Ann. Naturhist. Mus. Wien	96 B	19 - 27	Wien, Dezember 1994

Bryometopus hawaiiensis sp.n., a new colpodid ciliate from a terrestrial biotope of the Hawaiian Archipelago

(Protozoa: Ciliophora)

W. Foissner*

Abstract

Bryometopus hawaiiensis sp.n. was discovered in the upper layer of a grassland soil near a temporary brook in the Volcano National Park of Hawaii. Its morphology and infraciliature were studied in live cells using interference contrast optics and in specimens impregnated with silver carbonate and protargol. The new species has two unique characters which clearly distinguish it from the congeners, viz. 4 x 2 μm sized extrusomes (mucocysts) forming a distinct shiny seam beneath the pellicle, and paroral dikinetids which are conspicuously more widely spaced in the distal than in the proximal half of the paroral membrane. It is unknown whether *B. hawaiiensis* is a true soil inhabitant or a limnetic species which developed from resting cysts deposited in the mud of the brook area during its desiccation. 16 other ciliate species occurred together with *B. hawaiiensis* of which 5 are new for the fauna of Hawaii: *Amphisiella australis* BLATTERER & FOISSNER, 1988, *Corallocolpoda pacifica* ALEKPEROV, 1991, *Cyrtolophosis elongata* (SCHEWIAKOFF, 1892), *Pseudocyrtolophosis alpestris* FOISSNER, 1980, *Spathidium longicaudatum* BUITKAMP, 1977.

Key words: *Bryometopus hawaiiensis*, new species, Bryometopia, Colpodea, Hawaii, infraciliature.

Zusammenfassung

Bryometopus hawaiiensis sp.n. wurde im Volcano National Park von Hawaii entdeckt, und zwar in der oberen Zone eines Wiesenbodens, der sich im Überflutungsbereich eines temporären Bächleins befindet. Die Morphologie und Infraciliatur der neuen Art wurden an lebenden Zellen mit dem Interferenzkontrast und an Silberkarbonat und Protargol imprägnierten Individuen untersucht. *Bryometopus hawaiiensis* hat zwei Merkmale, die ihn eindeutig von den anderen Arten der Gattung unterscheiden, nämlich 4 x 2 μm große Extrusome (Mucocysten), die einen auffallend hyalinen Saum unter der Pellicula bilden, und eine parorale Membran, bei der die Dikinetiden in der distalen Hälfte deutlich lockerer stehen als in der proximalen. Es ist unbekannt, ob *B. hawaiiensis* ein euedaphisches oder ein limnisches Ciliat ist, das sich aus Ruhezysten entwickelte, die sich während der Austrocknung des Bächleins bildeten. Von den 16 anderen Ciliaten-Arten, die in der gleichen Probe wie *B. hawaiiensis* gefunden wurden, sind die folgenden neu für die Fauna von Hawaii: *Amphisiella australis* BLATTERER & FOISSNER, 1988, *Corallocolpoda pacifica* ALEKPEROV, 1991, *Cyrtolophosis elongata* (SCHEWIAKOFF, 1892), *Pseudocyrtolophosis alpestris* FOISSNER, 1980, *Spathidium longicaudatum* BUITKAMP, 1977.

Introduction

The soil protozoan fauna of the Hawaiian Archipelago is almost unknown. During a vacation I collected some soil samples and found them to be inhabitated by many interesting new species two of which have already been described (FOISSNER 1993a, b). In this paper I shall report on a new *Bryometopus* species.

* Prof. Dr. Wilhelm Foissner, Universität Salzburg, Institut für Zoologie, Hellbrunnerstr. 34, A-5020 Salzburg, Austria.

Material and Methods

Bryometopus hawaiiensis was discovered on 8.03.1993 in a sample of grassland soil collected on 6.07.1992 in the Volcano National Park of Hawaii, Big Island, near the crater rim road (Kilauea Caldera) where the Sandalwood trail branches off (155°20' W, 19°25' N). This site is about 1200 m above sea-level and very likely aperiodically flooded by a small, flat brook because the soil is covered with mosses and algal crusts (*Nostoc* etc.). However, at the sampling date the site was virtually dry and the top soil layer (0 - 5 cm) was collected, together with some mosses and algal crusts. This sample was air-dried for 14 days in August 1992.

