PL ISSN 0065-1583

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

VOLUME 25
Number 1

WARSZAWA 1986

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

International Journal of Protozoology

Manuscripts may be submitted to the Editorial Office: Acta Protozoologica, M. Nencki Institute of Experimental Biology, 02-093 Warszawa, 3 Pasteur Street, Poland, or to each member of the Editorial Board.

Subscription orders for all the magazines published in Poland available through the local press distributors or directly through the

Foreign Trade Enterprise
ARS POLONA
00-068 Warszawa, Krakowskie Przedmieście 7, Poland.

Our bankers:
BANK HANDLOWY WARSZAWA S.A.

ACTA PROTOZOOLOGICA appears quarterly. The indexes of the previous volume will appear in No. 1 of the next volume.

Indexed in Current Contents and in Protozoological Abstracts.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 1-14 (1986)

Revision der Gattung *Stegochilum* Schewiakoff, 1892

Wilhelm FOISSNER

Institut für Zoologie der Universität Salzburg, Akademiestrasse 26, A-5020 Salzburg, Austria

Received on 15 April 1985

Synopsis. Stegochilum schoenborni sp. nov. unterscheidet sich von den anderen Arten der Gattung in der Anzahl der Somakineten und durch die fast zentrale Lage der kontraktilen Vakuole. Die allgemeine Körperorganisation, der Oralapparat und das Silberliniensystem sind tetrahymenid. Das erfordert eine Einordnung in die Unterordnung *Tetrahymenina*. Das nächst verwandte Genus ist *Spirozona*, was durch folgende Apomorphien belegt wird: (1) isolierte Basalkörperpaare am anterioren Ende der paroralen Membran; (2) Verkürzung der beiden rechten Basalkörperreihen der dritten adoralen Membranelle; (3) kreisförmig angeordnete Caudalcilien. Die Genera *Stegochilum* Schewiakoff, 1892 und *Spirozona* Kahl, 1926 werden daher in der Familie *Spirozonidae* Kahl, 1926 vereinigt. Für sie und die beiden Genera werden verbesserte Diagnosen gegeben. Die Revision der Gattung *Stegochilum* zeigt, daß sie bisher nur 2 sichere Arten enthält: *S. fusiforme* Schewiakoff, 1892 und *S. schoenborni* sp. nov. Unsicher ist der taxonomische Status von *S. sphagnetorum* Šrámek-Hušek, 1954. *Stegochilum acutum Bürger*, 1908 wird mit *Tetrahymena pyriformis* (Ehrenberg, 1830) Lwoff, 1947, *S. ovale* Ghosh, 1921 mit *Colpidium colpoda* (Ehrenberg, 1831) Stein, 1860 synonymisiert. Alle Arten der Familie sind sehr selten, freilebend und auf das Süßwasser beschränkt und bevorzugen stark mesosaprobe oder sapropelische Biotope.

Schewiakoff fand am 30. August 1889 am veralgten Ufer des Taupo-Sees in Neuseeland ein schlankes Ciliat, für das er die Gattung *Stegochilum* errichtete, weil es im Unterschied zu *Glaucoma* keinen Schlund und keine adoralen Membranellen besitzen sollte. Erst Jankowski (1964) stellte diesen offensichtlichen Irrtum richtig und zeigte die tetrahymenide Struktur des Oralapparates der Typusart, *Stegochilum fusiforme* Schewiakoff, 1892. Eine genaue Darstellung der Infraciliatur findet sich jedoch weder bei Jankowski (1964) noch bei

anderen Autoren, weshalb die von Corliss (1961, 1979) vorgenommene Einordnung in die *Tetrahymenidae* nicht als gesichert gelten konnte. Die Entdeckung einer neuen Art bot daher Gelegenheit, diese Einordnung mit modernen taxonomischen Methoden zu überprüfen und richtig zu stellen.

Material und Methoden

Stegochilum schoenborni, *Spirozona caudata*, *Turaniella vitrea* und *Colpidium colpoda* wurden am 6. 11. 1983 im Faulschlamm einer kleinen lenitischen Zone des Drau-Flusses bei der Staustufe Amlach in Lienz, Osttirol, Österreich gefunden. Die Populationen waren mäßig stark entwickelt und lebten in der mit Diatomeen und Fadenbakterien bewachsenen oberen Zone des Faulschlammes.

Die Körperform der lebenden Tiere ist nach Präparaten gezeichnet, die nicht mit einem Deckglas bedeckt waren. Feinere Details wurden mit dem Ölimmersionsobjektiv an mehr oder weniger stark gequetschten Tieren im Hellfeld und Phasenkontrast studiert. Alle übrigen Zeichnungen sind mit einem Zeichenapparat angefertigt und nur sehr wenig schematisiert. Zur Darstellung des Silberliniensystems und der Infraciliatur dienten die bei Corliss (1953) und Foissner (1976, 1982) angeführten Silberimprägnationsmethoden. Zur Methodik der biometrischen Charakterisierung siehe Foissner (1982) und Berger et al. (1984).

Die Revision der Gattung basiert auf der Durchsicht des "Zoological Record". Um einen übersichtlichen Vergleich der Arten zu ermöglichen, sind alle bisher veröffentlichten Zeichnungen aufgenommen worden.

Ergebnisse und Diskussion

(1) Beschreibung von *Stegochilum schoenborni* sp. nov.
(Abb. 1 - 7, Taf. I 16 - 18, Tab. 1)[1]

Diagnose: *In vivo* etwa 95 × 22 µm großes, spindelförmiges *Stegochilum*, dessen kontraktile Vakuole dicht unterhalb der Körpermitte in Somakinete 7 ausmündet. Durchschnittlich 25 Somakineten. Am posterioren Körperende ein Kranz verlängerter Caudalcilien.

Locus typicus: Mäßig häufig im Faulschlamm der lenitischen Zonen des Drau-Flusses bei der Staustufe Amlach (Lienz, Osttirol, Österreich).

Aufbewahrungsort des Typenmaterials: Ein Holotypus- und ein Paratypuspräparat sind in der Sammlung der mikroskopischen Präparate des Oberösterreichischen Landesmuseums in Linz deponiert.

Beschreibung: Größe *in vivo* etwa 80 - 110 × 20 - 30 µm, sehr schlank spindelig, nicht abgeflacht, häufig leicht S-förmig gebogen und

[1] Diese Art widme ich Herrn Dr. habil. Wilfried Schönborn, in Anerkennung seiner grundlegenden Arbeiten zur Produktionsbiologie der Ciliaten.

anderen Autoren, weshalb die von Corliss (1961, 1979) vorgenommene Einordnung in die *Tetrahymenidae* nicht als gesichert gelten konnte. Die Entdeckung einer neuen Art bot daher Gelegenheit, diese Einordnung mit modernen taxonomischen Methoden zu überprüfen und richtig zu stellen.

Material und Methoden

Stegochilum schoenborni, *Spirozona caudata*, *Turaniella vitrea* und *Colpidium colpoda* wurden am 6. 11. 1983 im Faulschlamm einer kleinen lenitischen Zone des Drau-Flusses bei der Staustufe Amlach in Lienz, Osttirol, Österreich gefunden. Die Populationen waren mäßig stark entwickelt und lebten in der mit Diatomeen und Fadenbakterien bewachsenen oberen Zone des Faulschlammes.

Die Körperform der lebenden Tiere ist nach Präparaten gezeichnet, die nicht mit einem Deckglas bedeckt waren. Feinere Details wurden mit dem Ölimmersionsobjektiv an mehr oder weniger stark gequetschten Tieren im Hellfeld und Phasenkontrast studiert. Alle übrigen Zeichnungen sind mit einem Zeichenapparat angefertigt und nur sehr wenig schematisiert. Zur Darstellung des Silberliniensystems und der Infraciliatur dienten die bei Corliss (1953) und Foissner (1976, 1982) angeführten Silberimprägnationsmethoden. Zur Methodik der biometrischen Charakterisierung siehe Foissner (1982) und Berger et al. (1984).

Die Revision der Gattung basiert auf der Durchsicht des "Zoological Record". Um einen übersichtlichen Vergleich der Arten zu ermöglichen, sind alle bisher veröffentlichten Zeichnungen aufgenommen worden.

Ergebnisse und Diskussion

(1) Beschreibung von *Stegochilum schoenborni* sp. nov.
(Abb. 1 - 7, Taf. I 16 - 18, Tab. 1)[1]

Diagnose: *In vivo* etwa 95 × 22 µm großes, spindelförmiges *Stegochilum*, dessen kontraktile Vakuole dicht unterhalb der Körpermitte in Somakinete 7 ausmündet. Durchschnittlich 25 Somakineten. Am posterioren Körperende ein Kranz verlängerter Caudalcilien.

Locus typicus: Mäßig häufig im Faulschlamm der lenitischen Zonen des Drau-Flusses bei der Staustufe Amlach (Lienz, Osttirol, Österreich).

Aufbewahrungsort des Typenmaterials: Ein Holotypus- und ein Paratypuspräparat sind in der Sammlung der mikroskopischen Präparate des Oberösterreichischen Landesmuseums in Linz deponiert.

Beschreibung: Größe *in vivo* etwa 80 - 110 × 20 - 30 µm, sehr schlank spindelig, nicht abgeflacht, häufig leicht S-förmig gebogen und

[1] Diese Art widme ich Herrn Dr. habil. Wilfried Schönborn, in Anerkennung seiner grundlegenden Arbeiten zur Produktionsbiologie der Ciliaten.

posterior stärker zugespitzt als anterior. Nicht kontraktil, aber auffallend wurmartig biegsam. Sehr empfindlich, die typische Körperform verschwindet unmittelbar nach dem Auflegen des Deckglases. Schrumpft bei der Präparation ziemlich stark (etwa 25%; Tab. 1). Makronucleus zentral gelegen, meist ellipsoid, selten fast kugelförmig. Mikronucleus dem Makronucleus dicht anliegend. Kontraktile Vakuole wenig bis deutlich unterhalb der Körpermitte, mit 1 Exkretionsporus, der sich meist in der 7., selten in der 6. oder 8. Somakinete befindet. Cytopyge am posterioren Ende der rechten postoralen Kinete. Pellicula zart, durch die 8 - 10 µm langen Cilien deutlich gekerbt. Entoplasma farblos, in der anterioren Körperhälfte meist auffallend klar, posterior mit mäßig vielen etwa 1 µm großen, stark lichtbrechenden Granula und meist nur wenigen 3 - 5 µm großen Nahrungsvakuolen, die Bakterien enthielten. Bewegung sehr rasch unter Rotation um die Längsachse.

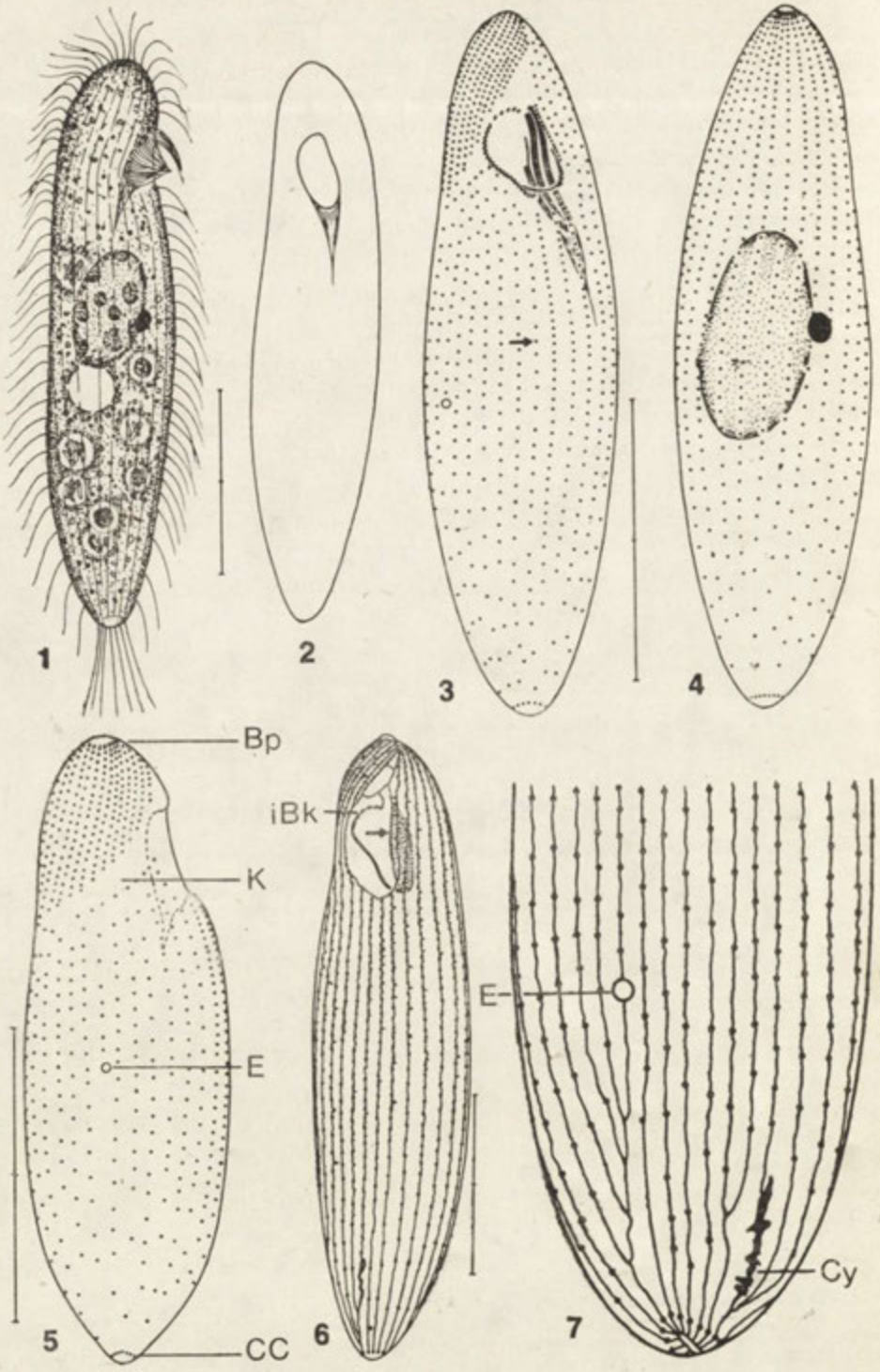
Somakineten meridional angeordnet, stoßen praeoral entlang einer spitzwinkeligen Nahtlinie zusammen, posterior zum Teil deutlich verkürzt, weshalb nur etwa 15 Kineten zur Bildung der ungefähr 20 µm

Tabelle 1

Biometrische Charakteristik von *Stegochilum schoenborni*

Merkmal	$\bar{x}$	*M*	*s*	$s_{\bar{x}}$	*V*	*Min*	*Max*	*n*
Länge in µm	70.31	67.0	8.40	2.33	11.95	60	90	13
Breite in µm	17.92	17.0	2.14	0.59	11.94	15	20	13
Distanz vom anterioren Körperende bis zum Beginn der adoralen Membranellen in µm	8.38	8.0	0.77	0.21	9.16	8	10	13
Distanz vom anterioren Körperende bis zum Ende der adoralen Membranellen in µm	21.23	21.0	1.17	0.32	5.49	20	24	13
Distanz vom anterioren Körperende bis zum Beginn des Makronucleus in µm	22.54	22.0	4.77	1.32	21.17	16	31	13
Distanz vom anterioren Körperende bis zum Exkretionsporus der kontraktilen Vakuole in µm	40.77	38.0	6.23	1.73	15.29	32	50	13
Länge des Makronucleus in µm	17.54	17.0	2.73	0.76	15.55	13	21	13
Breite des Makronucleus in µm	12.08	11.0	1.66	0.46	13.71	10	15	13
Anzahl der Somakineten in Tiermitte	25.23	25.0	0.93	0.26	3.67	23	27	13
Anzahl der Basalkörper in Somakinete 1	27.15	26.0	6.01	1.67	22.14	20	38	13
Anzahl der Basalkörper in Somakinete 4	41.54	40.0	6.08	1.69	14.63	32	52	13
Anzahl der Basalkörper in Somakinete 15	41.31	41.0	6.51	1.81	15.76	33	51	13
Anzahl der Basalkörper in der Kinete links von Kinete 1	36.31	36.0	5.12	1.42	14.11	26	47	13
Lage des Exkretionsporus der kontraktilen Vakuole in Somakinete	7.00	7.0	0.41	0.11	5.83	6	8	13

Alle Daten basieren auf protargolimprägnierten Individuen. *M* — Median, *Min* — Minimum, *Max* — Maximum, *n* — Stichprobenumfang, *s* — Standardabweichung, $s_{\bar{x}}$ — Standardfehler des Mittelwertes, *V* — Variationskoeffizient, $\bar{x}$ — arithmetisches Mittel



langen Caudalcilien beitragen. Ciliatur rechts oberhalb des Oralapparates stark verdichtet, postoral und besonders im posterioren Drittel deutlich aufgelockert. Rechts des Oralapparates eine kahle Zone, links 2 Kinetenfragmente, beim inneren stehen die Basalkörper sehr dicht, beim äußeren dagegen so wie bei den normalen Somakineten ziemlich locker. Rechts oberhalb des Oralapparates ebenfalls 1 Kinetenfragment, das im Silberliniensystem keine Verbindung zu den postoralen Kineten besitzt (Taf. I 18). Anzahl der postoralen Basalkörper in Kinete 1 beträchtlich geringer ($\bar{x} = 27.1$) als in der links von ihr befindlichen postoralen Kinete ($\bar{x} = 36.3$). Alle Somakineten beginnen mit einem Basalkörperpaar.

Oralapparat im anterioren Viertel, *in vivo* eher unscheinbar, da sich die untere Hälfte der adoralen Membranellen in der tiefen Buccalhöhle befindet. Mundeingang klein, schräg oval. Pharynxfibrillen kurz, *in vivo* kaum erkennbar. Am rechten Mundrand eine bogenförmige parorale Membran, deren Basalkörper in den hinteren Dreivierteln zickzackförmig angeordnet sind, im vorderen Viertel ist sie dagegen in 4 - 6 isolierte Basalkörperpaare aufgelöst. Links 3 dreireihige adorale Membranellen, bei der inneren sind die beiden rechten Basalkörperreihen vorne leicht verkürzt.

Silberliniensystem tetrahymenid, die links lateralen Ausläufer der Meridiane 1. Ordnung sind sehr klein und fehlen im posterioren Viertel, weshalb hier die Silberlinien fast gerade sind. Anterior und posterior je 1 kreisförmige Silberlinie, in welche die meridional verlaufenden Silberlinien einmünden, die Basalkörper der Somakineten verbinden.

Ökologie: *Stegochilum schoenborni* ist nach dem Fundort als Indikator für die Polysaprobie einzustufen. Auch geringe Mengen H_2S werden wohl vertragen. Sie ist aber sicher nicht an H_2S gebunden, da sie einige Tage in der Kahmhaut der Probe überlebte. Interessant ist die Vergesellschaftung mit *Spirozona caudata* und *Turaniella vitrea*, beide sehr seltene Ciliaten, die ich an diesem Fundort zum ersten Mal persönlich fand.

Artvergleich: Ähnelt hinsichtlich der Körper- und Kernform *Stegochilum fusiforme* Schewiakoff, 1892, unterscheidet sich von dieser

---

Abb. 1 - 7. *Stegochilum schoenborni* nach Lebendbeobachtungen (1, 2), Protargolimprägnation (3 - 5), nasser Silberimprägnation (6) und trockener Silberimprägnation (7). 1, 2 — rechts laterale und ventrale Ansicht eines typischen Individuums. 3, 4 — Infraciliatur der Ventral- und Dorsalseite. Der Pfeil weist auf die Somakinete 1. 5 — Infraciliatur der rechten Seite. 6 — Silberliniensystem und Infraciliatur der Ventralseite. Der Pfeil weist auf die Kinetensegmente links des Oralapparates. 7 — Silberliniensystem und Infraciliatur in der posterioren Körperhälfte stärker vergrößert. Bp — Basalkörperpaare am Beginn der Somakineten, CC — Caudalcilien, Cy — Cytopyge, E — Exkretionsporus der kontraktilen Vakuole, iBk — isolierte Basalkörperpaare am anterioren Ende der paroralen Membran, K — kahle Region. Maßstriche: 30 µm

aber durch die Lage der kontraktilen Vakuole und die Anzahl der Somakineten (Schewiakoff 1893, Jankowski 1964). *Stegochilum ovale* Ghosh, 1921, bei der die kontraktile Vakuole ebenfalls etwa in Körpermitte liegt, weicht in der Gestalt so erheblich von *S. schoenborni* ab, daß es sicher nicht mit ihr identifiziert werden kann (vgl. Abb. 1 mit Abb. 14).

(2) Die systematische Stellung der Gattung *Stegochilum* Schewiakoff, 1892

Corliss (1979) stellt *Stegochilum* in die *Tetrahymenidae* und subsummiert die *Spirozonidae* zu den *Trichospiridae*, deren systematische Position aber noch unklar ist (Foissner et al. 1981).

Die vorliegenden Untersuchungen weisen mehrere Apomorphien zwischen den Genera *Spirozona* und *Stegochilum* aus (Abb. 3, 8): die isolierten Basalkörperpaare am anterioren Ende der paroralen Membran, die Verkürzung der beiden rechten Basalkörperreihen der 3. adoralen Membranelle und die kreisförmig angeordneten Caudalcilien. Die praeorale Verdichtung der Somakineten und die Kinetenfragmente links des Oralapparates findet man auch bei der Gattung *Colpidium* (Taf. II 19 und Foissner und Schiffmann 1980). Die angeführten Apomorphien belegen nicht nur das Schwestergruppenverhältnis zwischen *Stegochilum* und *Spirozona* sondern auch die Eigenständigkeit der Familie *Spirozonidae* Kahl, 1926, was bereits Foissner et al. (1981) ausführlich diskutiert haben.

(3) Verbesserte Diagnosen für die Familie *Spirozonidae* und die Genera *Stegochilum* und *Spirozona*

Nach den vorliegenden und den Untersuchungen von Jankowski (1964) und Foissner et al. (1981) ist eine klare Abgrenzung der *Spirozonidae* und der Genera *Spirozona* und *Stegochilum* von den übrigen Familien und Genera der *Tetrahymenina* möglich (Corliss 1979).

Familie *Spirozonidae* Kahl, 1926: Mittelgroße (50 - 100 µm) *Tetrahymenina* mit meridional bis spiralig verlaufenden, zumindest praeoral rechts lateral sehr dicht bewimperten Somakineten, mehreren isolierten Basalkörperpaaren am anterioren Ende der paroralen Membran und mehreren Kinetensegmenten auf der linken Seite des Oralapparates. Silberliniensystem tetrahymenid.

Typusgattung: *Spirozona* Kahl, 1926

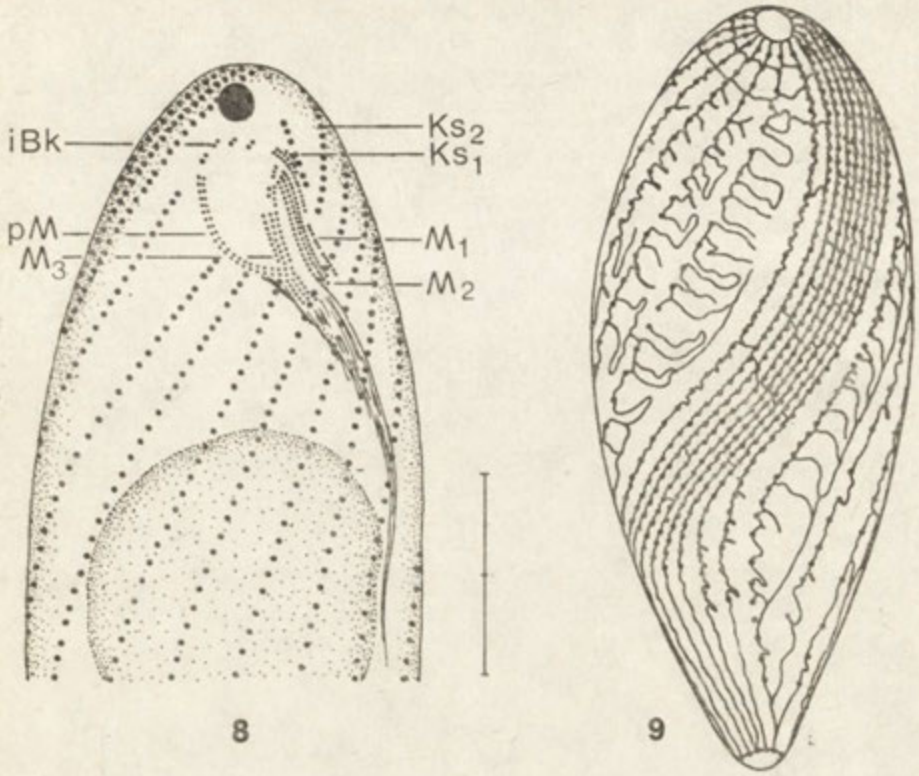


Abb. 8, 9. *Spirozona caudata* nach Protargolimprägnation (8) und trockener Silberimprägnation (9). 8 — Infraciliatur der Oralregion in Ventralansicht (aus Foissner et al. 1981). 9 — Silberliniensystem und Infraciliatur der Dorsalseite. iBk — isolierte Basalkörperpaare am anterioren Ende der paroralen Membran, Ks_1, Ks_2 — Kinetensegmente, M_1 - M_3 — adorale Membranellen, pM — parorale Membran. Maßstrich: 15 µm

Die nächst verwandten Familien sind die *Tetrahymenidae* (ohne isolierte Basalkörperpaare) und die *Turaniellidae* (vielleicht mit isolierten Basalkörperpaaren, jedoch mit peniculina-artigen adoralen Membranellen; Iftode et al. 1969, Foissner und Schiffmann 1980).

Gattung *Stegochilum* Schewiakoff, 1892: *Spirozonidae* mit meridional verlaufenden Somakineten, bei denen die Bewimperung praeoral rechts lateral auffallend verdichtet ist. Silberliniensystem schwach tetrahymenid (Abb. 6, 7, Taf. I 16 - 18).

Typusart: *Stegochilum fusiforme* Schewiakoff, 1892

Gattung *Spirozona* Kahl, 1926: *Spirozonidae* mit deutlich links spiralig verlaufenden Somakineten, die rechts lateral bandartig verdichtet und sehr dicht bewimpert sind. Silberliniensystem ausgeprägt tetrahymenid (Abb. 9, Taf. II 20).

Typusart: *Spirozona caudata* Kahl, 1926.

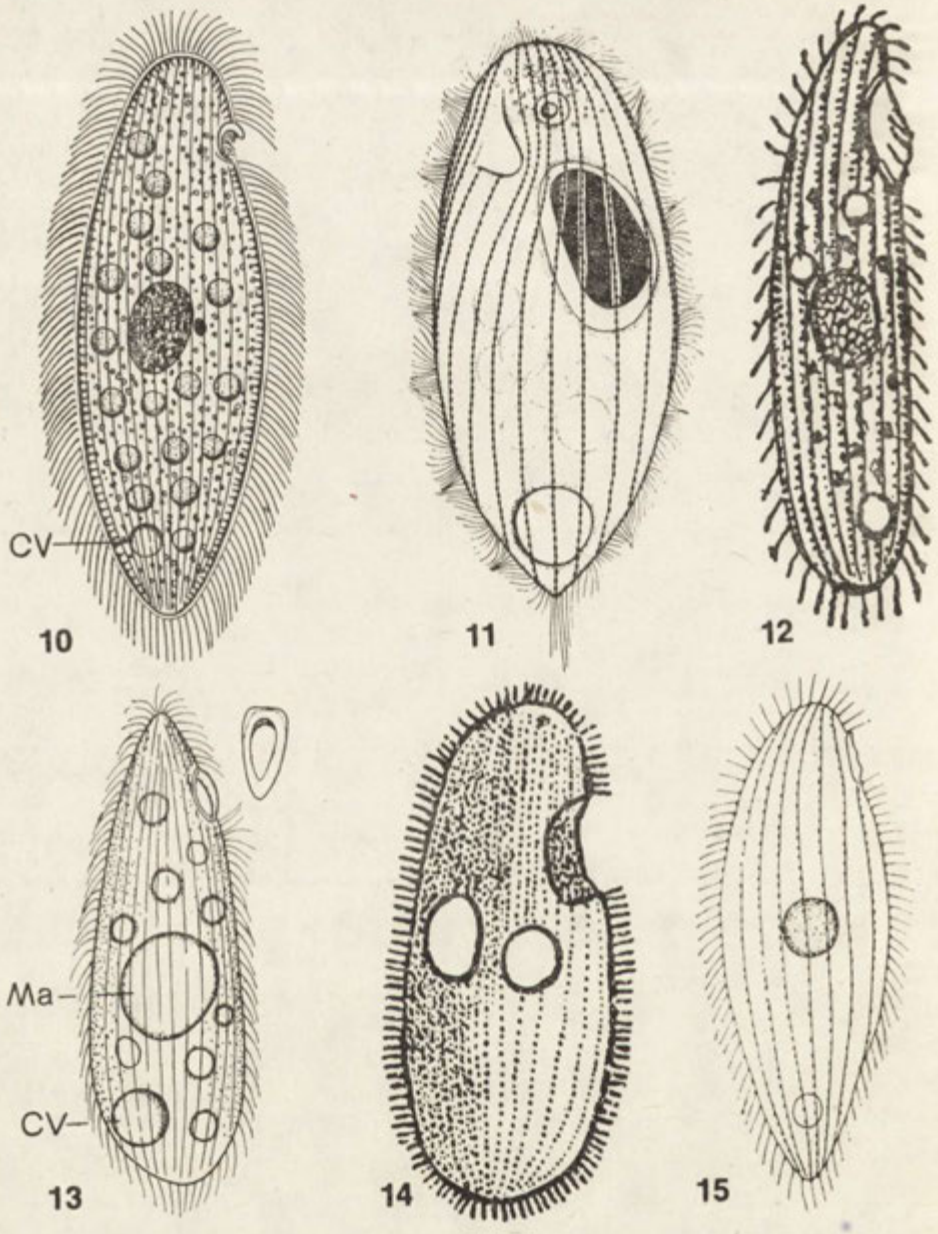


Abb. 10 - 15. Revision der Gattung *Stegochilum*. 10 — *S. fusiforme* (nach Schewiakoff 1893). 11 — *S. fusiforme* (nach Jankowski 1964). 12 — *Stegochilum* sp. (nach Vuxanovici 1963). 13 — *S. acutum* (nach Bürger 1908). Rechts oben der Mund in Aufsicht. 14 — *S. ovale* (nach Ghosh 1921 aus Bhatia 1935). 15 — *S. sphagnetorum* (nach Šrámek-Hušek 1954). CV — kontraktile Vakuole, Ma — Makronucleus

(4) Revision, Faunistik und Ökologie der Gattung *Stegochilum* Schewiakoff, 1892

Die erste Revision verdanken wir Kahl (1931). Er führt 3 Arten an, 1 Species wurde seitdem neu beschrieben. *Stegochilum* gehört vermutlich zu den seltenen tetrahymeniden Genera, da sich in der Literatur nur vereinzelt faunistische Daten finden und lediglich die Typusart ist mehr als einmal gefunden worden. Bisher nur im Süßwasser nachgewiesen. Alle Arten freilebend, bevorzugen stark mesosaprobe oder sapropelische Biotope.

Stegochilum fusiforme Schewiakoff, 1892 (Abb. 10, 11). Größe nach Schewiakoff (1893) 63 - 78 × 20 - 27 µm, nach Jankowski (1964) 72 - 78 × 26 - 30 µm. Körper länglich, mehr oder weniger spindelförmig, an beiden Polen verengt, anterior breiter und abgerundet, posterior etwas zugespitzt. Beim Mund leicht konvex. Makronucleus annähernd zentral gelegen, ellipsoid, nach Jankowski (1964) aber nur 18 × 17 µm, was nicht mit seiner Zeichnung übereinstimmt, nach der er deutlicher ellipsoid ist. Nucleolen netzförmig. Mikronucleus etwa 2 µm im Durchmesser, ziemlich weit vom Makronucleus entfernt. Kontraktile Vakuole subterminal dorsolateral. Pellicula mit deutlicher "Alveolarschicht", vermutlich Mucocysten. Entoplasma klar, farblos, besonders posterior mit vielen stark lichtbrechenden kristallähnlichen Einschlüssen. Frißt Zooflagellaten, einzellige Algen und Nostocaceen, die in kugelförmigen grauen bis gelblichen Nahrungsvakuolen verdaut werden. Bewegung lebhaft, wobei es langsam um die Längsachse rotiert. Zuweilen dreht es sich an einem Platze, fortwährend hin und her stoßend. Teilung im freischwimmenden Zustand.

18 - 20 meridional verlaufende Somakineten, die so wie bei *S. schoenborni* rechts oberhalb des Mundes besonders dicht bewimpert sind und praeoral auf der Ventralseite spitzwinkelig zusammenstoßen (Jankowski 1964). Wimpern lang, zart und dicht stehend. Am posterioren Körperende nach Jankowski (1964) ein Schopf verlängerter Caudalcilien, die Schewiakoff (1893) wohl übersehen hat. Mund nahe dem vorderen Körperende. Mundöffnung klein, oval, vorne breiter als hinten, rechts eine parorale Membran, links 3 lange adorale Membranellen (Jankowski 1964), die Schewiakoff (1893) irrtümlich als Fortsetzung der paroralen Membran interpretiert hat.

Faunistik und Ökologie: Taupo-See in Neuseeland, zwischen Uferalgen (Schewiakoff 1893); Baradla-Höhle in Ungarn, abgekratztes getrocknetes und wiederbefeuchtetes Wandmaterial (Varga 1963); Faulschlammtümpel in der Nähe von Leningrad, häufig und typisch für die sapropelische Lebensgemeinschaft (Jankowski 1964);

Canale Maggiore in Parma (Italien), auf verschlammten Kieselsteinen in 40 cm Wassertiefe bei 14°C Wassertemperatur und 40 cm/s Strömungsgeschwindigkeit (Madoni und Ghetti 1977). Die ökologische Valenz dieser Species scheint also ziemlich groß zu sein, eine Vorliebe für schlammige, nährstoffreiche Biotope zeichnet sich aber klar ab.

Taxonomischer Status: Die Beschreibungen von Schewiakoff (1893) und Jankowski (1964) stimmen weitgehend überein, die Wiedererkennbarkeit der Art kann daher als gesichert gelten. Eine Neubeschreibung ist jedoch notwendig, da die Infraciliatur noch nicht genauer untersucht worden ist.

Stegochilum acutum Bürger, 1908 (Abb. 13). Größe 42 - 53 µm, sehr metabolisch. Lanzettlich, von hinten nach vorne gleichmäßig verjüngt, anterior ganz spitz, posterior breit gerundet. Makronucleus leicht ellipsoid, zentral gelegen. Kontraktile Vakuole subterminal dorsal. Oralapparat auf dem ersten Viertel, soll ganz dem von *S. fusiforme* in der Beschreibung von Schewiakoff (1893) entsprechen.

Faunistik und Ökologie: Bisher nur vom Erstbeschreiber in einem Makrophyten-reichen Tümpel bei Santiago (Chile) gefunden, und zwar zahlreich in der Gallerte eines Arthropoden-Eierpaketes.

Taxonomischer Status: Der Habitat, die Größe und die allgemeine Körperorganisation weisen sehr darauf hin, daß es sich um eine *Tetrahymena*-Art handelt. Man wird sie in Zukunft am besten als Synonym von *Tetrahymena pyriformis* (Ehrenberg, 1830) Lwoff, 1947 betrachten.

Stegochilum ovale Ghosh, 1921 (Abb. 14). Größe fehlt. Körper ellipsoid (7 : 3), vorne etwas schmäler gerundet als hinten. Mund groß, auf dem anterioren Drittel, soll jenem von *S. fusiforme* in der Beschreibung von Schewiakoff (1893) gleichen. Makronucleus ellipsoid, so wie die kontraktile Vakuole zentral in der Körpermitte gelegen. Mikronucleus dem Makronucleus anliegend.

Faunistik und Ökologie: Bisher nur von Ghosh (1921) in einem Pflanzenaufguß bei Calcutta (Indien) gefunden.

Taxonomischer Status: Nach der Körperform, der Lage der kontraktilen Vakuole und dem Habitat ist Identität mit *Colpidium colpoda* (Ehrenberg, 1831) Stein, 1860 als sicher anzunehmen. Sie wird daher als synonym mit dieser Species betrachtet, zumal auch die Beschreibung sehr oberflächlich ist.

Stegochilum sphagnetorum Šrámek-Hušek, 1954 (Abb. 15). Größe 53 - 60 × 18 - 29 µm (n = 20), wenig metabolisch. Körper spindelig, beim Mund leicht konkav, anterior schmal gerundet, hinten fast zugespitzt, in Opalblau-Präparaten wird die Gestalt *Tetrahymena pyriformis*-ähn-

lich. Makronucleus zentral gelegen, kugelförmig, 10 - 12 µm im Durchmesser, in der Zeichnung von Šrámek-Hušek (1954) daher um mindestens 3 µm zu klein gezeichnet! Kontraktile Vakuole subterminal. An jeder Körperseite etwa 9 Somakineten, die Ansatzstellen der Cilien sollen durch perlenartige Verdickungen der Pellicula stark hervortreten. Mund nahe dem anterioren Körperende, rechts mit einer undulierenden Membran.

Faunistik und Ökologie: Bisher nur von Šrámek-Hušek (1954, 1957) in einem alten Moortümpel im Lipno-Talsperrengebiet bei Horni Planá (obere Moldau, Böhmerwald, Tschechoslowakei) gefunden.

Taxonomischer Status: Hinsichtlich Größe und Körperform vielleicht eine *Stegochilum*-Art, dann aber vermutlich identisch mit *S. fusiforme*, von der sie sich nur durch den kugelförmigen Makronucleus und die charakteristische "Perlung" der Pellicula unterscheidet. Diese Perlung wird von Šrámek-Hušek (1954) sicher falsch aufgefaßt, da die Cilien bei den Ciliaten immer in kleinen Grübchen stehen. Daher handelt es sich vermutlich um Mucocysten, was darauf hinweist, daß *S. sphagnetorum* mit *Tetrahymena rostrata* (Kahl, 1926) Corliss, 1952 synonym ist, bei der die Mucocysten ebenfalls häufig perlenartig hervortreten und die man meist in Moosen findet (Kahl 1931, Corliss 1974, eig. Beob.). Das für *T. rostrata* typische Caudalcilium mag Šrámek-Hušek (1954) übersehen haben, da es sehr klein ist. Eine Synonymisierung verbietet vorerst aber die fast terminale Lage der kontraktilen Vakuole.

Stegochilum sp. Brodksy, 1935. Gefunden in einem Boden in Zentralasien. Keine Beschreibung und Zeichnung. Vermutlich *Tetrahymena rostrata*, die man häufig in terrestrischen Biotopen findet.

Stegochilum sp. Vuxanovici, 1963 (Abb. 12). Länge 40 - 50 µm, sehr schlank zylindroid (7 : 1.7), beim Mund leicht eingebuchtet, anterior fast zugespitzt, posterior schmal gerundet. Makronucleus ellipsoid, sehr groß, zentral gelegen. Kontraktile Vakuole subterminal lateral. Etwa 7 Wimperreihen je Körperseite, kerben die Pellicula deutlich. Zwischen den Wimperreihen kleine glänzende Kügelchen, vermutlich Mucocysten (Verf.). Entoplasma mit kristallinen Einschlüssen und kleinen, leer erscheinenden nicht kontraktilen Vakuolen. Oralapparat im vorderen Viertel, groß, nicht genauer beobachtet. Einige Exemplare in einem Aufguß mit Pflanzen aus einem Teich des botanischen Gartens in Bukarest (Rumänien), vom November bis Jänner.

Wahrscheinlich auch eine *Tetrahymena* Species, bei der die kontraktile Vakuole etwas zu weit nach hinten gezeichnet worden ist.

DANKSAGUNG

Mit dankenswerter finanzieller Unterstützung des "Fonds zur Förderung der Wissenschaftlichen Forschung, Projekt P 5226" und der Hydrologischen Untersuchungsstelle Salzburg (Dipl.-Ing. Reinhold Haider). Frau Karin Anrather danke ich für die fototechnische Assistenz.

