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GLAUCONEMA BERMUDENSE N. SP. (SCUTICOCILIATIDA, OLIGOHYMENOPHOREA), A TROGLOBITIC CILIOPHORAN FROM BERMUDIAN MARINE CAVES

BY

EUGENE B. SMALL, JOHN HEISLER, JAMES SNIEZEK

Department of Zoology, University of Maryland, College Park, Md. 20742, U.S.A.

&

THOMAS M. ILIFFE

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

SUMMARY

Glauconema bermudense n. sp. is herein described from protargol stained preparations of cultured cells originally recovered from a dead fish resting on the floor of the interior of Green Bay Cave and also recovered from fish baited traps at other sites in Green Bay Cave as well as sites in Church Cave, Wonderland Cave, and Tuckerstown Cave — all caves containing marine waters. Further study of this *Glauconema* sp. has revealed its presence only in interior cave waters and never external to or at the entrances of the caves. The ciliate undergoes a tomont to tomite morphogenetic transformation when well fed and in light. Cultured tomites, when placed in darkness with freshly bacterized canned tunafish revert to the trophont morphotype. These criteria lead the authors to consider this species a true protistan troglobite, the first record of such an occurrence for marine ciliate protists.

RÉSUMÉ

On décrit *Glauconema bermudense* n. sp. sur des préparations colorées au protargol de cellules cultivées, originellement récoltées sur un cadavre de poisson trouvé dans Green Bay Cave et aussi sur du poisson ayant servi à l'amorçage de pièges dans d'autres parties de la même grotte ainsi que dans Church Cave, Wonderland Cave et Tuckerstown Cave (toutes grottes contenant de l'eau marine). Des recherches effectuées sur cette *Glauconema* ont démontré sa présence uniquement dans les eaux à l'intérieur de grottes. Dans de bonnes conditions d'alimentation, et à la lumière, le cilié passe par une transformation morphogénétique de tomont à tomite. Des tomites placés *in vitro* à l'obscurité et en présence de thon en conserve depuis peu infecté par des bactéries, reviennent au morphotype trophont. Ces observations sont considérées par les auteurs comme permettant de voir dans cette espèce de protozoaire un troglobie vrai (première découverte d'un tel élément parmi les ciliés marins).

INTRODUCTION

One of us (T. Iliffe), during the course of collecting microinvertebrate crustaceans from the marine waters of the numerous submerged anchialine caves that fenestrate the Karst limestone on which the Islands of Bermuda sit, noted

the presence of ciliated protistans swimming amongst the concentrated crustaceans and called these observations to the attention of E. B. Small (pers. comm.) and others (see Iliffe, 1979). Subsequently, dives were made into the labyrinthian passages of Green Bay Cave wherein a one week old dead fish had been noted from an earlier dive and samples were slurped from its mucoid surface into plastic syringes. In the laboratory microscopic examination revealed several different morphotypic ciliates which were cultivated in the sample and then fixed in Bouin. Subsequent silver-staining revealed eight different ciliate genera, probably all new to science. Herein, we report our finding from the above and subsequent samples for one of the recovered ciliates, *Glauconema bermudense* Small n. sp.

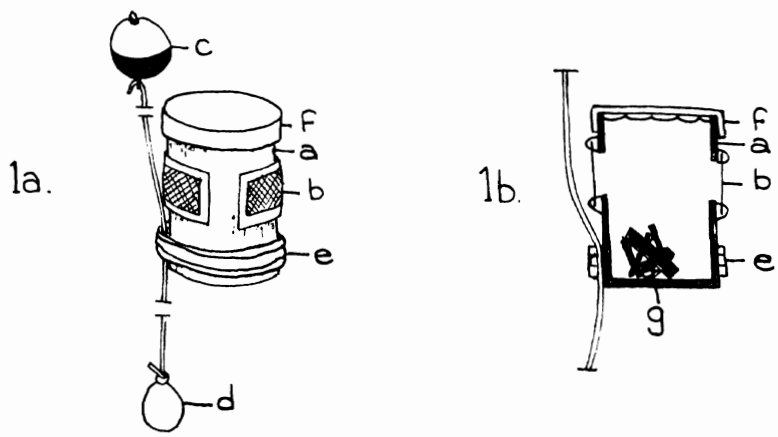

Fig. 1a, b. Diagrams of tissue-containing traps. Diagrams of the exterior (1a) and interior (lb) of a trap showing its principal features: a plastic cannister (a) (into which two 300 μm nytex screened windows (b) are situated) is equipped with a small plastic float marker (c) above and a small lead sinker weight (d) below-secured to the cannister by nylon line and girdling rubber band (e). The snapcap (f) is secured after the trap has had bait (g) placed inside and has been filled with cave water.

