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Euplotes iliffei n.sp.: A new species of *Euplotes* (Ciliophora, Hypotrichida) from the marine caves of Bermuda.

Bruce F. Hill

Department of Biology, Georgetown University,
Washington, D.C. 20057

Eugene B. Small

Department of Zoology, University of Maryland,
College Park, MD 20742

Thomas M. Iliffe

Bermuda Biological Station for Research,
Ferry Reach 1-15, Bermuda

ABSTRACT

Euplotes iliffei n.sp., a new anchialine species of *Euplotes* from the marine caves of Bermuda is described. *E. iliffei* has a dorsal interkinetal argentophilic reticulum of the multiple to complex type with a tendency toward 4 interkinetal polygonal areas. Like other members of the group of *Euplotes* that have a frontoventral cirri in pattern I the VI/2 cirrus is missing. *E. iliffei* also has a very pronounced notch in the upper border of the dorsal surface.

Introduction

The limestone platform that makes up the Bermuda Islands is composed of Pleistocene and recent, marine and eolian limestones which overlay a mid-ocean volcanic sea mount. Most of Bermuda's caves were formed when sea level lowered during periods of glaciation as a result of dissolution by slightly acidic percolating ground waters. The caves were subsequently flooded by marine waters when

sea level rose during postglacial periods (1,2). Extensive horizontal cave passages, some being more than 2.0 km in length, have been explored and mapped utilizing sophisticated cave diving techniques (3,4).

Recent studies on marine animals inhabiting these subterranean anchialine habitats has revealed the presence of diverse endemic macro-invertebrate faunas (5,6,7). However, during these earlier cited comprehensive cave faunal surveys, samples containing possible cave protozoa were not collected. Newer studies are currently examining these same caves for protozoa, and a rich and diverse anchialine ciliated protozoa fauna has been established (8). Included among the new ciliated protozoa are several species of *Euplotes*, one of which is described here.

In the literature over 80 species and varieties of *Euplotes* have been described in the last 200 years, many of which are now considered junior synonyms as reviewed by Hill, 1980 (9). Curds (10) in his 1975 guide to the genus listed 51 different species of *Euplotes*. In the last few years several new species have been described. Jones and Owen (11) described *E. nana* and Ten Hagen (12) characterized *E. palustris*. *E. terricola* originally described by Penard (13) is no longer considered a member of the genus *Euplotes* because of the spatial arrangement of the frontoventral and transverse cirri and the presence of many left marginal cirri. Thus, we now consider there to be 52 valid species in the genus *Euplotes*. This paper describes the first anchialine species of *Euplotes* (*Euplotes iliffei* n.sp.) from the marine caves of Bermuda.

Materials and Methods

Euplotes iliffei n.sp. was collected along with many other protozoa in Wonderland Cave. This cave, located in the Hamilton Parish, Bermuda, was previously known as Whitby Cave. The cave was open to the public until the 1940's when it was closed as a commercial tourist cave. A small entrance building gives access to a steep set of stairs which lead to the first room of the cave. This large room contains a sea level lake which is about 60 m long by 12 m wide. A 50 m long underwater passage connects this room to a second smaller air chamber. No known human-sized passageways connect the Wonderland Cave system with Castle Harbour, the nearest body of water which is 420 m from the inland entrance of the cave (14).

Ciliated protozoa were collected in the surface waters of the entrance room of Wonderland Cave using small protozoan traps baited with tuna fish (15). At the time of collection the water temperature ranged from 20.2°–21.2°C and the surface salinity was 12‰. *E. iliffei* was maintained in Millipore filtered sea water (20‰) with wheat grains at 20°C after initial isolation on tuna fish and associated decay bacteria from the protozoan traps.

For light microscope observations of cortical ciliary structures and their morphogenesis during cell division, the cells were stained by a modification (16) of the protargol method of Jerka-Dziadosz and Frankel (17). To demonstrate specific cortical structures of the argyrome, preparations were made using Corliss' (18) modification of the Chatton-Lwoff technique of silver impregnation. Borror's nigrosin-$HgCl_2$-formalin stain and fixative (19) was used to observe cortical sculpturing. For determining nuclear shape, the cells were fixed in 2.0% glutaraldehyde, washed in distilled water and afixed to cover slips with Mayer's albumin and feulgen stained following the procedures of DiStephano (20). Drawings were prepared with a Nikon drawing instrument and the terminology of the ventral ciliary structures were based upon the topographical and developmental characteristics as previously outlined for other *Euplotes* species (9,21,22).