SUMMARY

Stegochilum schoenborni sp. nov. differs from the other species of the genus by the number of ciliary rows and by its contractile vacuole which is located near the mid-region of the body. The general organization of the somatic and oral infraciliature and the silverline system of *Stegochilum* are tetrahymenal. This demands its classification in the suborder *Tetrahymenina*. The nearest related genus is *Spirozona* which is proved by the following apomorphic characters: (1) isolated pairs of basal bodies at the anterior end of the paroral membrane; (2) shortening of the right basal body rows of the third adoral membranelle; and (3) a ring of caudal cilia. Thus, the genera *Stegochilum* Schewiakoff, 1892 and *Spirozona* Kahl, 1926 are united in the family *Spirozonidae* Kahl, 1926. Improved diagnoses are given for the family and the genera contained therein. The revision of the genus *Stegochilum* show that it contains only 2 reliable species: *S. fusiforme* Schewiakoff, 1892 and *S. schoenborni* nov. spec. *Stegochilum sphagnetorum* Šrámek-Hušek, 1954 is of uncertain systematic position. *Stegochilum acutum* Bürger, 1908 is synonymized with *Tetrahymena pyriformis* (Ehrenberg, 1830) Lwoff, 1947, and *S. ovale* Ghosh, 1921 is synonymized with *Colpidium colpoda* (Ehrenberg, 1831) Stein, 1860. All species of the family are rare free-living fresh-water forms which prefer heavy mesosaprobic or sapropelic habitats.

LITERATUR

Berger H., Foissner W. und Adam H. 1984: Taxonomie, Biometrie und Morphogenese einiger terricoler Ciliaten (*Protozoa: Ciliophora*). Zool. Jb. Syst., 111, 339 - 367.

Bhatia B. L. 1936: *Protozoa: Ciliophora*. In: The Fauna of British India, Including Ceylon and Burma, Sewell R. B. S., ed., Taylor and Francis, London pp. 1 - 493.

Brodsky A. L. 1935: Soil protozoa and their relative importance on soil activity. (Soil protozoa of the Central Asia). Uzbekistan Commitee of Sciences, Tashkent, 20, 99 - 182 (in Russian).

Bürger O. 1908: Nuevos estudios sobre protozoos chilenos del aqua dulce. An. Univ. Chile, 122, 137 - 204.

Corliss J. O. 1953: Silver impregnation of ciliated protozoa by the Chatton-Lwoff technic. Stain Technol., 28, 97 - 100.

Corliss J. O. 1961: The Ciliated Protozoa. Characterization, Classification, and Guide to the Literature. Pergamon Press, London and New York, 310 pp.

Corliss J. O. 1973: History, taxonomy, ecology, and evolution of the species of *Tetrahymena*. In: Biology of *Tetrahymena*, (Elliott A. M., ed.) Dowden, Hutchinson and Ross, Stroudsburg, Pa pp. 1 - 55.

Corliss J. O. 1979: The Ciliated Protozoa. Characterization, Classification and Guide to the Literature. 2nd ed. Pergamon Press, Oxford and Frankfurt, 455 pp.

Foissner W. 1976: Erfahrungen mit einer trockenen Silberimprägnationsmethode zur Darstellung argyrophiler Strukturen bei Protisten. Verh. zool.-bot. Ges. Wien, 115, 68 - 79.

Foissner W. 1982: Ökologie und Taxonomie der *Hypotrichida* (*Protozoa: Ciliophora*) einiger österreichischer Böden. Arch. Protistenk., 126, 19 - 143.

Foissner W. und Schiffmann H. 1980: Taxonomie und Phylogenie der Gattung *Colpidium* (*Ciliophora, Tetrahymenidae*) und Neubeschreibung von *Colpidium truncatum* Stokes, 1885. Naturk. Jahrb. Stadt Linz, 24, 21 - 40.

Foissner W., Czapik A. und Wiackowski K. 1981: Die Infraciliatur und das Silberliniensystem von *Sagittaria hyalina* nov. spec., *Chlamydonella polonica* nov. spec. und *Spirozona caudata* Kahl, 1926 (*Protozoa, Ciliophora*). Arch. Protistenk., 124, 361 - 377.

Ghosh E. 1921: Infusoria from the environment of Calcutta. — I. Bull. Carmichael Med. Coll., 2, 6 - 17.

Iftode F., Versavel G. und Didier P. 1969: Morphologie, morphogenèse de bipartition et ultrastructures de *Turaniella vitrea* Brodsky (Cilié Hymenostome Peniculien) I — Structures infraciliaires et morphogénèse. Protistologica, 5, 523 - 533.

Jankowski A. W. 1964: Morphology and evolution of *Ciliophora*. III. Diagnoses and phylogenesis of 53 sapropelebionts, mainly of the order *Heterotrichida*. Arch. Protistenk., 107, 185 - 294.

Kahl A. 1926: Neue und wenig bekannte Formen der holotrichen und heterotrichen Ciliaten. Arch. Protistenk., 55, 197 - 438.

Kahl A. 1931: Urtiere order *Protozoa* I: Wimpertiere oder *Ciliata* (*Infusoria*) 2. *Holotricha* außer den im 1. Teil behandelten *Prostomata*. Tierwelt Dtl., 21, 181 - 398.

Madoni P. und Ghetti P. F. 1977: Indagine preliminare sulla distribuzione dei ciliati e altri protozoi nei corsi d'acqua della Val Parma. Riv. Idrobiol., 16, 35 - 53.

Schewiakoff W. 1892: Über die geographische Verbreitung der Süsswasser-Protozoën. Verh. naturw.-med. Ver. Heidelb. (N.S.), 4, 544 - 567.

Schewiakoff W. 1893: Über die geographische Verbreitung der Süsswasser-Protozoën. Mém. Acad. impér. Sci. St. Pétersb. (Sér. 7), 41, 1 - 201.

Šrámek-Hušek R. 1954: Neue und wenig bekannte Ciliaten aus der Tschechoslowakei und ihre Stellung im Saprobiensystem. Arch. Protistenk., 100, 246 - 267.

Šrámek-Hušek R. 1957: K poznání nálevníků ostravského kraje (Zur Kenntnis der Ciliaten des Ostrauer-Gebietes Tschechoslovakei). Věst. čsl. zool. Spol., 21, 1 - 24 (in tschechisch mit deutscher Zusammenfassung).

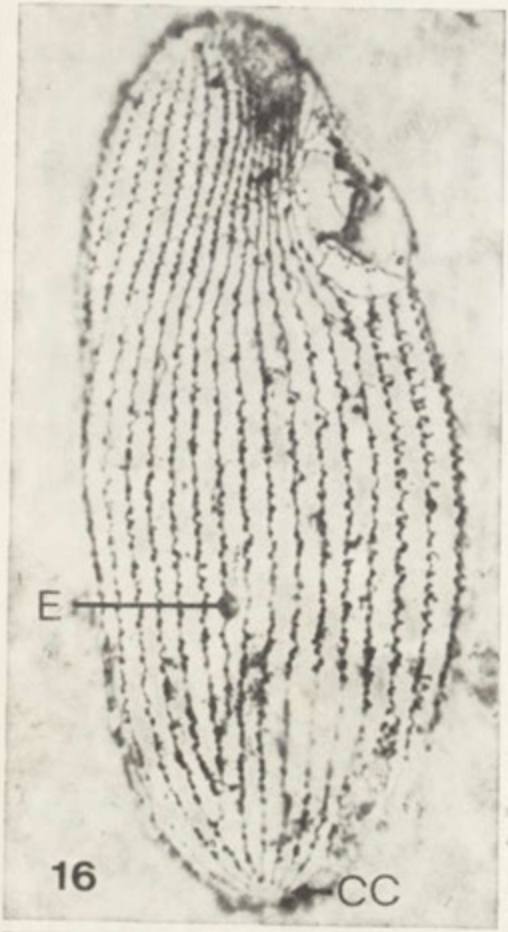
Varga L. 1963: Weitere Untersuchungen über die aquatile Mikrofauna der Baradla-Höhle bei Aggtelek (Ungarn) (Biospeologica Hungarica, XVII.). Acta Zool. Hung., 9, 439 - 458.

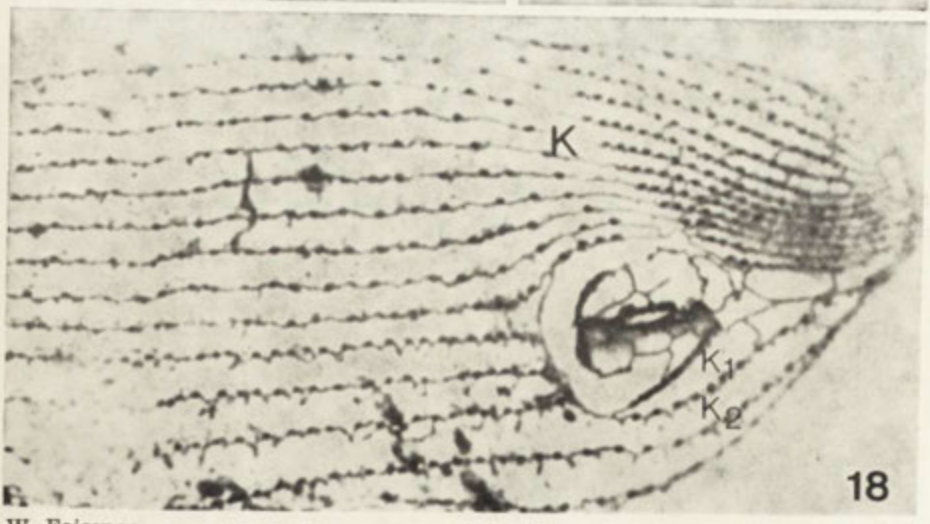
Vuxanovici A. 1963: Contributii la sistematica ciliatelor (Nota IV). Studii Cerc. Biol. (Biol. Anim.), 15, 65 - 93.

LEGENDEN ZU DEN TAFELN I UND II

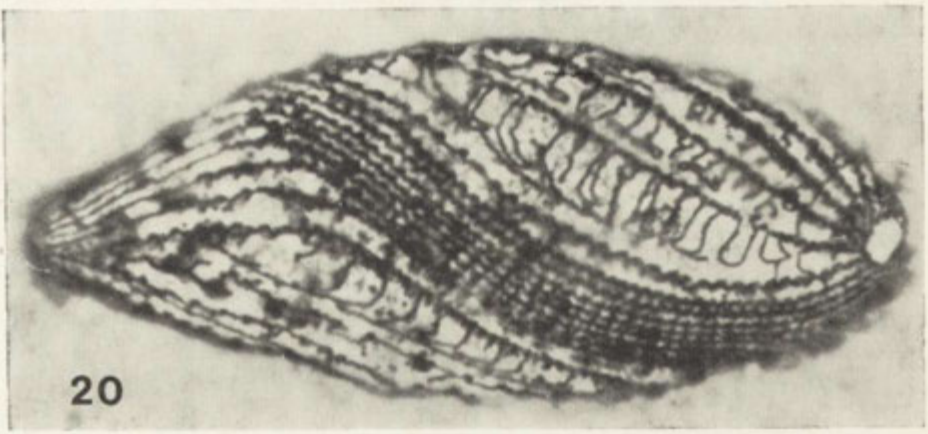
16 - 18: *Stegochilum schoenborni*. Infraciliatur und Silberliniensystem nach trockener Silberimprägnation. 16 — rechts laterale Ansicht. 17 — Dorsalansicht. 18 — anteriores Körperende in Ventralansicht stärker vergrößert. CC — Caudalcilien, E — Exkretionsporus der kontraktilen Vakuole, K — kahle Region, K_1, K_2 — Kinetensegmente

19, 20: Infraciliatur und Silberliniensystem von *Colpidium colpoda* und *Spirozoma caudata* nach trockener Silberimprägnation. 19 — Ventralansicht. Der Pfeil weist auf das Kinetensegment rechts des Oralapparates. 20 — Dorsalansicht





W. Foissner auctor phot.



W. Foissner auctor phot.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 15 - 22 (1986)

Ultrastructure de la frange aborale d'*Opisthonecta henneguyi* Fauré-Fremiet, 1906 (*Ciliophora Peritrichida*)

Almudena GUINEA, Rosario GIL
et Dimas FERNANDEZ-GALIANO

Instituto de Inmunologia y Biologia Microbiana, C.S.I.C., Valázquez 144, Madrid 28006, España

Received on 15 November 1984, *revised on* 11 April 1985

Synopsis. La frange aborale chez l'*Opisthonecta henneguyi* c'est un anneau ciliaire formé par cinéties obliques de six cinétosomes. De chaque cinétosome sort une fibre cinétodesmique qui se dirige vers le pôle aboral. De chaque cinétie, du cinetosome plus oral, sort une fibre orale, et aussi avec de chaque cinétie, sort aboralément, une fibre aborale qui est liée la baguette intercinétique. Oralement, la ceinture est limitée par un pli pelliculair et une rangée de sacs parasomaux, et aboralement, par deux plis et deux rangées de sacs parasomaux. On observe le système mionémique au-dessous des cinétosomes.

Opisthonecta henneguyi (un cilié Péritriche) se trouve dans les eaux douces oú il se deplace, le bout du corps orienté vers l'avant.

Des Fauré-Frémiet qui l'a decrit le premier, *O. henneguyi* a été etudié par de nombreux auteurs (Lynch et Noble 1931, Kofoid et Rosenberg 1940, Jahn et Hendrix 1969 et Foissner 1975, mais deux auteurs seulement ont observé ce cilié en microscopie électronique (Bradbury 1965 et Rosenberg et Grim 1966).

La structure la plus caractéristique de l'*O. henneguyi* est la frange ciliaire aborale (ou ceinture aborale) permanente. C'est elle qui est l'objet essentiel de ce travail, à cause de sa signification pour la systématique des ciliés Péritriches.

Matériel et Méthodes

Opisthonecta henneguyi a été récolté dans une petite flaque non permanente de la Dehesa de la Villa (Madrid) au mois d'avril, 1981. Après cette date, elle a été cultivée dans notre laboratoire dans un milieu de laitue.

Pour la microscopie électronique les cellules de *O. henneguyi* ont été fixées pendant 20 min dans une mélange de 1 volume de glutaraldéhyde 2.5% dans un tampon phosphate 0.05M (pH 7.4) + 1 volume du Os O_4 1% dans le même tampon. Les ciliés ont été inclus dans l'Epon. Les coupes ont été effectuées avec de l'ultramicrotome Reichert OMU 2, contrastées avec l'acétate d'uranyle (2%) et au citrate de plomb selon Reynolds (1963) et examinées au microscope électronique Siemens Elmiskop I (Kv 80).

Pour la microscopie photonique, on a employé le carbonate d'argent piridiné de Fernández-Galiano (1976).

Résultats

La bande trochale d'*Opisthonecta henneguyi* est une ceinture ciliée large de 1.5 μm qui entoure la cellule dans son tiers postérieur (Pl. I 1, 2) formé par de courtes cinéties obliques de six cinétosomes. Nous en avons observé les structures suivantes:

(1) Systèmes fibrillaires

Fibres périodiques (K D). Dans la microcopie photonique on observe ces fibres comme "fibres obliques", perpendiculaires aux cinéties, qui surgissent du côte droit de chaque cinétosome de la frange aborale et qui se dirigent obliquement vers le pôle aboral (Pl. I 2).

En microscope électronique, nous pouvons constater qu'il s'agit de fibres périodiques avec une périodicité de 15 nm environ, qui naissent de l'extrémité proximale de chaque cinétosome au côte droite et se dirigent vers le pôle aboral, en formant un angle d'environ 90° avec les cinéties de la ceinture (Pl. I 3 et 4, Pl. II 5 et 8) le plus souvent on observe ces fibres formant des faisceaux de trois unités (Pl. I 3), constitués des fibres de cinéties adjacentes; en outre, en raison de l'orientation oblique de ces fibres, les faisceaux se forment avec les fibres qui surgissent des cinétosomes plus aborales. Ce système fibrillaire tapisse la ceinture aborale à niveau de l'extremité proximal de ses cinétosomes et s'etend aboralément à environ 1.5 μm (Pl. I 2 et 3).

Fibres orales (F O). Ces fibres sont observées à microscope photonique comme petites fibres de 2 μm de longueur qui surgissent à gauche de chaque cinétie et qui se dirigent vers la zone orale (Pl. I 2).

Dans la microscopie électronique nous observons que ces fibres naissent à gauche du cinétosome le plus oral de chaque cinétie, de son bout proximal et en contact avec une masse electrondense qui entoure une grand partie du côté gauche du cinétosome (Pl. I 3 et Pl. II 6). Dans leur parcours, elles s'inclinent d'abord à gauche et après elles s'orientent vers la région orale et vers la pellicule (Pl. II 6) tout en diminuant leur épaisseur.

Fibres aborales (FA). Elles forment un système que avec notre méthode argentique, apparaît beaucoup plus évident que les deux systèmes déja decrites. Il s'agit de longues fibres qui surgissent à la suite des cinéties de la ceinture, en formant un anneau d'environ 15 μm de large, autour du pôle aboral du cilié (Pl. I 1 et 2).

En microscope électronique, nous observons que ces fibres sont continuation directe de la baguette dense placée à gauche de chaque cinétie. Ces baguettes intercinétiques (B) sont des bandes minces de 0.03 μm d'épaisseur et 0.2 μm de profondeur, parallèles aux cinéties de la ceinture (Pl. I 4 et Pl. II 6). Dans la partie orale antérieure, ces baguettes entrent en contact avec la fibre orale de chaque cinétie (Pl. I 4 et Pl. II 6); Aboralément, quand elles ont depassé le dernier cinétosome, on voit qu'elles sont formées par deux fibres parallèles: l'une d'elles, la plus grosse, a une structure semblable à la baguette intercinétique, tandis que l'autre, plus mince, et placée plus près de la pellicule du cilié est un peu plus opaque aux électrons (Pl. I 4).

(2) Autres structures en rapport avec la bande trochale

Dans cette zone existent d'autres structures qui ne sont pas toutes visibles en microscopie photonique. Ces structures sont: les crêtes et les plis pelliculaires, l'argyrome, les sacs parasomaux et le système myonémique.

Crêtes (C) et plis pelliculaires (P). On les observe a la surface du cilié (Pl. II 7). Aux proximités de la ceinture aborale, ces crêtes deviennent plus grandes et forment des "plis pelliculaires" qui limitent oralément et aboralément la ceinture; l'un du côte oral (PO) et deux du côté aboral (PA 1 et PA 2), (Pl. II 7). L'épaisseur de ces plis est d'environ 140 nm (Pl. I 4) et dans son intérieur on vois des microfibrilles (↑ Pl. II 7).

L'argyrome (A). Les minces lignes argyrophiles qui entourent le cilié et qui dans son ensemble forment l'argyrome (Pl. I 1) apparaissent dans la microscopie électronique formant l'axe des crêtes pelliculeuses (Pl. II 7).

Dans les plis pelliculaires ces axes sont remplacés par des microfibrilles allongées que nous avons décrit (Pl. II 7).

Sacs parasomaux (SP). On observe trois rangs de cacs parasomaux; limitant la frange aborale, un rang dans la partie orale et deux autres dans la région aborale. Dans la région orale on observe les sacs parasomaux très bien rangés avec les cinéties de la ceinture. Ils sont localisés exactement au devant du cinétosome plus oral de chaque cinétie, avant le pli pelliculaire (Pl. I 3). Dans la région aborale on obser-

ve parmi les cinéties, un rang avant le premier pli postérieur et un autre parmi les deux plis (Pl. I 4).

Système myonémique (SM). Dans la microscopie optique on observe très nettement deux systèmes myonémiques chez *O. henneguyi*: un puissant système myonémique antérieur et un autre, postérieur, beaucoup plus faible et délicat. L'un et l'autre systèmes se dirigent vers la ceinture aborale. Avec l'aide du microscope électronique, nous avons pu vérifier que la frange aborale est tapissée à l'intérieur par des faisceaux microfibrillaires, àu niveau peu inférieur aux fibres cinetodésmiques (Pl. I 3 et 4 Pl. II 7 et 8).

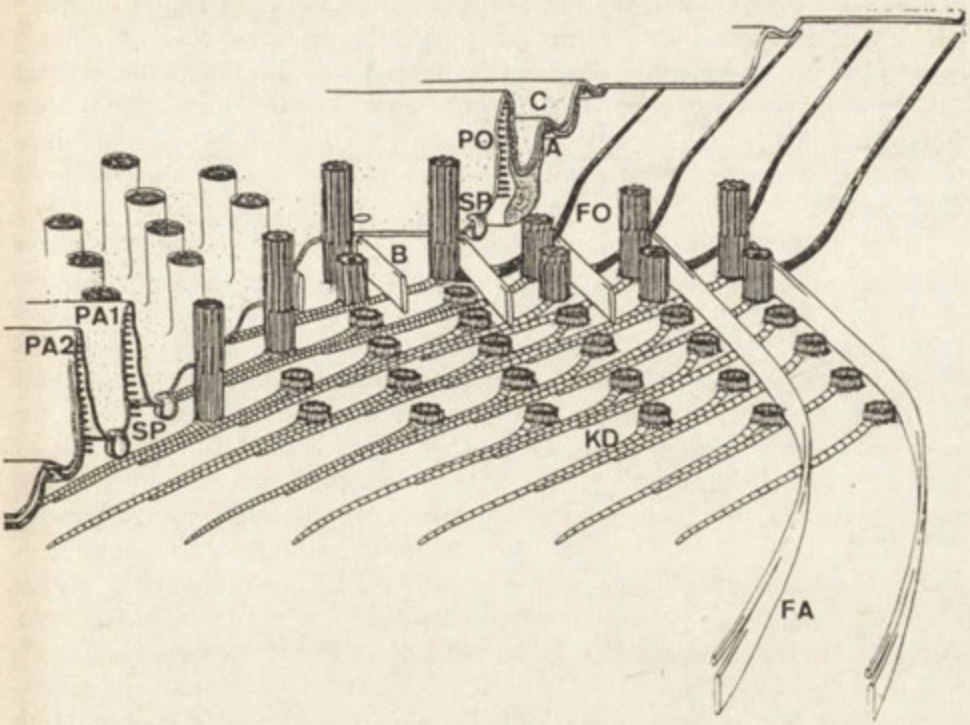
Discussion

Les systèmes fibrillaires annexés à la frange aborale de *Opisthonecta henneguyi* ont été à peine cités par les auteurs qui ont travaillé en microscope photonique; seulement Kofoid et Rosenberg (1940) décrivent des "membranelles obliques connectives" et aussi des "fibres radiales" qu'intègrent un complexe "appareil neuromoteur".

Nous avons décrit (Guinea et Fernández-Galiano 1981), les différents systèmes fibrillaires de la bande trochale de *O. henneguyi* visibles en microscope photonique et Fernández-Leborans (1982) a décrit dans *Opisthonecta* sp. des systèmes fibrillaires qui coincident partiellement avec ceux que nous avons observé chez *O. henneguyi*.

Bradbury (1965), en utilisant la technique de Chatton-Lwoff n'observe pas en microscope optique ces systèmes fibrillaires, mais à niveau électronique elle décrit en *O. henneguyi* une baguette placée entre les "cinéties" de la ceinture, qui s'allonge oralément 0.2 μm et aboralément 15 μm; cette auteur observe, aussi, les fibres périodiques à niveau de l'extrémité proximale des cinétosomes; mais elle ne peut pas préciser ni leur origine ni leur extension, et elle est d'avis que ces fibres pourraient être pareilles aux petites "racines" de la ceinture ciliaire de *Trichodina* et *Trichodinopsis*.

Nous croyons que les "fibres périodiques" que nous decrivons à niveau électronique ("fibres obliques" à niveau optique) sont le même que les "membranes obliques connectives" que décrivent Kofoid et Rosenberg (1940) et les "fibres périodiques" qu'observe Bradbury (1965). Nous avons pu constater que ces fibres ont une structure périodique avec une périodicité de 15 nm environ, nous avons aussi précisé leur origine et leur étendue, ce qui confirme notre avis, qu'il s'agit de fibres cinétodesmiques de chaque cinétosome de la bande trochale (Text-fig. 1).



Text-fig. 1. Figuration schématique de la frange aborale d'*Opisthonecta henneguyi* montrant son structure génerale: argyrome (A), baguette intercinétique (B), crêtes pelliculaires (C), fibres orales (FO), fibres aborales (FA), fibres cinétodesmales (KD), plis aborales (PA1 et PA2), pli oral (PO) et sacs parasomaux (SP)

A notre avis, Fernández-Leborans (1982) interprète mal ce système fibrillaire, chez *Opisthonecta* sp. car il écrit que ces fibres surgissent chacune de cinétie du bord aboral de la ceinture, exactement comme il montre dans son dessin. Cependant dans une de ses photographies (Fig. 5 de son travail) on peut s'appercevoir que quelques unes de ces fibres parcourent une partie de la ceinture aborale même et ne naissent pas au même bord.

Ce système fibrillaire est semblable au "système de cinétodesmes" (fibres F_2) que Rouiller et Fauré-Fremiet (1958) décrivent dans un autre Péritriche, *Ophrydium versatile*, qui forment un angle droit avec d'autres fibres, F_1, parallèles aux cinéties de la ceinture; et aussi au système de "fibres cinétodesmiques" que Hausmann et Hausmann (1981 a, b) observent dans *Trichodina pediculus*.

Avec la méthode de Fernández-Galiano nous avons pu montrer en utilisant le microscope optique deux autres systèmes fibrillaires: un système oral et un autre aboral. Avec le microscope électronique nous pouvons constater que les "fibres orales" ne sont pas, comme

affirme Bradbury (1965), la prolongation orale de la baguette intercinétique; elles surgissent au niveau plus profond, de la base du cinétosome plus oral de chaque cinétie (Text-fig. 1) et leur longueur est dix fois supérieure à celle qui fut signalée par Bradbury (1965). Les fibres ascendantes (ASF) qu'observe Fernández-Leborans dans *Opisthonecta* sp. en utilisant cette même méthode, sont un peu mois évidentes et un peu plus courtes que celles de *O. henneguyi*.

Nous observons, comme Bradbury (1965), le "système de fibres aborales" en connexion avec la baguette intercinétique, mais nous y trouvons (comme nous avons décrit avant) deux fibres parallèles (Text-fig. 1). Nous croyons que ces fibres sont celles que Kofoid et Rosenberg (1940) décrivent comme des "fibres radiales" qui mettent en rapport la bande trochale avec l'anneau aboral.

En ce qui concerne les pores pelliculaires, Bradbury (1965) et Foissner (1975) décrivent chez *O. henneguyi* un rang qui limite aboralément la ceinture; nous avons observé qu'il s'agit de trois rangs de pores, et pas seulement d'un ces trois rangs limitent la bande trochale: il y en a un dans la région orale et deux autres dans la région aborale (Text-fig. 1). Nous croyons, comme Corliss (1979 p. 25), que tous les trois doivent être interprétés comme des "sacs parasomaux". Ces sacs parasomaux ne se manifestent pas avec notre méthode argentique, mais on les vois en utilisant des autres méthodes d'impregnation argentique. Les aboraux n'etant pas rangés avec les cinéties de la ceinture, ont été observés facilement par les auteurs, tandis que les pores du rang oral, placés exactement au-dessus du cinétosome oral de chaque cinétie, ont été confondus avec de cinétosomes.

Le système myonémique des Péritriches est connu depuis longtemps. Lynch et Noble (1931) décrivent dans ce *Protozoa* un fort système myonémique antérieur, Kofoid et Rosenberg (1940) ont observé, en plus, un autre, postérieur, qui entoure la scopula. Nous avons décrit (Guinea et Fernández Galiano 1981) en détail les deux systèmes myonémiques au niveau optique, mais avec les grossisements utilisés nous n'avons pas pu vérifier la présence de myonémes dans la bande trochale du cilié. Le système myonémique observé par nous en microscope optique, coincide, dans les traits généraux, avec les systèmes décrits par les dits auteurs, mais celui qu'a observé Fernández-Leborans (1982) en *Opisthonecta* sp. est totalement différent. Cet auteur décrit d'épais paquets fibrillaires ramifiés qui sortent de la bande trochale et se dirigent oralement et aboralement (ARFB et PRFB).

Bien qu'auparavant personne n'a décrit de paquets mionémiques dans *O. henneguyi* nous avons vérifié leur existence même à niveau de la bande trocale, où ils sont placés au dessous du cinétosomes à niveau légèrement inférieur que des fibres cinétodesmiques. Les faibles

faisceaux microfibrillaires que nous observons dans cette zone sont semblabes à ceux qu'ont décrit Hausmann et Hausmann (1981 a, b) dans la bande locomotrice de *Trichodina pediculus*.

A notre avis, la structure de la ceinture aborale de *O. henneguyi*, est semblable, dans leurs traits généraux, à le bande locomotrice de *Trichodina pediculus* (Hausmann et Hausmann 1981 a et b): une frange ciliaire, qui est limité oralement par un septum ou un pli antérieur et aboralement par un septum basal et le disque adhésif.

En ce qui concerne la position systèmatique nous croyons, comme Foissner (1977), que d'aprés leurs caractéristiques, on ne peut pas inclure *O. henneguyi* ni parmi les *Mobilina* ni parmi les *Sessilina*, mais dans un sous-ordre indépendant, peut-être dans le sous-ordre *Natantina* selon Jankowski, 1967.

BIBLIOGRAPHIE

Bradbury P. C. 1965: The infraciliature and argyrome of *Opisthonecta henneguyi* Fauré-Fremiet. J. Protozool., 12, 345 - 363.

Corliss J. O. 1979: The Ciliated *Protozoa*. Characterization, Classification and Guide to the Literature, 2nd ed. Pergamon Press, London and New York, 455 pp.

Fauré-Fremiet E. 1906: Sur une nouvelle vorticellide, *Opisthonecta henneguyi*. C. R. Seances Soc. Biol. Fil., 60, 922 - 923.

Fernández-Galiano D. 1976: Silver impregnation of ciliated *Protozoa*: procedure yielding good results with the pyridinated silver carbonate method. Trans. Am. Microsc. Soc., 95, 557 - 560.

Fernández-Leborans G. 1982: Some characteristics of the fibrilar systems in *Opisthonecta* sp. (*Ciliophora, Peritrichida*). Arch. Protistenk., 126, 221 - 227.

Foissner W. 1975: *Opisthonectidae* (*Ciliata, Peritricha*) nov. fam. und Revision der Genera *Telotrochidium* (Kent) und *Opisthonecta* Fauré-Fremiet). Protistologica, 11, 395 - 414.

Foissner W. 1977: Revision der Genera *Astylozoon* (Engelmann) und *Hastatella* (Erlanger) (*Ciliata, Natantina*). Protistologica, 13, 353 - 379.

Guinea A. et Fernández-Galiano D. 1981: Infraciliación y sistema mionémico de *Opisthonecta henneguyi*. Communication a la V Reunión Bienal de la Real Soc. Esp. Hist. Nat., Oviedo no. 118.

Hausmann K. et Hausmann E. 1981 a: Structural studies on *Trichodina pediculus* (*Ciliophora, Peritricha*). I. The Locomotor Fringe and the oral Apparatus. J. Ultrastruct. Res., 74, 131 - 143.

Hausmann K. et Hausmann E. 1981 b: Structural studies on *Trichodina pediculus* (*Ciliophora, Peritrichida*). II. The adhesive disc. J. Ultrastruct. Res., 74, 144 - 155.

Jahn T. L. et Hendrix E. M. 1969: Locomotion of the telotroch ciliate *Opisthonecta henneguyi*. Rev. Soc. Mex. Hist. Nat., 30, 103 - 131.

Kofoid Ch. A. et Rosenberg L. E. 1940: The neuromotor system of *Opisthonecta henneguyi* (Fauré-Fremiet). Proc. Am. Philos. Soc., 82, 421 - 436.

Lynch J. E. et Noble A. E. 1931: Notes on the genus *Endosphaera* Engelmann and on its occasional host *Opisthonecta henneguyi* Fauré-Fremiet. Univ. Calif. Publ. Zool., 36, 96 - 114.

Rosenberg L. E. et Grim J. N. 1966: Ultrastructure of some buccal organelles of *Opisthonecta*. J. Microsc. Oxford, 787 - 790.

Rouiller Ch. et Fauré-Fremiet E. 1958: Ultrastructure des cinétosomes à l'état de repos et à l'état cilifère chez un cilié péritriche. J. Ultrastruct. Res., 1, 289 - 294.

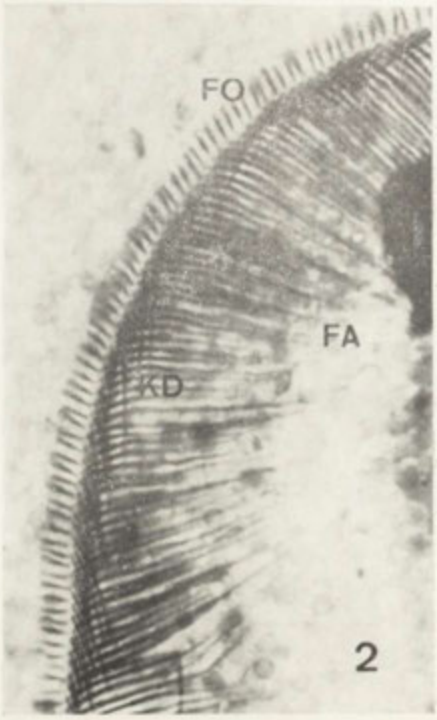
EXPLICATION DES PLANCHES I - II

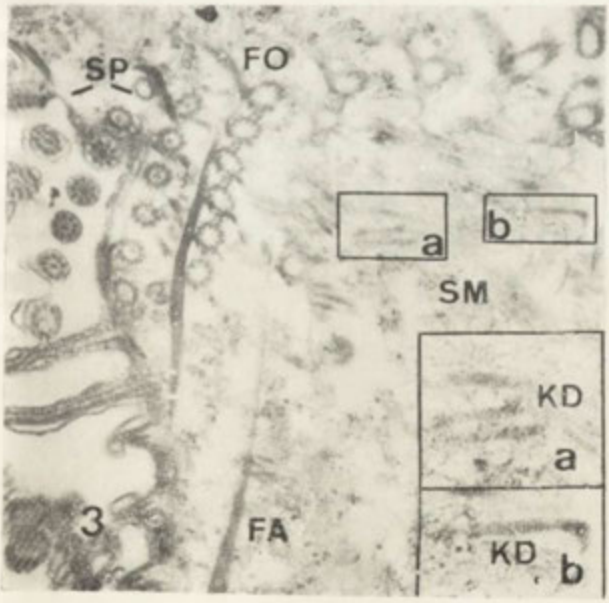
PLANCHE I

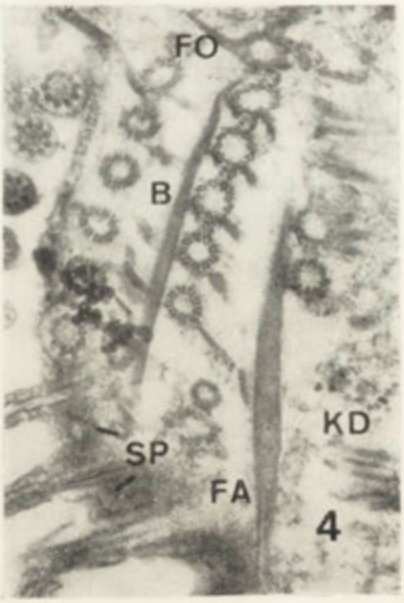
1: Microscopie photonique. Aspect général d'un indivudu d'*Opisthonecta henneguyi* coloré selon la méthode de Fernández-Galiano. × 750
2: Microscopie photonique. Détail de l'infraciliature de la frange aborale. × 1700
3: Coupe tangentielle légèrement oblique à la frange aborale, vue d'intérieur du cilié, qui montre les differents systèmes fibrillaires; fibres cinétodesmales (KD), fibres orales (FO), et fibres aborales (FA). On observe aussi les sacs parasomaux orales (SP) et le système myonémique (SM) × 21000; a et b detail des fibres cinétodesmales (KD) (× 40 000)
4: Section tangentielle de la frange aborale, vue d'intérieur de la cellule, montrant les sacs parasomaux aborales (SP) et les fibres (KD, FA, FO et B) × 29 000

PLANCHE II

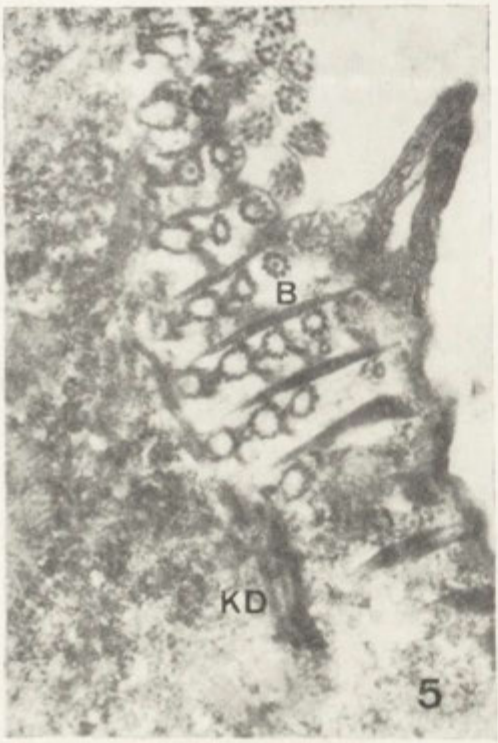
5: Section oblique aux cinéties de la ceinture vue d'extérieur du cilié, qui montre les fibres périodiques (KD) et la baguette intercinétique (B) × 20 000
6: Coupe tangentielle à la frange aborale vue d'exterior de la cellule. On observe la disposition des cinéties de la ceinture, des fibres orales (FO), qui naissent du cinétosome orale de chaque cinétie, des baguettes intercinetiques (B) et des fibres aborales (FA) × 21 000
7: Section perpendiculaire à la ceinture, qui montre les crêtes (C), les plis pelliculaires (PO, PA 1 et PA 2), avec microfibrilles dans son intérieur, (↑) le système mionémique (SM) × 21 000
8: Coupe grosse tangentielle à la frange aborale, vue d'extérieur du cilié. On peût observer les differents systèmes fibrillaires: fibres cinétodesmiques (KD), fibres orales (FO), baguette intercinetique (B), fibres aborales (FA) et système myonémique × 21 000

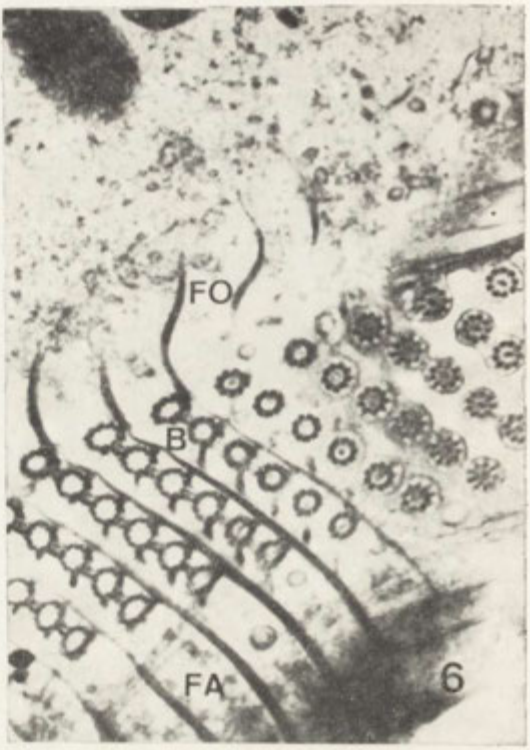


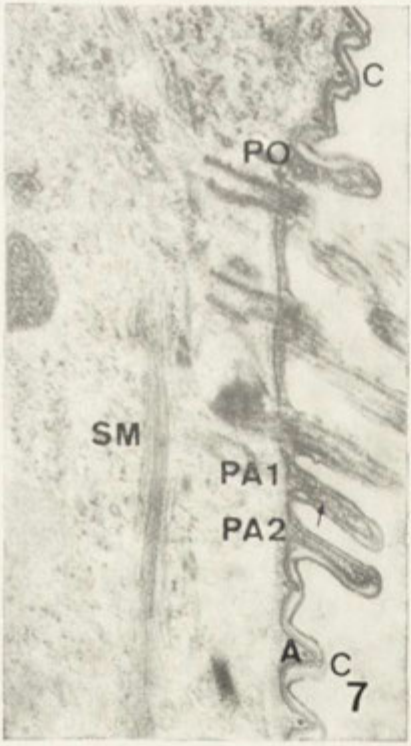


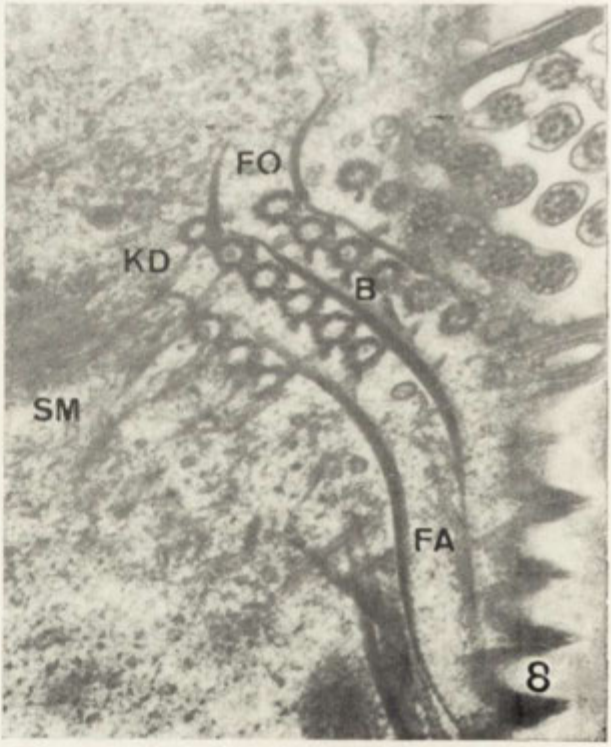


A.. Guinea et al. auctores phot.