MATERIALS AND METHODS

Glauconema bermudense Small n.sp. was first encountered in its marine underwater cave environment from whence it was collected by cave divers, T. Iliffe and M. Van Soeren. By use of a 50 ml slurp syringe samples were taken from the surface of a decomposing dead fish situated on the floor of Green Bay Cave in a connection passage near "the desert." Fixation was accomplished with a modified Bouin fixative and subsequently, at the University of Maryland Protist Laboratory of E. B. Small, the fixed material was silver-stained utilizing the protargol procedure of Small and Lynn (see Lee et al., 1984). Living cells were examined in the laboratory at the Bermuda Biological Laboratories with Zeiss Nomarski optics. Photo-microscopy was accomplished by combining the Zeiss Nomarski optics with a Zeiss microflash and an Exacta Varex 35 mm camera back.

Subsequently, in the same time period as well as in the first weeks of April, August, and October, 1984, these and other ciliate species were trapped in "tissue traps" (see fig. 1a and b) baited with minced previously canned tuna fish which were placed at various sites and depths in Green Bay Cave as well as at the Green Bay Entrance and Cliff Pool opening (see fig. 2). The

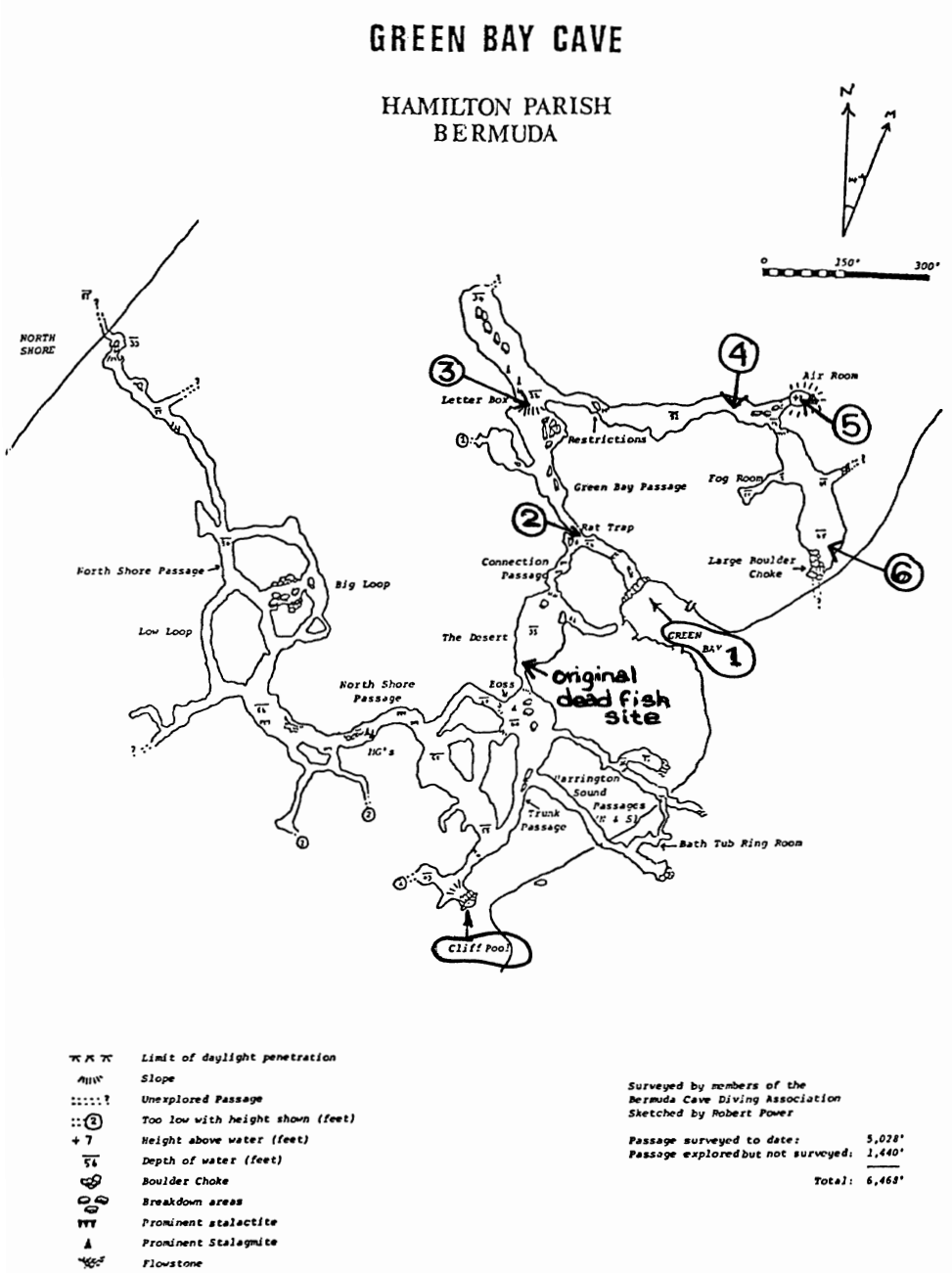

Fig. 2. Map of Green Bay Cave passages surveyed and prepared by the Bermuda Cave Diving Association. Sketches by Robert Power. Collecting sites are indicated by incircled numbers and labels.

traps were placed in residence for 48-96 hour periods by the cave diver team who subsequently recovered them by carefully enclosing the traps at their residence sites in clear plastic sterile bags which were promptly sealed in situ. On return to the Bermuda laboratories the traps contents were carefully removed to sterile petri-dishes wherein the ciliates continued to feed and multiply. Clonal isolations of the ciliates were made by mouth pipetting single cell isolates into well slides containing Millipore filtered (0.2 μm pore size) water from the cave site as well as the same tuna fish medium on which a luxuriant bacterial flocculum readily forms. Specimens were fixed from both mixed and colonally isolated populations and on return to Maryland these specimens were also silver-stained as described above.

During the same time frame, setting of traps, recovery, live observations and photomicrography, subsequent silverstaining and identification procedures were followed for sites visited in Church, Wonderland, and Tuckerstown Caves, as well. From all of these caves some of the sites also included among several ciliates recovered, the same morphotypic *Glauconema* sp. Unlike Green Bay Cave, these latter caves lack any direct connection to epigean marine waters.