On 1.03.1993 the dry sample was saturated with distilled water according to the non-flooded petri dish method (FOISSNER 1993c). The rewetted soil/litter-mixture had pH 5.1 and did not contain unusual amounts of salts. *Bryometopus hawaiiensis* appeared one week after rewetting and a few individuals were still alive when the sample was discarded three weeks later.

Cells were carefully studied in vivo using a high-power oil immersion objective, differential interference contrast, and video-microscopy (FOISSNER 1993c). Extrusomes were stained with methyl green-pyronin (FOISSNER 1993c). The silver carbonate method, as described in FOISSNER (1993c), was used to reveal the infraciliature; it yielded excellent, non-permanent preparations. Morphometry was done on protargol (FOISSNER 1993c, protocol 1) impregnated cells. However, the results with this method were rather mediocre, as in other members of the genus (FOISSNER 1993c).

Counts and measurements on silvered specimens were performed at a magnification of X 1,000. In vivo measurements were conducted at a magnification of X 250 - 1,000. Although these provide only rough estimates, it is convenient to give such data as specimens usually shrink in preparations or may even contract during fixation. Standard deviation and coefficient of variation were calculated following textbooks on statistics.

Terminology is according to the monograph by FOISSNER (1993c). All data are based on "field material" cultured with the method mentioned above.

Acknowledgements

The technical assistance of Maria Waldhör, Andreas Zankl and Mag. Eric Strobl is greatly acknowledged.

Description of *Bryometopus hawaiiensis* sp.n.

Data shown in Table 1 are not repeated in the description, which follows the pattern used by FOISSNER (1993c) in his monograph on colpodid ciliates.

D i a g n o s i s: In vivo about 60 x 40 μm, ellipsoid. 1 macronucleus and 1 micronucleus. Extrusomes very conspicuous, about 4 x 2 μm, form distinct shiny seam beneath pellicle. Paroral membrane with 28 dikinetids on average, those in distal half conspicuously more widly spaced than those in proximal half. 27 somatic kineties and 35 adoral organelles on average.

T y p e l o c a t i o n: Grassland soil near the entrance to the Sandalwood trail in the Volcano National Park, Big Island, Hawaiian Archipelago, 155°20' W, 19°25' N, about 1200 m above sea-level.

