen from a pure culture prepared as described in the method section. Measurements in μm . *CV*, coefficient of variation in %; *M*, median; *Max*, maximum; *Min*, minimum; *n*, number of specimens investigated; *SD*, standard deviation; *SE*, standard error of arithmetic mean; $\bar{x}$, arithmetic mean.