Examination of stained cells derived from the subcultured cell populations, all of which had originated both in Bermuda as well as in Maryland laboratories followed a regular lighted room regimen, revealed the absence of trophic cells. Rather much smaller cells were encountered.

These small cultured cells were then placed in fresh culture containers within constant temperature boxed for periods of 96 hours and longer. Upon subsequent fixation and staining following live observation and photomicrography, we observed the return of the normal trophic morphology.

The systematic treatment of the herein described ciliate is solely the work E. B. Small who accepts full responsibility for the taxonomic identification and is the sole author of the species. Concerning questions about ciliophoran protist terminology and systematics, the reader is refered to Small & Lynn (1984).

Description of **Glauconema bermudense** Small n.sp. — The trophont.

G. bermudense as a trophic organism may be described as having features characteristic of all other members of the class Oligohymenophorea (Order Scuticociliatida) in which it should be included (see Small & Lynn, 1984). Like the other two species in the genus, *G. trihymene* Thompson, 1966, the type species, and *G. pacificum* Small & Lynn, 1984, *G. bermudense* posseses a set of distinctive preoral polykinetids that gently curve anteriorwards between kinety 1 and kinety n (see figs. 3, 4), and the first and second oral polykinetids merge together at their respective posterior and anterior margins so that they form an inverted and tapered comma-shaped organellar complex. Just posterior to the third oral polykinetid lies an inverted slender, crescent shaped third oral polykinetid just anterior to the ovoid orifice of the cytostome. To the organism's right side of these organellar complexes and curved posteriorly around the cytostome lies the oral dikinetid: the first segment a curves anteriorly to a level beyond the second oral polykinetid and posteriorly to its apex angle with oral dikinetid segment b just to the right of the space between OPK3 and OPK2; oral dikinetid b segment from its juncture with ODKa forms a gentle curve around the cytostome and there it terminates; posterior to this termination in the space between kinety 1 and kinety n lies a linear file of 4-5, mostly paired non-ciliated kinetosomes, the oral dikinetid c segment.