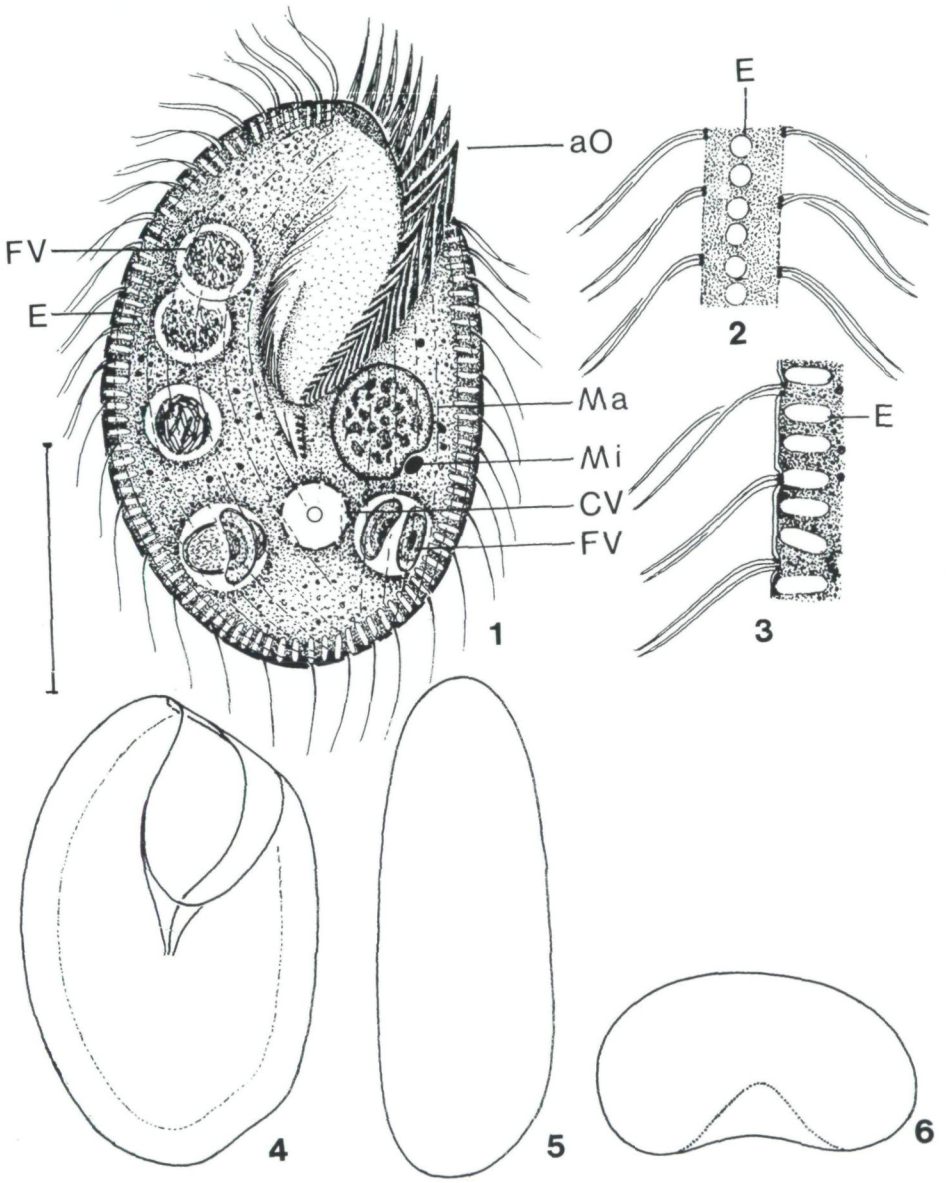


Fig. 1 - 6: *Bryometopus hawaiiensis* from life. (1) Right lateral (ventral) view of typical, ellipsoid specimen. (2) Surface and (3) lateral view of cortex at high magnification showing the conspicuous extrusomes. 4 - 6. Video record of a thylakidiform specimen in (4) right lateral, (5) dorsal and (6) transverse view. aO - adoral organelles, CV - contractile vacuole, E - extrusomes (mucocysts) forming distinct hyaline seam beneath pellicle, FV - food vacuoles, Ma - macronucleus, Mi - micronucleus.

T y p e s p e c i m e n s: Holotype and one paratype of *B. hawaiiensis* as two slides of protargol impregnated cells have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz, Austria. One paratype slide has been deposited in the Naturhistorisches Museum in Vienna.

E t y m o l o g y: Named after the location it was found, i.e. Hawaii.

D e s c r i p t i o n: Size in vivo about 50 - 70 x 35 - 45 μm. Ellipsoid to ovoid or slightly block-shaped, distal half of adoral zone of organelles extends more or less obliquely truncate at left anterior portion, near posterior end often slightly indented; up to 2:1 flattened laterally (Text Fig. 1, 4 - 6; Plate 1, Fig. 1 - 3). Macronucleus globular to slightly ellipsoid, always in triangular area deliminated by proximal portion of adoral zone of organelles, contractile vacuole and left (ventral) body margin, with many irregular nucleoli forming reticulate pattern (Text Fig. 1, 7 - 8; Plates 1 - 2, Fig. 5 - 6, 8, 11). Single, possibly abnormal specimen with two macronuclear segments and micronucleus interposed (Plate 2, Fig. 12). Contractile vacuole in median of posterior third, close below proximal end of adoral zone of organelles. Extrusomes ellipsoid, about 4 x 2 μm, blister-like, i.e. without dense content, form conspicuous, shiny seam beneath pellicle, released if cell is pressed between cover glass and slide or treated with methyl green-pyronin, when they extend to about 10 μm long threads (Text Fig. 1 - 3; Plates 1, 2, Fig. 1 - 5, 13). Cytoplasm colourless, contains many small granules and 7 - 12 μm sized food vacuoles with bacteria, flagellates (*Polytoma* sp.), ellipsoid green algae, and detritus. Movement without peculiarities.

Somatic infraciliature as in other members of genus, i.e. composed of paired, rather evenly spaced and inclined dikinetids forming slightly spirally arranged ciliary rows (Text Fig. 1, 7; Plates 1 - 2, Fig. 6 - 10). Both basal bodies of dikinetids ciliated in anterior body half and along adoral zone of organelles, anterior cilium lacking in dikinetids of posterior body half. 5 - 7 dikinetids form short kinety right of paroral membrane in about 50% of specimens (Plates 1 - 2, Fig. 7, 10). Postoral suture distinct, cilia not condensed below adoral zone of organelles (Text Fig. 7; Plates 1, 2, Fig. 6 - 10). Fibrillar associates of dikinetids as in *B. atypicus* (FOISSNER 1993c; Text Fig. 9; Plate 2, Fig. 9 - 10).