A. Guinea et al auctores phot.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 23 - 32 (1986)

Ultrastructure chez *Histriculus similis* (Cilié hypotriche)

P. CALVO, A. TORRES, C. FEDRIANI, R. M. RIOS et J. PEREZ SILVA

Departamento de Microbiologia, Facultad de Biologia, Universidad de Sevilla, Sevilla-41080, Ap. 1095, España

Received on 12 July 1984, *revised on* 11 April 1985

Synopsis. L'ultrastructure du *Histriculus similis* répond à celle décrite chez les autres Hypotriches aussi bien pour l'organisation de la ZAM et des cirres que pour l'appareil nucléaire. Les differences apparentes signalées dans l'organisation de la membrane parorale externe (Stichomonade ou Polystichomonade) des Hypotriches sont attribuées à l'orientation distincte de l'axe des couples de cinétosomes en rapport à l'axe longitudinal de la parorale.

Les protozoaires ciliés appartenent à l'orde Hypotrichide se caractérisent par un système complexe des structures ciliées. La zône adorale de paramembranelles (ZAM) délimite le bord droit de la cavité bucale, et dans la plupart des Ciliés Hypotriches étudiés (Albaret et Grain 1973, Bąkowska and Jerka-Dziadosz 1978, Gortz 1982, Grim 1972, Grimes 1972, Grimes and Adler 1976, Grimes and L'Hernault 1978, Jerka-Dziadosz 1980, 1981, 1982, Luporini et Magagnini 1970, Nobili 1971, De Puytorac et al. 1976, Ruffolo 1976 a, b, Wicklow 1979, 1981, 1982), est constitué par paramembranelles de 4 rangées des cinétosomes ciliés. Seulement *L. acuminata* (pas publié) a des paramembranelles composées par 5 rangées de cinetosomes. A droite de la ZAM est localisée, la ciliation parorale formée par une parorale interne (PI) et une parorale externe (PE). Dans tous les cas etudiés la parorale interne est composé d'une rangée de cinétosomes (Stichomonade). Cependant le nombre des rangées de cinétosomes de la parorale externe varie selon les espèces. Le reste de la ciliation de la face ventrale est constitué par des cirres, groupements de cinétosomes ciliés formant un parallélograme. Dans ce travail nous avons étudié l'ultrastructure de la face ventral du Cilié Hypotriche *Histriculus similis*.

Matériel et Méthodes

Comme matériel de travail nous avons utilisés le Cilié Hypotriche *Histriculus similis* maintenue dans l'eau minérale à 20±1°C, comme nourriture on a utilisé une algue verte unicellulaire, *Chlorogonium* sp.

Pour les observations au microscope optique nous avons réalisé des préparations colorées avec du proteinate d'argent (Nieto et al. 1981). Pour l'étude ultrastructurelle, les ciliés furent fixés avec un mélange de glutaraldéhyde à 4% (lv) et tétroxyde d'osmium à 2% (lv) tamponés avec du tampon cacodylate 0.1M pH = 7.4, pendant 40 min à 4°C, deshydrates et inclues dans le Spurr.

Le matériel a été coupé dans un ultramicrotôme Ultracut Jung et contrasté avec l'acétate d'uranyle (2 h) et citrate de plomb (7 min). Les observations ont été effectué au microscope de transmission Siemens Elmiscop 102.

Resultats

Chez *Histriculus similis* la zône adorale de paramembranelle (ZAM), qui délimite le bord gauche du peristome, présente 40 paramembranelles (Pl. I 1) chacune d'elles étant constituée par quatre rangées de cils de longueur différente. Dans la ZAM on peut distinguer les paramembranelles ventrales qui parcourent la plupart de la moitié antérieure gauche du cilié et les paramembranelles frontales (ou collier) qui se courbent vers l'extréme antérieur de l'organisme.

Tous les paramembranelles sont constituées par quatre rangées parallèles de cils (Pl. I 2 et 3). Dans les paramembranelles ventrales les deux plus larges rangées de cilies sont les rangées posterieures. On désigne conventionellement comme rangées 1 et 2, toutes les deux présentent un nombre identique de cinétosomes. La troisième rangée est un peu plus courte, et la quatrième (la plus antérieure dans le paramembrane ventrale) présente une réduction drastique de nombre de cils, étant donné que normallement elle est constituée par seulement trois ou quatre cinétosomes (Pl. I 2 et 3).

Les études au microscope électronique montrent que les cinétosomes qui constituent les paramembranelles de la ZAM présentent les caractéristiques générales décrites chez les autres ciliés. Les 3 ou 4 cinétosomes qui apparaissent dans la rangée 4 présentent 4 - 5 microtubules transverses (T) sur son côté libre (antérieur en rapport à l'axe antérieur-postérieur de la cellule) (Pl. I 2). Chaque cinétosome de la rangée 1 a une rangée de 4 - 6 microtubules postciliaires (Pc) (Pl. I 3). Les microtubules transverses (T) et postciliaires (Pc) permettent d'assigner une orientation à la paramembranelle. Ces fibres transverses servent donc comme indicateurs du côté gauche de la paramembranelle, ainsi la zône adoral a une orientation antérieure-postérieure propre, déterminée par la disposition des cinétosomes.

L'orientation de la paramembranelle par rapport à la cellule est indiquée dans la Pl. I 3. La partie antérieure correspond à la plus courte rangée qui porte les fibres transverses, la zône postérieure à la rangée qui porte de microtubules postciliaires (Pc), celle de droite serait, donc, la zône où se trouvent linéallement ordonnés tous les cinétosomes des 4 rangées, et celle de gauche sur le côté opposé.

La seule différence entre les paramembranelles ventrales et frontales c'est que dans ces dernières, la rangée 4 est un peu plus longue et ne commence pas sur la marge droite de la paramembranelle mais, au niveau du troisième cinetosome de la rangée 3 (Pl. I 3).

Les cinétosomes paramembranellaires sont liés par une réseau du matériel electrodense (Pl. I 3) qui s'étend sur différents niveaux. En sections transversales au niveau de la zône du "cartwheel" des cinétosomes, on a trouvé des connexions longitudinales antérieures (AC) et postérieures (PC) et des connexions transversales (TC). Au dessous, de cette zone, se trouvent les connexions postmembranellares (PMC), doubles postérieures (DPC) et diagonales (DC) — à ce niveau là on observe aussi le matériel electrodense (dm) associée aux microtubules postciliaires des cinétosomes de la rangée 1. De même que dans *L. acuminata* (pas publié) on n'a pas détecté içi des connexions obliques ni transversales gauches ni droites.

Les paramembranelles sont séparées par quelques crêtes cytoplasmatiques ou "crêtes intermembranellaires" (Pl. I 2) qui sont formés par des microtubules subpelliculaires croisés et formant une cape, et au niveau plus profond des faisceaux de microtubules transverses et postciliaires provenant de paramembranelles voisines.

On a observe une grande quantité de mitochondries approchés aux paramembranelles.

A droite de la zône adorale des paramembranelles se trouve la ciliation parorale (CP) (Pl. I 1) formée par la membrane parorale interne (PI) et la membrane parorale externe (PE) qui se insérent respectivement sur le côté droit et gauche d'un plis cytoplasmatique qui délimite le bord droit du péristome, séparant ainsi le cortex ventral de l'oral.

La parorale interne (PI), la plus proche de la ZAM est constituée par une seule rangée de cinétosomes (Pl. II 4) chacune d'eux a une faisceau de 6 - 7 microtubules postciliaires (Pc) dans la zône gauche et 4 - 5 microtubules transverses (T) à droite, les cinétosomes se trouvent rassemblés par des connexions obliques.

La parorale externe (PE) est constituée par deux rangées de cinétosomes, disposées selon un pattern en zig-zag (Pl. II 5, 6). Les cinétosomes sont couplés et l'axe de chaque paire forme avec l'áxe longitudinal de la rangée un angle aigu. Du cinétosome gauche de chaque couple, c'est à dire du cinétosome plus proche de la parorale interne, surgissent des

microtubules transverses (Pl. II 5). On n'a pas détecté des fibres postciliaires dans la parorale externe.

Le reste de la ciliation ventrale est constituée par les cirres, alignements de cinétosomes normalement ciliés et distribués, formant un parallelograme (Pl. II 7 - 9). Le nombre de cinétosomes varie suivant le cirre étudié, ceux de grandes tailles qui ont 7 - 8 cinéties de 6 cinétosomes chacune, ce sont les cirres frontaux et transversaux, pendant que ceux de taille inférieure ou plus petite avec 6 cinéties et 3 - 4 cinétosomes par cinétie sont les cirres marginaux.

Au microscope électronique tous les cirres présentent la même organisation de base. Tous les cinétosomes de la rangée externe ont 8 - 10 microtubules postciliaires (Pc) (Pl. II 7, 9) la dite rangée peut être considerée comme une cinétie, et ce côté est le côté droit du cirre. Les cinétosomes de la ligne externe opposée qui correspondent à la marge gauche du cirre, ont des microtubules transverses (T) (Pl. II 8). Certains cirres presentent une fibre cinétodesmale (Kd) associée au cinétosome antérieure droit (Pl. II 7).

Tous les cinétosomes du cirre sont fermement unis par une matériel fibrilaire electrodense (Pl. II 7 - 10). Les cinétosomes dans la même position par rapport à des cinéties voisines sont liés par les connexions doubles et parfois triples. Il existe aussi des connexions obliques simples et doubles. Tous les cinétosomes de la même cinétie sont unis par des connexions simples (Pl. II 8).

Les connexions se prolongent sur la péripherie du cirre. Les cirres sont entourés d'un matériel fibrilaire électrodense qui est la prolongation de la substance cimentante intercinétosomale et qui parvient à entrer en contact avec la pellicule du protozoaire.

Des faisceaux de microtubules partent de ce matériel electrodense et se dirigent vers la superficie du protozoaire. Au debut ces faisceaux sont perpendiculaires, mais plus tard elles courent parallèlement à la pellicule et contribuent à la couche microtubulaire subpelliculaire.

La pellicule de *Histriculus similis*, comme celle d'autres ciliés hypotriches, présente 3 unités de membrane (Pl. III 11). En dessous de la membrane la plus externe il existe une cape alvéolaire discontinue, qui dans la zône interne présente une couche de microtubules, aux bras latéraux parallèle à la superficie (Pl. III 11, 12). Dans certains zônes ont peut trouver des faisceaux de microtubules postciliaires et transverses provenants des cirres.

L'appareil nucléaire de *Histriculus similis* presente 4 fragments macronucléaires et 4 - 7 micronoyaux.

Les études au microscope électronique indiquent que l'enveloppe macronucléaire présente 2 et quelquefois 3 unités de membrane (Pl. III

13) avec une grande quantité de pores (P) (Pl. III 13, 14). La chromatine est distribuée en grandes accumulations et on observe des faisceaux de microtubules dans le nucléoplasme (Pl. III 15, 16). Ces faisceaux probablement jouent un rôle important dans le processus d'elongation et de division du macronoyaux. Les nucleoles sont trés nombreux.

Chez *H. similis* pendant la période de synthèse d'ADN macronucléaire, de même que dans la reste des ciliés hypotriches étudiés, apparaissent les bandes de réplication (Pl. IV 17). Dans ces bandes on peut distinguer clairement, deux zônes; zone antérieure où la chromatine condensée se désorganise et produit des fibres, et une zone posterieure, où ces fibres disparaissent et où l'on trouve seulement des très fins filaments. A l'extrême de la zône supérieure de la bande, des corps chromatiniques apparaissent de nouveau. Les nucléoles (nu) présentent un aspect distinct suivant le côté de la bande où ils se recontrent. On détecte sur le côte postérieur, des nucléoles avec une grande zône centrale granulaire, ce qui indique qu'ils sont en transcription active.

Les micronoyaux n'ont pas de nucléoles, contients de la chromatine disperse et dans l'enveloppe, composé de deux unités de membrane — il n'y a pas de pores pendant l'interphase.

Discussion

L'ultrastructure de l'appareil bucal de *Histriculus similis* est très similaire à celle décrite chez *Gastrostyla steinii* (Grim 1972), *Oxytricha fallax* (Grimes 1972), *Stylonychia mytilus* (De Puytorac et al. 1976), *Paraurostyla weissei* (Bąkowska et Jerka-Dziadosz 1978) et *Laurentiella acuminata* (pas publié). Chaque paramembranelle est constituée par 4 rangées de cinétosomes ciliés, tous les cinétosomes de la rangée 1 portent des microtubules postciliaires (Pc) et ceux de la rangée 4 portent des microtubules transverses (T). Les cinétosomes de la 2ª et 3ª rangée ne portent pas de microtubules transverses, sauf chez *Stylonychia mytilus* (De Puytorac et al. 1976) où on les a trouvé sur le côté libre des cinétosomes de la 3ª rangée. Il existe très peu des informations sur l'ultrastructure des paramembranelles frontales, on la décrits seulement chez *Paraurostyla weissei* (Bąkowska et Jerka-Dziadosz 1978); ces informations sont en rapport avec celles signalées pour *Histriculus similis*. Les paramembranelles frontales sont orientées parallélement à l'axe longitudinal de la cellule, tandis que les paramembranelles ventrales sont orientées perpendiculairement. D'autre part dans les paramembranelles frontales la 4^{iéme} rangée est constituée par 4 ou 5 cinétosomes qui commencent

au niveau du 3^{ième} ou 4^{ième} cinétosome de la rangée 3. Le système de connexion entre les cinétosomes des paramembranelles, chez *Histriculus similis* est le même que celui décrit chez les autres Ciliés Hypotriches.

Le complexe paroral de *Histriculus similis* en état végétatif, a une parorale interne constituée par une seule rangée de cinétosomes (Stichomonade) et une parorale externe de deux rangées de cinétosomes (Polystichomonade). *Laurentiella acuminata* (pas publié) et *Histriculus similis* montrent une parorale externe constituée par deux rangées de cinétosomes, alors que *Gastrostyla steinii* (Grim 1972) a 2-4 rangées et *Paraurostyla weissei* (Bąkowska et Jerka-Dziadosz 1978), *Pseudourostyla cristata* (Bąkowska et Jerka-Dziadosz 1978) et *Keronopsis rubra* (Bąkowska et Jerka-Dziadosz 1978) ont une polystichomonade de 4-5 rangées de cinétosomes. Chez *Stylonychia mytilus* (De Puytorac et al. 1976) la parorale externe a une rangée de cinétosomes, mais les microtubules transverses apparaissent alternants chaque deux cinétosomes. Cette observation jointe à la disposition en zig-zag des cinétosomes de la parorale externe de *Histriculus similis*, suggére, comme ont dejá signale Bąkowska et Jerka-Dziadosz (1978), que chez *Stylonychia mytilus* (De Puytorac et al. 1976) existe aussi une polystichomonade, mais masquée par la courbe des couples de cinétosomes. On peut donc supposer que l'organisation en polystichomonade de la parorale externe est commune dans tous les Hypotriches, et les seules variations dans cet groupe sont au niveau des rangées de cinétosomes.

Dans tous les Hypotriches étudiés, la membrane parorale interne est formée par une rangée de cinétosomes qui sont invertis dans le plan dorsoventral, aproximativement 180° par rapport aux cinétosomes de la membrane parorale externe. Cette inversion est provoquée par la formation d'un plis cytoplasmatique entre les deux membranes parorales (Bąkowska et Jerka-Dziadosz 1978), les microtubules transverses de la membrane parorale interne et la membrane parorale externe etant dirigées vers le plis cytoplasmatique.

Le reste de la ciliation de la face ventrale est constituée par les cirres. L'ultrastructure des cirres chez *Histriculus similis* est la même que celle décrite chez les autres Ciliés Hypotriches. Chez *Histriculus similis* nous avons trouvé, dans certains cirres marginaux, une fibre cinétodesmale associée au cinétosome antérieur droit, pendant que chez *Oxytricha fallax* (Grimes 1972) il y a une fibre cinétodesmale associée a chacun des cinétosomes de la cinétie externe droite dans le cirre ventral 1, frontal 7 et dans quelques cirres marginaux. Chez *Stylonychia mytilus* (De Puytorac et al. 1976) les fibres cinétodesmales sont absents sauf dans certains cirres marginaux où ils sont très courts.

L'organisation du cortex chez *Histriculus similis* est le même que celui décrit chez les autres Ciliés Hypotriches; 3 unités de membrane, et une couche alvéolaire renforcée par un système de microtubules aux bras latéraux.

Le macronoyau est morphologiquement et ultrastructuralement pareille à celui d'autres Ciliés Hypotriches. L'enveloppe macronucléaire est composée par 3 unités de membrane — une observation similaire a été dejá fait par Matsusaka (1981) chez *Histriculus muscorum*. Les bandes de réplication observées chez *Histriculus similis* ont ultrastructurellement le même aspect que ceux observées chez *Euplotes* (Ruffolo 1978) et d'autres Hypotriches (Walker and Goode 1976, Chadha 1980) chaque bande a deux zônes: une zone antérieure où la chromatine macronucléaire condensée se désintégre produisant des fibres de chromatine et une zône postérieure où la chromatine se condense graduellement formant à nouveau des corps chromatiniques. Prescott (1962) signalait que la synthèse d'ADN a lieu sur le bord antérieur de la zône postérieure de la bande, et que dans cette bande il n'y a pas de synthèse d'ARN. Cependant, nous avons observés chez *Histriculus similis*, quelques nucléoles localisés dans la bande de réplication comme a été signalé par Ruffolo (1978) chez *Euplotes*. Les nucléoles ont un aspect différent d'une part à l'autre de la bande, étant donné leur activité distincte. Une fois que la bande de réplication est passée, les nucléoles ont une grande granulaire zône central ce qui indique qu'il sont transcriptionellement actifs (Matsusaka and Kimura 1981).

Dans les macronoyaux de *Histriculus similis* nous avons observés des faisceaux des microtubules qui facilitent probablement l'élongation pendant la fragmentation macronucléaire comme il a été décrit chez *Dileptus* (Bohatier 1977), *Paramecium* (Tucker et al. 1980), *Tetrahymena* (Davidson and La Fountain 1975), *Stylonychia mytilus* (Walker and Goode 1976), *Gastrostyla steinii* (Walker and Goode 1976) et *Euplotes* (Ruffolo 1979).

BIBLIOGRAPHIE

Albaret J. L. et Grain J. 1973: L' ultrastructure de *Plagiotoma lumbarici* Dujardin (Cilié, hétérotriche). Protistologica, 9, 221 - 234.

Bakowska J. and Jerka-Dziadosz M. 1978: Ultrastructural analysis of the infraciliature of the oral apparatus in *Paraurostyla weissei* (*Hypotrichida*). Acta Protozool., 17, 285 - 301.

Bohatier J. 1977: Incorporation D'Actinomycine D ^{3}H et D'Uridine ^{3}H pendant la régénération et la division du Cilié *Dileptus anser*. Etude autoradiographique en microscopie électronique. Protistologica, 13, 89 - 99.

Chadha R. 1980: Fine structure of the chromatin during the micronuclear mitotic cycle in *Stylonychia mytilus* Ehrenberg. Arch. Protistenk., 123, 406 - 421.

Davidson L. and La Fountain J. R. 1975: Mitosis and early meiosis in *Tetrahymena pyriformis* and the evolution of mitosis in the Phylum *Ciliophora*. Biosystems, 7, 326 - 336.

Gortz H. D. 1982: The behavior and fine structure of the dorsal bristles of *Euplotes minuta, E. aediculatus* and *Stylonychia mytilus* (*Ciliata, Hypotrichida*). J. Protozool., 29, 353 - 359.

Grim J. N. 1972: Fine structure of the surface and infraciliature of *Gastrostyla steinii*. J. Protozool., 19, 113 - 126.

Grimes G. W. 1972: Cortical structure in nondividing and cortical morphogenesis in dividing *Oxytricha fallax*. J. Protozool., 19, 428 - 445.

Grimes G. W. and Adler J. A. 1976: The structure and development of the dorsal bristle complex of *Oxytrich fallax* and *Stylonychia pustulata*. J. Protozool., 23, 135 - 143.

Grimes G. W. and L'Hernault S. W. 1978: The structure and morphogenesis of the ventral ciliature in *Paraurostyla hymenophora*. J. Protozool., 25, 65 - 74.

Jerka-Dziadosz M. 1980: Ultrastructural study on development of the hypotrich ciliate *Paraurostyla weissei*. I. Formation and morphogenetic movements on ventral ciliary primordia. Protistologica, 16, 571 - 580.

Jerka-Dziadosz M. 1981: Ultrastructural study on development of the hypotrich ciliate *Paraurostyla weissei*. II. Formation of the adoral zone of membranelles and its bearing on the problems of ciliate morphogenesis. Protistologica, 17, 68 - 81.

Jerka-Dziadosz M. 1982: Ultrastructural study on development of the hypotrich ciliate *Paraurostyla weissei*. IV. Morphogenesis of dorsal bristles and caudal cirri. Protistologica, 18, 237 - 251.

Luporini P. et Magagnini G. 1970: Recherches sur la structure fine et la biologie reproductive du cilié hypotriche *Swedmarkia arenicola* Dragesco. Protistologica, 1, 113 - 127.

Matsusaka T. and Kimura S. 1981: Changes in macronuclear ultrastructures during encystment in a ciliate, *Histriculus muscorum*. Kumamoto J. Sci. Biol., 15, 49 - 58.

Nieto J. J., Calvo P., Torres A. et Perez Silva J. 1981: Régénération chez *Gastrostyla steinii*. Acta Protozool., 20, 373 - 384.

Nobili R. 1971: The fine structure of the oral apparatus of some ciliate hypotrichs. Monit. Zool. Ital., (N.S.) 5, 65 - 80.

Prescott D. M. 1962: Nucleic acid and protein metabolism in the macronuclei of two ciliated Protozoa. J. Histochem. Cytochem., 10, 145 - 153.

Puytorac P. de, Grain J. et Rodriguez de Santa Rosa M. 1976: A propos de l'ultrastructure corticale du Cilié Hypotriche *Stylonychia mytilus* Ehrbg., 1838: les caracteristiques du cortex buccal adoral et paroral des *Polyhymenophora* Jankowski, 1967. Trans. Am. Microsc. Soc., 95, 327 - 345.

Ruffolo J. J. 1976a: Cortical morphogenesis during the cell division cycle in *Euplotes*. An integrated study using light optical, scanning electron, and transmission electron microscopy. J. Morphol., 148, 489 - 528.

Ruffolo J. J. 1976b: Fine structure of the dorsal bristle complex and pellicule of *Euplotes*. J. Morphol., 148, 469 - 488.

Ruffolo J. J. 1978: Fine structure of the macronucleus during the cell division cycle of *Euplotes eurystomus*, a ciliate protozoon. J. Morphol., 157, 211 - 222.

Ruffolo J. J. Jr. 1979: The intranuclear helices of *Euplotes*: their substructure, localization, and apparent migration to the cytoplasm. J. Morphol., 159, 81 - 88.

Tucker J. B., Beisson J., Roche D. L. J. and Cohen J. 1980: Microtubules and control of macronuclear "amitosis" in *Paramecium*. J. Cell. Sci., 44, 135 - 151.

Walker G. K. and Goode D. 1976: The role of microtubules in macronuclear division in the hypotrichous ciliate *Gatrostyla steinii* and *Stylonychia mytilus*. Cytobiologie, 14, 18 - 37.

Wicklow B. J. 1979: Ultrastructure and cortical morphogenesis of the marine hypotrich ciliate *Certesia quadrinucleata*: relationships with *Discocephalus* and *Euplotes*. Trans. Am. Microsc. Soc., 98, 159 - 160.

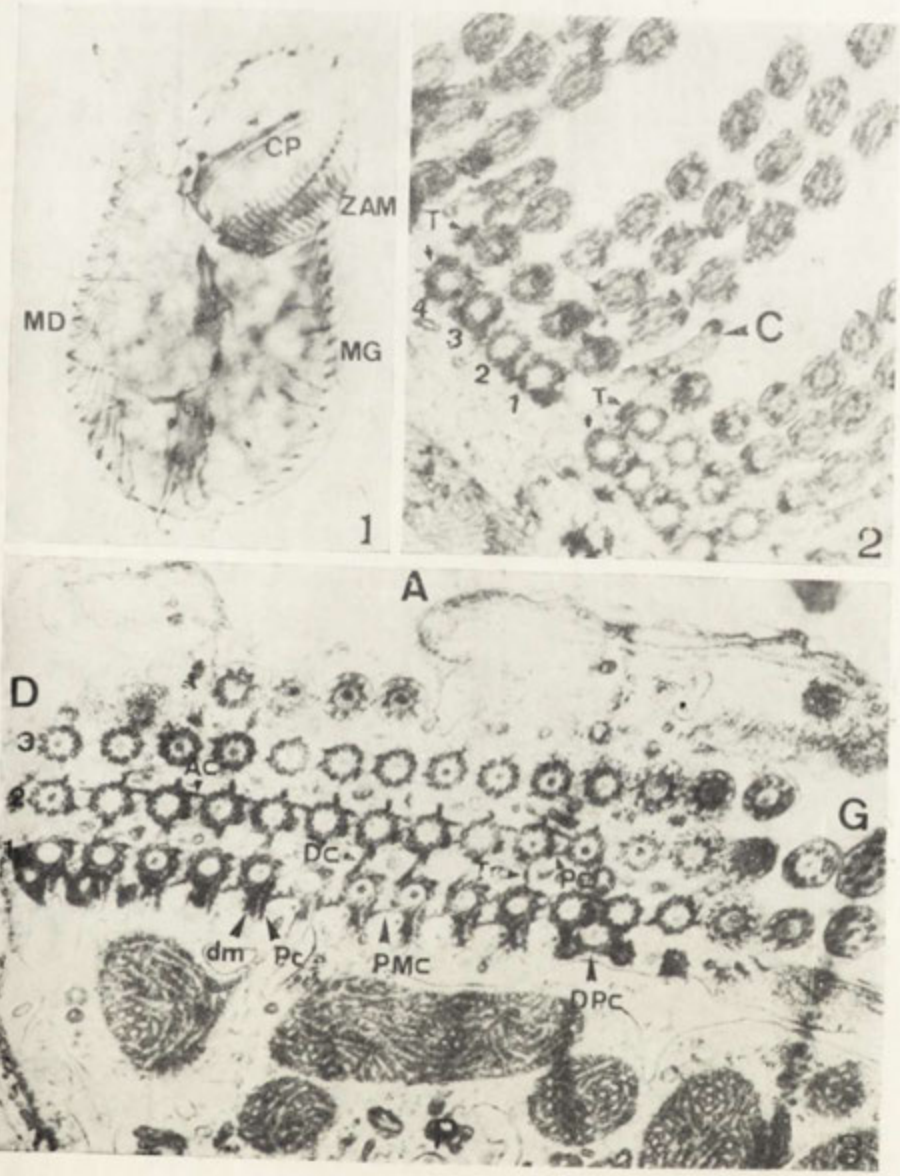
Wicklow B. J. 1981: Evolution within the order *Hypotrichida* (*Ciliophora, Protozoa*): ultrastructure and morphogenesis of *Thigmokeronopsis jahodai* (n.

gen., n. sp.); phylogeny in the *Urostylina* (Jankowski, 1979). Protistologica, 17, 331 - 335.

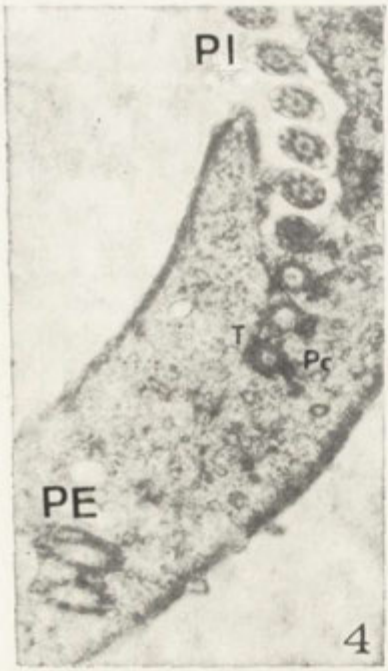
Wicklow B. J. 1982: The *Discocephalina* (n. subord.): Ultrastructure morphogenesis and evolutionary implications of a group of endemic marine interstitial hypotrichs (*Ciliophora, Protozoa*). Protistologica, 18, 299 - 330.

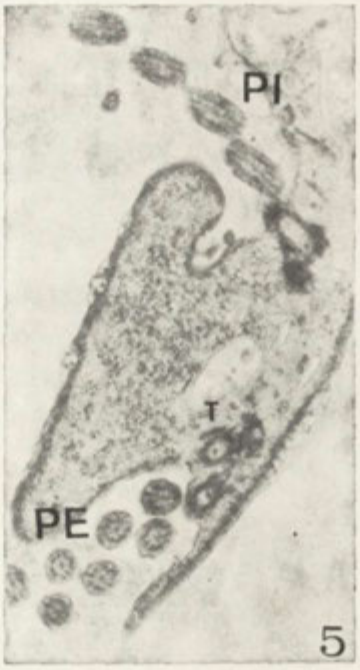
EXPLICATIONS DES PLANCHES I - IV

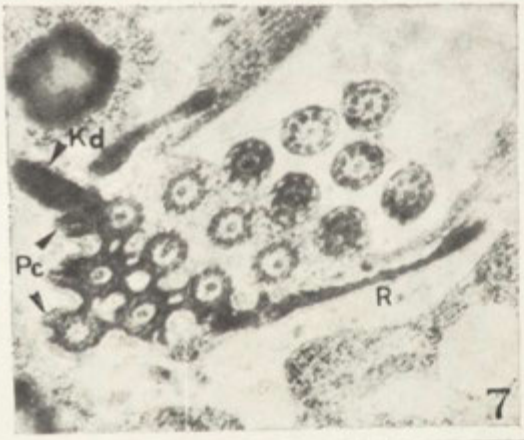
1: *Histriculus similis*. Micrographie de la face ventrale d'un individu végétatif, teint suivant la technique du proteinate d'argent. (1000 ×)
2: Paramembranelle ventrale. T — microtubules transverses, C — crêtes intermembranellaires. (22.500 ×)
3: Paramembranelle. Chaque cinétosome de la rangée 1 porte des microtubules postciliaires (Pc). On observe des connexions longitudinales antérieures (AC), des connexions longitudinales postérieures (PC), des connexions transversales (TC), des connexions diagonales (DC), des connexions postmembranellaires (PMC), des doubles connexions postérieures (DPC) et un matériel electrodense associé aux microtubules postciliaires des cinétosomes de la rangée 1. A, P, D, G, indiquent la région antérieure, postérieure, droite et gauche de la cellule. (30 000 ×)
4 - 5: Ciliation parorale. PI — parorale interne, PE — parorale externe, Pc — microtubules postciliaires, T — microtubules transverses. (25 000 ×), (17 000 ×)
6: Parorale externe (PE) avec ses cinétosomes disposés selon un pattern en zig-zag. (26 000 ×)
7 - 9: Coupe transversale d'un cirre au niveau de la plaque basale montrant les connexions intercinétosomales. Pc — microtubules postciliaires, T — microtubules transverses, Kd — fibre cinetodesmale, R — maille envelopée de matériel electrodense. (25 000 ×), (30 000 ×), (24 000 ×)
10: Coupe longitudinale d'un cirre. A l'intérieur des cinétosomes on observe des granules electrodenses (fléche) (20 000 ×)
11: Pellicule avec trois unités des membrane (fléche) m — microtubules (25 000 ×)
12: Microtubules (m) paralléles à la pellicule (40 000 ×)
13 - 14: Macronoyau. L'enveloppe présente une grande quantité de pores (P). (60 000 ×), (37 500 ×)
15 - 16: Faisceaux de microtubules (mt) à l'intérieur du macronoyau. nu — nucléole. (26 000 ×), (24 000 ×)
17: Macronoyau présentant une bande de réplication. nu — nucléole (10 000 ×)
18: Micronoyaux. (12 000 ×)

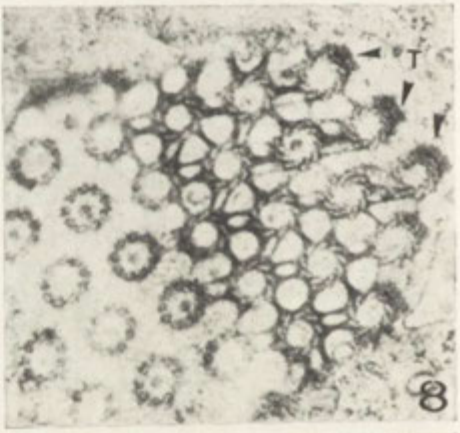


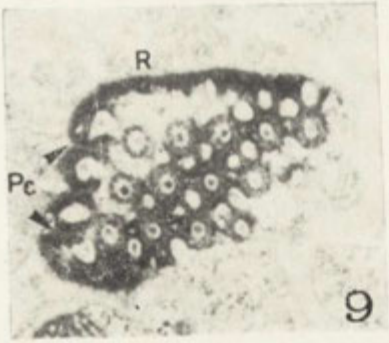
P. Calvo et al. auctores phot.





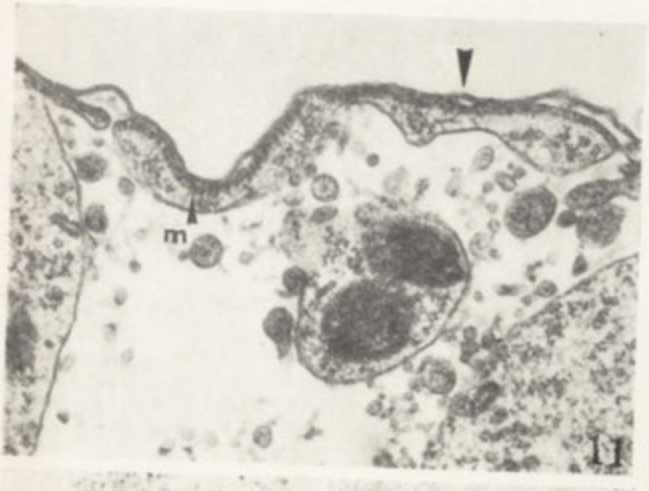


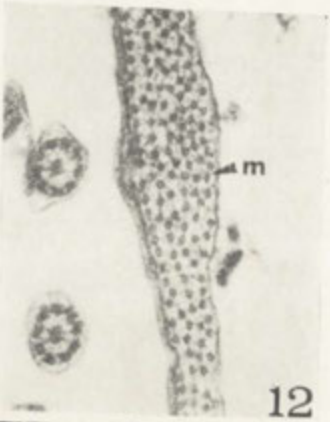


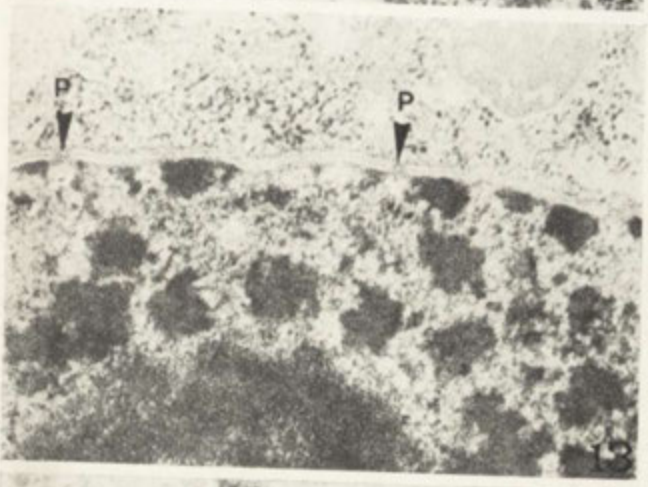


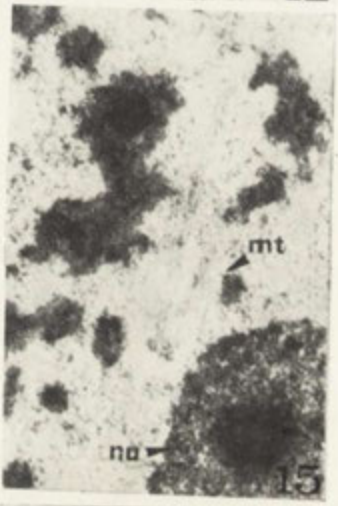
P. Calvo et al.

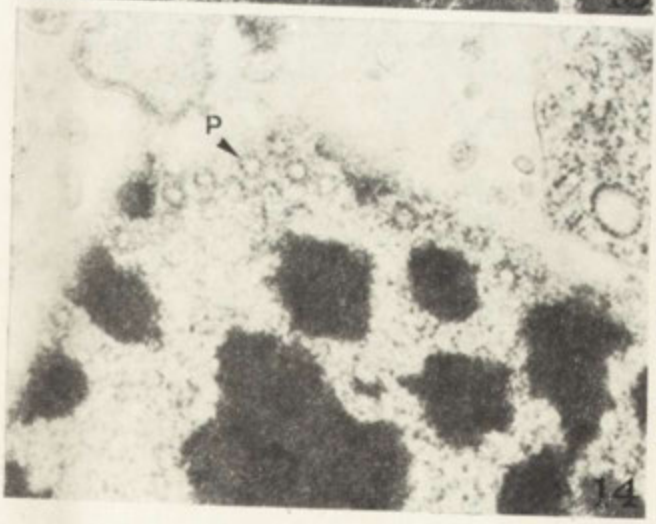
auctores phot.