In the living-cell the anterior end is slightly twisted to the right with its cytostome and immediately surrounding oral area tucked into a medially located inpocket. The deformation results in the first oral polykinetid lying on

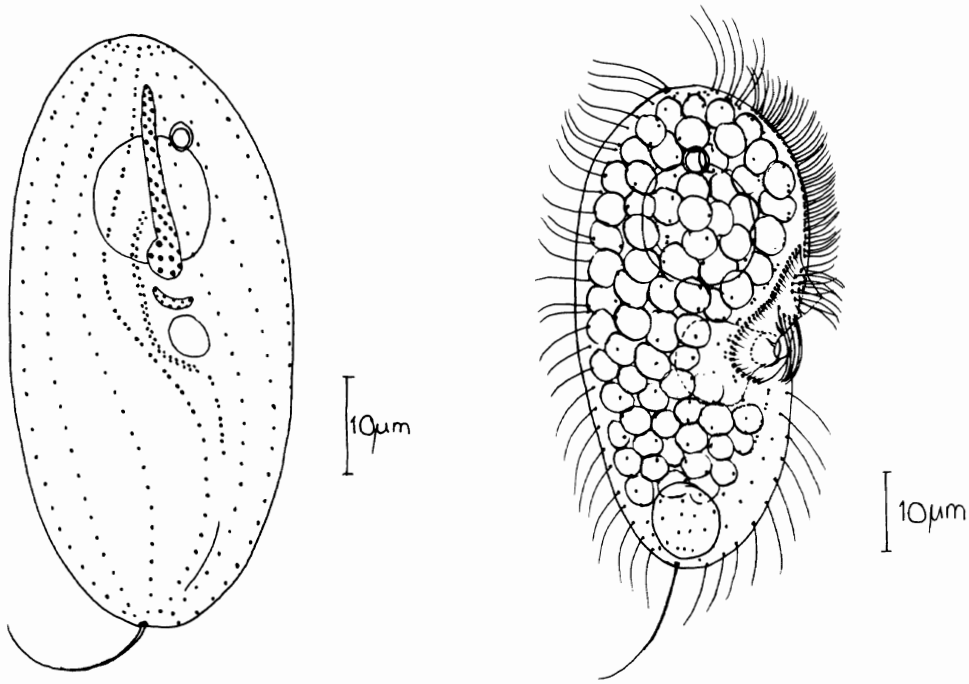

Fig. 3. (left). *Glauconema bermudense* Small, n.sp. composite figure based on protargol stained trophont specimen in ventral view. Fig. 4. (right). Reconstructed semidiagrammatic figure of living *Glauconema bermudense* Small, n.sp. trophont illustrating distribution and relative density of somatic and oral ciliation as well as the relative number and size of the numerous internal food vacuoles in a stuffed trophic cell.

a slight bulge in the anterior cell half between kineties 1 and n (fig. 3). In the fixed and silver-stained cell, this skewness is apparent, although the normally ovoid cell becomes slightly inflated and forshortened with kineties 1 and n twisted leftwards in the cytostomial and postcytostomial cell area (fig. 4).

The somatic kinetome is composed of 15-16 (mode 16, $N = 20$) bipolar kineties in which kinety 1 is clearly sigmoid and others on the ventral surface are less but similarly skewed. Aboral kineties are relatively straight. Kinety 1 immediately to the right of the oral area possesses paired kinetosome kinetids to the terminal level of the oral dikinetid c segment. The anterior most paratene of all other kineties contain paired kinetosome kinetids. Except for scattered paired kinetosome kinetids in the right anterio-ventral kinetomal region, the remainder of the kinetids contain only single kinetosomes. A 12-14 µm long caudal cilium is found terminally and slightly to the right of the posterior polar axis.

In the anterior 1/2 of the cell, slightly anterior to the level of the cytostome is situated a 10 μm^3 globular macronucleus with a 2 μm^3 single micronucleus nested on its anterior surface.

A cytoproct has been observed to expulse particulate wastes situated between kineties 1 and n posterior to the oral dikinetid c segment in the posterior curving end of the cell.

A single contractile vacuole has also been observed in the posterior end of the cell to the right of the cytoproct region. Protargol stained preparatios have failed to reveal the position or number of the contractile vacuole (expulsion vesicle) pore/s.