Results

Measurements. Total body length 90–115 μm (average 101 μm); body width 70–100 μm (average 85 μm); buccal cavity length 68–88 μm (average 72 μm). (n = 25).

Body Shape (Figs. 1–5). *E. iliffei* is a medium size marine *Euplotes* with an ellipsoidal body shape. The right margin is more convex than the left with the widest point being slightly posterior to the equator of the cell. There is a prominent notch in the upper border of the aboral surface. The posterior end is rounded. The buccal cavity is narrow, extending about ⅗ of the length of the body with the right buccal overture extending from the left most frontoventral cirrus ventro-laterally in a convex curve ending at the anterior most left marginal cirrus. From a mid-point along the right buccal overture, the buccal cavity cuts a medial recess that extends posteriorly to the cytosome. The aboral zone of membranelle (AZM) extends along ⅔ of the left side of the ventral surface in a prominent convex curve turning more dextrally near the cytosome. The AZM archs over the anterior end of the cell with a thin browlike extension bordering the AZM antero-dorsally. On the dorsal surface are 5 prominent single-edged ridges with the left most sixth ridge being double-edged. Each of the ridges are associated with a single kinety. Also a single kinety is associated with a very prominent double-edged ridge that separates the right lateral surface and the ventral surface. On the ventral surface is a wide prominent ridge that extends along the right side and four small ridges that extend anteriorly from between the transverse cirri with the most prominent ridge being between cirri, 1/III and 1/IV. The contractile vacuole pore is ventral, posterior of transverse cirrus 1/VII.

Surface organelles. (Figs. 1–3). There are nine frontoventral, five transverse, two left marginal and two caudal cirri. The number of frontoventral and transverse cirri was constant in over 100 specimens and less than 4% variation in the number of left marginal (1 left marginal cirrus) and caudal (3 caudal cirri) cirri. There is a longitudinal group of endoral cilia in a rectangular field along the posterior part of the buccal cavity. The AZM possesses 28 to 36 membranelles (average = 33). The kinetosomes of the dorsal kinety are variable in number and located in eight kinetal rows (16% of the organisms have 9 kinetal rows). Kinetal row number 1 (found on the left ventrolateral

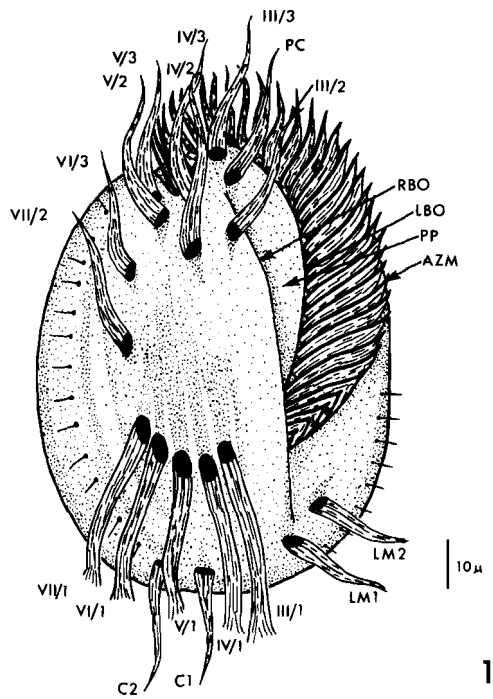

Fig. 1. Ink line diagram of the ventral aspect based on a protargol stained specimen.

Figs. 1–7. Line diagrams of *Euplotes iliffei* n.sp. Key: frontoventral cirri III/2, III/3, IV/2, IV/3, IV/3, V/2, V/3, VI/3, VII/2; PC, paroral cirrus or cirrus II/1; transverse cirri III/1, IV/1, V/1, VI/1, VII/1; right caudal cirri C1, C2; left marginal cirri LM1, LM2; EC, endoral cilia; AZM, adoral zone of membranelles; K1–K8, kinetical rows 1 thru 8. RBO, right buccal overture; LBO, left buccal overture; PP, peristomial plate; CVP, contractile vacuole pore; CS, cytostome; Ma, macronucleus; Mi, micronucleus.