Oral aperture in left anterior quadrant of cell, oriented at about 30° to longitudinal body axis. Vestibulum broad-elliptical, shallow, opens into short, tubular pharynx with entrance bordered by indistinct lip on right side. Anterior region of right vestibular wall overhangs distal portion of adoral zone of organelles (Text Fig. 1, 4, 8). Paroral membrane loosely alinged with somatic kinety 1, consists of distinctly inclined dikinetids widely spaced in distal and narrowly spaced in proximal portion of organelle, appears very short in live cells because loosened portion looks like a somatic kinety (Text Fig. 7 - 8; Plates 1 - 2, Fig. 6 - 7, 9 - 10); indeed, in some specimens a few somatic kinetids occur at anterior end of paroral membrane, as indicated by their fibrillar associates (Plates 1 - 2, Fig. 7, 10). Adoral zone of organelles commences on anterior pole of cell and extends along obliquely truncate left anterior body margin, where it curves to centre of cell (Text Fig. 1, 4, 7 - 8; Plates 1 - 2, Fig. 1 - 2, 5 - 7, 9). Adoral organelles each composed of 2 equally long kineties and 1 short row forming small knob at left side of organelles; those within pharynx have short projections, possibly fibrills, extending to and surrounding right pharyngeal wall (Text Fig. 7 - 8; Plates 1 - 2, Fig. 6, 9 - 11).

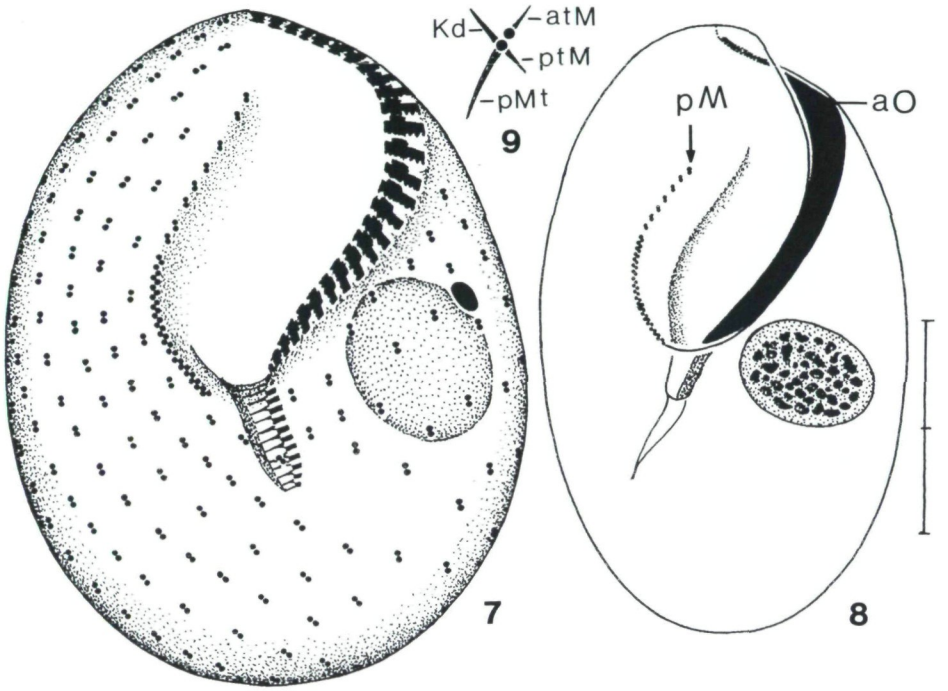


Fig. 7 - 9: *Bryometopus hawaiiensis* after silver carbonate (7, 9) and protargol (8) impregnation. (7) Right lateral (ventral) view of slightly squashed specimen. (8) Right lateral (ventral) view of a specimen with impregnated paroral membrane and adoral zone of organelles. (9) Fibrillar associates of a somatic kinetid. aO - adoral organelles, atM - transverse fibre of the anterior basal body of the dikinetid, Kd - kinetodesmal fibre, pM - paroral membrane, pMt - postciliary fibres, ptM - transverse fibre of the posterior basal body of the dikinetid.