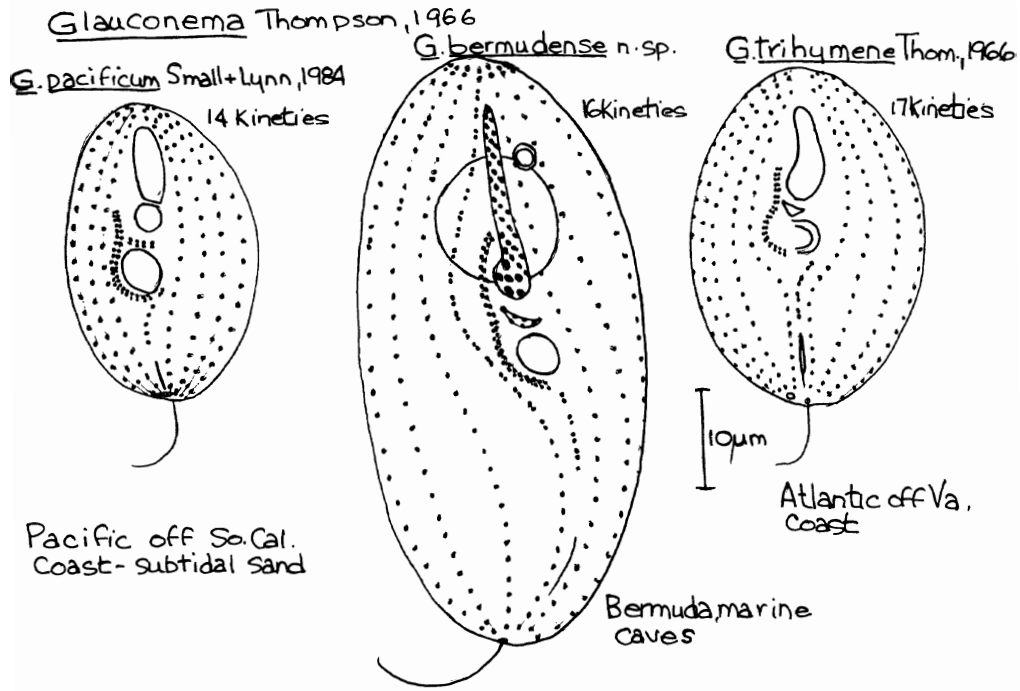

Fig. 5. A comparison of *Glauconema* species.: *G. pacificum* Small & Lynn, 1984; *G. trihymene* Thompson, 1966 *G. bermudense* Small, n.sp. Note the similarity of the oral organellar systems and the differences of the somatic kineties, their shapes, and the included kinetids.

The trophic organism measures 64-56 µm (mean = 60 µm, $N = 20$) long by 24-28 µm (mean = 26 µm, $N = 20$) wide whereas the protargol stained cells are considerably decreased in size: length 32-46 µm (mean = 35 µm, $N = 20$) and width 15-16 µm (mean = 15.5 µm, $N = 20$). Since the other two described species have in the past been silver-stained by Chatton-Lwoff procedure, which causes the cells to swell, and the living dimensions of *G. bermudense* are larger than the Chatton-Lwoff stained related species, the living cell dimensions (accurately measured) are important. As one can see in fig. 5, *G. bermudense* although larger than either of its congeners has slightly fewer kineties and a greater degree of posterior kinetal skew.

TABLE I.

A list of sites from the four caves from which *Glauconema bermudense* Small, n.sp. has been recovered as either trophonts, tomites, or both. Note that the depths and salinities of these sites is also listed (see also Iliffe, 1981 for a general description of the caves).

Cave & Site	*Depth,*	*Salinity*	*Remarks*
1. Green Bay Cave (see fig. 8)			
a. dead fish site	55′	?	Numerous ciliates recovered including *Glauconema* trophonts
b. Green Bay entrance tissue trap site (1)	2′	36‰	*Glauconema* not recovered among the 12 different ciliates
c. "Rat trap" tissue trap site (2)	56′	?	*Glauconema* trophonts recovered within trap; one of 14 different ciliates.
d. "Letter Box" tissue trap site (3)	24′	36‰	*Glauconema* trophonts the only ciliate found and trapped.
e. Bottom slope to "air room" tissue trap site (4)	50′	36‰	*Glauconema* tomites found in one of three sampling periods.
f. "Air Room" tissue trap site (5)	2′	23.5‰	*Glauconema* tomites only found in one sampling period.
g. "Far Room" tissue trap site (6)	36′	36‰	Few *Glauconema* tomites found in initial samples only
h. "Cliff pool" various sites and in tissue traps	20′	22‰	No *Glauconema*, trophonts or tomites found in repeated sampling.
2. Church Cave			
a. surface sites	1′	16‰	*Glauconema* trophonts, tomites and tomite conjugants
b. mid depth site	30′	33‰	No *Glauconema*
c. deepest site	60′	34‰	No *Glauconema*
3. Wonderland Cave			
a. main pool front room site 1	1′	12‰	No *Glauconema*
b. main pool back ledge site 2	23′	24‰	*Glauconema* present
c. main pool bottom site 3	58′	36‰	*Glauconema* tomites only
d. air room surface site 4	1′	12‰	*Glauconema* trophonts & tomites
4. Tuckerstown Cave — one pool			
a. site 1	1′	27‰	No *Glauconema*
b. site 2	10′	34‰	No *Glauconema*
c. site 3	50′	36‰	*Glauconema* trophonts
d. site 4	70′	36‰	*Glauconema* trophonts