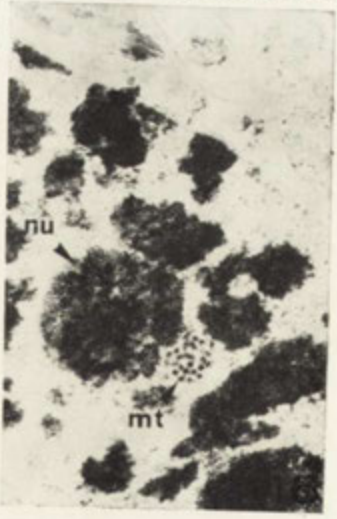






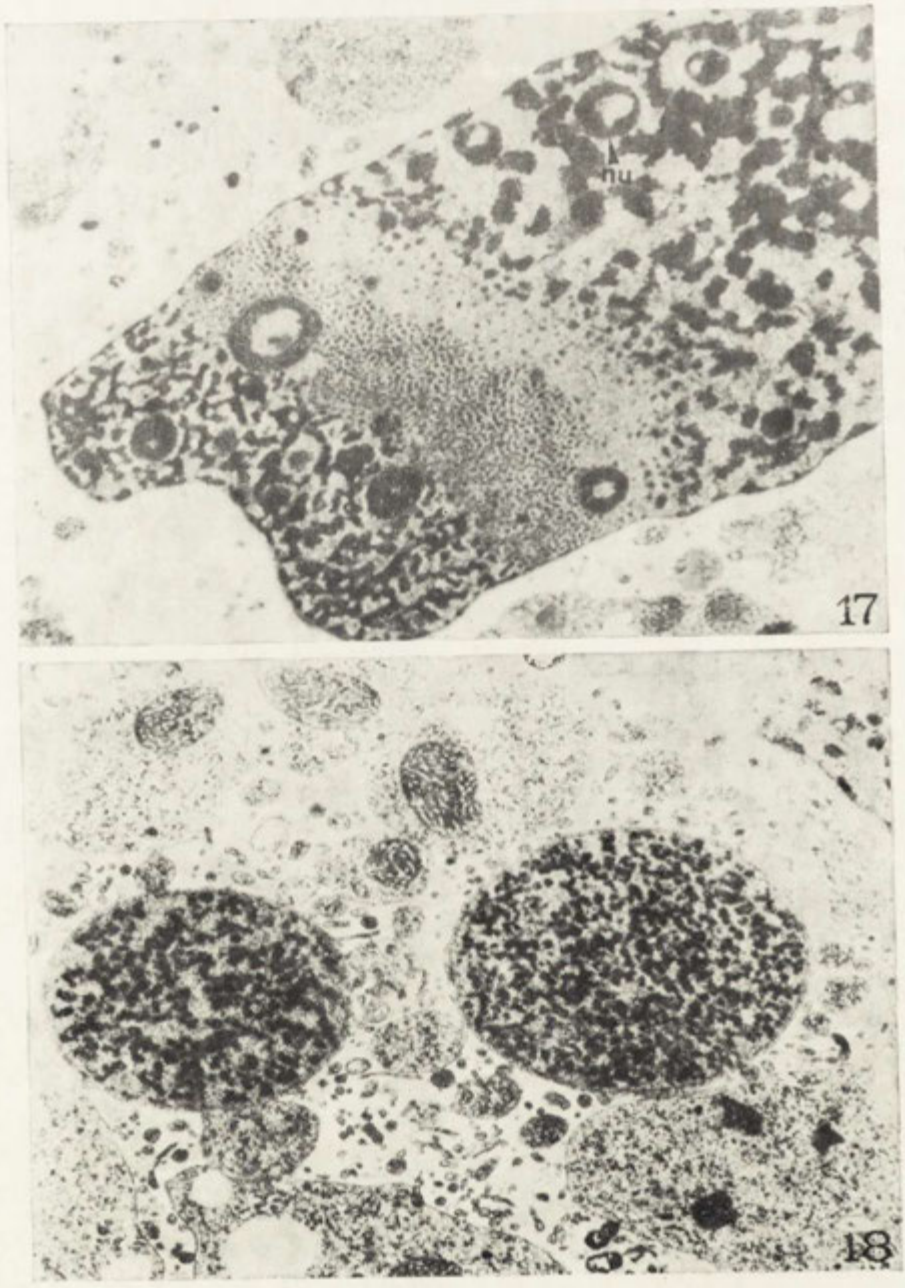






P. Calvo et al.

auctores phot.



P. Calvo et al. auctores phot.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 33 - 39 (1986)

Formation d'éléments reproducteurs endogènes chez *Histriculus similis* (*Ciliophora Hypotrichida*)

Didier CHARDEZ

Laboratoire de Zoologie Générale et Faunistique (Prof. J. Leclercq) Faculté des Sciences Agronomiques de l'Etat, 3, Chaussée Romaine, 4252 Omal, Belgique

Received on 13 March 1985

Synopsis. Dans ce travail, nous décrivons la formation d'éléments reproducteurs endogènes chez le Cilié *Histriculus similis*. Nous donnons également le schéma de son cycle vital complet. Parallelement à la reproduction classique par bipartition, il existe aussi un autre mode de reproduction asexuel chez certaines espèces de ce groupe.

Il semble que la formation d'éléments reproducteurs, soit répandue chez certaines espèces d'Hypotriches, Curds (1966), l'a observé chez *Histriculus vorax*, nous l'avons observé également chez *Histriculus similis*. Ce type de reproduction asexuelle a déjà fait l'objet d'études chez d'autres groupes de Protozoaires, en particulier chez les Rhizopodes, Bonnet (1964), Elpatiewsky (1907), Swarczewsky (1908) et Chardez (1965).

Nous avons étudié le cycle complet en élevages, en grande partie sur le vivant, et à l'aide de techniques cytologiques simples.

Nous préférons le terme "éléments reproducteurs" à ceux de spores ou bourgeons qui se rapportent aux végétaux.

Généralités

D'une façon générale, l'ordre des *Hypotrichida* rassemble de très nombreuses espèces dont la détermination reste difficile par la simple observation sur le vivant, non seulement en raison de leur grande mobilité, mais surtout de la diversification structurale qui met en jeu la disposition des différents groupes de cirres. En effet, chez les Hypotriches les organites vibratiles se répatissent en plusieurs groupes.

Dans l'ensemble, on distingue les organisations suivantes: Les membranelles adorales, la membrane parorale, les cirres frontaux, les cirres frontaux ventraux, les cirres ventraux médiants, les cirres transversaux, les cirres marginaux droits et gauches enfin les soies ou cirres caudaux.

Certaines formations ne sont pas toujours présentes suivant les espèces.

Matériel et méthode

Cet Hypotriche a été trouvé dans un échantillon d'eau prélevé dans la rivière "La Vesdre" à Verviers (Belgique) au moi de novembre 1983).

La première souche a été obtenue à partir de quelques individus triés à la micropipette, et déposés dans un milieu composé à volume égal d'un bouillon de laitue stérilisé et d'une solution de Ringer, après trois jours, les Ciliés ont été rassemblés dans une solution inorganique, par la méthode de Soldo et van Wagtendonk (1967). Les espèces ensuite réparties dans une solution de Ringer maintenue à 18°C, en boîtes de Petri, contenant trois grains de blé lavés et une souche bactérienne représentée par *Bacillus subtilis* obtenue par le Bactisubtil sec. Des repiquages clonaux ont été pratiqués toutes les deux semaines, des élevages mixtes ont également été entretenus avec *Colpidium campylum* et *Tetrahymena pyriformis*.

Les études ont été faites sur le vivant et après anesthésie par la solution de sulfate de Nickel à 0,60‰. Cette opération se pratique en salière, dans 1 CC d'eau contenant les Ciliés dans laquelle on ajoute 0,50 CC de la solution anesthésiante; après trois minutes, les Ciliés sont immobilisés sans déformation.

La mise en évidence de l'appareil nucléaire est obtenu par la coloration directe "C1" (Vert de methyl aceto-orceine-chlorhydrique), cette coloration est progressive et temporaire.

La formation expérimentale de kystes de protection; s'obtient par l'adjonction de deux goutes d'une solution d'hydrate de chloral à 0,01‰ dans 1 CC d'eau contenant les Ciliés.

Cette méthode rapide, rend de grands services dans ce type d'étude, qui nécessite de très nombreux examens de Protozoaires dont les imprégnations sont extrêmement difficiles.

Description de l'espèce

Le genre *Histriculus*, est largement représenté dans les Mousses, les Sphaignes et l'eau douce. Le genre compte actuellement neuf espèces.

Histriculus similis (Quennerstedt) Corliss 1960 mesure 85 à 95 µm pour les petites formes et 100 à 130 µm pour les grandes.

La forme générale du corps est ovalaire, avec un péristome assez développé, atteignant souvent la moitier du corps (Fig. 1).

Le péristome est bordé à gauche par une série de 35 à 40 membranelles adorales puissantes; la paroi droite est délimitée par une fine membrane parorale.

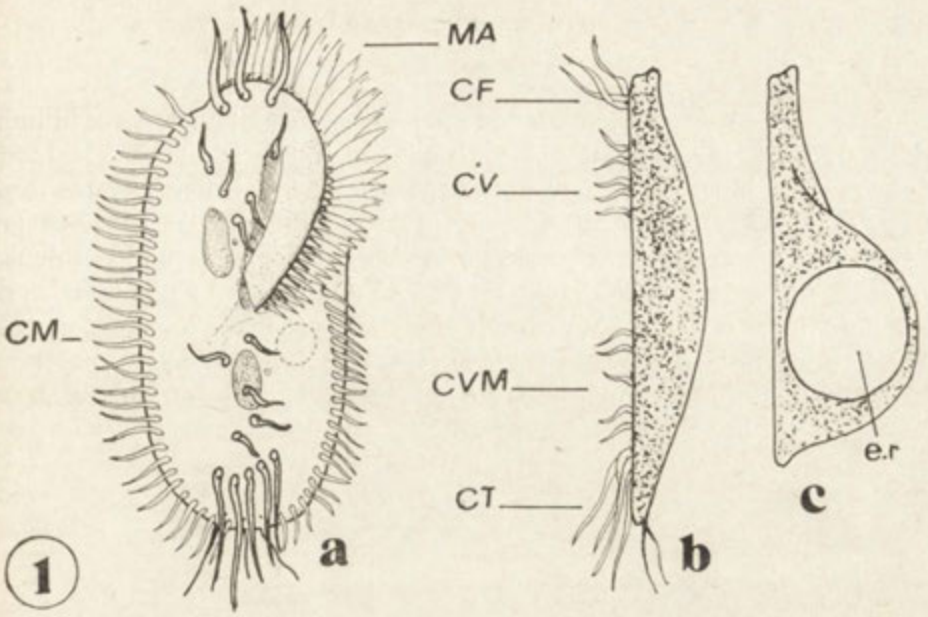


Fig. 1. *Histriculus similis*. a — face ventrale (individu anesthésie calqué sur écrant de microprojection), b — vue schématique de profil, c — vue schématique de profil d'un individu contenant un élemént reproducteur (er)

L'ensemble de la ciliature est constituée par 3 cirres frontaux robustes, 5 cirres frontaux ventraux, 5 cirres ventraux médiants, 5 cirres transversaux très puissants, les cirres marginaux de la rangée droite sont au nombre de 29 à 32, tandis que la gauche est contituée de 22 à 27 cirres. Les deux rangées se rejoignent à la partie postérieure, 3 ou 4 cirres différents plus longs et plus minces sont implantés légèrement sur la surface dorsale, certains individus en sont dépourvus.

L'appareil nucléaire est formé de deux macronoyaux de 16 à 20 μm et par deux micronoyaux.

Une vacuole est visible sur le côté gauche du corps.

Observé sur le vivant, *H. similis* nage rapidement en pleine eau en tournant sur lui même et en décrivant de larges spirales a l'aide des membranelles adorales seules.

Au contact d'un substrat, il marche en se servant des cirres marginaux et ventraux comme pattes. Les trois cirres frontaux, généralement courbés vers l'avant, tout en participant à la marche, semblent posséder un certain pouvoir tactile.

La marche est polarisée vers l'avant très rapide, quelquefois, le Cilié effectue une sorte de piétinement sur place avec de brusques reculs, cette opération est déterminée par les trois cirres frontaux qui exercent une poussée vers l'avant.

Le cycle vital

La première population obtenue en élevage, était composée d'individus de 95 à 100 µm qui se sont rapidement reproduits par le mode classique de bipartition, après deux jours, les premiers kystes sont apparus, ces kystes étaient de deux types: (a) kyste de 38 à 45 µm à membrane épaisse bien séparée de la masse cytoplasmique granuleuse, (b) kyste de protection sphérique de 40 à 45 µm, entouré d'une membrane hyaline plissée entourant un cytoplasme clair contenant les deux macronoyau souvent bien visibles (Fig. 2). C'est ce type de kyste que nous obtenons expérimentalement par la solution d'hydrate de chloral.

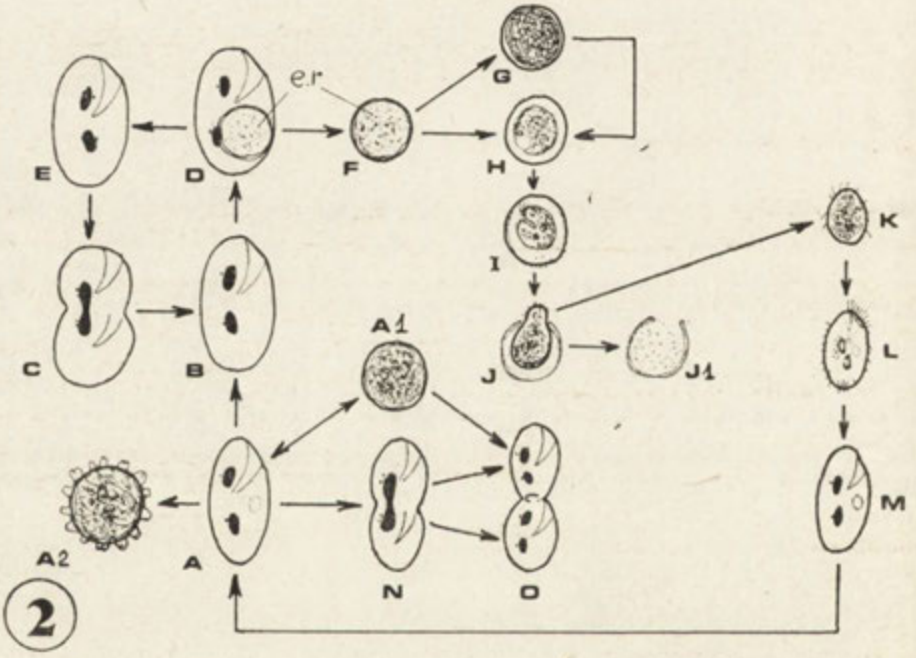


Fig. 2. Cycle vital d'*Histriculus similis*. A — adulte de petite taille, A 1 — kyste d'attente, A 2 — kyste de protection, B — adulte de grande taille, C — bipartition d'un adulte de grande taille, D — développement d'un élément reproducteur "er", E — adulte de grande taille après l'exclusion de l'élément reproducteur, F — élément reproducteur après l'exclusion, G — élément reproducteur en attente en milieu défavorable, H — élément reproducteur en développement en milieu favorable, I — élément reproducteur contenant un embryon du cilié, J — exuviation du cilié, J 1 — membrane vide de l'élément reproducteur, K — embryon libre, L — stade embryonaire possédant une indication de membranelle adorale, M — *H. similis* de petite taille reformant le cycle, N, O — adulte de petite taille en fission binaire

Dans cette population, sont apparus après vingt jours des individus sensiblement plus grands, plus larges et plus foncés, dont le cytoplasme est rempli de granules, environ 20% de ces grandes formes contenaient des embryons d'éléments reproducteurs d'un diamètre variant de 18 à 35 µm, bien visibles dans la partie médiane du corps (Fig. 1 c et Pl. I 3).

Ces éléments grossissent jusqu'a atteindre à leur maturité 38 à 40 µm de diamètre. A ce stade, l'Hypotriche est fortement déformé et pratiquement immobile, jusqu'a l'expulsion de l'élément reproducteur (Pl. I 4). Une fois expulsés, ces éléments se présentent sous la forme d'une sphère à membrane lisse bien différenciée, contenant un cytoplasme granuleux et opaque; au deuxieme stade très souvent une pulsole apparaît et l'ensemble du cytoplasme se contracte, se détachant nettement de la paroi, rapidement, cette masse effectue un mouvement de rotation alternatif et de contractions. Cette phase dans certains cas, peut durer plusieurs jours, elle est suivie par la sortie de l'organisme embryonaire (Pl. I 6 et 7).

Certains *H. similis*, possèdent deux éléments reproducteurs.

Le cycle complet, tel que nous l'avons observé en élevage clonal, s'établit comme suit:

Les adultes de petite taille se reproduisent par bipartition, ils peuvent former des kystes d'attente en milieu peu nutritif et des kystes de protection en milieu défavorables. Ayant atteint la grande taille, ils se reproduisent soit par fission binaire (Pl. I 13 et 14), soit en formant des éléments reproducteurs endogènes ces éléments une fois expulsés s'enkystent si le milieu est défavorable (cas d'élevages trops vieux), dans de bonnes conditions, ils forment un embryon dont les mouvements de rotation sont visibles sous l'enveloppe (Pl. I 5). Après un temps variable, il perce l'enveloppe (Pl. I 6 et 7) et nage librement sous la forme d'une petite masse ciliée (Pl. I 8 et 9), plusieurs heures après l'exuviation, les membranelles adorales et les cirres sont reconnaissables (Pl. I 10), il grossit rapidement pour prendre progressivement la forme d'*H. similis* de petite taille (Pl. I 12).

La durée de l'ensemble du cycle est fort variable, elle dépend certainement de différents facteurs. A ce sujet, il n'est pas possible d'extrapoler des données obtenues par des élevages de laboratoire avec ce qui se passe dans la nature.

Il est certain que l'abondance de la nourriture et la température jouent un rôle important dans ces phénomènes.

L'expérience nous a montré que les élevages maintenus à une température de 18°C, étaient plus réguliers que ceux soumis aux fluctuations de la température ambiante.

Discussion

La production d'éléments reproducteurs chez certains Protozoaires, peut avoir plusieurs buts, mais le plus important semble être un pouvoir particulier de dissémination et de survie comparable à la sporulation chez les végétaux.

Les résultats obtenus en élevages clonaux et en élevages mixes sont identiques. Les grandes formes apparaîssent généralement dans des élevages à population nombreuse, ce qui signifie un appauvrissement de la nourriture. Des repiquages très fréquents ne favorisent pas la prolifération des grandes formes génératrices d'éléments reproducteurs.

Dans l'ensemble, nos observations confirment celles de Curds (1966), a savoire qu'il existe chez certains Hypotriches un cycle vital comportant un stade embryonaire.

SUMMARY

Formation of endogenous reproductive elements in *Histriculus similis* is described, as well as the entire life cycle of this ciliate.

It has been found that in some species of this group another kind of asexual reproduction occurs parallelly with classical bipartite reproduction.

BIBLIOGRAPHIE

Bonnet L. 1959: Dekystement, phase tropique et enkystement chez *Plagiopyxis minuta* Bonnet. C. R. Acad. Sci., 249, 2617 - 2619.

Bonnet L. 1964: Le peuplement Thecamoebien des sols. Rev. Ecol. Biol. Sol., 1, 123 - 408.

Chardez D. 1965: Sur un mode particulier et peu connu de reproduction chez les Thecamoebiens aquatiques. Bull. Inst. Agron. Stn. Rech. Gembloux, 33, 26 - 34.

Curds C. R. 1966: Endogenous bud formation in *Histriculus vorax*, a new asexual reproductive process in the *Hypotrichida*. J. Protozool., 13, 155 - 164.

Elpatiewsky W. 1907: Zur Fortpflanzung von *Arcella vulgaris* Ehrbg. Arch. Protistenk., 10, 441 - 600.

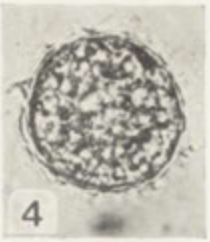
Swarczewsky B. 1908: Über die Fortpflanzungengerscheinugen bei *Arcella vulgaris* Ehrbg. Arch. Protistenk., 12, 173 - 312.

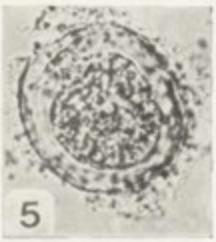
Soldo A. T. and Van Wagtendonk W. J. 1967: A method for the mass collection of axenically cultivated *Paramecium aurelia*. J. Protozool., 14, 497 - 498.

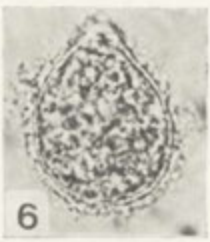
EXPLICATION DE PLANCHE I

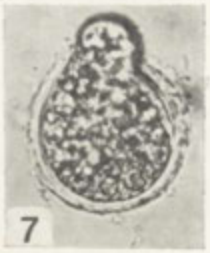
3: *Histriculus similis*, grande forme contenant un élément reproducteur
4: Elément reproducteur libéré
5: Apparition des mouvements internes
6: Début du percement de la membrane
7: Sortie de l'embryon cilié
8, 9: Petit cilié libre après 30 min
10: Apparition des membranelles après 1 h
11: *H. similis* après 12 h
12: *H. similis* petite forme
13: *H. similis* grande forme en fission binaire (fixé et coloré par C 1)
14: Le meme individu après deux heures de coloration par C 1
15, 16: Kystes de protection provoqués par la solution d'hydrate de chloral
17: Elément reproducteur en attente dans un milieu non nutritif

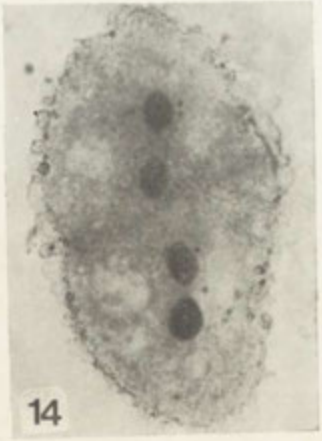
Note: Les figures 3, 11 - 14 (× 400), les autres (× 600)

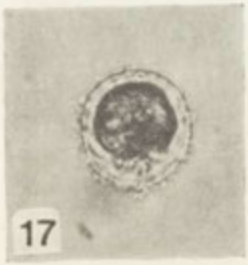












D. Chardez auctor phot.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 41 - 53 (1986)

Polyploid Cells in *Amoeba proteus* Culture

S. Yu. AFON'KIN

Laboratory of Cytology of Unicellular Organisms, Institute of Cytology of the Academy of Science of the USSR, 4 Tikhoretsky prosp., Leningrad 194064, USSR

Received on 11 March 1985, *revised on* 5 June 1985

Synopsis. The original ploidy level in *Amoeba proteus* is not known. It may be assumed arbitrarily as diploid. The precise number of chromosomes (several hundreds) in this species is difficult to count. The enlarged nuclear DNA content in this case may be used as indirect evidence in favour of the existence of polyploid cells in amoebae mass culture. Clones with two- and four-fold increase of the DNA content were grown from mass culture cells of *A. proteus* strain B. The proportion of polyploid cells in the mass culture was no less than 1.8%. Artificially mixed cultures with equal numbers of normal and polyploid amoebae were produced. During growth of the population the proportion of the latter steadily decreased, in some cases to the original 1.8% and even less in others. However, the mean generation time of polyploid amoebae remained unchanged. The phenomenon can be explained on the assumption of a lesser probability to grow clones from polyploid cells than from the control ones. In other words, polyploid cells show a decreased probability of accomplishing the cell cycle. The data on cloning efficiency in polyploid clones fit this hypothesis. During cultivation the mean DNA content in polyploid B7 clone was reduced from the octo- to tetraploid level. This kind of spontaneous depolyploidization was described earlier in artificially produced polyploid amoebae clones (Afon'kin 1984). The present results indicate that in *A. proteus* mass cultures there are cells with different ploidy levels. The study supports the hypothesis that the poly- and depolyploidization of amoeba cells may occur in nature and play a role in genetic variability of these asexually growing organisms.

Polyploidy played a primary part in the evolution of some protozoan groups (Poljansky and Raikov 1960, 1977) and, up to now, it is an important source of genetical variation among them. There are many kinds of polyploidy in *Protozoa*, for instance such as cyclic, hyperploid and generative ones (Raikov 1982). The latter is characteristic of

all stages in the species life cycle and is supposed to occur spontaneously. It was shown in the case of *Pandorina morum* (Coleman and Zollner 1977, hypermastigida (*Holomastigotoides* (Cleveland 1949, Grasse and Holland 1963), mastigophora *Opalina ranarum* (Kaczanowski 1968) and some ciliata with polyploid micronuclei (see the review Ossipov and Borchsenius 1973).

There is every reason to suppose that in asexually growing *Protozoa* one may find many polyploid forms. The fresh water amoebae belong to this category, the world-known *Amoeba proteus* among them. The original ploidy level of this species is not known but there is indirect evidence of the existence of polyploid forms in *A. proteus*. (1). We have found cells with the enlarged nuclear DNA content in samples (Sopina et al. 1982). (2). The polyploid cells induced experimentally by injecting colchicine solution are viable and may give clones (Afon'kin 1984).

The present study demonstrates that there are viable polyploid cells in *A. proteus* mass culture from which polyploid clones are grown. Our data provide a strong support for the possibility of a spontaneous polyploidization in *Amoeba proteus* cultures. The study speaks in favour of the hypothesis that the polyploidization may occur in nature and plays some role in the genetic variability of asexually growing amoebae.

Materials and Methods

Culturing and cloning of amoebae. *Amoeba proteus* strain B were cultured at 25°C according to Prescott and Carrier (1964). Fresh culture medium and food (*Tetrahymena pyriformis* GL) were changed three times a week. Clones were grown in microplates for immunological reaction (Afon'kin and Yudin 1985). Each plate consists of 60 depressions; the volume of one depression is about 0.02 ml. In this case amoebae were fed on a mixture consisting of *Chilomonas* sp. and *Colpidium* sp. (Ord 1979). Clones were transferred to new microplates once a week. To obtain clones with a large number of cells amoebae were placed into microaquaria (0.2 ml) and then into glass containers.

Cell samples. We used two kinds of amoebae samples. To obtain partly synchronized samples a group of cells was taken at random from a mass culture or a clone and cultured individually for 24 h with food. At the end of this period the divided cells were discarded. As a result, the sample consisted of cells in the second half of the interphase. To obtain a synchronized sample 20 - 30 dividing amoebae were picked up from a mass culture. The whole procedure took about 10 - 15 min. The end of cytokinesis was regarded as the "zero" time. Taking into account also the time needed for isolation of the nuclei the maximum age difference between nuclei in the synchronized samples was estimated to be about 30 min.

Isolation of nuclei. Nuclei were isolated one by one from cells in the culture medium. Amoebae were destroyed by means of a micropipette. Its diameter was slightly larger than the nuclear one. Each 5 nuclei were transferred in the tip of a pipette onto a slide. Each sample was placed onto a separate square drawn by means of diamond pencil and fixed with a drop of 96° ethanol.

Measurement of nuclear areas. Isolated and fixed nuclei were dyed with toluidine blue and $Na_2B_4O_7$ during 3 min. Then, the slides were washed off with distilled water. The nuclear areas were measured by means of an automatic TV device "Izmeritel-1" and were expressed in arbitrary units. In this paper 1 arb. unit is 1×10^{-5} mm². The accuracy of measurement of the same nucleus was 0.3% (N = 25).

Measurement of nuclear DNA content. The relative DNA content in nuclei isolated onto slides was estimated cytofluorimetrically by recording the fluorescence of the Auramine OO bound to DNA in a Feulgen-like reaction (Kudriavtsev and Rosanov 1974). For this purpose the slides were incubated in 6N HCl (18 - 20°C, 8 min) and stained in Auramine solution (0.3%, 2 - 5°C, 1.5 h) with thionyl chloride (0.2%). The intensity of fluorescence was measured with a cytofluorimeter "Lumam-IUF". The excitation light with the waves length of 365, 404 and 436 nm was isolated from the mercury lamp spectrum by means of light filters FC-1 (8 mm) and CSC-24 (4 mm). The fluorescent light was isolated with JC-18 and JSC-19 light filters. The objective × 20 and probes with 1 and 1.5 mm holes were used. The measurement time of une nuclei was 0.2 s.

To avoid systematic errors caused by drift in the instrument sensitivity in time the intensity of nuclei fluorescence was expressed in arbitrary units. The fluorescence intensity of rat hepatocyte tetraploid nuclei (G_2 phase) was assumed for 1 arb. unit. The groups of rat and amoebae nuclei were measured in turn.

It is known that the measurements of the DNA content in the nuclei of the same age from amoebae of one strain, fixed on different slides and stained at different time, may differ significantly (Makhlin et al. 1979). To render the comparison of the DNA content between different samples more reliable, we correlated only the data produced by the nuclei fixed on the same slide, but also in this case the mean values (one clone and one time) could differ (Afon'kin 1983, 1984). It is likely to result from an uneven distribution of cells of different age between the samples. To eliminate this factor, several samples from the same clone were studied and the second order mean value calculated.

Measurement of the cell number in culture. To determine the number of amoebae in a culture the number of cells per 0.04 cm² was counted. For this purpose the amoebae in a Petri dish were thoroughly suspended and then allowed to settle down. The procedure was repeated three times. In each case a new area of the bottom was choosen for counting. Thereupon the average number of amoebae in the culture and the standard error were calculated for the whole area of the Petri dish.

Measurement of cloning efficiency. To evaluate the cloning efficiency 60 amoebae were cloned in a microplate (see above). The culture medium contained food organisms throughout this experiment. After 168 h the proportion of holes in which the cell divisions took place was estimated. This value was considered as cloning efficiency.

It may by suggested *a priori* that there are cells in a clone that are already uncapable of dividing but, none the less, survive for some period of time. To reject such cells a random sample of the clone under test was subcloned. Only amoebae from subclones in which the cell division did occur were taken for further experiments. So, when measuring cloning efficiency we defined the probability of completing the next cell cycle.

Results

Screening for polyploid clones. Production of polyploid clones is the most convincing evidence for the existence of polyploid cells in amoebae culture. To work out a schedule for screening such clones some characteristics of normal (strain B) and tetraploid amoebae (clone Bcol-6) were studied once again. Clone Bcol-6 was produced by injecting colchicine into dividing cells of B strain (Afon'kin 1984).

It is known now that the average DNA amount in polyploid clones sometimes appears to be reduced after the prolonged cultivation (Afon'kin 1986). The mean DNA content of B and Bcol-6 amoebae was therefore remeasured just before the onset of the experiment (Table 1). Seven samples of partly synchronized amoebae of each type were taken. The second order means for Bcol-6 and B amoebae were 4.57 ± 0.06 and 2.29 ± 0.07 arb. units, respectively. The ratio of these values is 1.99. Therefore, it could be concluded that the DNA amount in Bcol-6 amoebae remained twice as high as the normal level at the very start of the experiment. It must be emphasized that in B samples there were four nuclei (2%) with two-fold increase of the DNA content (4.0 - 4.9 arb.

Table 1

Average DNA amount in the B and Bcol-6 partly synchronized samples at the very start of the experiments

DNA content, $\bar{X} \pm S_{\bar{x}}$ arb. units	
Strain B	Clone Bcol-6
2.11 ± 0.06	4.53 ± 0.08
2.23 ± 0.05	4.76 ± 0.10
2.24 ± 0.05	4.64 ± 0.10
2.14 ± 0.04	4.35 ± 0.12
2.25 ± 0.05	4.77 ± 0.11
2.61 ± 0.07	4.44 ± 0.07
2.45 ± 0.05	4.48 ± 0.08

Each sample consists of 25—34 nuclei.

units). These values do not statistically belong to other B nuclei in samples. One such nucleus with enlarged DNA content (7.3 arb. units) was found in Bcol-6 nuclei.

Two-fold increase of the DNA content in *A. proteus* is now a reliable index of the enhanced ploidy level. However, this criterion proved to be inconvenient when large number of cells or clones are being studied. In this case the nuclear size is a more suitable though indirect index. The mean nuclear area of Bcol-6 polyploid cells is 1.57-fold increased in respect to the control (Table 2). None the less the size of one single nucleus is not a good indication of ploidy level, since the maximum B nuclear area in partly synchronized cells (61 arb. units) is greater than the minimum Bcol-6 area (40 arb. units). The mean nuclear area of five partly snychronized cells yields better results. This character varies in B amoebae from 25.0 to 49.6 arb. units (123 clones were studied) and in Bcol-6 amoebae, from 50.6 to 81.2 arb. units (in 44 clones).

These figures indicate that the two distributions are not overlapping and that the mean nuclear area of five cells may be used as a criterion of ploidy level.

Table 2

Size of partly synchronized nuclei in B strain and in tetraploid Bcol-6 clone

Nuclear area, $\bar{X} \pm S_{\bar{x}}$ arb. units	
Strain B	Clone Bcol-6
39.5±1.4	64.1±1.9
38.3±1.3	67.1±1.9
39.7±1.0	57.0±1.7
36.3±1.0	58.3±1.8
45.0±1.7	69.3±2.0
38.4±1.2	61.8±2.6
42.3±1.2	63.1±1.7

Each sample consists of 25—30 nuclei.

720 amoebae cells were taken from B culture at random and 660 clones were grown. Only 26 of them, consisting of large amoebae, were chosen for further research. Recent observations have shown that the volume of polyploid cells is to some extent greater than in the control (A f o n' k i n 1984). Thus, the large cells in a clone may be considered as a first indication of its polyploid nature.

The measurements of the mean nuclear areas in five partly synchronized cells of chosen clones show that 14 of them are normal (31.4 - 44.4 arb. units) and 12 are polyploid (51.5 - 76.0 and 102 arb. units). The value of 102.0 arb. units is not characteristic of the tetraploid Bcol-6 clone and is typical of octoploid clones (A f o n' k i n, unpublished data).

DNA content in polyploid clones. To make sure of the polyploid nature of 12 recognized clones the nuclear DNA amount in some of them was measured. For this purpose we chose six supposed tetraploid clones marked B1 - B6 and one clone (B7) which was supposed octoploid. Five partly synchronized samples of 20 - 33 nuclei were isolated from each of them and the second order means of the DNA content

Table 3

DNA content in partly synchronized samples of B1-B6 clones 1 month after their isolation

Clone	DNA content Second order mean $\bar{X} \pm S_{\bar{x}}$ arb., units	Ratio of second order mean to the control value
Strain B (control)	1.57±0.05	—
B1	3.26±0.10	2.07
B2	2.90±0.06	1.85
B3	3.25±0.08	2.07
B4	3.18±0.08	2.02
B5	3.03±0.06	1.96
B6	2.96±0.06	1.88

Each second order mean was obtained as a result of the calculations of five independent mean values.

were calculated (Table 3). In B1, B3, B4 and B5 clones these values were two-fold increased as compared with the nuclei of the original strain. In B2 and B6 this ratio was only 1.85 and 1.88, respectively. Taking into consideration the confidence intervals of the second order means it may be shown that the ratio in the case of B2 clone is between 1.62 and 2.11, and in the case of B6 clone, between 1.65 and 2.16. It means that a deviation from the two-fold ratio in these two cases may be accidental. To show this, the DNA content in B2 and B6 clones was measured for the second time in the synchronized samples of 1 h old nuclei. The results show that the DNA content of B2 and B6 clones are two-fold increased in respect to the original strain (Table 4).

The DNA content was also measured in partly synchronized nuclei of B7 clone 1 month after it has been isolated. At that time the sample consisted of two kinds of nuclei. Eleven nuclei had large amount of DNA (5.5 - 7.3 arb. units; mean value was 6.10 ± 0.16) and 14 had lesser amounts (3.20 ± 0.13 arb. units only). In the first group the DNA amount was 1.9-fold increased as compared to the second one and exceeded 3.9 times that of the control. Taking into consideration the confidence intervals it may be assumed that the ratio of the DNA amount between the two groups of B7 clone and the control is 4 : 2 : 1.

It may be supposed: (1) that other clones with increased nuclear area are also polyploid and (2) that such clones were grown from polyploid cells present in a mass culture. In that case the proportion of the polyploid individuals may be determined. In B strain it was no less than 1.8% (95% confidence interval was 0.9 - 2.2%).

Table 4

DNA content in synchronized nuclei of B2 and B6 clones 1 month after their isolation

Clone	DNA content $\bar{X} \pm S_{\bar{x}}$ arb. units	
	In the samples	Second order mean
Strain B (control)	0.94±0.02	
	0.83±0.02	
	0.87±0.02	0.85±0.02
	0.82±0.02	
	0.80±0.02	
	1.75±0.04	
	1.74±0.04	
B2	1.71±0.03	1.70±0.02
	1.71±0.03	
	1.61±0.03	
	1.90±0.04	
	1.76±0.04	
B6	1.68±0.03	1.73±0.05
	1.67±0.03	
	1.64±0.03	

Each sample consists of 20 nuclei

Combined cultivation of normal and polyploid cells. Polyploid clones were grown in culture. It is clear that polyploid cells are viable. The question arises why the proportion of polyploid amoebae in original culture is not large. The hypothesis which has been advanced (Afon'kin 1986) that polyploid cells are less competitive under laboratory conditions may be accepted as explanation.

To verify this assumption, artificially mixed cultures were produced. In each of six glasses (the volume was 35 ml, the bottom area was 17.8 cm^2) 1000 polyploid amoebae of B1 - B6 clones were placed and then 1000 B cells were added. In this way six combined cultures were produced with 2000 cells in each glass.

Within two months the number of cells increased rapidly to fluctuate from 40 to 55 thousands (Fig. 1). One month after the onset of the experiment the DNA content was measured in partly synchronized samples (131 - 159 amoebae) taken from each combined culture.

In all the cases the distribution comprised two separate groups of values. The typical distribution is presented in Fig. 2. Under assumption

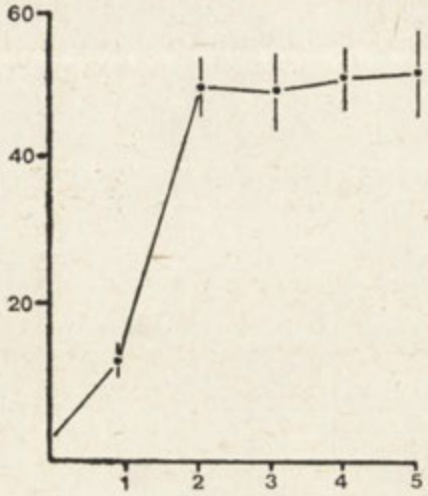


Fig. 1. Mean number of cells in six mixed cultures of polyploid and normal amoebae. *Abscissa*: time in months from the beginning of the experiment, *ordinate*: cell number in thousands. The vertical dashes designate the 95% confidence intervals

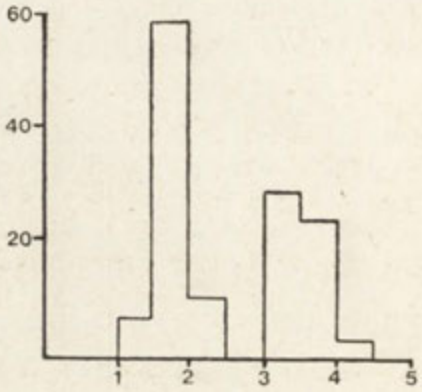


Fig. 2. Distribution of values of the DNA content of nuclei in a partly synchronized cell sample from a mixed culture (B4 + B) 1 month after the start of the experiment. *Abscissa*: DNA content in arb. units, *ordinate*: number of nuclei

that cells with the lesser DNA amount are B amoebae and cells with larger DNA content are polyploids, the proportion of the latter may be estimated in the sample (Table 5). The ratio of polyploid and normal amoebae in B1 + B and B4 + B cultures did not differ reliably from 1 : 1. In all the other cases the proportion of polyploid cells was lower.

Four months later the samples (140 - 160 amoebae) were taken again from the combined cultures. In this case only a few polyploid cells were detected in most of the cultures. The proportion of polyploid

Table 5

Proportion of polyploid amoebae in combined cultures 1 and 5 months after the onset of the experiment

Combined culture	Proportion of polyploid amoebae (%)	
	1 month	5 months
B1 + B	45.2	6.3
B2 + B	35.6	0.7
B3 + B	23.0	1.2
B4 + B	45.6	0
B5 + B	36.9	0
B6 + B	29.7	1.4

Each sample consists of 130—160 nuclei.

amoebae was about the control level in two cultures (1.2 - 1.4%), being lower (0.7%) or larger (6.3%) in others. Two cultures contained no polyploid cells.

Thus it was shown that the percent of polyploid cells decreased during their prolonged cultivation together with normal amoebae.

Cloning efficiency and the generation time of polyploid amoebae.

The results of three independent experiments show that the cloning efficiency (see Material and Methods) of polyploid B1, B2 and B3 amoebae is lower than in the control (Table 6).

To take this fact into account in measurement of the mean generation time three assumptions were made. (1) The generation time in a clone is constant. (2) At the end of this period an amoeba divides with a probability q ($q - 1$). (3) Undivided amoebae further do not divide but remain viable during the experiment (168 h).