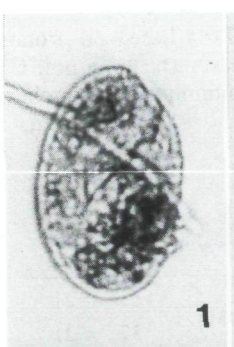
O c c u r r e n c e and e c o l o g y: As yet found only at type location, together with the following species of which those marked with an asterisk are new for the fauna of Hawaii: **Amphisiella australis* BLATTERER & FOISSNER, 1988, *Bryometopus triquetus* FOISSNER, 1993 (a typical and individual-rich population), *Colpoda inflata* (STOKES, 1885), *C. steinii* MAUPAS, 1883, **Corallocolpoda pacifica* ALEKPEROV, 1991 [a small-sized population like that described by FOISSNER (1993) from South Africa], *Cyclidium muscicola* KAHL, 1931, **Cyrtolophosis elongata* (SCHEWIAKOFF, 1892), *C. mucicola* STOKES, 1885, *Drepanomonas pauciciliata* FOISSNER, 1987, *Leptopharynx costatus* MERMOD, 1914, *Nivaliella plana* FOISSNER, 1980, *Platyophrya vorax* KAHL, 1926, **Pseudocyrtolophosis alpestris* FOISSNER, 1980, *Pseudoplatyophrya nana* (KAHL, 1926), *Sathrophilus muscorum* (KAHL, 1931), and **Spathidium longicaudatum* BUITKAMP, 1977 (with extrusomes as described in type population, i.e. anchored not only in oral apparatus but also in somatic cortex). This list of species is based on 5 inspections during 4 weeks.

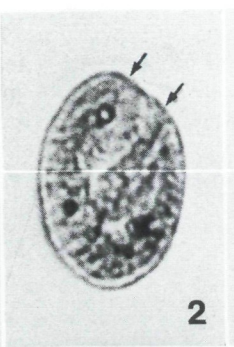
Table 1. Morphometric characteristics from *Bryometopus hawaiiensis*. Data based on protargol (P) or silver carbonate (S) impregnated specimens from raw culture. Measurements in μm. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of specimens investigated, SD - standard deviation, SD$\bar{x}$ - standard deviation of the mean, $\bar{x}$ - arithmetic mean.

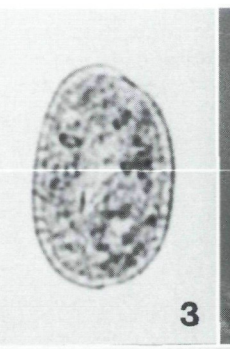
Character	$\bar{x}$	M	SD	SD$\bar{x}$	CV	Min	Max	n
Body, length (P)	51.6	52.0	4.4	1.0	8.5	45	63	21
Body, width in lateral view (P)	30.1	30.0	2.6	0.6	8.6	27	37	21
Distance anterior end to macronucleus (P)	23.5	23.0	3.0	0.7	12.9	19	29	21
Distance anterior end to vestibular vertex (P)	29.4	30.0	2.2	0.5	7.3	26	34	21
Distance anterior end to proximal end of adoral zone (P)	33.9	33.0	2.5	0.5	7.3	30	39	21
Macronucleus, length (P)	11.4	11.0	1.6	0.3	14.0	8	11	21
Macronucleus, width (P)	9.6	9.0	1.3	0.3	13.4	8	11	21
Macronucleus segments, number (S)	1.0	1.0	0	0	0	1	1	11
Micronuclei, number (S)	1.0	1.0	0	0	0	1	1	11
Somatic kineties, number (S)	27.3	27.5	1.9	0.6	7.1	25	30	10
Dikinetids in left lateral (dorsal) kinety, number (S)	14.9	14.0	2.1	0.7	14.3	12	19	10
Adoral organelles, number (S)	35.1	35.0	2.9	0.9	8.3	31	42	11
Paroral dikinetids, number (S)	29.2	28.0	3.5	1.0	11.8	24	36	11

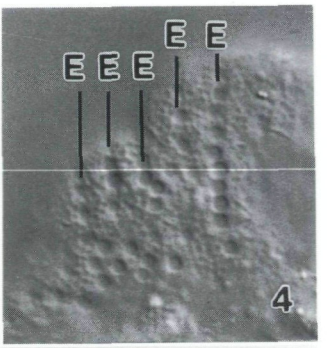
Bryometopus hawaiiensis must be a rare species since I have not found it in about 1000 other soil and moss samples collected worldwide; it is probably endemic to the Hawaiian Archipelago. The ciliate fauna at the site investigated is composed of few species and most (66%) of them are r-selected colpodids (*Bryometopus* spp., *Colpoda* spp., *Corallocolpoda*, *Cyrtolophosis* spp., *Nivaliella*, *Platyophrya*, *Pseudocyrtolophosis*, *Pseudoplatyophrya*), indicating extreme conditions (FOISSNER 1993c). However, it is unknown whether *B. hawaiiensis* is a true soil inhabitant or a limnetic species which developed from resting cysts deposited in the mud of the brook area during its desiccation.