When the trophic organism is in an actively growing and dividing culture with an ample food supply it is completely filled with food vacuoles (fig. 3). Uniform in size and quite opalescent in Nomarski microscopy. Perhaps the change to these many vacuoles at the time of fixation is responsible for the striking shrinkage observed in the stained cells.

Ecology of *Glauconema bermudense*, trophonts.

Table I lists the sites in caves with site depths and salinities from which *Glauconema* trophic, large trophic organisms were isolated or were not found. *Glauconema* was only found in the interior of the caves, not at entrances, or for that matter in any of the many other epigean and psammobiotic sites samples by Small or Heisler. The ciliate appears at different depths (and salinities) in different caves. Furthermore, the *Glauconema* trophic cells from these sites, all of which have been protargol silver-stained by Heisler or Sniezek and examined by Small, all fall into the dimensions recorded for the cells as enumerated above. Note that in different caves the ciliate may be found at different depths and different salinities. In all of these sites the ciliates were trapped using the tissue containing traps discussed earlier.

Glauconema bermudense, the tomites.

As is also indicated in table I (see remarks) in addition to the large trophic tomonts [and particularly in the collections from Church Cave surface waters (taken on four different months of collection) in the laboratory after no more than 24 hours in Petri dishes in the lighted room] Small discovered that rapid divisions ensued and small, morphologically reduced forms, tomites, resulted. As time progressed to 2-3 days from the initial times of isolation, an increasingly larger proportion of the total population became tomites so that by 1 week's end, no large tomonts were any longer visible. These smaller tomites also were capable of conjugation, a sexual process of nuclear recombination. These transformations are figured diagramatically in fig. 6.

The tomites have been shown experimentally to return to the tomont condition when raised in the darkness of a culture chamber from which all light was excluded for 48 hours minimally. Tomite cells derived from Church Cave originally collected samples had been maintained in low numbers on tuna fish and bacterial infusions for 8 weeks when the idea emerged that perhaps light was inhibitory to their growth and culture. Since the cultured tomites had been grown in Millipore filtered cave water (at appropriate salinity) and at temperatures of 18-20°C (approximating those of the cave waters), the one environmental variable that had not been dealt with was that of light — especially since the ciliates had never been found except in the totally dark habitats of the underground cave waters at varying depths.

The tomite cells (see fig.7) differ from the tomonts by their diminutive size: length in protargol stained specimens, 18-26 μm (mean = 22 μm, $N = 10$) and width 12-20 μm (mean = 15 μm, $N = 10$). The oral apparatus is situated mid ventrally and is much reduced, particularly the anterior a segment of the oral dikinetid, the kinetid number and length of the oral dikinetid b segment as well as the whole of the anterior-most oral polykinetid number 1. Four linear sets of barren paired kinetosome kinetids are still to be seen comprising the oral dikinetid c segment. Additionally in all stained specimens thus far observed the