Table 6

Cloning efficiency and the generation time of polyploid amoebae

Clone	Cloning efficiency $\bar{X} \pm S_{\bar{x}}$, %	Final number of cells in 60 subclones after 168 h of culturing $\bar{X} \pm S_{\bar{x}}$, %	Calculated generation time $\bar{X} \pm S_{\bar{x}}$, h
Strain B (control)	97±2	961±37	40.6±0.5
B1	74±2	401±70	44.8±5.3
B2	56±3	171±20	64.3±13.2
B3	67±4	301±25	45.0±3.2

All mean values were obtained in three independent experiments.

The assumption that the cloning efficiency means here the probability of cell division in the next cell cycle (see Material and Methods) allows us to formulate the following: if we take into the experiment N_0 cells, N_0q of them divide at the end of the first cell cycle and N_0 ($q - 1$) remain undivided. At the end the second cell cycle $2N_0q^2$ cells divide and $N_0(q - 1) + 2N_0(q - 1)q$ remain undivided. After K cell generations we may derive the equation:

$$\frac{N_f}{N_0} = \left(1 - \frac{\lambda}{2}\right)\left(\frac{\lambda^k - 1}{\lambda - 1} + \lambda^k\right)$$

where $K = \frac{T}{t}$; $\lambda = 2q$; N_0 is the number of cells at the beginning of an experiment; N_f is the final number at the end of the experiment; T is the time of culturing; t is the generation time; q is the probability of

cell division. By solving the equation one may define the generation time. The mean value estimated in such a way for B1 - B3 clones did not significantly differ (95% significance level) from the control one (Table 6).

Four months later the cloning efficiency of B1 - B3 cells was estimated for the second time. The obtained values (76 ± 1; 64 ± 3; 71 ± 1%) were also lower than in the control (95 ± 1%). Thus, during this period the cloning efficiency of polyploid cells did not increase.

Stability of polyploid clones. As shown earlier, during prolonged cultivation the mean DNA content in polyploid clones grown from cells with colchicine blocked mitoses may be reduced (Afon'kin 1986). It is due to the appearance of cells with two-fold decreased DNA amount and a steady increase of their number in clones.

To determine the stability of polyploid clones obtained in the present study the DNA content was measured five months after they had been produced. One partly synchronized sample from each of the polyploid clones was made (100 nuclei in each). The mean values (3.9 - 4.2 arb. units) were increased 1.9 - 2.0 times in respect to the control (2.0 arb. units, $N = 25$). The cells with decreased DNA amount were not detected.

Essentially, the sample of B7 clone consisted of nuclei with only two-fold increased DNA content (the mean value is 4.06 ± 0.05 arb. units). It must be noted that 1 month after isolation the clone was found to consist of two kinds of nuclei, with two- and four-fold increased of the DNA amount. Thus, it may be ascertained that within this period the DNA content in B7 clone decreased from the octo- to tetraploidy level. In all the other clones the ploidy level underwent no change.

Discussion

Among *Amoeba proteus* strains of our collection one was detected (strain A) to have a two-fold decreased DNA content as compared to the strain B (Afon'kin 1983). However, this is not a proof of the existence of a polyploidy series since *A. proteus* have no sexual process and we cannot apply the biological species criterion to B and A strains. The existence of polyploid clones obtained by injecting colchicine solution into divided amoebae also is not an evidence because hitherto we knew nothing about the spontaneous polyploidization in *A. proteus*.

In this study the cells with unusually enlarged nuclei were successfully picked out from a mass culture of the strain B. The analysis of

the DNA content in clones grown from such cells showed that they are genuine polyploids. The screening method asserts that the proportion of polyploid amoebae in the mass culture was no less than 1.8%. It coincides with the number of nuclei with high DNA levels found in the samples in other studies (Sopina et al. 1982 and our observations).

Strain B was imported to our laboratory from England in 1960. Since then the B culture was recloned many times. The culturing conditions render impossible any contamination by foreign cells (for instance by the cells from artificially produced polyploid clones). All this leads to the inevitable conclusion that the occasional polyploidization does occur in the B culture. We have no information about the frequency of this phenomenon. The octoploid amoebae in tetraploid samples and the composition and behaviour of B7 clone indicate that the two-step polyploidization may also take place.

In the artificially mixed cultures the polyploid cells are less competitive than the control ones. The proportion of the polyploids during cultivation decreased to the ordinary level characteristic of the B culture. This phenomenon may account for the low level of polyploid cells in cultures where the probability of polyploidization is relatively high. It is possible that conditions may be created under which the polyploid amoebae would have an advantage over the normal ones (high level of mutagenes and radiation).

The decreased competitiveness of polyploid amoebae may be dependent on the reduced cloning efficiency. The ability to produce clones is as a rule decreasing with the increase of cell ploidy level (Brodskij and Uryvaeva 1981).

The decreased cloning efficiency of polyploid amoebae and their uncompetitiveness in mixed cultures were described earlier, when artificially produced polyploid clones were studied (Afon'kin 1984, 1985). This led to the conclusion that many of the amoebae cell characteristics change simultaneously when the level of ploidy is increasing. The way of polyploidization is not in this respect essential.

In the present study, as well as in the previous publications, the mean generation time of polyploid amoebae was shown to remain unchanged. The proposed method of its measuring in clones with decreased cloning efficiency, however, may be inexact. The method leaves out of account the following: (1) The generation time usually varies within some limits. (2) Cells that die in subclones are not taken into consideration. (3) The value of cloning efficiency may not precisely correspond to the probability of the cell cycle completion by an amoeba in the clone. The latter may be due to a kind of group effect since single cells are less tolerant to overfeeding.

The analysis of the nuclear size and DNA content of the nuclei shows that originally the clone B7 isolated from mass culture was octoploid and then its ploidy fell to the tetraploid level. A similar phenomenon of depolyploidization has been described for "colchicine" polyploid clones (Afon'kin 1986). It should be emphasized that it occurs also in clones isolated from amoebae mass culture.

It is not excluded that poly- and depolyploidization of cells plays a role in the genetic variability of amoebae. This is illustrated by the data on the effects of mutagenes on *A. proteus*. N-methyl-N-nitrozourethane induces in *A. proteus* the appearance of cells with both small and large nuclei (Ord 1968, 1977). So called "mini-mutants" with decreased size of nuclei and shortened cell cycle were recently obtained under the influence of ethylmethansulfonate on *A. indica* (Gangopadhyay and Chatterjee 1984). ^{3}H-thymidine incorporation into the "mini-nuclei" of amoebae was two-times less intense than in the control.

A possibility of spontaneous polyploidization in amoebae should be taken in consideration in approaching some problems of systematics. Among others, such a character as the "nuclear size" must be handled with care. There is no doubt that the original amoebae and the amoebae of various ploidy degrees described in this study belong to the same species despite their variation in the nuclear size and DNA content.

ACKNOWLEDGEMENTS

The author is indebted to Dr A. L. Yudin for valuable advice and discussion of the results and to A. A. Staviskaya for the help in translating the article. The author is also indebted to A. I. Alexandrova and G. I. Stein for their technical assistance in nuclear size measurements.

REFERENCES

Afon'kin S. Yu. 1983: Soderžanie DNK v jadrah rasnych štammov *Amoeba proteus* po dannym citofluorometrii. Citologija, 25, 771 - 776.

Afon'kin S. Yu. 1984: Production of *Amoeba proteus* clones with increased nuclear DNA content. Acta Protozool., 23, 35 - 54.

Afon'kin S. Yu. 1986: Spontaneous "depolyploidization" of cells in *Amoeba* clones with increased nuclear DNA content. Arch. Protistenk., (in press).

Afon'kin S. Yu. and Yudin A. L. 1985: Ispol'zovanie mikroplat dlja immunologičeskih reaktsii dlja klonirovanija i kul'tivirovanija svobodno živuščih prostejših. Citologija, 27, 1207 - 1209.

Brodskij V. Ya. and Uryvaeva I. V. 1981: Kletočnaja poliploidija, proliferacija i differencirovka. Izd. "Nauka" Moskva, 260 pp.

Cleveland L. R. 1949: The whole life cycle of chromosomes and their coiling systems. Trans. Amer. Philos. Soc., 39, 1 - 100.

Coleman A. W. and Zollner J. 1977: Cytogenetic polymorphism within the species *Pandorina morum* Bory de St. Vincent (*Volvocidae*). Arch. Protistenk., 119, 224 - 232.

Gangopadhyay A. and Chatterjee S. 1984: A cell-cycle-phase-specific mutant of amoeba. J. Cell Sci., 68, 95 - 111.

Grassé P.-P. and Hollande A. 1963: Les flagellés des genres *Holomastigotoides* et *Rostronympha*. Structure et cycle de spiralization des chromosomes chez *Holomastigotoides* psammotermitidis. Ann. Sci. Nat. Zool., sér. 12, 5, 749 - 792.

Kaczanovski A. 1968: Mitosis and polyploidy of nuclei of *Opalina ranarum*. Experientia, 24, 846 - 847.

Kudryavtsev B. N. and Rosanov Yu. M. 1974: Citofluorimetrija. Obšče princypy. V kn.: Metody Biologii Rasvitija, Moskva, Nauka, 497 - 500.

Makhlin E. E., Kudryavtseva M. V. and Kudryavtsev B. N. 1979: Pecularities of changes in DNA content of *Amoeba proteus* nuclei during interphase. Exp. Cell Res., 118, 143 - 150.

Ord M. J. 1968: Immediate and delayed effects of N-methyl-N-nitrosourethane on *Amoeba proteus*. Exp. Cell Res., 53, 73 - 84.

Ord M. J. 1970: Mutation induced in *Amoeba proteus* by the carcinogen N-methyl-N-nitrosourethane. J. Cell Sci., 7, 531 - 548.

Ord M. J. 1979: A capillary technique for cloning *Amoeba proteus* from single cells. Cytobios, 21, 57 - 69.

Ossipov D. V. and Borchsenius O. N. 1973: Osobennosti poliploidii mikronukleusov u infusorii (*Protozoa*). Citologija, 15, 243 - 253.

Poljansky Yu. I. and Raikov I. B. 1960: Rol' poliploidii v evoljucii prostejših. Citologija, 2, 509 - 518.

Poljansky Yu. I. and Raikov I. B. 1977: Polimerizacija i oligomerizacija v evoljucii prostejših. Zh. Obshch. Biol., 37, 325 - 335.

Prescott D. M. and Carrier R. F. 1964: Experimental procedures and cultural methods for *Euplotes eurystomus* and *Amoeba proteus*. In: Methods in Cell Physiology. (ed. D. M. Prescott), Academic Press, New York - London, 1, 85 - 95.

Raikov I. B. 1982: The Protozoan Nucleus. Morphology and Evolution. Cell Biology Monographs. Springer-Verlag, Wien - New York, 9, 474 pp.

Sopina V. A., Afon'kin S. Yu. and Kudryavtsev B. N. 1982: Nuclear DNA content in *Amoeba proteus* cultured at different temperatures. Acta Protozool., 21, 203 - 212.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 55 - 69 (1986)

Antigenic and Immunogenic Properties of Pathogenic and Non-pathogenic Strains of *Naegleria* spp.

Jadwiga WINIECKA

Department of Biology and Medical Parasitology, Academy of Medicine, Fredry 10, 61-701 Poznań, Poland

Received on 25 February 1985, *revised* 12 June 1985

Synopsis. The subject of this work was to define the differences and similarities in the antigenic structure and immunogenic properties of five *Naegleria* strains: two pathogenic strains of *Naegleria fowleri* isolated from human fatal cases, two *Naegleria fowleri* strains, pathogenic and non-pathogenic, isolated from the environment and non-pathogenic strain of *Naegleria gruberi*. The results showed the close antigenic relationship between the pathogenic strains and some similarity of the non-pathogenic *Naegleria fowleri* strain to the strain of *Naegleria gruberi*. The high virulent human strains were the weakest immunogens, whereas the non-pathogenic strain of *Naegleria gruberi* the strongest one. Strong immunogenicity of the non-pathogenic strains, their antigenic similarity to pathogenic strains and frequent occurrence in the environment may be the reason for active immunization of people and animals to the pathogenic strains.

Naegleria amoebae receive increasing attention as they have proved dangerous to human health and life. As amphizoic organisms they can propagate in free environment, i.e., water, soil, air or artificial water reservoirs, as well as inside macroorganisms. Pathogenic strains enter the central nervous system via the nosopharynx causing primary amoebic meningoencephalitis (PAM). Although spontaneous infections of *Naegleria* in animals have not been found, the evidence of anti-*Naegleria* antibodies in animals gives rise to the assumption that such infections may occur (Červa 1981).

Since 1965, when Fowler and Carter described first cases of fulminating meningoencephalitis, over 150 cases of PAM in man have been reported (Martinez and Kasprzak 1980). In all the cases the host reaction to the parasite invasion was rather slight. Cain et al. (1979) and Cursons et al. (1979) observed normal or even slightly lowered level of all classes of immunoglobulins and a lack of

specific antibodies. In animals the existence of a factor inhibiting the migration of macrophages and delayed hypersensitivity have been reported (Cursons et al. 1980 b).

Weak and ineffective reaction of the host may be due to a rapid course of the disease leading to death within a few days. It may also be due to the parasite localization in the central nervous system where the production of antibodies is rather difficult; some specific antigenic properties of these amoebae may also be the cause.

The antigenic structure of amoebae strains isolated from the fatal cases of PAM in many countries has shown a considerable similarity. All this strains are classified as *Naegleria fowleri*. It is more difficult to determine the taxonomic position of many pathogenic and non-pathogenic *Naegleria* strains isolated from the free environment. Most of the environmental pathogenic strains because of their antigenic similarities to the so-called human strains, are also included in the *Naegleria fowleri* species. In 1981, however, De Jonckheere described *Naegleria australiensis*, a pathogenic species of antigenic and biochemical properties different from *Naegleria fowleri*. Environmental non-pathogenic strains different from *Naegleria gruberi*, are characterized by a great variety of their antigenic properties. They are classified as different species, i.e., *Naegleria fowleri* — De Jonckheere and Van de Voorde (1977), *Naegleria jadini* — Willaert and Le Ray (1973), *Naegleria lovaniensis* — Stevens et al. (1980).

It is assumed that regardless of the classification, non-pathogenic strains of *Naegleria* share in the aquisition of protective immunity to infection for they are common and it is easy to get in contact with them (John and Bush 1980). However, to confirm the above assumption, further investigation concerning the antigenic relationship between these strains and their immunogenic properties is necessary.

The aim of this study was a comparison of the antigenic structure and immunogenic properties of some chosen *Naegleria* strains, isolated from the fatal cases of PAM as well as from the environment. It is hoped that this research will be useful in the species and strains differentiation, between pathogenic and non-pathogenic strains in particular, and may thus contribute to the epidemiology of invasion and to the taxonomy of this group.

Material and Methods

Strains of amoebae: *Naegleria fowleri*, strain HB1, pathogenic for mice, isolated by Butt (1968); *Naegleria fowleri*, strain Vitek, pathogenic for mice, isolated by Červa et al. (1969); *Naegleria fowleri*, strain KIV, pathogenic for mice, isolated from the environment by Kasprzak and Mazur (1974); *Nae-*

gleria fowleri, strain 5D, non-pathogenic for mice, isolated from the environment by Kasprzak and Mazur (1978); *Naegleria gruberi* strain EG, non-pathogenic, isolated from environment by Schuster (1961).

Culture of amoebae: *Naegleria fowleri* strains were cultivated axenically in a liquid medium containing sheep serum; the temperature of growth was 37°C. *Naegleria gruberi* strain was cultivated in a medium containing calf serum, at 24°C.

Preparation of antigens: 7-day-long cultures were collected, washed with physiological saline three times, with cell sediment gathered. For soluble antigen, the suspension of amoebae in physiological saline was freezed and thawed, the homogenate was left out at 4°C for 18 h, then centrifugated; the sediment was removed. The protein content in supernatant was determined by the method of Lowry et al. (1951). For cell antigen, the washed suspension of amoebae was diluted to obtain the required number of cells.

Preparation of antisera: the immunization process was similar to this described by Dwyer (1972). Rabbits, each 2.5 kg of weight, were weekly injected intramuscularly with 1 ml of the antigen-complete Freund adjuvant mixture (1 : 1 vol/vol), (Table 1). Intact cells were given in the last dose according to Červa and Kramar (1973). During the immunization period blood was withdrawn for the follow up antibody formation. 7-days after the last injection, anesthetized with Brevinarcon, animals were bled by the cardiac puncture.

Table 1

The scheme of animal immunization with soluble antigen

Day of immunization	1	7	14	21	28	35	42
Dose of antigen in mg of protein to ml	2.5	3.5	5	7.5	9.5	12	10^6 cells

Serological tests: Indirect Immunofluorescent Test (IIF) was performed in accordance with the technique described by Goldman (1966) on teflon microscopical plates. Swine anti-rabbit fluorescent serum mixed with the Evans blue was used as a conjugate. Mounted in glycerin preparates were observed with Fluoval microscope with HBO 200 mercury lamp and with OG1 and BG12 filters. Double Immunodiffusion Test (ID) was based on the technique described by Dwyer (1972). 0.8% agarose and 1% agar were used as the medium. Precipitates were stained with 0.1% Amido Black and discharged with the mixture composed of acetic acid, ethanol and water in the ration of 1 : 7 : 2, respectively. Disc Immunodiffusion Test was based on a modified method of Seto and Hokama (1964). Soluble amoebae antigens (400 - 700 μg of protein) were separated by means of poliacrylamid gel electrophoresis (PAGE) according to the method described by Hadaś et al. (1977). The separation was performed on 7.5% poliacrylamid gel, at 4°C, for 60 min at 2.5 mA for each gel; the electrode buffer was tris-glicine, ph 8.5. After electrophoresis, non-stained gel columns were cut into slices of 2 mm and prepared into agar. Then wells were cut and filled with antiamoebic sera. Further procedure was the same as in ID test.

Results

The dynamism of growth of antibodies in animals immunized with five different antigens is presented in Table 2 and Fig. 1. The highest titres in IIF test were obtained for the group of animals immunized with EG strain of *Naegleria gruberi*, and the lowest for the group immunized with HB1 strain of *Naegleria fowleri*. In ID test no significant differences in the number of precipitin lines have been found in particular animal groups. The estimation of antigenic relationship was performed on the basis of a set of cross reactions in serological tests. The results are

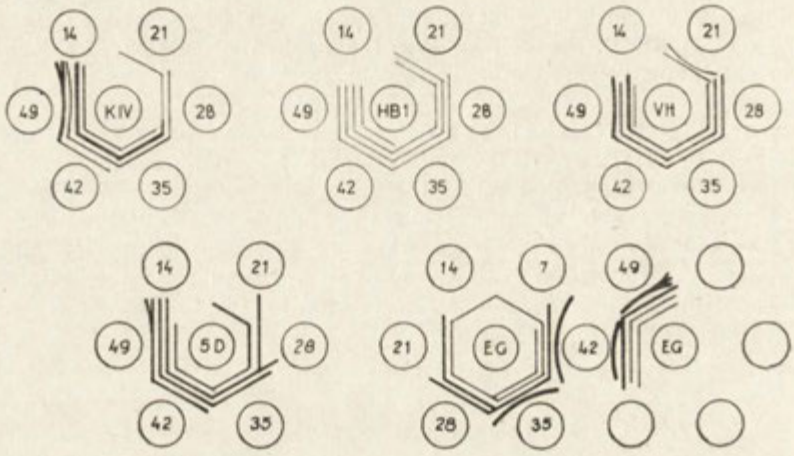


Fig. 1. Double Immunodiffusion Test. The growth of serum antibodies during the immunization period. Wells 7 - 49 — sera from different days of immunization, center wells — *Naegleria* antigens

Table 2

Immunization of animals with five soluble *Naegleria* antigens. The results of Indirect Immunofluorescent (IIF) and Double Immunodiffusion (ID) Tests

Day of immunization		1	7	14	21	28	35	42	49
Antigen	IIF*	–	32	64	128	256	512	512	1 024
KIV	ID**	–	–	–	1	2	3	4	4(5)
Antigen	IIF	–	16	64	128	256	256	512	1 024
Vitek	ID	–	–	–	2	3	3	3	4
Antigen	IIF	–	2	8	32	64	128	128	256
HB1	ID	–	–	–	2	3	3	4	4
Antigen	IIF	–	32	256	512	512	1024	2 048	2 048
5D	ID	–	–	–	1	2	3	4	4(5)
Antigen	IIF	–	512	1024	4096	4096	32 768	32 768	32 768
EG	ID	–	1	1	2	3	4	4	4(6)

* serum titre in IIF-Test
** number of precipitin lines in ID-Test

presented in Table 3 and Fig. 2. Pathogenic strains of *Naegleria fowleri* KIV, HB1 and Vitek pointed to a considerable antigenic likeness. The differences between reference and cross reactions in IIF test are comprised within the limits of 2 - 3 dilutions of sera, whereas in precipiting test the differences concern a single precipitin line (Fig. 2). The weakest cross reactions occurred between pathogenic strains and the strain EG of *Naegleria gruberi* (low titres in IIF — Table 3, and the lack of reaction between the anti-pathogenic strains sera and EG antigens in ID test — Fig. 2). Non-pathogenic strain 5D of *Naegleria fowleri* reacted with both, pathogenic strains of *Naegleria fowleri* and EG strain of

Table 3

The cross reactions between *Naegleria* antigens and sera from animals immunized with soluble antigens. The results of Indirect Immunofluorescent (IIF) and Double Immunodiffusion (ID) Tests

Antibodies	Anti-KIV		Anti-HB1		Anti-Vitek		Anti-5D		Anti-EG	
	IIF*	ID**	IIF	ID	IIF	ID	IIF	ID	IIF	ID
Antigens										
KIV	1024	4 (5)	128	3	512	3	32	2	32	1
HB1	128	2	256	4	256	4	16	2	16	1
Vitek	256	2	128	4	1024	4	64	2	128	1
5D	64	2	8	2	32	2	2048	4 (5)	512	3
EG	8	—	8	—	256	—	128	2	32 768	4(6)

* serum titre in IIF Test
** number of precipitin lines in ID Test

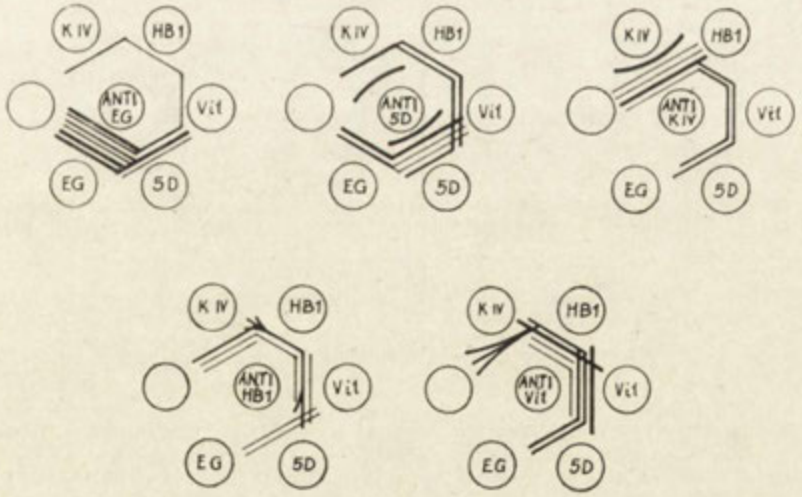


Fig. 2. Double Immunodiffusion Test. The cross reactions between antibodies (center wells) and antigens of different *Naegleria* strains (outside wells)

Naegleria gruberi. Figure 3 ilustrates the reactivity of immune sera collected from animals in different periods of immunization. The antigens of pathogenic strains reacted with the corresponding sera as early as after 7 days of immunization, whereas the antigens of non-pathogenic strains reacted with these sera much later, i.e., after 14 - 42 days. The longest non-reaction time was between antigens and sera of EG and HB1 strains.

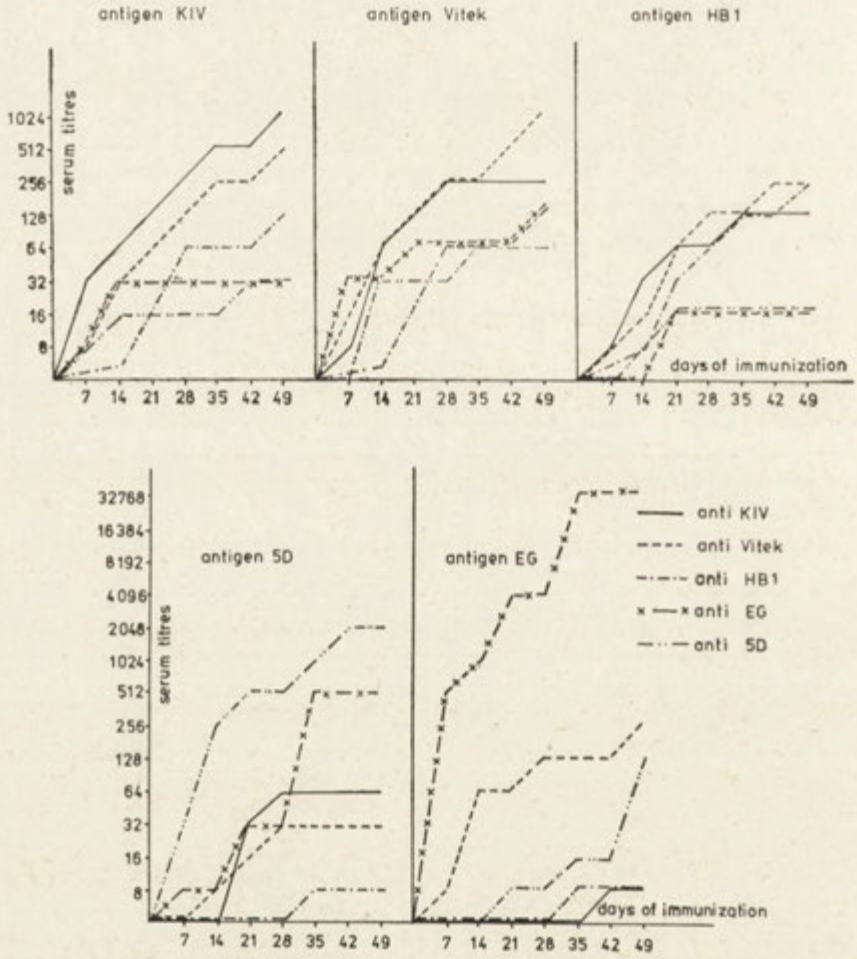


Fig. 3. The reactivity (in IIF-Test) of animal sera during the immunization period

Table 4 and Fig. 4 - 8 ilustrate the reactivity of amoebae protein fractions. In reference reactions of pathogenic, 5D and EG strains, the precipitates were formed with 8, 9 and 15 sections of gel, respectively. There were no cross reactions between fractions of pathogenic strains

and anti-5D or anti-EG sera and no reactions between EG protein fractions and anti-pathogenic strains sera. Protein fractions of 5D strain reacted faintly with sera against pathogenic strains and a little stronger with anti-EG serum.

Table 4

The reactivity of protein fraction in Disc Immunodiffusion Test

Antigens	Fractions / Sera	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
KIV	Anti-KIV	3*	4	4	2	3	2	1	1	–						
	Anti-Vitek	2	2	2	1	1	1	1	1	–						
	Anti-HB1	2	2	2	1	1	1	1	1	–						
	Anti-5D	–														
	Anti-EG	–														
Vitek	Anti-KIV	2	1	2	2	1	–									
	Anti-Vitek	3	4	3	2	3	3	1	1	–						
	Anti-HB1	3	3	2	2	1	2	1	1	–						
	Anti-5D	–														
	Anti-EG	–														
HB1	Anti-KIV	2	1	1	1	–										
	Anti-Vitek	3	3	3	2	2	2	1	1	–						
	Anti-HB1	3	3	3	2	1	2	1	1	–						
	Anti-5D	–														
	Anti-EG	–														
5D	Anti-KIV	1	–													
	Anti-Vitek	1	–													
	Anti-HB1	1	–													
	Anti-5D	4	3	4	4	3	1	2	2	2	–					
	Anti-EG	1	1	3	3	1	1	1	1	1	–					
EG	Anti-KIV	–														
	Anti-Vitek	–														
	Anti-HB1	–														
	Anti-5D	1	1	2	1	1	1	1	1	–						
	Anti-EG	5	2	4	4	2	2	2	1	2	2	1	1	1	1	1

*number of precipitin lines.

Discussion

The results of animal immunization proved that all strains under investigation may provoke the immunological response. Out of all pathogenic strains the environmental strain KIV of *Naegleria fowleri* proved to be the best immunogen. Environmental non-pathogenic strain 5D of *Naegleria fowleri* immunized animals to a similar extent. Surprisingly enough, the non-pathogenic strain EG of *Naegleria gruberi* was found

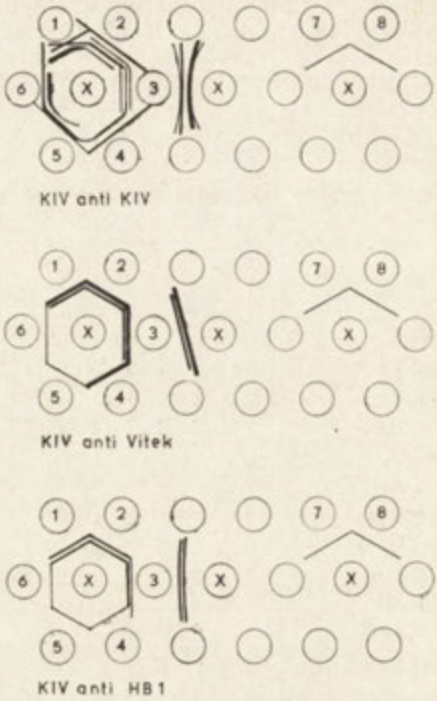


Fig. 4. Disc immunodiffusion of KIV antigens. x — animal serum, 1-8 — polyacrylamid gel slices

to cause the strongest immunological response. Similar results were reported by John et al. (1977) and John and Bush (1980) who immunized animals with different strains of *Naegleria fowleri* and *Naegleria gruberi*. Equally important is that the injection of intact amoeba cells to animals immunized with soluble antigen caused an increase of their immunological response. Stevens et al. (1977) observed a similar phenomenon in the related genus *Acanthamoeba*. They obtained the best immune sera by injecting the non-pathogenic strain Neff of *Acanthamoeba castellani* and sensitizing the animals with purified cell membranes. This seems to be due to particularly strong immunogenic properties of cell membranes and the presence of many surface antigens in these amoebae.

The results of comparative investigations point to the fact that human strains of *Naegleria fowleri* are considerably similar to one another. The differences observed were so slight that they might concern interspecies differences connected with the occurrence of particular antigens in varying quantities. KIV, the pathogenic environmental strain, though similar to human strains of *Naegleria fowleri*, shows some differences. Apart from antigens common to those in human strains, KIV is likely

to possess some new antigens which cannot be found in the others. Investigating the affinity between human and environmental strains, Willaert et al. (1974) similarly discovered among the latter, strains that showed a complete identity with the former and others that differed only slightly. The analysis of cross reactions between the pathogenic strains of *Naegleria fowleri* shows that the environmental strain KIV is more similar to Vitek than to HB1 (both are human strains). The above findings confirm the results of previous investigations carried by Hadaś et al. (1977) who pointed to a similar relationship in the protein content of these strains. Strain EG, representing non-pathogenic species *Naegleria gruberi*, proves to be definitely different from all the other strains of *Naegleria fowleri*. It is striking, however, that this strain is strongly involved in the cross reaction with the non-pathogenic environmental strain 5D of *Naegleria fowleri*, and relatively strongly reacts with the antigens of human strain Vitek. Similar results obtained by Van Dijck et al. (1974) made the authors say that there is some antigenic relationship between *Naegleria gruberi* and strain Vitek of *Naegleria fowleri*, which testifies to the existence of certain antigens common to both species.

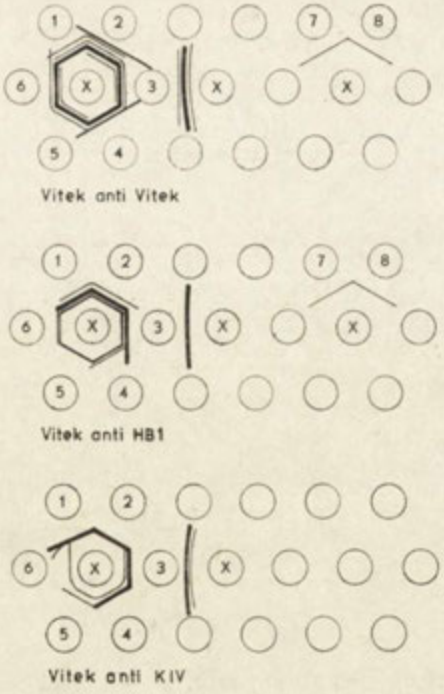


Fig. 5. Disc Immunodiffusion of Vitek antigens. x — animal serum, 1 - 8 — poliacrylamid gel slices

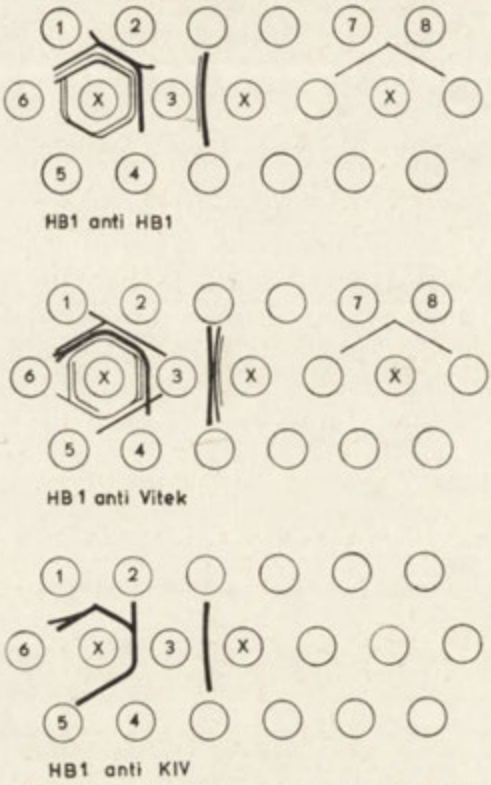


Fig. 6. Disc Immunodiffusion of HB1 antigens. x — animal serum, 1 - 8 — poliacrylamid gel slices

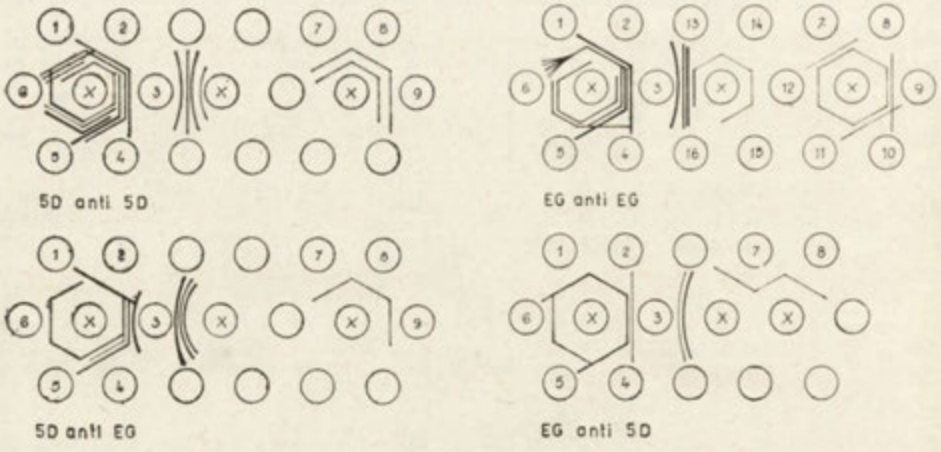


Fig. 7. Disc Immunodiffusion of 5D antigens. x — animal serum, 1 - 9 — poliacrylamid gel slices (on the right)

Fig. 8. Disc Immunodiffusion of EG antigens. x — animal serum, 1 - 16 — poliacrylamid gel slices (on the left)

The analysis of the reactivity of immune sera from different immunization periods makes the observation of mutual relationship between strains more accurate. Boyden (1970) and Fairbrothers (1966) state that the usefulness of serum in immunotaxonomic investigations varies according to different injection periods and the composition of antigens mixture. It may be assumed that in the first period of immunization the reaction comes from only a part of antigenic material such as proteins with strong immunogenic properties, markers of investigated taxons. When the process of immunization is prolonged, other proteins in the antigenic material get involved in the immunogenic activity and, in consequence, the immune serum loses its specificity and immunotaxonomical quality.

It is characteristic that the antigens of human strain HB1 of *Naegleria fowleri*, which had previously been described as different from all the other pathogenic strains, reacted with the anti-non-pathogenic sera after three weeks of immunization, whereas the other two pathogenic strains reacted with these sera immediately. Similarly, anti-HB1 serum reacted with antigens of non-pathogenic strains as late as after 35 days of animals immunization. That means that the greatest antigenic differences occur between HB1 and 5D strains as well as between HB1 and EG. Anti-EG serum, on the other hand, got immediately involved in positive reaction with antigens of all the investigated strains with the exception of HB1. This gives rise to the assumption that among the antigens of EG strain of *Naegleria gruberi* there were strongly immunogenic components, common to the antigens of *Naegleria fowleri* strains, which immunized the animal during the first days of immunization.

Assuming that the parasitism of *Naegleria* strains is of secondary character and that they originate from free-living forms, it can be inferred that in the process of acquiring pathogenic properties, certain antigens were lost or replaced by new ones which are not present in free-living strains. It can also be possible that the antigenic properties of these proteins underwent certain modifications. Such presumption explains the differences between the strains HB1 (typical virulent pathogen) and 5D or EG (non-pathogenic free-living strains). The environmental strain KIV, pathogenic for animals, has the antigenic structure much similar to that of human strains. On the other hand, it preserves many antigenic properties common to non-pathogenic strains, so it strongly reacts with anti-EG and anti-5D sera. Its antigens, however, were not able to induce the synthesis of antibodies which might equally strongly react with antigens of non-pathogenic strains. 5D, the environmental non-pathogenic strain, reacted in a similar way. The immediate reaction between its antigens and anti-EG serum supports the view that

the relationship between these species is a close one. However, the anti-5D antibodies reacted with EG antigens a little slower. The pathogenic human strain Vitek is quite exceptional among the investigated strains for it gets involved in strong cross reactions with strain EG immediately. This untypical behaviour of Vitek was earlier described by other authors (Van Dijck et al. 1974). It is possible that Vitek is a younger pathogen than strain HB1. Therefore, the investigated strains considered in terms of the degree of antigenic relationship form the following series:

EG → 5D → KIV → Vitek → HB1

↓ ↓

strong antigenic marker of free-living mode of life

weak antigenic marker of free-living mode of life, strong antigenic new marker of parasitism

The presence of a strong antigenic marker of free environment in free-living strains facilitates the reaction between the anti-non-pathogenic strains sera and the pathogenic strains antigens. On the other hand, the presence of a weak marker of free environment (a marker of smaller immunogenicity), requires a prolonged period of time necessary for the reaction between the antigens of non-pathogenic strains and the sera obtained by immunization with pathogenic strains.

The cross reactions of the pathogenic strains protein fractions confirmed a close antigenic relationship between them, especially between the human strains HB1 and Vitek. It is strange, however, that their protein fractions did not react with anti-5D serum. In ID test there were two precipitin lines developed by the whole soluble antigens. It is possible that there were too few antigenic protein in poliacrylamid gel slices to react with the serum. In accordance to the previous observations the sera anti-pathogenic strains did not react with EG and only faintly reacted with 5D protein fractions.