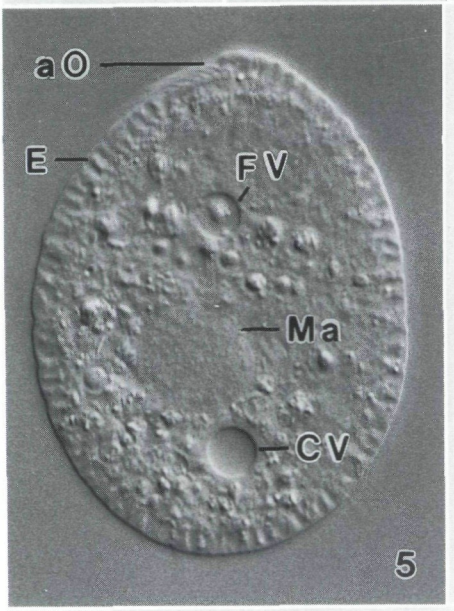
Plate 1. *Bryometopus hawaiiensis* from life (1 - 5) and after silver carbonate impregnation (6 - 8). (1 - 3) Bright field micrographs of typical cells showing the ellipsoid body shape and the hyaline extrusome seam underneath the pellicle. Arrows mark vestibular opening. (4) Interference contrast micrograph of cortex surface showing distinct extrusome row between each somatic kinety. (5) Interference contrast micrograph of a slightly squashed specimen showing the distinct extrusome seam underneath the pellicle and some main cell organelles. (6 - 8) Somatic and oral infraciliature in right lateral (ventral) and left lateral views. aO - adoral zone of organelles, CV - contractile vacuole, E - extrusomes (mucocysts), FV - food vacuole, Ma - macronucleus, Mi - micronucleus, pM - paroral membrane.