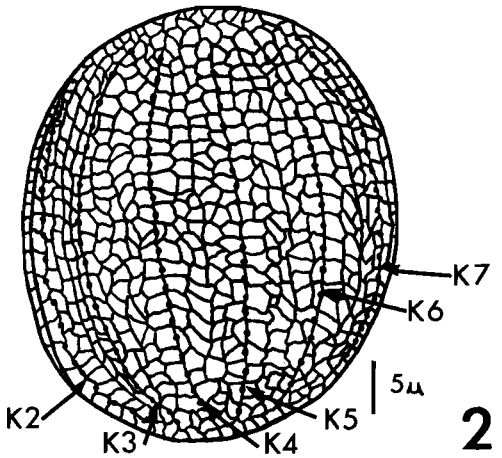

Fig. 2. Dorsal infraciliature, showing position of paired kinetic and argentophilic network.

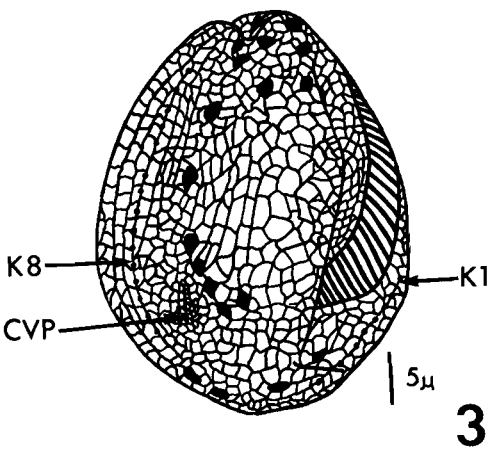

Fig. 3. Ventral infraciliature, indicating ciliary organelles, contractile vacuole pore and argentophilic network.

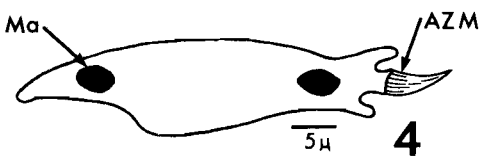

Fig. 4. Optical longitudinal-section at the level of midpoint of the cell.

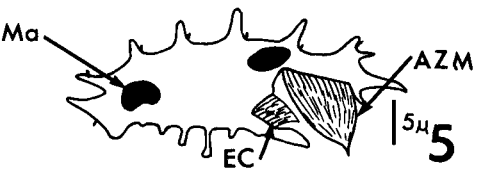

Fig. 5. Optical cross-section at the level of midpoint of the cell.

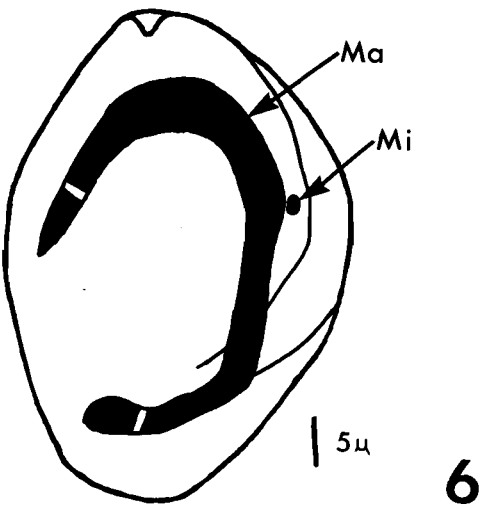

Fig. 6. Micronucleus and macronucleus; replication bands indicated on the macronucleus.

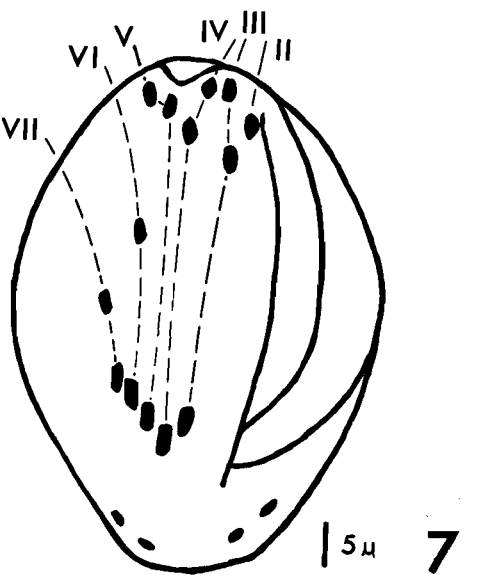

Fig. 7. Diagram of frontoventral cirrus development; occurring during curtical morphogenesis of cell division.

surface just to the left of the AZM) is the shortest row having from 4–10 kinetids of paired kinetosomes (average 7.3). The remaining kinetal rows are numbered consecutively to the cell's right with an increase modal number of kinetids (row 2, 17–24 (average 18.4); row 3, 18–23 (average 20.6); row 4, 17–24 (average 20.8); row 5, 18–23 (average 20.1); row 6, 16–22 (average 19.1); row 7, 16–21 (average 18.0); row 8, 11–17 (average 13.8)).