In disc electrophoresis on poliacrylamid gel proteins of amoebae get separated into about 27 fractions (Hadaś et al. 1977). The present study has found that a sections of gel column entering into reaction in disc immunodiffusion contains 12 fractions in the case of *Naegleria fowleri* strains. Other slices, even after elution and condensation do not react with immune serum. In the case of *Naegleria gruberi* the amount of antigenically active proteins is greater, i.e., 17. Not all protein fractions of the investigated strains, then, react as antigens. It can only be presumed that some of the antigenically active proteins common to both *Naegleria fowleri* and *Naegleria gruberi*, are of antigenic character and induce the protective antibody production. It seems that strong immunogenic properties of non-pathogenic strains and species of amoebae primarily deprived of the potential pathogenic properties and their

common occurrence in the environment make possible active immunization of humans and animals against pathogenic strains. This may be the cause of a relatively small number of cases of PAM in relation to the frequency of contacts with potentially pathogenic strains of *Naegleria* in the environment. The above statement finds support in experimental investigations in which animals, immunized by a single dose of amoeba, survived the intranasal invasion (Culbertson 1971, John et al. 1977, Thong et al. 1978 a, 1979, 1980, 1983). John and Bush (1980) found that the immunity which is best expressed by the number of surviving animals is conditioned by a particular amoebae strain used, the number of cells in the primary (immunizing) dose and the number of successive doses. The protection can be achieved not only by the intravenal injection of alive *Naegleria gruberi* and *Naegleria fowleri* amoebae (John et al. 1977), but also by the intraperitoneal administration of cell-free culture supernatant (Thong et al. 1979) or immune serum (Thong et al. 1978 b). These experimental data increase in value in the light of observations made by Cursons et al. (1980 a), who pointed to the existence of specific antibodies IgM and IgG to *Naegleria fowleri* and *Naegleria gruberi* in the sera of healthy humans. The titres of these sera were relatively low, but in view of the above experiments, it has to be noted that even a minimum level of antibodies in the sera of healthy humans may form a protective barrier against the *Naegleria* invasion.

ACKNOWLEDGEMENTS

The study was supported by a grant MR-II-4 from the Institute of Parasitology, Polish Academy of Sciences.

REFERENCES

Boyden A. 1970: A critique of some recent reports in the field of systematic serology. Serol. Mus. Bull., 44, 8.

Butt C. C. 1968: Primary amoebic meningoencephalitis. N. Engl. J. Med., 274, 1473.

Cain A. R. R., Mann P. G. and Warhurst D. C. 1979: IgA and PAME. Lancet, 1, 441.

Červa L. 1981: Spontaneous occurrence of antibodies against pathogenic amoeba of the limax group in domestic animals. Folia Parasitol., 28, 105 - 108.

Červa L., Ferdinandova M., Novak K., Ptackova M., Schrottenbaum M. and Zimak V. 1969: Meningoenzephalitis durch *Amoebida Naegleriidae*. Ein weiterer Fall in der Tschechoslovakei — Isolierung des Erregers. Münch'Med. Wochenschr., 111, 2029 - 2094.

Červa L. and Kramař J. 1973: Antigenic relationship among several limax amoebae isolates assessed with The Indirect Fluorescent Antibody Test (IFAT). Folia Parasitol., 20, 113 - 118.
Culbertson C. G. 1971: The pathogenicity of soil amebas. Annu. Rev. Microbiol., 25, 231 - 254.
Cursons R. T. M., Keys E. A., Brown T. J., Learmonth J., Campbell C. and Metcalf P. 1979: IgA and PAME. Lancet, 1, 223 - 224.
Cursons R. T. M., Brown T. J., Keys E. A., Moriarty K. M. and Till D. 1980 a: Immunity to pathogenic free-living amoebae: role of humoral antibody. Infect. Immun., Aug., 401 - 407.
Cursons R. T. M., Brown T. J., Keys E. A., Moriarty K. M. and Till D. 1980 b: Immunity to pathogenic free-living amoebae: role of cell-mediated immunity. Infect. Immun., Aug., 408 - 410.
Van Dijck P., De Jonckheere J., Reybrouch G. and Van de Voorde H. 1974: Rapid identification of *Naegleria* sp. by sero-agglutination and fluorescent antibodies. New possibilities for the water supply services. Zentralbl. Bakteriol. Parasitenkd. Infectionskr. Hyg., 158, 541 - 551.
Dwyer D. M. 1972: Analysis of the antigenic relationship among *Trichomonas*, *Histomonas*, *Dientamoeba* and *Entamoeba*. II. Gel diffusion methods. J. Protozool., 19, 326 - 332.
Fairbrothers D. E. 1966: Comparative serological studies in plant systematics. Serol. Mus. Bull., 35, 2 - 6.
Fowler M. and Carter F. R. 1965: Acute pyogenic meningitis probably due to *Acanthamoeba* sp. A preliminary report. Br. Med. J., II, 740 - 742.
Goldman M. 1966: Evaluation of a fluorescent antibody test for amebiasis using two widely differing amoeba strains as antigens. Am. J. Trop. Med. Hyg., 15, 694 - 700.
Hadaś E., Kasprzak W. and Mazur T. 1977: Electrophoretic characterization of small free-living amoebae. Tropenmed. Parasitol., 28, 35 - 43.
John D. T., Weik R. R. and Adams C. 1977: Immunization of mice against *Naegleria fowleri* infection. Infect. Immun., 817 - 820.
John D. T. and Bush L. E. 1980: Protection of mice against *Naegleria fowleri* following intranasal immunization. 2-nd Int. Conf. Biol. Path of Small Free-living Amoebae, March 23 - 25.
De Jonckheere J. F. and Van de Voorde H. 1977: Comparative study of six strains of *Naegleria* with special references to non-pathogenic variants of *Naegleria fowleri*. J. Protozool., 24, 304 - 309.
De Jonckheere J. F. 1981: *Naegleria australiensis* sp. nov., another pathogenic *Naegleria* from water. Protistologica, XVII, 423 - 429.
Kasprzak W. and Mazur T. 1974: Small free-living amoebae isolated from "warm" lakes: investigations on epidemiology and virulence of the strains. Proc. 3-rd Int. Congr. Parasitol., Abstr. A, 12, 12.
Kasprzak W. and Mazur T. 1978: Environmental strains of *Naegleria fowleri*. Proc. 4-th Int. Congr. Parasitol., Abstr. A, 29.
Lowry O., Rosenbrough N. J. and Randal J. R. 1951: Protein measurement with the Folin-fenol reagent. J. Biol. Chem., 193, 265 - 271.
Martinez A. J. and Kasprzak W. 1980: Patogenne pełzaki wolnożyjące — przegląd. Wiad. Parazyt., XXVI, 495 - 522.
Schuster F. L. 1961: Axenic cultivation of *Naegleria gruberi*. J. Protozool., 8, 19.
Seto J. T. and Hokama Y. 1964: Disc electrophoresis of sialidase from influenca virus. Ann. N. Y. Acad. Sci., 121, 640 - 650.
Stevens A. R., Killpatric T., Willaert E. and Capron A. 1977: Serologic analyses of cell-surface antigens of *Acanthamoeba* sp. with plasma membranes antisera. J. Protozool., 24, 316 - 324.
Stevens A. R., De Jonckheere J. F. and Willaert E. 1980: *Naegleria lovaniensis* — new species: isolation and identification of six thermophilic strains of a new species found in association with *Naegleria fowleri*. Int. J. Parasitol., 10, 10 - 64.
Thong Y. H., Shepherd Ch., Ferrante A. and Rowan-Kelly B. 1978 a: Protective immunity to *Naegleria fowleri* in experimental amebic meningoencephalitis. Am. J. Trop. Med. Hyg., 27, 238 - 240.
Thong Y. H., Ferrante A., Shepherd Ch. and Rowan-Kelly B. 1978 b:

Resistance of mice to *Naegleria meningoencephalitis* transferred by immune serum. Trans. R. Soc. Trop. Med. Hyg., 72, 650 - 652.

Thong Y. H., Ferrante A., Rowan-Kelly B. and O'Keefe D. 1979: Immunization with culture supernatant in experimental amoebic meningoencephalitis. Trans. R. Soc. Trop. Med. Hyg., 73, 684 - 685.

Thong Y. H., Ferrante A., Rowan-Kelly B. and O'Keefe D. 1980: Immunization with live amoebae, amoebic lysate and culture supernatant in experimental *Naegleria meningoencephalitis*. Trans. R. Soc. Trop. Med. Hyg., 74, 570 - 576.

Thong Y. H., Carter R. F., Ferrante A. and Rowan-Kelly B. 1983: Site of expression of immunity to *Naegleria fowleri* in immunized mice. Parasite Immun., 5, 67 - 76.

Willaert E. and Le Ray D. 1973: Caracteres morphologiques, biologiques et immunochimiques de *Naegleria jadini* sp. (*Amoebida, Vahlkamphidae*). Protistologica, IX, 417 - 426.

Willaert E., Jamieson A., Jadin J. B. and Anderson K. 1974: Epidemiological and immunoelectrophoretic studies on human and environmental strains of *Naegleria fowleri*. Ann. Soc. Belg. Med. Trop., 54, 333 - 342.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 71 - 74 (1986)

Cytochemical Studies on some Hydrolytic Enzymes in Gregarines. II. β-Glucuronidase Activity in Different Stages of the Gregarine *Stylocephalus conoides* Devdhar, 1962 (*Protozoa* : *Sporozoa*)

R. N. DESAI

Zoology Department, Karnatak Science College, Dharwar — 580 001, India

Received on 16 January 1985

Synopsis. The present article describes cytochemical localization of the hydrolytic enzyme β-glucuronidase in different stages of the life-cycle of the gregarine *Stylocephalus conoides*. In the light of our findings probable physiological roles of this enzyme in the parasite are suggested.

β-glucuronidase, one of the hydrolytic enzymes associated with hydrolysis of acid mucopolysaccharides, steroid metabolism, etc., has been studied cytochemically so far in only two protozoans, viz. *Paramecium caudatum* (Rosenbaum and Wittner 1962) and *Trichomonas vaginalis* (Sharma and Bourne 1964) and these studies are confined to the trophic stages only. The present study aims at detecting, by cytochemical methods, the presence/absence of this enzyme in different stages of the life-cycle of a gregarine *Stylocephalus conoides*, an intestinal parasite of the beetle *Opatrum* sp. abundantly available in and around Dharwar. On the basis of our findings the probable functional significance of this enzyme to the parasite has been discussed.

Materials and Methods

Trophozoites of *S. conoides* were obtained from the fore-gut of a few beetles while cysts were procured from the fecal matter of the beetles. The latter were rinsed briefly in 0.1% formaldehyde, thoroughly washed in distilled water and finally kept on moist paper in duly covered "embryo" cups for further development at room temperature, i.e., 28 - 30°C. Such cysts yield after a lapse of 24 - 30 h actively moving gametes easily visible through the cyst wall when examined under a com-

pound microscope. Subsequent to this zygotes, i.e., freshly fertilized oocytes, are produced. These bodies within 12 h of their formation get enveloped with a resistant coat and become spores. Finally sporozoites are formed within the spores. The entire process from the zygotes to the formation of sporozoites in *S. conoides* takes about four days.

To study the activity of β-glucuronidase in the gregarine life-cycle, smears of trophozoites from the freshly collected hosts and squash preparations of gametes, zygotes and sporozoites procured from cysts developed in the laboratory were used in the present work and post-coupling method of Seligman (see Pearse 1972) was followed. Acetone fixed above said preparations were incubated aerobically at 37°C for 3 h in a medium consisting of phosphate-citrate buffer (pH 4.95) 10 ml and a few drops of dimethyl formamide in which 2 mg of 6-bromo-2-naphthyl-B-D-glucupyrunoside was dissolved. After incubation the materials were washed in tap water briefly and treated with Fast blue B solution prepared in phosphate buffer (pH 7.5) at 4°C for 2 min and again were washed in cold distilled water at 4°C. After a brief rinsing in 0.1% acetic acid, the preparations were mounted in glycerol jelly. Samples incubated in substrate-free medium served as controls.

Observations

Trophozoites of *S. conoides* showed an intense enzyme activity as indicated by a uniform distribution of dark purple granules in the endoplasm (Pl. I 1). Control preparations showed only traces of the enzyme activity (Pl. I 2). Gametes and zygotes yielded negative results. Sporozoites showed a mild activity of the enzyme as indicated by one or two purple coloured granules in these bodies (Pl. I 3).

Discussion

Perusal of the literature reveals that so far β-glucuronidase activity has been cytochemically demonstrated in only two protozoans, viz., *Paramecium caudatum* (Rosenbaum and Wittner 1962) and *Trichomonas vaginalis* (Sharma and Bourne 1964). In both the cases the enzyme activity is uniform throughout the body of the organisms. In *S. conoides* a similar feature is observed too. Opinions regarding the localization of β-glucuronidase at ultrastructural level are at variance. Bimodal localization, i.e., lysosomal and endoplasmic reticular, has been proposed by a few workers (De Duve and Beanfay 1959, Greenbaum 1965, and Herveg et al. 1966 — all cited in Pearse 1972 and Oscar 1969). On the basis of parallelism between the neutral red stained granules and the cytochemically stained lysosomes in *P. caudatum* Rosenbaum and Wittner (1962) consider the localization

of this enzyme as lysosomal only. In *S. conoides* the activities of non-specific acid and alkaline phosphatase +ve granules (Desai 1985, in press) and the distribution of dark blue glucuronidase +ve granules show a similar parallelism suggesting the enzyme activity confined to lysosomes. However, our results based on the cytochemical techniques adopted, have their own limitations and do not permit us to further elaborate this controversial matter. From functional point of view, this enzyme has been associated with intracellular digestion in *P. caudatum* (Rosenbaum and Wittner 1962) and *T. vaginalis* (Sharma and Bourne 1964), with hydrolisis of acid mucopolysaccharides and steroidoglucuronic acid (Long 1968). It is also said to be involved in steroid metabolism, i.e., excretion (Pearse 1972). In *S. conoides* an intense activity of this enzyme in all trophic stages and its almost absence in reproductive stages, viz., gametes and zygotes, may indicate an active role of this enzyme in the organism's enabolic activities including intracellular digestion probably. Further, acid mucopolysaccharides have been demonstrated cytochemically in the gregarine *Stenoductus penneri* (Ramachandran 1976) and it may not be unreasonable to visualize their occurrence in other gregarines too. In such a case the enzyme β-glucuronidase may also be involved in the hydrolysis of these polysaccharides.

ACKNOWLEDGEMENTS

This work forms a part of Ph. D. Thesis accepted by Karnatak University, Dharwar and was supported by the University Grants Commission, New Delhi (India) under the Faculty Improvement Programme. The author is grateful to Dr. V. B. Nadkarni, Professor of Zoology, Karnatak University, Dharwar, for his guidance during the course of this work.

REFERENCES

Desai R. N. 1985: Cytochemical demonstration of some hydrolytic enzymes in some gregarines (*Protozoa*: *Sporozoa*). I. The non-specific acid and alkaline phosphatases. Acta Protozool. (in press).

Long C. 1968: Biochemist's Handbook. E. F. and N. Spon Ltd.

Oscar T. 1969: Aldonic and uronic acids. In: Comprehensive Biochemistry (eds. Florkin M. and Stotz E. H.), Elsevier Comp. Publ. Amsterdam, New York and London, Vol. 17.

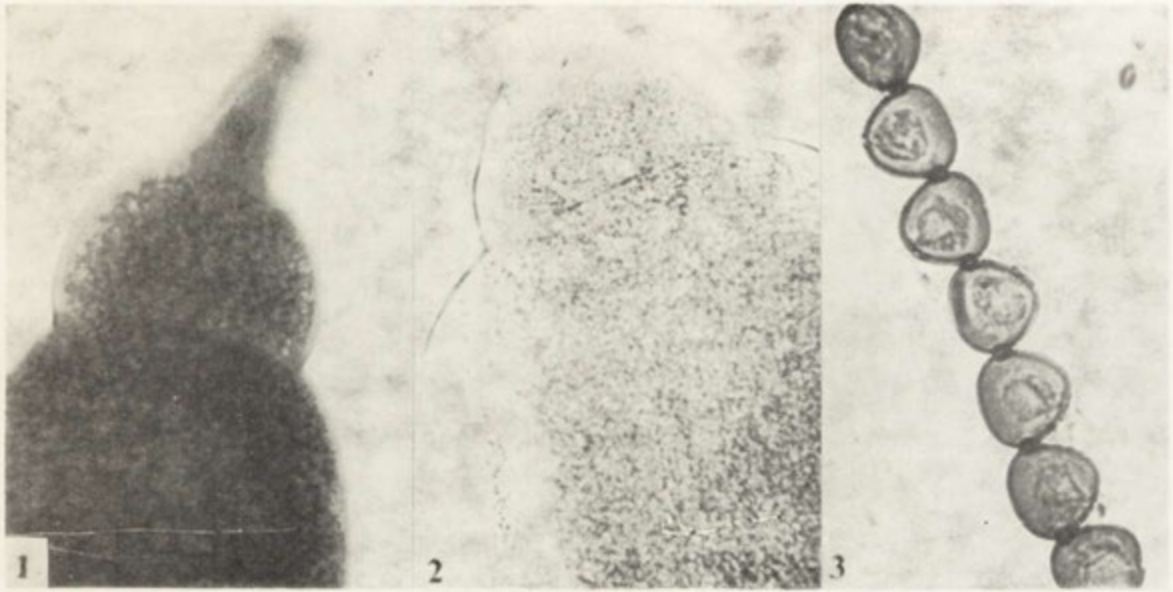
Pearse A. G. E. 1972: Histochemistry: Theoretical and Applied. 3rd ed. Little Brown and Co. Boston.

Ramachandran P. 1976: Cytochemical observations on *Stenoductus penneri* Ramachandran, 1975 (*Cephalina* : *Monoductidae*). Zool. Anz., 197 (3 - 4), 234 - 237.

Rosenbaum R. M. and Wittner M. 1962: The activity of intracytoplasmic

enzymes associated with feeding and digestion in *Paramecium caudatum*. The possible relationship to neutral red granules. Arch. Protistenk., 106, 223 - 240.

Sharma N. N. and Bourne G. H. 1964: Histochemical studies on the distribution of DPN and TPN diaphorases, β-glucuronidase and some enzymes associated with Kreb's cycle in *Trichomonas vaginalis*. Histochemie, 3, 487 - 494.



EXPLANATION OF PLATE I

β-glucuronidase activity in *Stylocephalus conoides*

1: Anterior portion of trophozoite × 200. Note an intense activity of the enzyme throughout the body

2: Anterior portion of trophozoite incubated in the substrate-free medium. A control preparation × 400. Note the traces of the enzyme activity

3: Sporozoites showing β-glucuronidase activity × 1000. Many sporozoites are visible through the spore capsules owing to an intense purple colour they have developed. In sporozoites outside the spore capsule dark purple granules can be seen

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 75 - 80 (1986)

Flagellates *Spironucleus mobilis* sp. n. in Eel, *Anguilla anguilla* (L.)

Jadwiga WIERZBICKA and Teresa EINSZPORN-ORECKA

Institute of Ichthyology, Faculty of Marine Fisheries and Sea Fisheries Institute, Szczecin Regional Branch, 4 Kazimierza Królewicza Str., 71-550 Szczecin, Poland

Received on 27 December 1984

Synopsis. A new species of flagellates, *Spironucleus mobilis* sp. n., is described. The protozoans were found in the intestine of eel, *Anguilla anguilla* (L.)

The genus *Spironucleus* Lavier, 1936 was described from amphibians and fishes. Einszporn-Orecka (1979) found the protozoans in eel, *Anguilla anguilla* (L.) with symptoms of *morbus anguillorum* and described the flagellates as *S. anguillae* sp. n. Hunninen and Wichterman (1938) encountered flagellates *Hexamina* sp. in the trematode *Deropristis inflata* parasitizing *Anguilla chryspa* [1]. The *Hexamita* sp. protozoans were also found in the intestine of *Ctenopharyngodon idella* by Chen-Chin-Leu (1956). Kulda and Lom (1964 b) transferred the flagellates reported in the two latter papers to the genus *Spironucleus*. Protozoans identified down to the genus level as *Spironucleus* were also reported by Molnar (1974) from a few fish species (*Hypophthalamichthys nobilis, Chondrostoma nasus, Barbus barbus, Cyprinus carpio, Pterophyllum scalare*). Detailed studies on *S. elegans* Lavier, 1936 and *Hexamita salmonis* Moore, 1923 can be found in Kulda and Lom (1964 a, b).

During comprehensive studies on eel in 1982 - 1983 parasites were encountered which differed from those known from the available literature. The differences observed prompted the authors to described the protozoans as a new species, *Spironucleus mobilis* sp. n.

---

[1] Berg (1949) considers *A. chrysypa* Raf. to be synonymous with *A. anguilla rostrata* (Le Sueur).

Materials and Methods

The fishes examined had been caught from various parts of the Szczecin Lagoon (Trzebież, Lubin, Stara Odra, Stepnica, Nowe Warpno, and Świnoujście). The studies were carried out in August 1982 and in July and August 1983. A total of 190 eels were examined, their total length and weight ranged within 34 - 84 cm and 70 - 1200 g, respectively.

Parasitologic examinations were made on fresh individuals, immediately after killing the fishes. Mounts were made of the material scraped off from the intestines, the mounts were then examined under the microscope. Whenever flagellates were found, smears were made on glass plates, dried, and May-Grünwald and Giemsa stained (Pappenheim's technique). Drawings and measurements were made from stained mounts. More than 20 specimens were measured. Apart from the intestine, the gall bladder content of live fish was examined. Additionally, for those eels which were found to contain intestinal flagellates, smears of the peripheral blood, liver, spleen, and kidney were made.

Results

Description of *Spironucleus mobilis* sp. n.

Morphology. Shapes of the protozoans in stained mounts differ. Fish slime was found to contain strongly elongated, narrow individuals of obliterated internal structure, somewhat stronger stained in their anterior part. Their width and length were 4.0 - 5.0 μm and up to 16.0 μm, respectively. The flagellates collected from places lacking slime as well as from the intestinal content were elongated, egg-shaped to oval or even circular (Fig. 1 and 2). The cell length and width ranged within 9.2 - 16.0 μm and 6.8 - 12.8 μm, respectively.

Two elongated nuclei are located in the anterior part of the cell and occupy about one-third of the cell's length. The nuclei anterior parts are often slightly narrowed — the nuclei are then tear-shaped (Fig. 1 and 2). Their length and width range within 3.6 - 4.4 μm and 1.8 - 2.4 μm, respectively.

Two oval, non-staining structures similar to vacuoles, frequently differing in size are situated between the nuclei, tightly attached to the nuclear membranes, or in their vicinity, depending on cell's position (Fig. 1 and 2). Their length and width range within 1.6 - 2.6 μm and 0.8 - 2.2 μm, respectively. These structures were invisible in numerous individuals, they might have been obscured by the nuclei or by slime. Each of these structures has two thin canaliculi, a shorter one directed forward and a much larger one running to the rear (Fig. 1 and 2). The posterior canaliculi run sometimes almost parallel, more often, how-

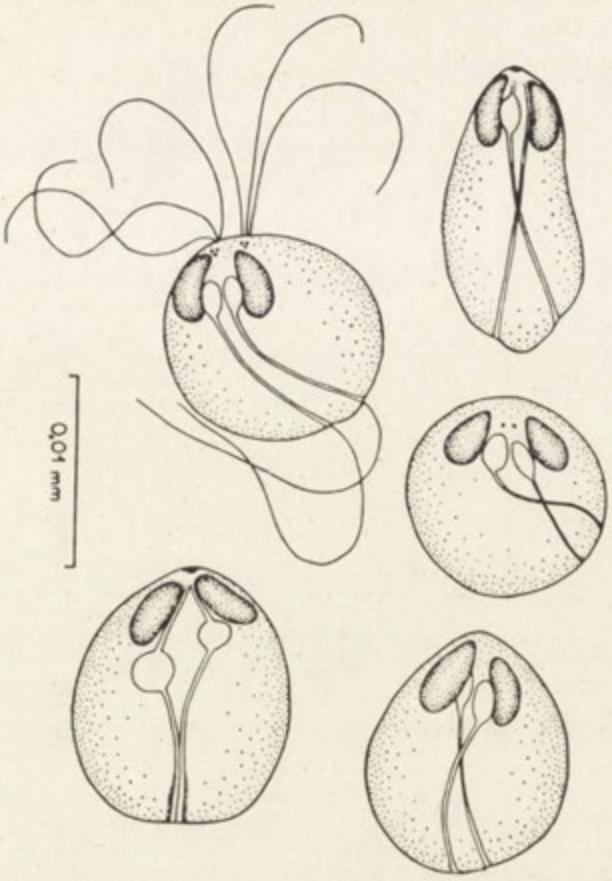


Fig. 1. *Spironucleus mobilis* sp. n.

ever, they cross at more or less half the cell's length. In the posterior part of the cell the canaliculi do not form any funnel-like broadening and there are no caudal apertures where the two posterior flagellae are let out. The posterior flagellae measure about 16 - 20 μm. The posterior flagellae basal bodies are located between the anterior ends of the nuclei.

Six anterior flagellae run from basal bodies situated close to the anterior margin of the nuclei (Fig. 1). The basal bodies are concentrated in two groups, three in each. The anterior flagellae length exceed somewhat that of the cell, the flagellae are, however, shorter than the posterior ones and measure about 15 - 17 μm.

Spironucleus mobilis sp. n. was found in the intestines of four eels, *Anguilla anguilla* (L.), out of 190 individuals examined. The infested fishes were caught in different areas of the Szczecin Lagoon. The in-

vasion intensity was not very high, single to numerous flagellates were observed. No flagellates were found in the gall bladder, peripheral blood, liver, spleen, and kidney.

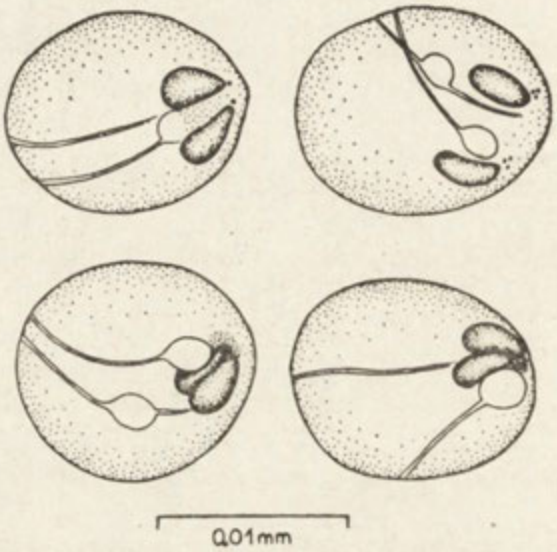


Fig. 2. *Spironucleus mobilis* sp. n.

Discussion

When the morphology of *Spironucleus mobilis* sp. n. found in eel, *A. anguilla* (L.) is compared with descriptions of other closely related protozoans, the highest similarity is assigned to the species *S. anguillae* Einszporn-Orecka, 1979, described from *A. anguilla*, too. The two species do show, however, certain differences.

The main character distinguishing *Spironucleus mobilis* sp. n. from *S. anguillae* is the shape and size of the nuclei. In *S. mobilis*, they are elongated, relatively wide, sometimes tear-shaped. They are 3.6 - 4.4 μm long, their length being about twice their width. On the other hand, *S. anguillae* has slim, sausage-shaped nuclei reaching 3.2 - 3.6 (4.0) μm in length. The drawings and photographs show the width: length ratio to be about 1 : 3 to 4 (Einszporn-Orecka 1979). Additionally, certain differences are noticed between the sizes of vacuole-like non-staining structures situated between the nuclei. In *S. mobilis* sp. n. they are somewhat smaller (1.6 - 2.6 μm long, 0.8 - 2.2 μm wide) than in *S. anguillae* (2.0 - 2.8 μm long, 1.6 - 1.8 μm wide).

The parasites' location in fish is different, too. *S. mobilis* sp. n. oc-

curred only in the intestine of *A. anguilla*. On the other hand, *S. anguillae* occurred in large numbers in the internal organs (liver, kidney, and spleen), in the peripheral blood, and in the necrotically changed (ulcerated) muscles of eels suffering of *morbus anguillarum* (Einszporn-Orecka 1979).

Common for the two *Spironucleus* species is the lack of any funnel-like broadening where the posterior flagellae are let out. Kulda and Lom (1964 b) also pointed to the lack of any caudal aperture in their redescription of *S. elegans* Lavier, 1936 occurring in *Pterophyllum scalare* and amphibians. *S. elegans* differs from the species discussed in the present paper by the shape of the cell and nuclei and by the absence of vacuole-like non-staining structures between the nuclei.

S. mobilis sp. n. is different from the flagellates observed in the trematode *Deropristis inflata* parasitizing the intestine of *Anguilla chrysspa*, the flagellates were found in eggs and uterus of the trematode and described by Hunninen and Wichterman (1938) as *Hexamita* sp. Kulda and Lom (1964 b) are of the opinion that the protozoans should be transferred to the genus *Spironucleus* Lavier, 1936. The cells contain strongly elongated, sausage-shaped nuclei with a bright outline of the membrane in the posterior part, there are no vacuole-like non-staining structures.

According to Kulda and Lom (1964 b), the intestine of some freshwater fish (*Salmo trutta m. fario*) is inhabitated by a parasitic species *Hexamita salmonis* Moore, 1923). Its morphology differs completely from the newly described species *Spironucleus mobilis*. The protozoans belonging to *H. salmonis* have oval nuclei with a large caryosome separated from the nuclear membrane by a bright zone. Additionally, the posterior end of the body shows funnel-like broadenings, i.e., caudal apertures, letting the posterior flagellae out. This is one of the major generic characters (Kulda and Lom 1964 b). Moreover, Šulman (1984) mentions another species of the genus, found also in fish, *H. truttae* Schmidt, 1920 which is morphologically close to *H. salmonis*.

The flagellates of this groups, occurring in fish, are rather poorly known. Further studies will perhaps render some morphological observations more precise.

REFERENCES

Berg L. S. 1949: Ryby presnyh vod SSSR i sopredelnyh stran. 3, Izd. AN SSSR, Moskva - Leningrad, 929 - 1382.

Chen Chin-Leu 1956: The protozoan parasites from four species of Chinese pond fishes: *Ctenopharyngodon idellus, Mylopharyngodon piceus, Aristichthys nobilis* and *Hypophthalamichthys molithrix*. I. The protozoan para-

sites of *Ctenopharyngodon idellus*. Acta Hydrobiol. Sin., 19 - 42.
Einszporn-Orecka T. 1979: Flagellates *Spironucleus anguillae* sp. n. parasites of eel (*Anguilla anguilla L.*). Acta Protozool., 18, 237 - 241.
Hunninen A. V. and Wichterman R. 1938: Hyperparasitism: A species of *Hexamita* (*Protozoa, Mastigophora*) found in the reproductive systems of *Deropristis inflata* (*Trematoda*) from marine eels. J. Parasitol., 24, 95 - 101.
Kulda J. and Lom J. 1964 a: *Spironucleus elegans* Lavier, parasite of fish. Česk. Parasitol., 11, 187 - 192.
Kulda J. and Lom J. 1964 b: Remarks on the diplomastigine flagellates from the intestine of fishes. Parasitology, 54, 753 - 762.
Molnár K. 1974: Data on the "octomitosis" (spironucleosis) of cyprinids and aquary fishes. Acta Vet. Acad. Sci. Hung., 24, 99 - 106.
Šulman S. S. (ed.) 1984: Opredelitel parazitov presnovodnyh ryb fauny SSSR. I. Parazitičeske prostejše. Izd. AN SSSR, Leningrad.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 81 - 91 (1986)

Some New and Rare Testate Amoebae from the Arctic

Louis BEYENS[1] and Didier CHARDEZ[2]
with the collaboration of Paul De BOCK[1]

[1]Dienst Plantkunde (Prof. Dr. R. Vanhoorne), R.U.C.A., Groenenborgerlaan 171-B-2020 Antwerpen, Belgium, [2]Laboratoire de Zoologie Générale et Faunistique (Prof. J. Leclercq), Faculté des Sciences Agronomiques dé l'Etat, Gembloux, Belgique

Received on 16 January 1985

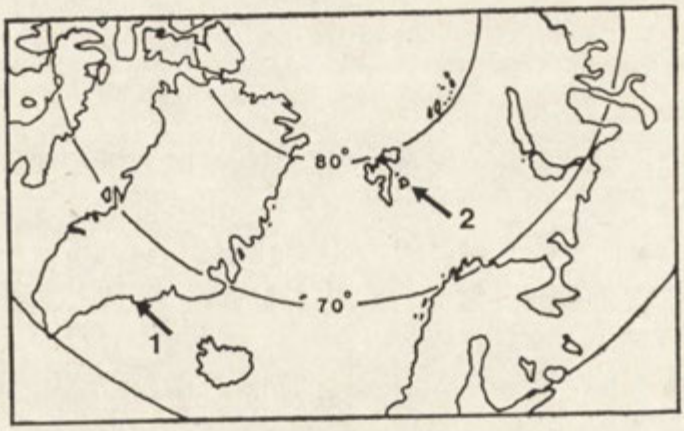
Synopsis. Four new species from the Arctic Regions are described and some ecological data are given: *Cyclopyxis lecercqi* sp. nov. and *Difflugiella vanhoornei* sp. nov. from S. E. Greenland, *Centropyxis pontigulasiformis* sp. nov. and *Cyphoderia perlucidus* sp. nov. from Edgeøya (Svalbard). Some rare species are also discussed.

For the past few years the authors have been running a project on the distribution, ecology and population structures of testate amoebae in the Arctic Regions. So far, fieldwork has been accomplished in S. E. Greenland (1978), Jan Mayen (1983), N. W. Spitsbergen (1983) and Edgeøya (Svalbard 1984). This paper presents four new species, and also mentions some rare and remarkable rhizopods. Details of climate, geology, geomorphology and the population structures will be given in further papers.

The New Species from S. E. Greenland

The study area is situated ±75 km north of the main settlement of Angmagssalik (map 1 and 2), at the head of the Tasîlaqfjord (Pl. I 1). This region has a low-arctic, oceanic climate. Topographically it is a wild mountainous area, deeply dissected by numerous fjords.

Authors names in alphabetical order.



Map 1. The positions of the Angmagssalik region in East-Greenland (1) and Edgeoya in Svalbard (2)

Map 2. The position of the Tasilaq-fjord area in the Angmagssalik region. Point 1 marks the site of *Centropyxis lecercqi* sp. nov. on the river delta, point 2 the locality of *Difflugiella vanhoornei* sp. nov.

Cyclopyxis leclercqi sp. nov. (Pl. II 6)

Description. The shell is circular in apertural view, dorsi-ventrally compressed with a swollen rim. It is composed of small polymorphous mineral particles. The aperture is slightly invaginated, circular and central, with three of four internal extensions reaching the dorsal part of the shell.

Measurements (in μm):

diameter of shell	diameter of aperture	depth of shell
116 - 118	55 - 58	33 - 38

n = 5

The Sampling Site: Description and Ecological Data

The habitat is situated in the river delta at the head of the fjord (Pl. I 2 and 3). It is a pool (11 m long and max. 4 m wide) partly overgrown by subaquatic mosses. The surrounding vegetation consists mainly of *Salix glauca* ssp. *callicarpaea* (Trauty) Böch., *Equisetum variegatum* Schleich., *Polygonum viviparum* L., *Salix herbacea* L. and *Bartsia alpina* L. Sampling occurred on 20.07.1978.

The temperature of the water, in bright weather, was 16°C at the surface, and 10°C on the bottom at 30 cm, with a pH of 6.8 to 7, and 12 mg dissolved oxygen.

The testate amoebae population consisted of only five species with *Cyclopyxis leclercqi* sp. nov. comprising 53% of the individuals. *Euglypha tuberculata* Dujardin (26.5%), *Centropyxis aerophila sphagnicola* Deflandre (8.8%), *Difflugia linearis* (Penard) Gauthier-Lièvre et Thomas (5.8%) and *Centropyxis aerophila* Deflandre (2%) were also observed. The diversity of this population is low, with a value of 1.2 according to the Shannon-Weiner formula, while maximum diversity (H max) equals 1.6.

Difflugiella vanhoornei sp. nov. (Pl. II 7)

Description. The shell is ovoid, with a circular cross-section. It is entirely chitinoid, with a yellowish hyaline tint. The aperture is circular, obliquely truncated, and the border shows a slight swelling.

Measurements (in μm):

diameter of shell	diameter of aperture	depth of shell
13 - 15.5	3.8 - 4.5	8 - 8.5

n = 5

The Sampling Site: Description and Ecological Data

On the east side of the fjord, small brooks intersect the heavily hummocked tundra (Pl. II 3). These brook vary between 30 to 70 cm in width, with the water level more than 40 cm under the soil surface.

The moss sample (*Sphagnum girgensohnii* Russ.) was taken from the steep bank of such a brook, approximately 10 m a.s.l. The surrounding vegetation is luxurious, reaching a height of almost 40 cm. The following plants were noted: *Salix glauca* ssp. *callicarpaea* (Trautv.) Böch., *Polygonum viviparum* L., *Carex bigelowii* Torr., *Salix herbacea* L., *Taraxacum* sp., *Poa alpina* L., *Bartsia alpina* L., *Veronica alpina* L. *Sibbaldia procumbens* L. *Sphagnum girgensohnii* Russ. prefers moist substrata (Andrus 1974).

The testate amoebae fauna from this sample is fairly rich with fourtheen species in all. The composition is as follows: *Centropyxis aerophila* Deflandre (22%), *Euglypha rotunda* Wailes (17%), *E. laevis* Perty (15%), *Difflugiella vanhoornei* sp. nov. (12%). *Trinema lineare* Penard (10%), *Euglypha strigosa glabra* Wailes (7%), *E. tuberculata* Dujardin (4%), *Trinema lineare truncatum* Chardez (4%), *Phryganella acropodia* (Hert. et Less.) Hopkinson (2%), *Nebela penardiana Deflandre* (2%), *N. tincta* (Leidy) Awerintzew (2%), *Trinema enchelys* (Ehrenberg) Leidy (2%), *Assulina muscorum* Greeff (2%), *Cyclopyxis ambigua* Bonnet et Thomas (2%). A moderate to high diversity is apparent: 2.3; H max. 2.6.

The New Species from Edgeøya

Edgeøya is the main southeastern island of the Svalbard archipelago (map 1 and 3). It has a high-arctic and more continental climate. Topographically, the island has mountains of the plateau-type, divided by broad valleys.

Centropyxis pontigulasiformis sp. nov. (Pl. III 1 - 5)

Description. The shell is formed of two distinct parts: the shell body, and a rather large semi-circular visor. The body is perfectly spherical and is perforated by a small circular aperture. The shell wall is composed of an organic matrix, incorporating fine plate-like polymorphous mineral particles. The visor which expands fan-like above and around the aperture, is mostly embossed with more voluminous quartz particles, in particular at the rim. Seen from an apertural view, this visor is as large as the shell body.

Measurements (in μm):	
diameter of the shell	diameter of the aperture
58–62	8–10
length of the visor	total length
24–28	82–90
$n = 5$	

Map 3. The present known distribution of the discussed species on Edgeøya. One should note that the best studied site is Rosenbergdalen/Kapp Lee, where most of the samples have been taken

■ *Centropyxis pontigulasiformis* sp. n. in pools

□ *Centropyxis pontigulasiformis* sp. n. in terrestric mosses

⊛ *Cyphoderia perlucidus* sp. n.

▲ *Nebela nobilis* (Cash.) Deflandre in pools

△ *Nebela nobilis* (Cash.) Deflandre in terrestric mosses

⊕ *Paraquadrula madarica* Valkanov

◎ *Centropyxis acuminata* Coûteaux et Chardez

The Sampling Site: Description and Ecological Data

Centropyxis pontigulasiformis sp. nov. has been found near the east coast, the north-east coast and the north-west coast of Edgeøya,

in very wet to wet terrestrial mosses and in mosses living in pools (map 3).

The Pools. The pools are situated in the moss-tundra; one of them (Site 45) being somewhat anomalous since it was a pool in a cracked rock, lying in the tundra.