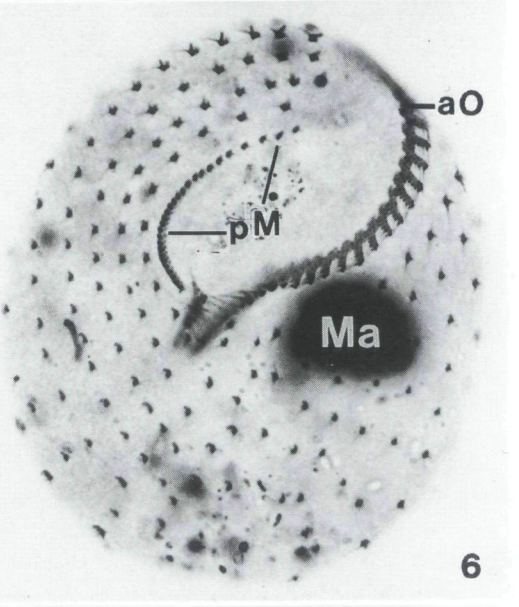


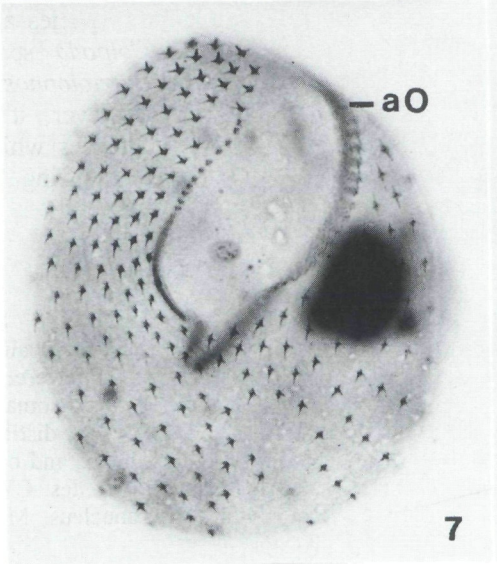


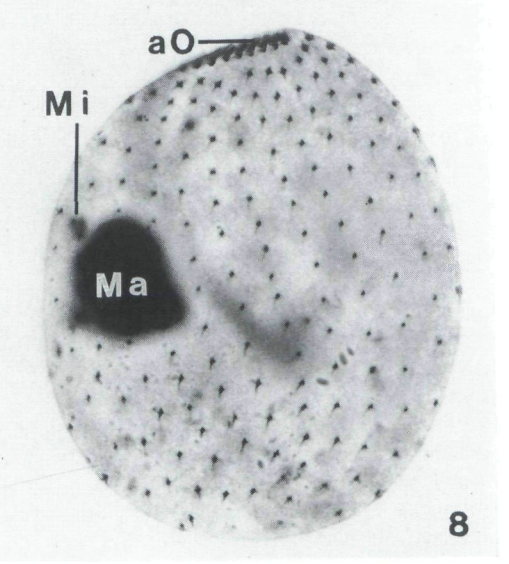


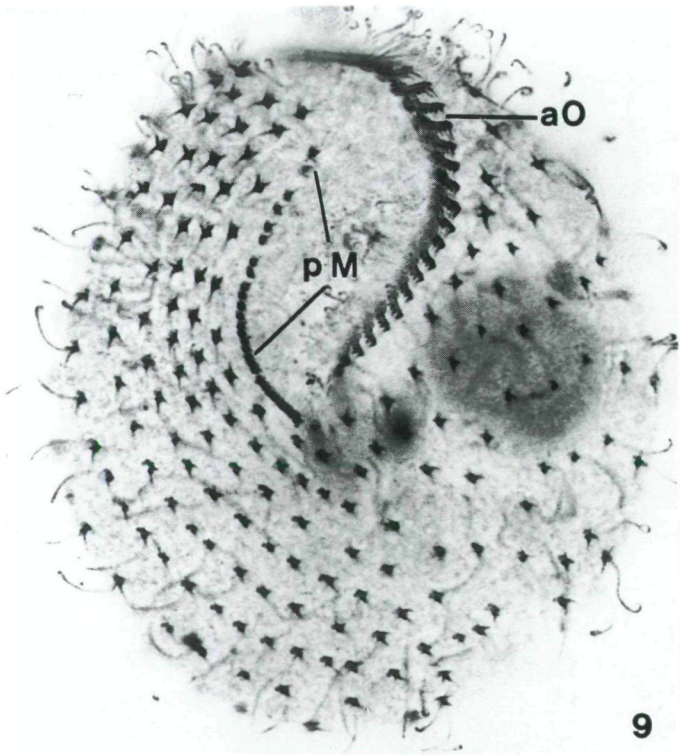




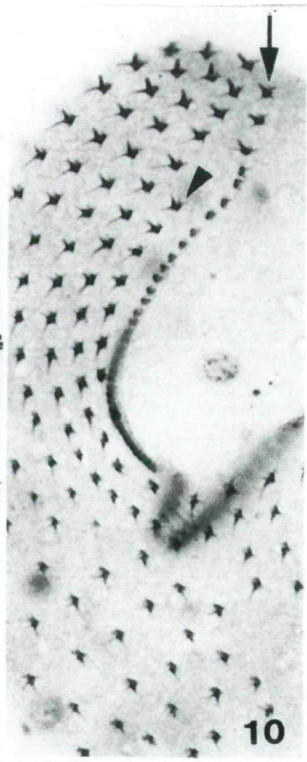




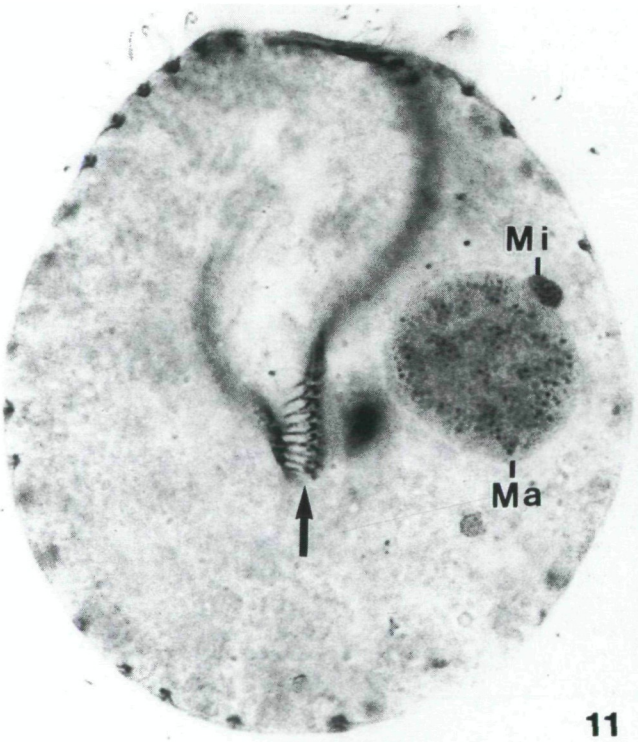




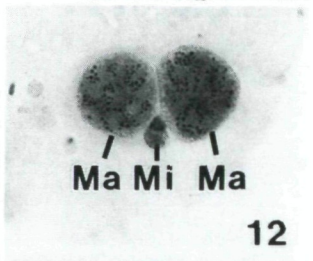
9



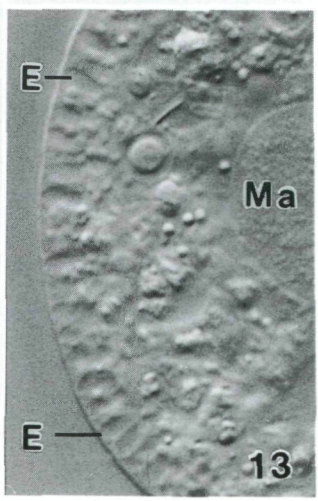
10



11



12



13

Discussion

FOISSNER (1993c) recognized 8 *Bryometopus* species in his revision of the genus. Very likely, all have extrusomes of the mucocyst type which are, however, smaller and thus less conspicuous than in *B. hawaiiensis*. It was in fact the distinct hyaline seam produced by the extrusomes which induced me to look at this species in more detail. An analogous situation is found in the genus *Colpoda*, where *C. lucida* has very conspicuous extrusomes which look quite similar to those of *B. hawaiiensis* (FOISSNER 1993c).

In 6 of the 8 *Bryometopus* species hitherto described the structure of the paroral membrane has been studied in silver prepared cells. All have the paroral dikinetids evenly and narrowly spaced (FOISSNER 1993). It is thus reasonable to use the distal loosening of the paroral dikinetids as a second main character of *B. hawaiiensis*.

Bryometopus hawaiiensis is very likely most closely related to *B. pseudochilodon* and *B. triquetus*, as indicated by the body size and location of the contractile vacuole as well as the number of somatic kineties and adoral organelles. The ellipsoid body shape and some details of the oral apparatus resemble *Thylakidium*, a genus closely related to *Bryometopus* (FOISSNER 1993c).