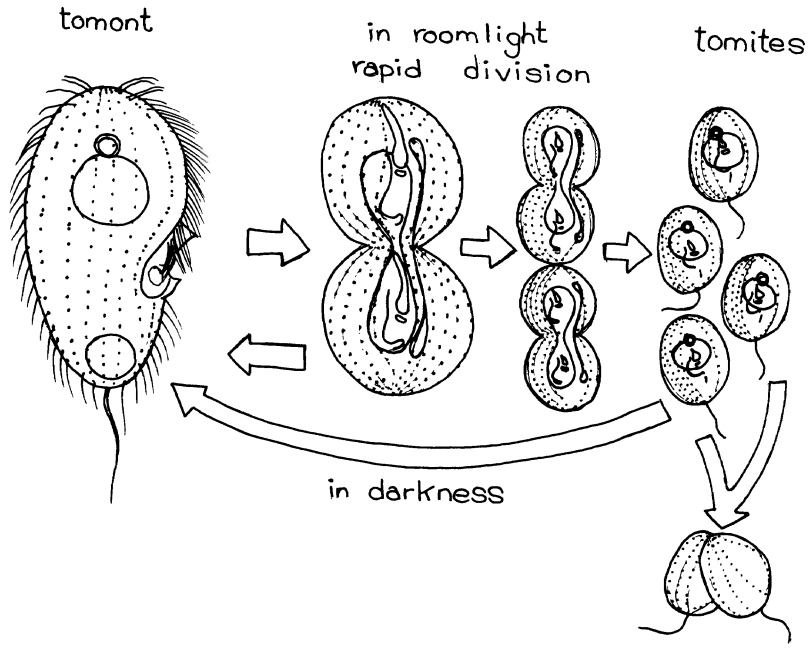

Fig. 6. A diagrammatic representation of the transformation of trophic tomonts to tomites by repetitive rapid division in the presence of light. Such tomites from one cave site set (Church Cave) have been observed to conjugate. Subsequent laboratory studies have confirmed that when these small tomite cell cultures are returned to darkness the tomites again transform directly back to trophonts.

cytostome is retained along with a connected internal "preparatory" food vacuole.

The somatic kinetome is reduced to 7-12 kineties in which only the anteriormost paratene contains paired kinetosome kinetids with paired cilia. All other kinetids are single kinetosomal. A caudal cilium still is noted.

The macronucleus is an oblate spheroid approximately 6 μm in height, 9 μm in width and 6 μm in depth. The micronucleus is approximately 2 μm^3 and sits on the anterior surface of the macronucleus in a shallow inpocket. Neither cytoproct, contractile vacuole, nor contractile vacuole pores have been observed.

The morphogenetic process by which the gradual or immediate (saltorial) transformation back to the trophont stage has not been observed, although some stages of binary division have been noted in stained cells and these do indeed resemble the division stages studied elsewhere by Small (unpublished) of other members of the ciliate family Parauronematidae Small & Lynn, 1984 to which the genus *Glauconema* Thompson, 1966 belongs.

Although encystment is known for other scuticociliate species encountered in the caves and a new euplotid species as well (being described elsewhere — Hill

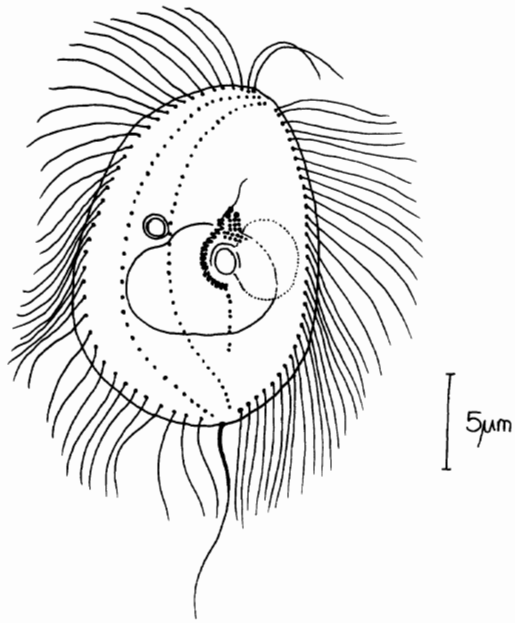

Fig. 7. A semidiagrammatic figure of a tomite based on protargol silverstained specimens from Church Cave sites. Both oral polykinetids and dikinetids are reduced along with kinetal complexity and number. In the cell interior the macronucleus, micronucleus and cytostomally connected preparatory food vacuole are also illustrated.

et al., in press) no encystment has been noted for *Glauconema* trophonts or tomites, either way.

Quite on the contrary, discolored sediments from the floor of Green Bay Cave and Tuckerstown Cave have been discovered to contain sparse, scattered tomites. Based on this discovery, tuna fish baited traps set back in the cave collecting sites on the discolored sediments directly after 48-72 hours contained on initial laboratory microscopic observation trophonts of *Glauconema.* Only tomites of *Glauconema* were recovered in sites 4, 5, and 6 of Green Bay Cave. It may be that the daylight exposure of these cells produced a very rapid transition to the tomites so that the trophont stages of these latter *Glauconemas* were missed.