No. site and sample locality	42, W71 Semenovfjella (Pl. I 5)	44, W76 Semenovfjella (Pl. I 6)	45, W78 Rosenbergdalen (Pl. II 1)
date of sampling	16.08.84; 11 h 00	16.08.84; 12 h 00	22.08.84; 14 h 30
height a.s.l.	100 m	100 m	± 20 m
appr. size depth	15 m×4 m 12 cm	3.5 m×2 m 11 cm	2 m×0.2 m 19 cm
aquatic vegetation	*Calliergon stramineum* (Brid.) Kindb. a moss species	*Calliergon stramineum* and *Ranunculus hyperboreus* Rottb.	*Calliergon giganteum* (Schimp.) Kindb. and *macroscopic Chlorophyta*
temperature (in °C)	7.5	8	3
pH	8.2	8.3	7.2
conductivity in (μm ho·cm^{-1})	43	39	110

The following species have been observed in these pools (given in percentages):

	W 71	W 76	W 78
Centropyxis sylvatica (Deflandre) Thomas	23.8	19.4	—
Centropyxis aerophila Deflandre	—	—	33.3
Centropyxis pontigulasiformis sp. nov.	19	13.9	26.2
Centropyxis gibba gibbosa (Rampi) Godeanu	—	19.4	—
Difflugia globulus (Ehrenberg) Hopkinson	—	30.5	—
Difflugia globularis (Wallich) Leidy	—	—	23.8
Difflugia penardi (Penard) Hopkinson	—	—	9.5
Trinema lineare Penard	57.1	16.6	7.1
Number of species	3	5	5
Diversity index (H)	0.96	1.6	1.5
Maximum diversity (H max)	1.1	1.6	1.6

The similarity index (according to the formula of Spatz), suggests a separation between the eastern populations and the one from the western part of the island:

Samples	71–76	71–78	76–78
IS_s (ranging from 0 to 100)	27.5	7.7	4

The Aerophitic Terrestric Mosses. The moss samples originate from the moss-tundra on the east coast and the northwest coast.

No. site and sample locality	36, M 54 Blådfjordflya (Pl. I 4)	49, M 59 Rosenbergdalen (Pl. II 2)	51, M 75 Rosenbergdalen (Pl. II 5)
date of sampling	14.08.84, 14 h 05	23.08.84, 14 h 30	24.08.84 12 h 30
height a.s.l.	no data	15 m	10 m
moss species	*Drephanocladus revolvens* (sw.) Warnst.	*Bryum cryophilum* Mårt.	*Calliergon giganteum* (Shimp.) Kindb.
moisture content-class (according to Jung 1936)	very wet F : III	wet F : IV	very wet F : III
temperature (in °C)			
+1 m above soil level	7	5.5	2
soil level	8	11	4
relative humidity			
at + 1 m	72%	69%	72%
at soil level	72%	78%	74%

The following populations were found living in these mosses (expressed as percentages):

	M 54	M 59	M 75
Centropyxis sylvatica (Deflandre) Thomas	–	20.8	–
Centropyxis aerophila Deflandre	34.8	17	26
Centropyxis pontigulasiformis sp. nov.	26	11.3	11.1
Centropyxis plagiostoma Bonnet et Thomas	–	7.5	–
Difflugia dujardini Chardez	–	–	11.1
Difflugia globularis (Wallich) Leidy	–	–	14.8
Difflugia minuta Rampi	27.5	–	–
Difflugia penardi (Penard) Hopkinson	–	5.7	14.8
Nebela wailesi Deflandre	–	15	–
Trinema lineare Penard	11.6	22.6	22.2
Number of species	4	7	6
Diversity index (H)	1.3	1.8	1.7
Maximum diversity (H max)	1.4	2	1.8

In this case the similarity index also seems to indicate a difference between the population on the east and those from the west coast.

Cyphoderia perlucidus sp. nov. (Pl. III 8 - 10)

Description. The shell is, as characteristic for the genus, retort-shaped and circular in transverse section. The bent collar at the apertural end makes an angle of 30 - 40° with the longitudinal axis. The aperture is circular, the aboral region rounded. The shell is composed of numerous very small circular shell plates, the whole being transparent and fragile.

Measurements (in μm):		
length of shell	diameter of shell	diameter of aperture
50–58	25–27	10–11
n = 5		

The Sampling Site: Description and Ecological Data

Cyphoderia perlucidus sp. nov. was present in three moss samples from the moss-tundra in Rosenbergdalen.

No. site and sample	51, M 69	51, M 76	51, M 77
locality	Rosenbergdalen	Rosenbergdalen (Pl. II 5)	Rosenbergdalen (Pl. II 4)
date of sampling	24.08.84, 12 h 30	24.08.84, 12 h 30	24.08.84, 12 h 30
height a.s.l.	10 m	10 m	10 m
moss species	*Distichium capillaceum* (Hedw.) B.S. et G	*Homalothecium nitens* (Hedw.) Robins	*Calliergon giganteum* (Schimp.) Kindb.
moisture content	fairly wet	fairly wet	wet
class (cfr. Jung 1936)	F : V	F : V	F : IV
temperature (in °C)			
+1 m above soil level	2	2	2
soil level	4	4	4
relative humidity			
+1 m	72%	74%	74%
soil level	74%	74%	74%

The following testate amoebae populations were found in these samples (expressed as percentages):

	M 69	M 76	M 77
Centropyxis aerophila Deflandre	7.7	25	25
Plagiopyxis callida Penard	2.1	–	2
Plagiopyxis labiata Penard	–	–	1.4
Difflugia bryophila (Penard) Jung	–	–	11.6
Difflugia dujardini Chardez	–	–	2.7
Difflugia lucida Penard	–	10.4	1.4
Difflugia lata Jung	–	–	2.7

Difflugia paulii Ogden	—	—	2.0
Nebela dentistoma oblonga Gauth.-Lievre et Thomas	—	—	7.5
Nebela penardiana Deflandre	2.8	6.2	13
Nebela tubulosa Penard	—	—	15.8
Nebela tincta (Leidy) Awerintzew	—	6.2	—
Paraquadrula globulosa (Penard) Ferlan	33.8	—	—
Paraquadrula irregularis Archer	5	—	—
Paraquadrula madarica Valkanov	12.6	—	—
Euglypha rotunda Wailes	7	—	—
Trinema lineare Penard	12.6	43.7	20.5
Trinema penardi Thomas et Chardez	2.1	—	—
Cyphoderia perlucidus sp. nov.	14.1	8.3	4.1
Number of species	10	6	13
Diversity index (H)	2	1.5	2.2
Maximum diversity (H max)	2.3	1.8	2.6

The similarity between these populations is low:

	69–76	69–77	76–77
IS_s	8.2	6.8	9.4

Some Rare Species Encountered in Svalbard

Nebela nobilis (Cash) Deflandre (Pl. III 7) syn.: *Hyalosphenia nobilis* Cash, 1908. In his monograph of 1936, Deflandre put this species in the group of "Species inquirendae vel incertae".

The shell is pyriform with a circular cross-section. The elongated neck is cylindrical, the aperture truncated at right angles. The shell is transparent, with small reworked scales, inlaid in an organic matrix. These shell scales of different sizes are not contiguous.

Measurements (in μm):

length of shell	length of neck
200–216	60–66
diameter of shell	diameter of aperture
90–106	38–43

$n = 5$

Geographical distribution: This species is known from Great Britain. Plaifair (1917) cited a variety compressa from Australia. We observed this species in three samples from Svalbard: one from Bockfjorden in N. W. Spitsbergen (79°27′N, 13°16′E), the other two being collected on Edgeøya. Some information on these samples is listed below:

locality:	Bockfjoren (N.W. Spitsbergen)	Rosenbergdalen (Edgeøya)	Rosenbergdalen (Edgeøya)
habitat	pool	pool	moss: *Aulacomnium turgi-*

			dum (Weihlenb.) Schwaegr.
date of sampling	27.06.1983	22.08.1984	23.08.1984
height a.s.l.	40 m	~ 10 m	15 m
dimensions	80 cm × 40 cm	20 cm × 10 cm	
depth	max. 10 cm	3 cm	
temperature	6° C	3.5° C	
pH	7.8	7	
conductivity in μm ho·cm^{-1}	80	120	
moisture content			almost dry (F : VII)
number of species	14	10	18
dominant	*Difflugia pulex* Penard (19.8%)	*Difflugia bryophila* (Penard) Jung (25.9%)	*Centropyxis aerophila* Deflandre (16.9%)
relative frequency of *N. nobilis*	5.8%	3.5%	2%
diversity of the population	2.3	2	2.5
maximum diversity	2.6	2.3	2.8
similarity	—	non existent	—

Centropyxis acuminata Coûteaux et Chardez, 1981 (Pl. III 6)

This species which has been described from a forest soil humus of French Guyane, must be considered as an intermediate form between *C. aerophila* and *C. cassis*. It is distinguished by the shape of the aboral region of the shell which is always pointed by an accumulation of exogenic particles. Length: 70 μm to 100 μm

We have found this species in soil (pH : 5) under a reindeer antler, and in the soil (pH : 5) beneath a moss species and an unidentified mushroom (*Basidiomycetes*).

Paraquadrula madarica Valkanov, 1962

Amongst the numerous species of *Paraquadrula* we noticed the presence of *P. madarica* in some moss samples (*Distichium capillaceum* (Hedw.) B. S. et G., *Homalothecium nitens* (Hedw.) Robins) from Rosenbergdalen (Edgeøya). Typical for this species is the obliquely truncated aperture, but in contrast to the descriptions of Valkanov, the aperture is only slightly ovoid, and mostly even perfectly circular.

ACKNOWLEDGEMENTS

The authors wish to thank Drs. P. Oosterveld for his hospitality at Kapp Lee scientific station, Drs. K. De Korte, the captain (A. Veldkamp) and the crew of the expedition vessel "Plancius" for their willing co-operation. Mrs. E. Van Cauteren is gratefully acknowledged for her enthusiastic help in the field. Prof. Dr. D. K. Ferguson corrected the English version of the text, while Miss S. Pooters typed the manuscript. Miss A. Torfs, Mr. F. Neefs and Mr. R. Neefs provided some technical assistance. One of us (L.B.) received grants from the National Science Foundation (Belgium) for these expeditions.

REFERENCES

Andrus R. E. 1974: The Sphagna of New-York State. Ph. D. dissertation, Sunny-Syracuse. (University Microfilms nr. 74-24,003. Ann. Arbor. Michigan).

Deflandre G. 1936: Etudes' monographique sur le genre *Nebela* Leidy. Ann. Protistol., 5, 201 - 286.

Playfair G. I. 1917 - 1918: Rhizopods of Sydney and Lismore. Proc. Linn. Soc. N.S.W., Vol. XLII, 635 - 675.

EXPLANATION OF PLATES I - III

Plate I

1: Aerial view of the Taxîlaq-fjord, seen from the North
2: The non-tidal part of the river delta, in the Tasîlaq-fjord. A pool in this plain represents the site of *Cyclopyxis leclercqi* sp. nov.
3: View of the delta with conspicuous *Eriophorum*-vegetation
4: Locality 36 on Blåfjordflya. A sample of the moss *Drepanocladus revolvens* (Sw.) Warnst (see arrow) contained *Centropyxis pontigulasiformis* sp. nov.
5: Locality 43 on Semenovfjella. Subaquatic *Calliergon stramineum* (Brid.) Kindb. mosses form the habitat of *Centropyxis pontigulasiformis* sp. nov.
6: Locality 42 on Semenovfjella (see arrow), *Centropyxis pontigulasiformis* sp. nov. was found in a sample of the subaquatic moss *Calliergon giganteum* (Schimp.) Kindb.
7: Moss-tundra near Rosenbergdalen, looking towards Kapp Lee. The arrow marks site 49, where *Centropyxis pontigulasiformis* sp. nov. was found
8: View from the north of the moss-tundra near Rosenbergdalen. The arrow indicates site 51, where *Centropyxis pontigulasiformis* sp. nov. and *Cyphoderia perlucidus* sp. nov. were sampled

Plate II

1: The pool with *Centropyxis pontigulasiformis* sp. nov. is situated in the crack of the rock (site 45)
2: *Bryum cryophilum* Mårt., another habitat of *Centropyxis pontigulasiformis* sp. nov.
3: The hummocked low-arctic tundra beside the Tasîlaq-fjord. *Difflugiella vanhoornei* sp. nov. was found here in *Sphagnum* samples
4: Moss-tundra (site 51) near Rosenbergdalen. The sample of *Calliergon giganteum* (Schimp.) Kindb. (arrow) contained *Cyphoderia perlucidus* sp. nov.
5: Moss-tundra (site 51) near Rosenbergdalen, the locality of *Centropyxis pontigulasiformis* sp. nov. (A) and *Cyphoderia perlucidus*
6: *Cyclopyxis leclercqi* sp. nov. Apertural view
7: *Difflugiella vanhoornei* sp. nov. A — lateral view, B — apertural view

Plate III

1 - 5: *Centropyxis pontigulasiformis* sp. nov. Apertural views
2: *Centropyxis pontigulasiformis* sp. nov. Lateral view
5: Shows the aperture (Ap) without the visor
6: *Centropyxis acuminata* Coûteaux et Chardez. Apertural view
7: *Nebela nobilis* (Cash) Deflandre. Lateral view
8 - 10: *Cyphoderia perlucidus* sp. nov. Lateral views
11: *Paraquadrula irregularis* Archer. Lateral view

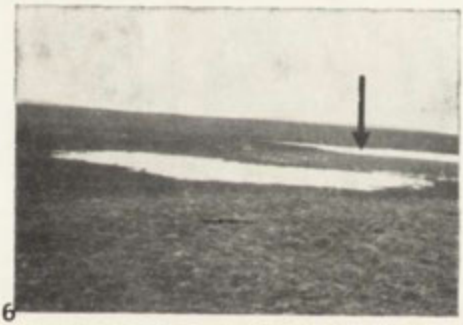
1

2

3

4

5



6

7

8

L. Beyens et D. Chardez

auctores phot.

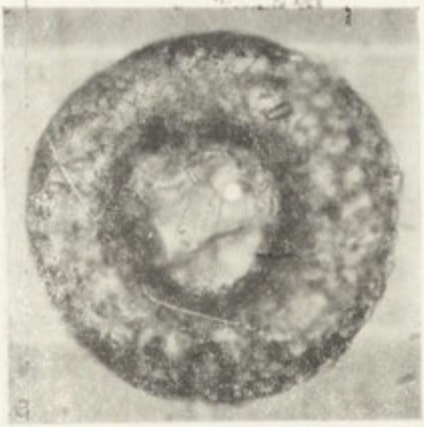
1

2

3

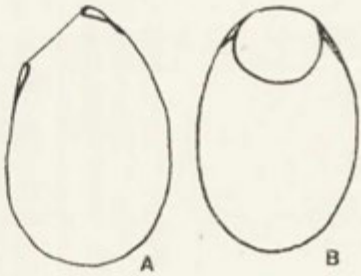
4

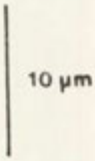
5



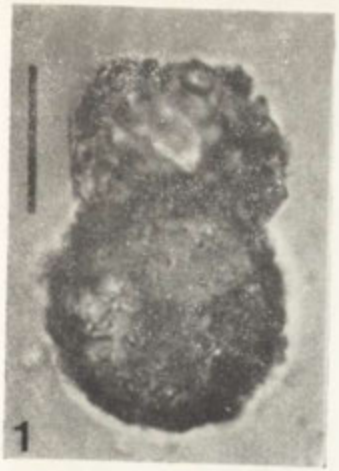
60 µm

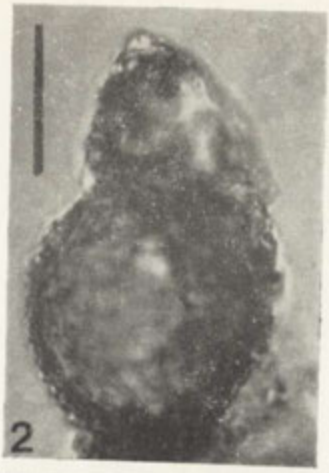
7

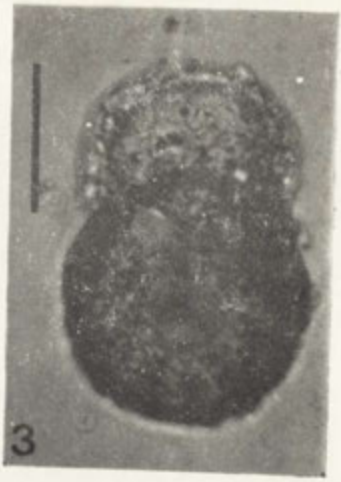


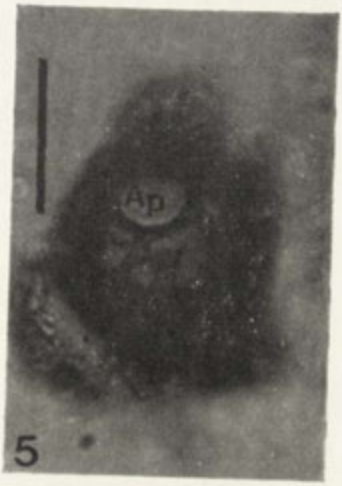


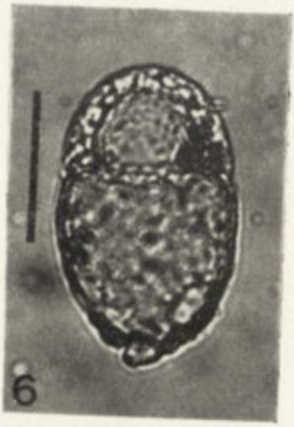
L. Beyens et D. Chardez auctores phot.

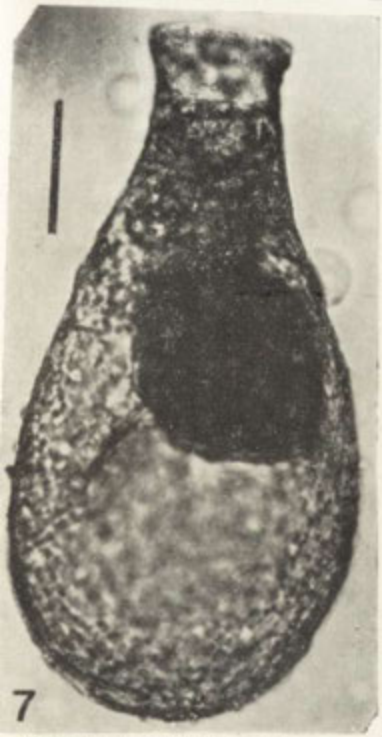


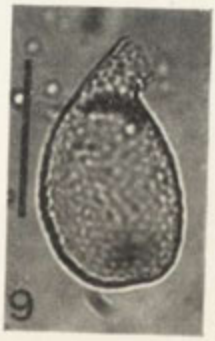


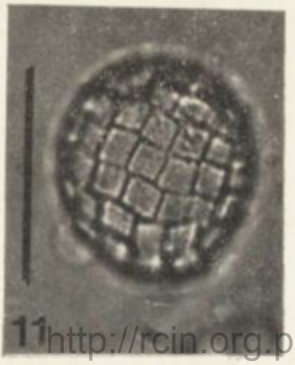












Scales: 40 μm

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 93 - 108 (1986)

Two New Septate Gregarines (*Apicomplexa : Sporozoea*), *Gregarina basiconstrictonea* sp. n. and *Hirmocystis oxeata* sp. n. from *Tribolium castaneum* (Herbst)

S. GHOSE, T. SENGUPTA and D. P. HALDAR

Protozoology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India

Received on 10 January 1985, *revised on* 3 June 1985

Synopsis. This paper deals with the morphology, life history, cytochemistry and seasonal intensity of two new species of septate gregarines (*Apicomplexa : Sporozoea*) in the gut of the coleopteran pest, *Tribolium castaneum* collected from nuts. These are *G. basiconstrictonea* sp. n., the ratios of which are LP : TL = 1 : 2.6 - 5.1 (3.85); WP : WD = 1 : 1.0 - 1.75 (1.25) and *H. oxeata* sp. n., the ratios of which are LP : TL = 1 : 3.3 - 5.5 (4.2); WP : WD = 1 : 0.8 - 1.75 (1.06).

The common flour beetle *Tribolium castaneum* (Herbst) is known to harbour a number of protozoan parasites (Sokoloff 1972, Haldar et al. 1984). Some of these protozoans produce pathological symptoms upon the host and as such their importance as the microbial agents for controlling insect pests is immense. Besides flour, *Tribolium castaneum* also infests a number of other stored grains and nuts. The present communication records two new species of septate gregarines (*Apicomplexa : Sporozoea*) in the midgut of this beetle infesting groundnuts and walnuts.

Gregarina basiconstrictonea sp. n. has been obtained in the beetle from groundnuts while *Hirmocystis oxeata* sp. n. is reported from the beetle infesting walnuts. Descriptions of the detailed life history, localization of some important cytochemical substances and seasonal intensity of infection have been incorporated in the paper.

Material and Methods

The host insects *Tribolium castaneum* (Herbst) were collected from groundnuts and walnuts from a grocer's shop at Chinsurah, West Bengal and brought alive to the laboratory for investigation. The insects were decapitated, their guts carefully

dissected out under a dissecting binocular and gently pressed for the parasites to come out from the gut lumen. Thin smear preparations were fixed in Schaudinn's fluid and subsequently stained with Heidenhain's haematoxylin. Portions of midgut of infected hosts were fixed in Bouin's fluid and 5 μm thick sections were stained as above for studying any intracellular stages of development of the parasites. Cysts were collected from the hind gut of the infected hosts and cultured in moist chamber for sporulation (Sprague 1941). Standard cytochemical techniques have been followed for detecting the patterns of localization and the degrees of concentration of various substances like general and mucopolysaccharides, general protein, simple lipid, alkaline phosphatase and nucleic acids (Pearse 1976). For studying the seasonal intensity the hosts were collected and examined throughout the year, the percentage of infection were noted and plotted in histograms. The following abbreviations have been used:

TL — Total length: LE — Length of epimerite; LP — Length of protomerite; LD — Length of deutomerite; LN — Length of nucleus; WE — Width of epimerite; WP — Width of protomerite; WD — Width of deutomerite; WN — Width of nucleus.

The ratios used in this paper are those of length of protomerite to total length (LP : TL) and the width of protomerite to width of deutomerite (WP : WD). The holotype and the paratype materials are deposited at the Department of Zoology, University of Kalyani.

Observations

Life History

Gregarina basiconstrictonea sp. n.

Host: *Tribolium castaneum* from groundnuts.

Incidence: 123 out of 332 hosts examined are infected with this gregarine.

Development: Early development of this gregarine takes place within the epithelial cells of the midgut of the insect. The earliest stage has an ovoidal body with a spherical nucleus. The infected cell shows a clear area around the parasite. Subsequently the parasite acquires a protomerite and a deutomerite and leaves the infected epithelial cell.

Trophozoite: The trophozoite is an elongated organism appearing opaque white in colour when viewed freshly. It measures 45.9 to 48.6 μm in total length. The body contains the usual three segments: the epimerite, the protomerite and the deutomerite. The epimerite is small, papilla-like (Fig. 1) and measures 3.2 μm to 18.9 μm in length and 3.2 μm to 16.2 μm in width. Occasionally, the epimerite expands greatly giving it a bulbous appearance (Fig. 2). The protomerite is tongue-shaped measuring 8.1 μm to 13.5 μm × 8.1 μm to 17.6 μm while the deutomerite is the largest segment of the body and is cylindrical to obese in shape with a rounded posterior extremity. The cytoplasm is

uniformly granulated throughout the protomerite and deutomerite. The nucleus is variable in shape and measures 4.1 μm to 13.5 μm × 5.4 μm to 9.5 μm in dimension. The pellicle is thick and the epicyteal striations cannot be observed.

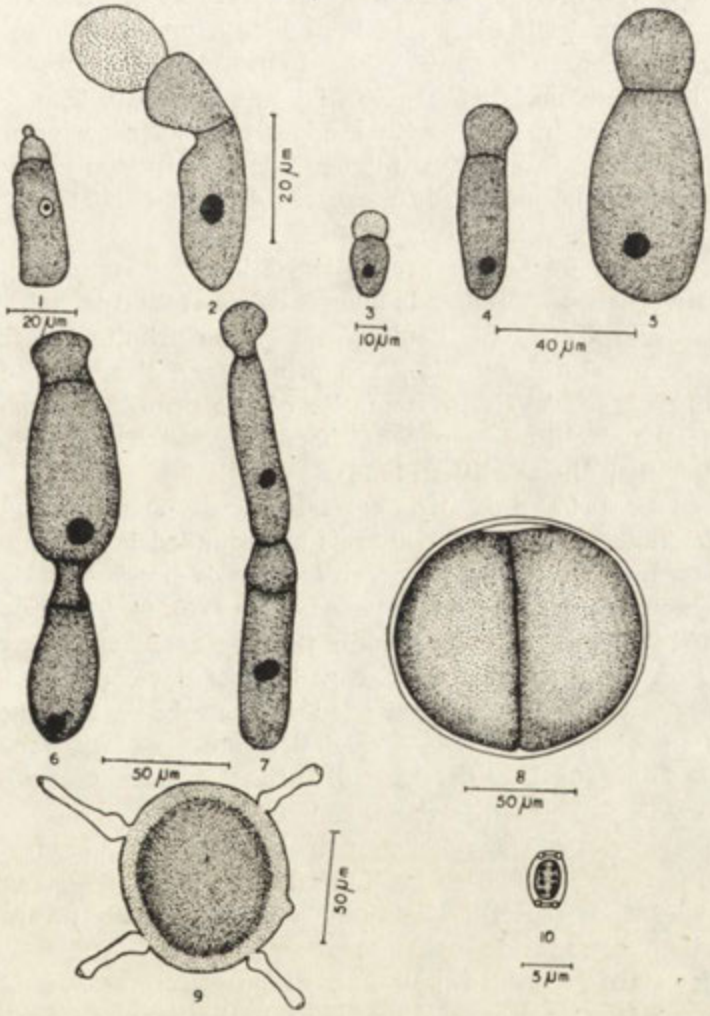


Fig. 1 - 10 — Camera lucida drawings of *Gregarina basiconstrictonea* sp. n. 1 - 2 — Trophozoite from stained smear, 3 — Young sporadin, 4 - 5 — Mature sporadins in different shapes and sizes, 6 - 7 — Sporadins in syzygy, 8 — A gametocyst with two equal gametocytes, 9 — Gametocyst with four sporoducts, 10 — Barrel-shaped spore with eight sporozoites and two knob-like projections at each pole

Sporadin and association: The young solitary sporadins (Fig. 3, 4) are oval in shape and measure 21.6 μm in length and 10.8 μm in width. The protomerite is spherical measuring 8.1 μm × 9.5 μm while the deutomerite is obese, its width being maximum at the middle. A deeply stained elliptical nucleus is present at the posterior portion of the body. The mature sporadins (Fig. 5) are cylindrical in shape and measure 35.1 μm to 166.1 μm in total length and 13.5 μm to 43.2 μm in width. The protomerite is dome-shaped and measures 8.1 μm to 32.4 μm in length and 10.3 μm to 31.1 μm in width. The deutomerite is long cylindrical in shape with a slightly broader posterior end. The nucleus is rounded to elliptical in shape and is situated in the posterior portion of the deutomerite. It measures 4.1 μm to 13.5 μm in length and 5.4 μm to 13.5 μm in width.

The sporadins in syzygy are obtained in large numbers. The association between two sporadins is always caudo-frontal in nature (Figs. 6, 7). The posterior tip of the deutomerite of the primite fits firmly with the protomerite of the satellite. The primite and the satellite are morphologically different. The protomerite of the primite is expanded fan-like, while the satellite is somewhat barrel-shaped. The primite is sometimes larger than the satellite (Fig. 6).

Gametocyst and spore: Gametocysts are collected from the hindgut of infected hosts. Gametocysts are rounded in shape and enclose two equal gametocytes. A sharp constriction is present at the junction of two gametocytes in freshly collected cysts (Fig. 8). The cysts measure 100.8 μm × 98.6 μm in dimension in the average. Four sporoducts are developed on the cyst surface at about 30 h of development. The sporoducts are characteristically constricted at their bases and then become broader immediately after the constriction and gradually taper towards the free end (Fig. 9). Forty eight hours after the formation of the sporoducts, spores are liberated in short chains.

The double-walled, barrel-shaped spores (Fig. 10) measure 6.3 μm × 4.2 μm in the average. There are four distinct knob-like structures, two in each pole of the spores. The eight rounded sporozoites are arranged in two oblique rows inside each spore.

Measurements (in μm): The summary of measurements of 20 specimens of trophozoites and sporadins with the mean within parenthesis is given below:

Trophozoite

TL = 45.9 - 48.6 (47.7);
LE = 3.2 - 18.9 (21.9); WE = 3.2 - 16.2 (10.1);
LP = 8.1 - 13.5 (10.8); WP = 8.1 - 17.6 (12.1);
LD = 20.3 - 34.6 (26.4); WD = 13.5 - 20.3 (17.6);
LN = 4.1 - 13.5 (6.8); WN = 5.4 - 9.5 (6.7);

Sporadin

TL = 21.6 - 166.1 (54.3);
LP = 8.1 - 32.4 (13.8); WP = 9.45 - 31.1 (15.3);
LD = 13.5 - 133.6 (38.9); WD = 10.3 - 43.2 (19.4);
LN = 4.1 - 13.5 (6.7); WN = 5.4 - 13.5 (6.9);
LP : TL = 1 : 2.6 - 5.1 (3.85)
WP : WD = 1 : 1.0 - 1.75 (1.25)

Details of measurements of individual specimens are given in Table 1.

Table 1

Showing details of measurements (in μm) of different parts of 20 individuals of *Gregarine basiconstrictonea* sp. n.

Sl. No.	TL	LE	LP	LD	LN	WE	WP	WD	WN	LP : TL	WP : WD
(1)	45.9	3.24	8.1	34.56	4.05	3.24	10.8	18.9	5.4	1 : 5.6	1 : 1.75
(2)	48.6	17.55	10.8	20.25	7.56	16.2	8.1	13.5	6.75	1 : 4.5	1 : 1.6
(3)	48.6	18.9	13.5	24.3	6.75	10.8	17.55	20.25	6.75	1 : 3.6	1 : 1.15
(4)	35.1	—	9.45	25.65	7.56	—	10.8	14.85	7.56	1 : 3.7	1 : 1.4
(5)	56.7	—	17.55	39.15	6.75	—	17.55	17.55	6.75	1 : 3.2	1 : 1.0
(6)	35.1	—	12.15	22.95	6.75	—	12.15	13.5	6.75	1 : 2.8	1 : 1.08
(7)	48.6	—	16.2	32.4	4.05	—	12.15	22.95	5.4	1 : 3.0	1 : 1.0
(8)	24.3	—	8.1	16.2	5.4	—	10.26	10.26	5.4	1 : 3.0	1 : 1.0
(9)	86.4	—	16.2	70.2	8.1	—	21.6	24.3	8.1	1 : 5.3	1 : 1.1
(10)	81.0	—	21.6	59.4	9.45	—	24.3	35.1	9.45	1 : 3.7	1 : 1.4
(11)	51.3	—	14.85	36.45	6.75	—	17.55	20.25	8.1	1 : 3.45	1 : 1.15
(12)	23.76	—	7.56	16.2	4.59	—	9.72	10.8	5.4	1 : 3.1	1 : 1.1
(13)	59.4	—	16.2	43.2	6.75	—	12.15	16.2	6.75	1 : 3.6	1 : 1.3
(14)	43.2	—	14.85	28.35	4.86	—	13.5	14.85	4.86	1 : 2.9	1 : 1.1
(15)	35.1	—	10.8	24.3	5.4	—	16.2	18.9	5.4	1 : 3.2	1 : 1.16
(16)	166.05	—	32.4	133.65	13.5	—	31.05	43.2	5.5	1 : 5.1	1 : 1.3
(17)	67.5	—	16.2	51.3	6.75	—	21.6	24.3	8.1	1 : 4.1	1 : 1.1
(18)	72.9	—	9.45	63.45	9.45	—	12.15	20.25	9.45	1 : 7.7	1 : 1.6
(19)	35.1	—	11.34	23.76	5.4	—	16.2	16.2	5.4	1 : 3.0	1 : 1.0
(20)	21.6	—	8.1	13.5	5.4	—	9.45	10.8	5.4	1 : 2.6	1 : 1.1

Material:

(a) Holotype: Trophozoite on slide No. B/18 prepared from the contents of the midgut of *Tribolium castaneum*, from groundnut at Chinsurah, Hooghly, West Bengal, India, by S. Ghose on May 11, 1983.

(b) Paratype: Many, on the above numbered slide and on other slides; other particulars are the same as for the holotype material.

Affinities: The characters like globular or knob-like epimerite, caudo-frontal association, dehiscence of cysts through sporoducts and barrel-shaped spores place the gregarine under the genus *Gregarina*. A very careful comparison of the parasite with those enlisted by Watson (1916), Kamm-Watson (1922) and others, however, reveals that it has no resemblance with the earlier described species. It somewhat

comes closer with *G. blattarum* Siebold, *G. pillinensis* Watson and *G. passali* Lankester in the ratios of different body parts; the barrel shaped spore is a common feature in every species. The most striking character which separates the present one from all other species is that each of the four sporoducts shows a distinct constriction at the base. In this respect it can also be differentiated from the *Gregarina* spp. from *Tribolium* (Ishii 1914, Lipa 1967). It is, therefore, considered as a new species, for which the name *Gregarina basiconstrictonea* is given (L. basis — base; constrictan — constriction).

A comprehensive discussion on the differences between the present form and *G. minuta* Ishii, 1914 has been made later in this communication.

Hirmocystis oxeata sp. n.

Host: *Tribolium castaneum* from walnuts.
Incidence: 120 out of 257 hosts examined are infected with this gregarine.

Development: The earliest stage of the parasite found in the paraffin sections of epithelial cell of the host is a spherical body. Further developmental stages could not be seen in our histological preparations.

Trophozoite: Only one trophozoite has so far been obtained in smears. This is solitary, elongated and milky white in colour when freshly collected. The trophozoite measures 40.5 μm in total length. It contains the usual three segments: epimerite, protomerite and deutomerite. The epimerite is a hyaline structure with a characteristic oxeate shape (Fig. 11) measuring 6.6 μm in length and 4.1 μm in width. The protomerite is elongated and broadest at the middle, measuring 9.5 μm in length and 10.8 μm in width. The deutomerite is the largest segment of the body measuring 24.3 μm in length and 16.2 μm in width. The posterior extremity of the deutomerite is rounded. The cytoplasm is uniformly granulated throughout the protomerite and deutomerite. The nucleus is spherical with an indistinct nuclear membrane. It is present at the centre of the deutomerite and measures 6.8 μm in length and 5.4 μm in width. The pellicle is thin and the epicyteal striations are not observable.

Sporadin and Association: Solitary sporadins are elongated, cylindrical or broadly ovoidal. Structurally the sporadins differ markedly from the trophozoite. The young solitary sporadin (Figs. 12, 13) is globular in shape and measures 27.0 μm in length and 9.5 μm in width. The protomerite of the sporadin is hemispherical measuring 8.1 μm × 9.5 μm. The deutomerite is somewhat barrel-shaped measuring 18.9 μm × 9.5 μm. The mature sporadin (Fig. 14) is cylindrical in shape and measures 27.0 μm to 121.5 μm in total length and 8.1 μm to 24.3 μm in width. The protomerite is hemispherical with distinct epicyteal stria-

tions and measures 8.1 μm to 24.3 μm in length and 8.1 μm to 32.4 μm in width. The deutomerite is separated from protomerite by a distinct concave septum. The deutomerite is long, cylindrical in shape with broader posterior end. The nucleus is rounded to elliptical in shape and situated at the posterior portion of the deutomerite. It measures 4.1 μm to 13.5 μm in length and 4.1 μm to 12.2 μm in width. The cytoplasm is granulated and uniformly distributed in both protomerite and deutomerite.

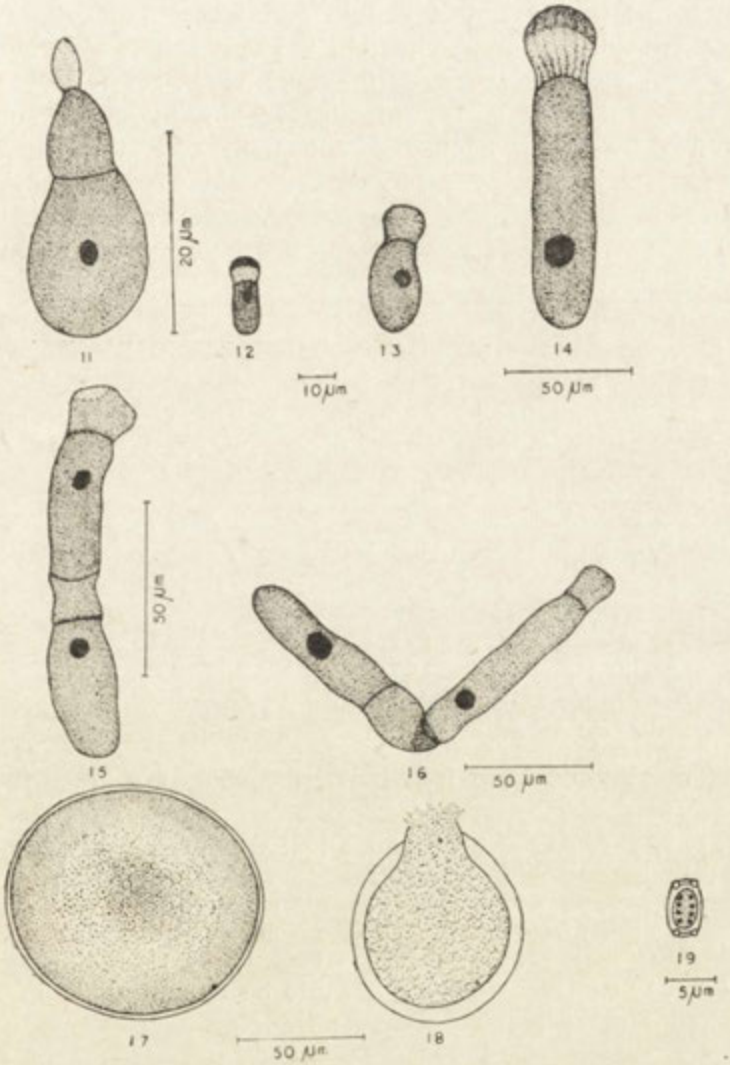


Fig. 11 - 19. Camera lucida drawings of *Hirmocystis oxeata* sp. n. 11 — A fully grown trophozoite, 12 - 13 — Young sporadins in different shapes, 14 — Mature sporadin, 15 - 16 — Sporadins in syzygy, 17 — Gametocyst, 18 — Showing dehiscence of gametocyst through simple rupture, 19 — Barrel-shaped spores with eight sporozoites and two knob-like projections at each pole

The sporadins in caudo-frontal syzygy are obtained in large numbers (Fig. 15, 16). Morphologically both primite and satellite are similar. The two sporadins in syzygy gradually bend upon themselves resulting in a head-tail attachment. Ultimately these become enclosed in a cyst wall.

Gametocyst and Spore: Gametocysts are collected from the hind gut of the infected hosts. A freshly collected gametocyst is greyish white in colour, rounded (Fig. 17) and measures 116.9 μm in length and 104.0 μm in width. Cyst wall is thin and transparent. After 18 h of development inside the moist chamber the cyst brusts by a simple rupture (Fig. 18) liberating spores in short chains. The barrel-shaped, double-walled spores (Fig. 19) measure 5.9 μm in length and 4.3 μm in width. There are four knob-like projections, two at each pole, of the spores. These are probably made of a mucoid substance (Sprague 1941), and perhaps help spores to attach endwise and come out through short chains. Formation of eight sporozoites is completed at 50 h inside the moist chamber.

Measurements (in μm): The summary of measurements of 20 specimens of trophozoite and sporadins with the mean within parenthesis is given below·

Trophozoite

TL = 40.5;
LE = 6.6; WE = 4.1;
LP = 9.5; WP = 10.8;
LD = 24.3; WD = 16.2;
LN = 6.8; WN = 6.7;

Sporadin

TL = 27.0 - 121.5 (64.5);
LP = 8.1 - 24.3 (15.1); WP = 8.1 - 27.0 (15.9);
LD = 18.9 - 97.2 (49.0); WD = 10.8 - 24.3 (17.5);
LN = 4.1 - 13.5 (7.5); WN = 4.1 - 12.1 (6.5);
LP : TL = 1 : 3.3 - 5.5 (4.2);
WP : WD = 1 : 0.8 - 1.75 (1.06).