Live and silver impregnated cells must be studied for a reliable determination of *B. hawaiiensis* because the extrusomes do not stain with silver carbonate and protargol, whereas the distal loosening of the paroral dikinetids is difficult to recognize in vivo.

References

FOISSNER, W. 1993a: *Idiocolpoda pelobia* gen. n., spec. n., a new colpodid ciliate (Protozoa, Ciliophora) from an ephemeral stream in Hawaii. - Acta Protozool. 32: 175-182.

FOISSNER, W. 1993b: *Corticocolpoda kaneshiroae* n. g., n. sp., a new colpodid ciliate (Protozoa, Ciliophora) from the bark of Ohia trees in Hawaii. - J. Euk. Microbiol. 40: 764-775.

FOISSNER, W. 1993c: Colpodea (Ciliophora). G. Fischer, Stuttgart, Jena, New York. 798 pp.

Plate 2. *Bryometopus hawaiiensis* after silver carbonate impregnation (9 - 12) and from life (13). (9, 11) Ventral view of a heavily squashed specimen photographed in two levels to show details of the oral and nuclear apparatus. Arrow marks pharyngeal adoral organelles. (10) Details of the somatic infraciliature (see text figure 9 for explanation of fibrillar associates). Arrow marks 3 somatic dikinetids aligned with paroral membrane; arrowhead points to short kinety right of paroral membrane. (12) Abnormal specimen having two macronuclear segments with micronucleus interposed. (13) Interference contrast micrograph showing the distinct extrusome seam underneath the pellicle. aO - adoral zone of organelles, E - extrusomes (mucocysts), Ma - macronucleus, Mi - micronucleus, pM - paroral membrane.