Silverline system. In wet-silver Chatton-Lwoff preparations, the dorsal interkinetal argentophilic reticulum is of the multiple to complex type consisting of an assemblage of polygenes which have a tendency toward 4 regular rows between the kinetis. The argentophilic meshwork on the ventral surface consists of an irregular assemblage of polygones.

Nuclear configuration. (Fig. 6). The interphase macronucleus is usually C-shaped with the posterior end being flattened and more irregular posterior of the AZM. The micronucleus is small, nearly spherical and located in the upper right half of the cell adjacent to the flattened back of the macronucleus.

Morphogenesis. (Fig. 7). The buccal and frontal ciliature, with the exception of the AXM, develop from an orderly series of ciliary streaks labeled with Roman numerals from the ciliate's left to right. The endoral cilia develop from streak I while the paroral cirrus (II/1) from streak II. The other frontoventral and transverse cirri develop from streaks III–VII. After distinct fields of cirri have formed for both the proter and opisthe from the five original ciliary streaks each field consists of five transverse cirri (III/1–VII/1) and nine frontoventral cirri (II/1 (paroral cirrus), III/2, III/3, IV/2, IV/3, V/2, V/3, VI/3 and VII/2). As the new ciliary structures of the developing daughter cells migrate to their final position and parental cirri are dedifferentiated and resorbed, an equatorial cleavage furrow forms that will result in the cytokinesis.

Discussion

There are 22 described species of *Euplotes* which have a 9 frontoventral cirrotype. Fifteen of these species belong to the type one frontoventral cirrotype pattern where cirrus VI/2 is absent from the frontoventral arrangement (9,21,23). The 9 marine, 2 euryhaline and 4 freshwater species that belong to this group all have a double to complex dorsal argyrome. Several members of this group have 8 or fewer frontoventral cirri. *E. parkei*, when grown in a marine environment, is missing cirrus IV/2 which is present when grown in fresh water (24). *E. poljanskyi* has eight frontoventral cirri with a cirrus missing from row V or VI (25). *E. raikovi*, which has 7 or 8 cirri, is always missing cirri III/2 and IV/2; however in some populations, cirrus VI/2 is present (25) and in others, it is only an argentophilic plaque (21). *E. strelkovi* has eight frontoventral cirri and six transverse cirri which are in the same cirral pattern as *E. raikovi* except that an additional cirral primordia streak develops between streaks IV and V, thus giving rise to the additional frontoventral and transverse cirri (26). *E. parkei* and *E. elegans* are both euryhaline species while *E. affinis, E. gracilis, E. muscicola* and *E. muscorum* are all from fresh water. Both *E. parkei* and *E. affinis* have a double dorsal argyrome silver system whereas *E. elegans, E. gracilis, E. muscicola* and *E. muscorum* have a complex dorsal argyrome system where there are from four to many polygonal areas within each interkinetal area. *E. apsheronicus, E. bisuleatus, E. dogieli, E. latus, E. nana* and *E. zenkewitchi* are all marine species that have a double argyrome silver-line system. *E. elegans* however is smaller both in length (80 μm) and width (55 μm) and has more oral membranelles (AZM) in it, 40–45. Also in *E. elegans* the central kineties have more dikinetids (40–45) and the dorsal argyrome has many more polygonal areas in each interkinetal zone.

Seven species of *Euplotes* have been described that have the second type of frontoventral cirrotype pattern where cirrus VI/3 is missing. All these species are from freshwater and have double dorsal argyrome system. The silver-line systems have not been described in six undefined but recognizable species; *E. novemcarinata*, *E. rotunda*, *E. terricola*, *E. aberrans*, *E. roscoffensis* and *E. thononensis*. The first three of these species have only been found in freshwater. *E. roscoffensis* has 10 frontoventral cirri while *E. aberrans* has only eight. *E. thononensis*, a marine species, is about the same size as *E. iliffei* and has 9 frontoventral cirri. However *E. thononensis* has a very pronounced peristomial collar and does not have a prominent notch in the upper border of the aboral surface as is seen in *E. iliffei*.

E. identatus (28) described from an intertidal pool in Nassau, Bahamas resembles *E. iliffei* in that it has an anterior notch in the upper border of the dorsal surface. However, *E. identatus* is smaller than *E. iliffei*, has 10 frontoventral cirri and has a 3 polygonal dorsal interkinetal silver-line pattern.

Acknowledgments

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