DISCUSSION

A newly found marine cave scuticociliate possessing tomont and tomite morphological forms has been described and illustrated, and the life history stages as we presently know them from both field and laboratory observations have been presented and illustrated (see fig. 6). Light appears to be causal for the transformation from trophont to tomite, since in the presence of light the

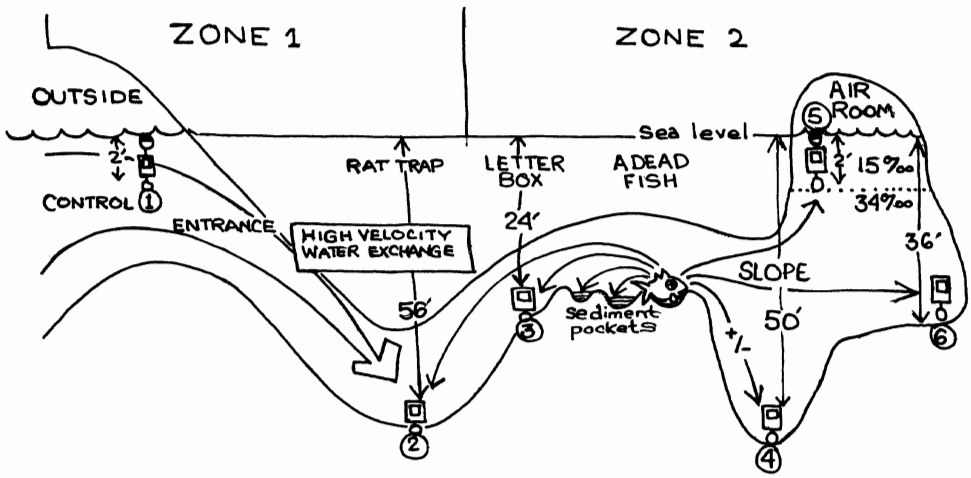

Fig. 8. A diagrammatic cross section of the Green Bay Cave collection sites serially arranged to illustrate the ciliate zonation within the cave. In Zone 1 many ciliates from epigean waters could penetrate the cave to the level of the "Rat Trap" site. In Zone 2, only ciliates adapted to the caves interior were discovered. *Glauconema bermudense* and other ciliates colonized sediments and traps at varying distances from a dead fish, but not beyond the "Rat trap" site.

trophonts divide rapidly and transform to tomites, and in the absence of light (under laboratory controlled conditions) the reciprocal transformation, from tomite to trophont, occurs. Among the Protista, one ciliate has been reported to move in marine tidally variant waters of sediments (Fauré-Fremiet, 1948) so that the ciliate maintains itself in the sediment-bound water at both low and high tide. If removed from the sediment and brought into the laboratory the ciliates still undergo their diurnal vertical migration.

Since no marine cavernicolous ciliates have heretofore been reported, the observations reported herein are new to science. We suggest that the morphogenetic transformation in response to light is a unique attribute of this ciliate's adaptation to the cave environment. The dark light transformations may be coincidental to the more important adaptive significance: the ciliates appears to survive in sediments in very low numbers in a presumably very reduced state of metabolic activity as tomites. In the presence of a fortuitous decaying fish, a crustacean ecdysis (and its subsequent bacterial decomposition), or the decay of other resident invertebrates (eg. sponges that normally cling to the walls in the most interior recesses of the caves), the ciliates then transform to feed and grow to become tomonts, gorged with food vacuoles. Tomite transformation could then ensue and some of the tomites would then survive until the next opportunistic feeding event takes place.

Trophont—tomite transformation in philasterine scuticociliates, reported for both anophryid and philasterid ciliates isolated from epigean coastal waters,

has earlier been reported by Mugard (1949) in France. Similar morphogenetic transformation from trophont to tomite has been observed in *Miamiensis avidis* as a part of an even more complex life history (Small & Meola, 1980). Polymorphism is also known in the estuarine species, *Potomacus pottsi* (see Ramsey et al., 1980). Both of these ciliate genera are with *Glauconema* members of the Parauronematidae.

Furthermore, *Glauconema bermudense* appears only in the inner recesses of the caves, and is not a part of the marine ciliate faunule that may invade the cave waters proximal to the cave entrance (see fig. 8). Of similar significance is the fact that this ciliate species was also found in Church, Wonderland, and Tuckerstown Caves in the same Walsingham karst limestone formation. These latter caves, unlike Green Bay Cave have no direct connection to the open sea although all the marine waters in the caves are under tidal influence.

We therefore conclude that *Glauconema bermudense* ought to be considered a true troglobite, an organism (protist in this case) especially adapted to live in a cave habitat. Its absence from the epigean marine habitats and its morphogenetic adaptation, we believe are two major reasons to support our contention. In so far as we are aware, this is the first report of a truly troglobitic protist (and ciliate, yet) having been discovered in a marine cave habitat.

ACKNOWLEDGMENTS

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