Details of measurements of individual specimens are given in Table 2.

Material:

(a) Holotype: Trophozoite on Slide No. AP/3 prepared from the contents of the midgut of *Tribolium casteneum*, from walnuts at Chinsurah, Hooghly, West Bengal, India by S. Ghose on June 7, 1983.

(b) Paratype: Many, on the above numbered slide and on other slides; other particulars are the same as for the holotype material.

Affinities: This gregarine has also been obtained from *Tribolium casteneum* but the hosts are collected from walnuts. Simple dehiscence of the gametocyst of this gregarine at once separates it from the genus *Gregarina* and places under the genus *Hirmocystis*. There are close proximities in the ratios of different body parts of this gregarine with

those of *H. ovalis* Crawley, *H. theodoridesi* Kundu and Haldar and *H. hoplasomae* Kundu and Haldar. The shape of the epimerite is very characteristic in the present form besides differences in measurements of body parts. We, therefore, believe it to be a new species for which the name *Hirmocystis oxeata* sp. n. is given after the oxeate shape of the epimerite.

Table 2

Showing details of measurements (in μm) of different parts of *Hirmocystis oxeata* sp. n.

Sl. No.	TL	LE	LP	LD	LN	WE	WP	WD	WN	LP : TL	WP : WD
(1)	40.5	6.75	9.45	24.3	6.75	4.05	10.8	16.2	5.4	1 : 4.3	1 : 1.5
(2)	33.75	—	9.45	24.3	6.75	—	10.8	18.9	5.4	1 : 3.6	1 : 1.75
(3)	121.5	—	24.3	97.2	10.8	—	27.0	24.3	12.15	1 : 5.0	1 : 0.9
(4)	56.7	—	16.2	40.5	6.75	—	14.85	13.5	6.75	1 : 3.5	1 : 0.9
(5)	43.2	—	10.8	32.4	5.4	—	13.5	13.5	5.4	1 : 4.0	1 : 1.0
(6)	72.9	—	13.5	59.4	8.1	—	18.9	18.9	6.75	1 : 5.4	1 : 1.0
(7)	70.2	—	18.9	51.3	8.1	—	16.2	21.6	6.75	1 : 3.7	1 : 1.3
(8)	75.6	—	18.9	56.7	8.1	—	18.9	16.2	5.4	1 : 4.0	1 : 0.9
(9)	37.8	—	10.8	27.0	4.05	—	8.1	8.1	4.05	1 : 3.5	1 : 1.0
(10)	86.4	—	21.6	64.8	8.1	—	17.55	24.3	6.75	1 : 4.0	1 : 1.3
(11)	56.7	—	16.2	40.5	8.1	—	12.15	13.5	6.75	1 : 3.5	1 : 1.08
(12)	78.3	—	18.9	59.4	9.45	—	16.2	16.2	6.75	1 : 4.1	1 : 1.0
(13)	29.7	—	8.1	21.6	5.4	—	10.8	10.8	4.05	1 : 3.6	1 : 1.0
(14)	56.7	—	13.5	43.2	8.1	—	16.2	13.5	6.75	1 : 4.2	1 : 0.8
(15)	54.0	—	13.5	40.5	5.4	—	13.5	14.85	6.75	1 : 4.0	1 : 1.1
(16)	51.3	—	10.8	40.5	5.4	—	13.5	13.5	5.4	1 : 4.7	1 : 1.0
(17)	27.0	—	8.1	18.9	4.05	—	9.45	9.45	4.05	1 : 3.3	1 : 1.0
(18)	116.1	—	24.3	91.8	13.5	—	32.4	24.3	10.8	1 : 4.7	1 : 0.85
(19)	102.6	—	18.9	83.7	9.45	—	18.9	16.2	6.75	1 : 5.5	1 : 0.8
(20)	78.3	—	16.2	62.1	8.1	—	20.25	18.9	8.1	1 : 4.9	1 : 0.9

Cytochemistry

General Polysaccharides: Both in *G. basiconstrictonea* (Fig. 20) and *H. oxeata* (Fig. 25) PAS-positive granules are present throughout the protomerite and deutomerite of the sporadins. However, in the former species the granules in the protomerite are more concentrated. In both the gregarines nucleus appears as a clear unstained area.

Mucopolysaccharide: The presence of this substance is detected by the metachromatic staining with toludine blue. In both the gregarines the metachromatic substances are scattered in the protomerite and deutomerite; the nucleus, the pellicle and major portion of the cytoplasm in the deutomerite stain orthochromatically indicating the absence of mucopolysaccharides in these sites (Figs. 21 and 27).

General Protein: Staining with Mercury bromophenol blue has revealed intense accumulation of general protein in the cytoplasm of protomerite and deutomerite as well as nucleus of the sporadins of both *G. basiconstrictonea* (Fig. 22) and *H. oxeata* (Fig. 28). The pellicle also gives a positive reactions to this stain.

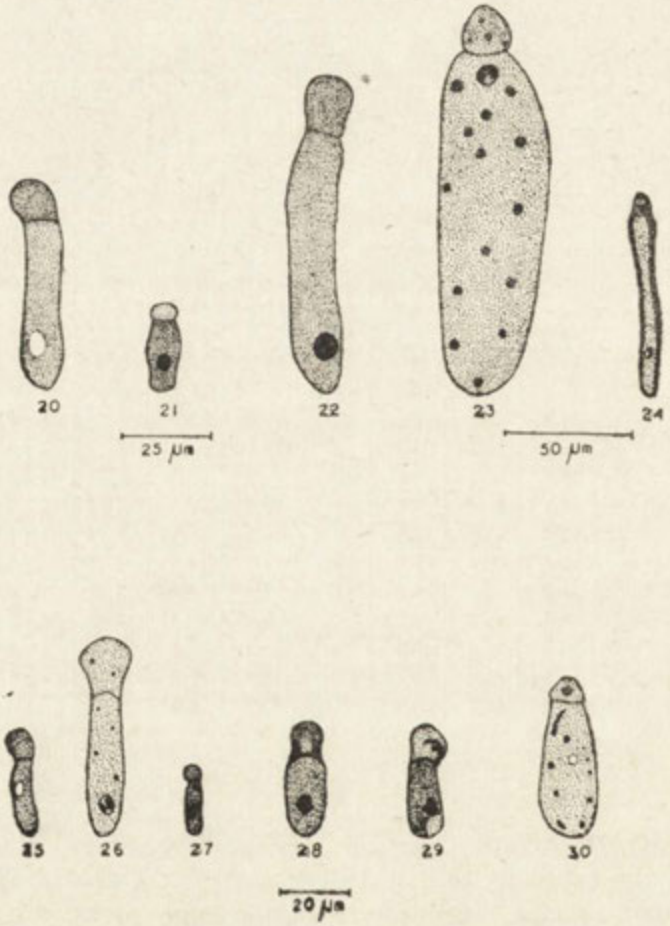


Fig. 20 - 24. Camera lucida drawings of various cytochemical reactions in *Gregarina basiconstrictonea* sp. n. 20 — Showing PAS-positive reaction in the sporadin, 21 — Showing metachromatic granules in the sporadin, 22 — Showing mercury bromophenol blue positive reaction in the sporadin, 23 — Exhibiting simple-lipid reaction in the sporadin, 24 — Alkaline phosphatase reaction in the sporadin

Fig. 25 - 30. Camera lucida drawings of various cytochemical reactions in *Hirmocystis oxeata* sp. n. 25 — PAS-positive reaction in sporadin, 26 — Showing the pattern of orientation of pyronin Y-positive substances in the cytoplasm of the sporadin, 27 — Exhibiting metachromatic granules in the sporadin, 28 — Mercury-bromophenol blue positive reaction in the sporadin, 29 — Showing simple lipid reaction in the sporadin, 30 — Alkaline phosphatase reaction in the sporadin

Simple lipid: The lipid granules are deposited in the form of patches in case of *G. basiconstrictonea* (Fig. 23) while in *H. oxeata* these are present throughout the cytoplasm (Fig. 29). However, the intensity of positive reaction in the nucleus is more or less identical.

Nucleic Acids: Slides were stained with PMG technique for simultaneous staining of DNA and RNA. While no concrete staining behaviour could be detected in case of *G. basiconstrictonea*, the reaction could be observed clearly in *H. oxeata* (Fig. 26). It has been observed that the cytoplasm, pellicle and the endosome indicate heavy deposition of RNA as revealed by their pink colour and very faint greenish granules are present in the nucleoplasm. This indicates that DNA materials are very scanty in the nuclei of sporadin. This has also been confirmed by the Feulgen technique.

Alkaline phosphatase: This enzyme is heavily concentrated in the protomerite and below the pellicle of the deutomerite in *G. basiconstrictonea* (Fig. 24). In the latter species this is present in the form of patches and scattered granules throughout the cytoplasm (Fig. 30).

It is thus observed that there is more or less a uniformity in the pattern of orientation and degree of concentration of various cytochemical substances. This may be due to the fact that the host of these gregarines take similar type of food.

Seasonal Intensity

The incidence of the gregarines in their hosts have been studied throughout the year and very interesting data have been obtained from this study. Figures 31 and 32 show the percentage of infection in *G. ba-*

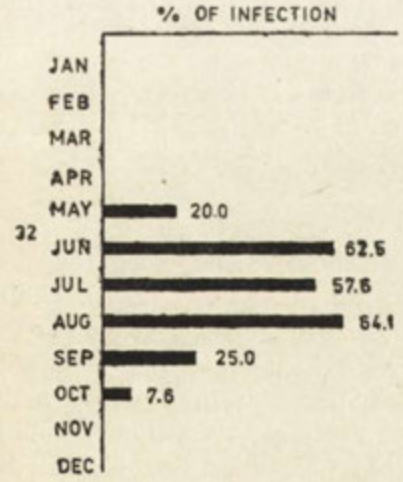


Fig. 31. Histogram showing the seasonal intensity of *Gregarina basiconstrictonea* sp. n.

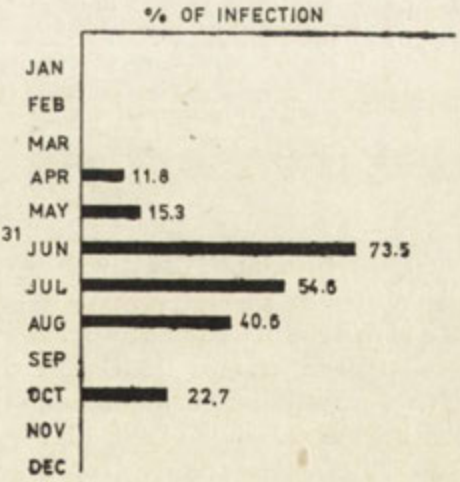


Fig. 32. Histogram showing the seasonal intensity of *Hirmocystis exeata* sp. n.

siconstrictonea and *H. oxeata* respectively. In both the cases no infection is obtained from November to March when general climatic condition is dry. The infection begins to appear in the month of April and reaches to a high peak in June and gradually declines from July onwards in *G. basiconstrictonea*. In case of *H. oxeata*, however, first indication of infection is observed in May, which reaches to a high peak in June and continues steadily upto August after which it gradually declines. Monsoon is very active in this part of the country from June to September, when humidity reaches even more than 95%. It is apparent from these findings that the gregarines require an optimum rainfall and humidity for sporulation.

Diagnosis

Gregarina basiconstrictonea sp. n.

Trophozoite elongated; epimerite small, papilla like; sporadins in syzygy; gametocysts spherical; sporoducts four in number each with a constriction at the base; spores double-walled, barrel-shaped; early development intracellular.

LP : TL = 1 : 2.6 - 5.1 (3.85);
WP : WD = 1 : 1.0 - 1.75 (1.25).

Hirmocystis oxeata sp. n.

Trophozoite elongated; epimerite characteristically oxeate shaped; sporadins in caudo-frontal association; gametocysts spherical; dehiscence by simple rupture; spores double walled and barrel-shaped; early development intracellular.

LP : TL = 1 : 3.3 - 5.5 (4.2);
WP : WD = 1 : 0.8 - 1.75 (1.06).

Discussion

Gregarina minuta has a worldwide distribution. It has been reported from different species of *Tribolium* from Japan (Ishii 1914), USSR (Wellmer 1911), Canada (Laird 1959), France (Théodoridès 1955), Poland (Lipa 1967) and Germany (Geus 1969). The species of *Gregarina* reported in this communication was at first provisionally identified as *G. minuta* Ishii, 1914 but upon detailed observations, a number of distinctive features could be noticed for which it could not be accommodated within the species range of *G. minuta*. The characters

of *G. minuta* as presented by Ishii (1914), Lipa (1967) and Geus (1969) as well those of present form have been summarised in the accompanying Table 3. It is clearly observed that *G. basiconstrictonea* sp. n. differs conspicuously from *G. minuta* in several respects, like the structure of the sporadin, general shape of the associating primite and satellite, appearance of the gametocyst and ratios of different body parts. Moreover, no information is available on the nature of development, structure of epimerite, mode of dehiscence of cyst and the structure of spore of *G. minuta*. Besides the differences listed above, the most striking feature of the present form is that the sporoducts always exhibit a sharp constriction at their bases for which the name *G. basiconstrictonea* has been suggested for it. It may be inferred from the above discussion that the present form is altogether different from *G. minuta*. Climatic variations might have produced distinct morphological changes in the present form necessitating the separate species status for it. However, further research in this line perhaps will answer this problem.

Watson (1916) in her monograph on the gregarines pointed out "Ishii (1914) has evidently confused two species of polycystid gregarines and designated them by the same name" (p. 408). Earlier in the same monograph the author pointed out "under the name *Gregarina minuta* the author described two gregarines belonging to widely different families, one, the larger, being a Didymophyes (*D. minuta*), for the absence of a protomerite in the satellite, and the other described above" (p. 392) (i.e., *G. minuta*). The problem was explained in detail in the Appendix in the said monograph by Watson (1916). In our preparations of the gut contents of *T. castaneum* we have never encountered any specimen in which the satellite was devoid of any septum.

As regards the taxonomic status of *Hirmocystis oxeata* it may be mentioned that the gametocyst of this species never produced any sporoducts. Even development of the gametocyst of *G. basiconstrictonea* and *H. oxeata* under simultaneous and identical laboratory conditions have shown similar results after repeated trials. Absence of sporoducts is one of the most diagnostic features of genus *Hirmocystis*. It is difficult to establish that *T. castaneum* population feeding on walnuts may have different pathogens than population feeding on groundnuts. But the absence of sporoducts in the pathogens feeding on walnuts leads one to the conclusion that it must belong to the separate genus other than *Gregarina*. Haldar and Chakraborty (1979, 1981) and Kundu and Haldar (1981) described a number of new species of *Hirmocystis* in which the sporoducts were never formed.

Table 3

Listing differences between *G. minuta* as given by various authors and *G. basiconstrictonea* sp. n. as revealed in the present study

	Gregarina minuta Ishii			*Gregarina basiconstrictonea* sp. n.
	Ishii (1914)	Lipa (1967)	Geus (1969)	Present study
(1) Development	–	–	–	Early development takes place within the epithelial cells of the midgut of the host insect.
(2) Epimerite	–	–	–	Epimerite small, papilla-like and measures 3.2 μm to 18.9 μm in length and 3.2 μm to 16.2 μm in width. Occasionally expands greatly giving it a bulbous appearance.
(3) Sporadin	Larger sporonts are usually in association, the smaller ones often solitary. Length of protomerite – 5–8 μm Length of deutomerite – 22–112 μm Breadth of body – 6–28 μm Total length of body – 27–120 μm	Maximum length 165 μm; width 50 μm	Solitary sporonts 110 μm long	Young solitary sporadins oval in shape. Protomerite spherical while deutomerite is obese. Mature sporadins cylindrical in shape, the protomerite is domeshaped and the deutomerite is long cylindrical in shape with a slightly broader posterior end.
(4) Association	Protomerite ovoid, sometimes more or less half-moon-shaped, broader than long. It is not large, especially so in the satellite, in which it is not infrequently hidden from view, being entirely imbedded in the deutomerite of the primite. Deutomerite elongate, cylindrical, rounded posteriorly. Nucleus large, spherical, usually situated near the middle of the	Primite: protomerite oval with flattened posterior end, wider than long. Deutomerite cylindrically elongate with oval end. Nucleus has one karyosome. Satellite: Protomerite twice wider than long. Deutomerite elonagate. Nucleus located in the anterior end of deutomerite.	Primite: Protomerite of the primite is egg or small cap-like. Satellite: Protomerite close to the septum. Deutomerite is long with rounded end. Nucleus rounded situated at the middle or in the hinder half of the deutomerite with central rounded karyosome. Maximumlength of association 180 μm	The association is always caudo-frontal in nature. The protomerite of the primite is expanded fan-like, while the satellite is somewhat barrel-shaped. The primite is sometimes larger than the satellite.

	deutomerite of both primite and satellite. Larger association Total length — 188 μm Smaller association Total length — 118 μm			
(5) Gametocyst	Small, spherical, 36–48 μm	Oval upto 160 μm in diameter	Rounded 36–48 μm in diameter.	Rounded and enclose two equal gametocytes. A sharp constriction is present at the junction of two gametocytes. The cysts measure 100.8 μm × 98.6 μm in dimension in the average.
(6) Sporoduct	—	—	—	Four sporoducts are developed on the cyst surface at about 30 h of development. The sporoducts are characteristically constricted at their bases and then become broader immediately after the constriction and gradually tapers towards the free end. Forty eight hours after the formation of the sporoducts, spores are liberated in short chains.
(7) Spore	—	—	—	Double-walled, barrel-shaped spores measure 6.3 μm × 4.2 μm in the average. There are four distinct knob-like structures, two in each pole of the spores. The eight rounded sporozoites are arranged in two oblique rows inside each spore.
(8) LP : TL	—	1 : 5–16	—	1 : 2.6–5.1 (3.85)
(9) WP : WD	—	1 : 1.7–2.7	—	1 : 1.0–1.75 (1.25)
(10) Host	*Tribolium castaneum* (= *ferrugineum*)	*Tribolium confusum*	*Tribolium castaneum* (= *ferrugineum*) and *Tribolium confusum*	*Tribolium castaneum* (= *ferrugineum*)
(11) Locality	Japan	Poland	Germany	India

ACKNOWLEDGMENTS

Part of the financial assistance has been received from the Department of Environment, New Delhi and also from the Council of Scientific and Industrial Research, New Delhi, for which sincere acknowledgements are made. Sincere thanks are also expressed to Prof. N. D. Levine for some literature and the Editor, Acta Protozoologica, for valuable suggestions.

REFERENCES

Geus A. 1969: Sporentierchen (*Sporozoa*), Die Gregarinides in Tierwelt Deutschlands, Fischer Verlag, 57, 9 Teil, Jena, 608 pp.

Haldar D. P. and Chakraborty N. 1979: Observations on three new species of cephaline gregarines (*Protozoa* : *Sporozoa*) of the genus *Hirmocystis* Labbé from coleopteran insects. Z. Parasitenkd., 59, 121 - 130.

Haldar D. P. and Chakraborty N. 1981: Observations on the morphology and life history of a cephaline gregarine *Hirmocystis lepropi* n. sp. from the gut of the beetle, *Lepropus* sp. Proc. Indian Acad. Sci. (Anim. Sci.), 90, 315 - 320.

Haldar D. P., Sengupta T. and Ghose S. 1984: An annotated list of septate gregarines (*Apicomplexa* : *Sporozoea*) from Indian arthropods. J. Beng. Nat. Hist. Soc. (in press).

Ishii S. 1914: On four polycystid gregarines from the intestine of *Tribolium ferrugineum* F. Annot. Zool. Jap., 8, 436 - 441.

Kamm M. W. 1922: Studies on gregarines. II. Synopsis of the polycystid gregarines of the world, excluding those from the *Myriapoda*, *Orthoptera* and *Coleoptera*. Ill. Biol. Monogr., 7, 1 - 104.

Kundu T. K. and Haldar D. P. 1981: Observations on the new species of cephaline gregarines (*Protozoa* : *Sporozoa*) of the genus *Hirmocystis* Labbé from insects, Acta. Protozool., 20, 323 - 335.

Laird M. 1959: Gregarines from laboratory colonies of flour beetles, *Tribolium castaneum* Herbst and *T. confusum* Duval at Montreal. Can. J. Zool., 37, 378 - 381.

Lipa J. J. 1967: Studies on gregarines (*Gregarinomorpha*) of arthropods in Poland. Acta Protozool., 5, 97 - 179.

Pearse A. G. E. 1976: Histochemistry — Theoretical and Applied, Vol. I, J. A. Churchill Ltd., London.

Sokoloff A. 1972: The biology of *Tribolium*, Oxford University Press, 1, 2 249 - 294.

Sprague V. 1941: Studies on *Gregarina blattarum* with particular reference to the chromosome cycle. Ill. Biol. Monogr., 18, 5 - 57.

Théodoridès J. 1955c: Grégarines parasites de coléopteres ténébrionides d'Israel. Ann. Parasitol. Hum. Comp., 30, 161-173.

Wellmer L. 1911: Sporozoen ostpreussischer Arthropoden. Schr. physik. ökonom. Ges. Königsberg, 52, 103 - 164.

Watson M. E. 1916: Studies on gregarines. Ill. Biol. Monogr., 2, 213 - 268.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 109 - 114 (1986)

Eimeria biswapatii sp. n., a New Coccidium from the Indian Bandicoot Rat, *Bandicota indica* (Bechstein)

S. BANDYOPADHYAY

Zoological Survey of India, 8 Lindsay Street, Calcutta-700 087, West Bengal, India

Received on 22 November 1984, *revised on* 27 March 1985

Synopsis. A coccidium belonging to genus *Eimeria* has been detected in an Indian bandicoot rat, *Bandicota indica* (Bechstein). The morphology of the parasite is described and compared in detail with other forms of *Eimeria* reported from different members of suborder *Myomorpha* (*Rodentia : Myomorpha*), to which the rats belong along with allied rodents. The species has been found new and named *Eimeria biswapatii* sp. n. after Professor Biswapati Dasgupta.

Until very recently, rat coccidiosis was a subject insignificantly dealt with in India. Kshirsagar (1980) described *Isospora aurangabadensis* and *Isospora krishnamurthyi* from rats, *Rattus rattus rattus* [1]. Subsequently, Krishnamurthy and Kshirsagar (1981) reported *Eimeria levinei* [2] and *Eimeria tuberculata* from rats, *Rattus rattus rattus* [1]. Bandyopadhyay and Dasgupta (1982) recorded *Eimeria bandicota* from a mole rat (bandicoot rat), *Bandicota bengalensis* for the first time in India.

Material and Methods

During the course of study, 22 specimens of the Indian bandicoot rat, *Bandicota indica* (Bechstein) were collected and examined in search of protozoan parasites. Of these 22 specimens only one was found to be infected with a coccidium of genus *Eimeria*. The faecal sample was placed in 2.5% potassium dichromate solution for sporulation of the oocysts. Unsporulated as well as sporulated oocysts were studied under low power and subsequently under oil immersion lens. Meas-

[1] Availability of this subspecies in India is doubtful, it is an European form.
[2] *E. levinei* is a preoccupied name (see Pellérdy 1974).

urements were taken with ocular micrometer. Camera lucida drawing have been made.

The type material will be deposited to the National Collection of Zoological Survey of India, Calcutta.

Description

Eimeria biswapatii sp. n. (Fig. 1 A - B)

Type-host: *Bandicota indica* (Bechstein).
Type-locality: South Howrah, District-Howrah, West Bengal, India.
Site of infection: Oocysts obtained in the faeces. Life-cycle: Unknown.

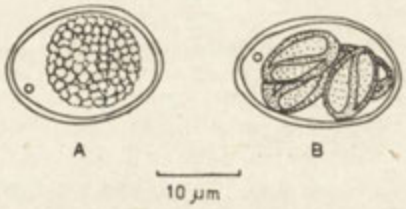


Fig. 1. A - B. Camera lucida drawings of *Eimeria biswapatii*. A — Unsporulated oocyst, B — Sporulated oocyst

The oocysts were ovoid in shape and scaled 16.5 - 19.5 µm (mean 18.18 µm) in length and 12 - 15 µm (mean 13.68 µm) in width. A 1 µm thick oocysts wall consisted of two layers of equal thickness. The layers of the oocyst wall were smooth and colourless. A distinct micropyle 1 µm in diameter could be seen. No polar granule or oocyst residuum were recorded. The spherical unsporulated cytoplasmic mass measured 10 - 13 µm in diameter and looked to be somewhat foamy in appearance. The ovoid sporocysts were devoid of the Stieda body and measured 8.25 - 10.5 µm (mean 9.37 µm) in length and 5.5 - 6.5 µm (mean 6.2 µm) in width. Sporocysts numbered four, each with two sporozoites. The sporozoites were broad comma-shaped with the narrow end sharply pointed and exhibiting head to tail orientation within the sporocyst. The sporozoites measured 6 - 7.5 µm (mean 6.93 µm) in length and 2.25 - 3 µm (mean 2.62 µm) in width. The sporocyst residuum was quite prominent and scattered within the sporocysts in the form of fine granules, with more accumulation in the space between the sporozoites.

Sporulation time was 24 to 36 h at room temperature, i.e., at 30 - 37°C.

Diagnosis

Ovoid oocysts measure 16.5 - 19.5 μm (18.18 μm) × 12 - 15 μm (13.68 μm). A 1 μm thick smooth and colourless oocyst wall consists of two layers of equal thickness. Micropyle prominent. Polar granule and oocyst residuum absent. Ovoid sporocysts without the Stieda body measuring 8.25 - 10.5 μm (9.37 μm) × 5.5 - 6.5 μm (6.2 μm). Comma shaped sporozoites with narrow end pointed sharply, lay head to tail and measure 6 - 7.5 μm (6.93 μm) × 2.25 - 3 μm (2.62 μm). Sporocyst residuum prominent.

Remarks

Pellérdy (1974) listed in all 30 species of *Eimeria* described from rats belonging to four genera, viz., *Rattus*, *Oryzomys*, *Neotoma* and *Cricetomys*. Later Mirza (1975) recorded another Eimerian species from a member of genus *Nesokia*. Krishnamurthy and Kshirsagar (1981) described two species of *Eimeria* from rats of genus *Rattus*. Subsequently, Bandyopadhyay and Dasgupta (1982) reported one Eimerian parasite from a member of genus *Bandicota*. The species under report was detected in an Indian bandicoot rat, *Bandicota indica* (Bechstein) and exhibited bilayered oocyst wall and a micropyle; and these two morphological features were considered as key characters for comparing the species with the described forms. During comparison, amongst the 34 species mentioned earlier only two resembled the present species in the key characters. Other parasites, therefore, could be safely discarded. The present species was also compared with seven other species of *Eimeria* having double-layered oocyst wall with a micropyle, described so far from other members of suborder *Myomorpha* (*Rodentia* : *Myomorpha*) to which the rats belong along with allied mammals. Thus, the present species when compared elaborately with those nine species of *Eimeria* (two of rats and seven of allied rodents other than rats) with bilayered oocyst wall and a micropyle, displayed certain resemblance with some of them in definite features but there were tangible points of departure as will be evident from the Table 1.

It is thus clear that the species under consideration does not correspond to any known species and is, therefore, declared new to science and named *Eimeria biswapatii* sp. n. after Professor Biswapati Dasgupta,

Table 1

Eimeria species	Oocyst characters					Sporocyst characters		
	Shape and size	Wall	Micropyle	Polar granule	Residuum	Shape and size	Stieda body	Residuum
1. *E. popovi* Matschoulsky (1949)	Oval, 21–25 μm ×17–21 μm (mean 23.4×19 μm)	Total thickness 2 μm, the inner layer colourless and the outer layer light brown	+ (3.8–5.7 μm wide)			Oval, 7.6–9.8 μm ×4–4.6 μm	+	+
2. *E. harbelensis* Levine et al. (1959)	Ovoid, 32×23 μm	Inner layer 1.5 μm thick at one pole, thinned to 9 μm at the other pole; outer layer 1 μm thick at thick end of the inner layer, after 2/3 of the oocyst it narrowed down and seemed to disappear anteriorly	+	–	–	Tapered at both the ends, 14×8 μm	+	+
3. *E. terristris* Musaev and Veisov (1960)	Ovoid or ellipsoid, 18–22 μm×12–16 μm (mean 21.3×15.6 μm)	Smooth, total thickness 1.5–2 μm, the inner layer darker than the outer one	+	–	–	Oval or sub-spherical, 6–12 μm×4–8 μm (mean 9 ×6.2 μm)	–	+
4. *E. egypti* Prasad (1960)	Oval, 27–28 μm ×15–16 μm (mean 28×16 μm)	The outer layer thicker than the inner one	+	–	+	Piriform, 7–9 μm×4–5 μm	+	+
5. *E. kolanica* Veisov (1963)	Oval, 16–26 μm ×14–22 μm (mean 21.2×18.8 μm)	Total thickness 2.5 μm; the inner layer dark brown and the outer layer colourless, each layer of 1 μm thickness	+	–	–	Round, 5–10 μm (mean 8 μm) in diameter	–	+
6. *E. micropiliana* Musaev et al. (1963)	Oval or round, 20–25 μm×16–21 μm (mean 23×19 μm)	Total thickness 2.5 μm, outer layer colourless and inner layer dark yellow	+	–	–	Ovoid, 6–11 μm ×4–7 μm (mean 9.9×5.5 μm)	–	+

7. *E. vahidovi* Veisov (1964)	Ovoid or ellipsoid, narrowed at the micropylar end; 25–32 μm ×21–23 μm (mean 29.1×21.8 μm)	3 μm in thickness, the outer layer colourless and the inner layer yellowish brown	+	+	–	Ovoid, 6–10 μm ×4–8 μm (mean 8.9×6.9 μm		+
8. *E. edwardsi* Colley and Mullin (1971)	Ovoid, tapered at micropylar end; 25–32 μm×21–23 μm (mean 29.1×21.8 μm)	The outer layer 1.5 μm thick, the inner layer 5 μm in thickness	+	–	–	Tapered at both the ends, 14×8 μm	+	+
9. *E. bandicota* Bandyopadhyay and Dasgupta (1982)	Subspherical, 24–25.5 μm ×21.5–23 μm (mean 26×22.5 μm)	The outer layer thin and yellowish brown in colour while the inner layer 1.5 μm in thickness	+	–	–	Ovoid, 10.5–13.5 μm×6–9 μm (mean 11.7×7.6 μm)	+	+
10. *E. biswapatii* sp. n.	Ovoid, 16.5–19.5 μm × 12–15 μm (mean 18.18×13.68 μm)	Bilayered, total thickness 1 μm; smooth and colourless layers equally thick	+	–	–	Ovoid, 8.25–10.5 μm×5.5–6.5 μm (mean 9.37×6.2 μm)	–	+

Research Guide of the author. This is, however, the second report of a coccidium occurring in the Indian bandicoot rat of genus *Bandicota* and the first of its kind in this host species viz., *Bandicota indica* (Bechstein).

ACKNOWLEDGEMENT

The author expresses sincere gratitude to Dr. B. K. Tikader, Director, Zoological Survey of India, Calcutta for providing laboratory facilities and financial assistance. Thanks are due to Sri S. R. Dey Sarkar, Zoological Survey of India, Calcutta for identifying the mammalian specimens. Thanks are also conveyed to Drs. A. K. Mandal, A. K. Das and R. Ray of the Zoological Survey of India, Calcutta for their help in this work.

REFERENCES

Bandyopadhyay S. and Dasgupta B. 1982: A new coccidium *Eimeria bandicota* n. sp. from *Bandicota bengalensis* (Gray) in Darjeeling. J. Beng. Nat. Hist. Soc. (N.S.), 1, 28 - 36.

Colley F. C. and Mullin S. W. 1971: New species of *Eimeria* (*Protozoa : Eimeriidae*) from Malaysian rats. J. Protozool., 18, 601 - 604.

Krishnamurthy R. and Kshirsagar H. S. 1981: Two new species of *Eimeria* Schneider, 1875 from rats in India. Arch. Protistenk., 123, 215-220.

Kshirsagar H. S. 1980: Two new species of *Isospora* Schneider, 1881 from rats in India. Arch. Protistenk., 123, 267 - 271.

Levine N. D., Bray R. S., Ivens V. and Gunders A. E. 1959: On the parasitic protozoa of Liberia. V. *Coccidia* of Liberian rodents. J. Protozool., 6, 215 - 222.

Matschoulsky S. N. 1949: K voprosu o koksidioze grizunov juznih raionov Buryat-Mongolskoi ASSR. Tr. Buryat-Mong. Zool. Ing. 5, 40 - 56.

Mirza Y. M. 1975: Three new species of coccidia (*Sporozoa : Eimeriidae*). Bull. Nat. Hist. Res. Cent. Univ. Baghdad, 6, 39 - 53.

Musaev M. A. and Veisov A. M. 1960: Novye vidy koksidii iz Volyanih polevok *Arvicola terristris* L. Izv. Akad. Nauk Az. SSR. Ser. Biol.-Med. Nauk., 1, 51 - 61.

Musaev M. A., Veisov A. M. and Alieva F. K. 1963: Pyat' novyh vidov koksidii roda *Eimeria* iz obščestvennoi polevki (*Microtus socialis* Pall.). Zool. Zh., 42, 809 - 813.

Pellérdy L. P. 1974: Coccidia and Coccidiosis, 2nd ed. Akademiai Kiado, Budapest, 959 pp.

Prasad H. 1960: Studies on the coccidia of some rodents, of the families *Muridae, Dipodidae*. Ann. Trop. Med. Parasit., 54, 321 - 330.

Veisov A. M. 1963: Koksidii obyknovennoi polevki *Microtus arvalis* Pall. (1778) v Azerbaidžanie. Izv. Akad. Nauk Az.-SSR. Ser. Biol. Med. Nauk., 4, 61 - 75.

Veisov A. M. 1964: Koksidii nekotoryh vidov grizunov sem *Muridae* i *Cricetidae*. Nakh. ASSR. Diss. Kand.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 115 - 118 (1986)

On the Occurrence of a New Coccidium, *Dorisa indica* sp. n. in a Common House Rat, *Rattus rattus arboreus* (Horsfield) from India

S. BANDYOPADHYAY, R. RAY[1]
and A. BHATTACHARJEE[2]

Zoological Survey of India, 8 Lindsay Street, Calcutta — 700 087, West Bengal, India

Received on 22 November 1984, *revised on* 27 March 1985

Synopsis. A species of Dorisa has been encountered in a common house rat, *Rattus rattus arboreus* (Horsfield), collected from South of Howrah town, District-Howrah, West Bengal, India. The morphology of the oocysts is described and compared with those of earlier reported species of *Dorisa* of mammals. The coccidium has been found distinct from any known species and is designated as *Dorisa indica* sp. n. to mark its occurrence India.

Only three species of *Dorisa*[3] have so far been reported from mammalian hosts. In the United States of America, Levine et al. (1955) described *D. arizonensis* from a desert wood rat, *Neotoma lepida*. In India, Sinha and Dasgupta (1978) observed a *Dorisa* species in Himalayan hairy winged bat, *Harpiocephalus harpia lasyurus*, which was later designated as *D. harpia* by Sinha (1979). Subsequently, Bandyopadhyay and Ray (1982) reported the occurrence of *D. bengalensis* in a five-striped palm squirrel, *Funambulus pennanti* Wroughton from India.

---

[1] Present address: Post Graduate Dept. of Zoology, Parasitology Laboratory, Darjeeling Govt. College, Darjeeling 734101, India.

[2] Present address: Z. S. I. Shillong, Meghalaya, India.

[3] All these species of *Dorisa* were actually reported under genus *Dorisiella*, created by Ray (1930) after obtaining oocysts from a marine polychaete. As Levine (1980) established *Dorisa* n. gen. for the coccidian parasites earlier described under genus *Dorisiella* from the vertebrate hosts, the genus of the said parasites was emended accordingly to genus *Dorisa*.

Material and Methods

During the course of investigation of protozoan parasites of small mammals, a common house rat, *Rattus rattus arboreus* (Horsfield) was found infected moderately with a coccidium of genus *Dorisa*. The host specimen was trapped in June, 1984 from South of Howrah town, District-Howrah, West Bengal, India. The faecal sample was kept in 2.5% potassium dichromate solution. Both the unsporulated and sporulated oocysts were studied under low power and under oil immersion lens. Camera lucida drawing of the oocyst was made. Measurements were taken. Sporulation time was also recorded. The mammalian specimen was identified by the staff members of Mammal and Osteology Section of the Zoological Survey of India, Calcutta. The type material will be deposited to the National Collection of the Zoological Survey of India, Calcutta.

Description

Dorisa indica sp. n. (Fig. 1)

Fig. 1. Camera lucida drawing of *Dorisa indica*. Sporulated oocyst

Type-host: *Rattus rattus arboreus* (Horsfield).
Type-locality: South Howrah, District-Howrah, West Bengal, India.
Site of infection: Oocysts in faeces.
Prevalence: Of 18 rats examined only one was found to be infected with this coccidium.
Life-cycle: Unknown.

The oocysts were almost subspherical in shape and measured 19.5 - 22.5 µm (mean 20.8 µm) in length and 18 - 19.5 µm (mean 18.6 µm) in width. The oocyst wall consisted of two equally thick, colourless and smooth layers. The total thickness of the oocyst wall reached 1.5 µm on average. A 1.8 µm wide micropyle could be seen. A polar body could be detected but no oocyst residuum was present. Sporocysts numbered two, each with eight sporozoites. Bimembraneous sporocysts with a very prominent knob-like Stieda body at the narrow end were ovoid in shape and ranged 14.25 - 16.5 µm (mean 15.4 µm) in length

and 8.25 - 9.3 μm (mean 8.5 μm) in width. Sporozoites were ellipsoid in shape and measured 4.2 - 4.5 μm (mean 4.4 μm) in length and 2.7 - 3 μm (mean 2.9 μm) in width. The sporozoites exhibit no definite pattern of orientation though sometimes lay in pairs. Sporocyst residuum in the form of numerous fine globules as seen to scatter throughout the sporocyst in the space between the sporozoites.

Sporulation time to be recorded was 48 h at room temperature i.e., at 30 - 37°C.

Diagnosis

Subspherical oocysts measure 19.5 - 22.5 μm (20.8 μm) × 18 - 19.5 μm (18.6 μm). The 1.5 μm thick bilayered oocyst wall smooth and colourless. A 1.8 μm wide micropyle present. Polar body present. Oocyst residuum absent. Ovoid, bimembraneous sporocysts with a knob-like Stieda body scaled 14.25 - 16.5 μm (15.4 μm) × 8.25 - 9.3 μm (8.5 μm). Sporozoites ellipsoid, measured 4.2 - 4.5 μm (4.4 μm) × 2.7 - 3 μm (2.9 μm) and occassionally lay in pairs. Fine globules of sporocyst residuum abundant.

Remarks

Amongst the three previously recorded species of *Dorisa* of mammals, two were reported from rodents and the third was described from a chiropteran host. The parasite under discussion was detected in *Rattus rattus arboreus*, i.e., in a rodent. The species was compared elaborately with the three earlier available species of *Dorisa*.

The present species is close to *D. arizonensis* in the shape of the oocyst (in some cases), in the presence of smooth, colourless and bilayered oocyst wall, in the absence of oocyst residuum, in the presence of polar granule and Stieda body, but departed from it in the shape (in some cases) and size of the oocyst, in the shape and size of the sporocyst, in the presence of micropyle in the present case, in the orientation of the sporozoites and also in the morphology of sporocyst residuum. It resembled *D. harpia* in the shape and size (to some extent) of the oocyst, in the presence of smooth, bilayered oocyst wall, in the presence of polar granule, refractile body and sporocyst residuum, and in the absence of oocyst residuum, but differences were noted in the detailed morphology of the oocyst wall, in the presence of micropyle in the present form, in the size of the sporocysts and sporozoites. The

present parasite exhibited similarities with *D. bengalensis* in the shape (in some cases) and size (to some extent) of the oocyst, in the presence of smooth, colourless, bilayered oocyst wall, in the absence of oocyst residuum, in the presence of Stieda body and conspicuous sporocyst residuum, while diversities were noticed in the shape (in some cases) of the oocyst, in the absence of micropyle and polar granule in *D. bengalensis*, in the size of the sporocyst, in the morphology of the sporozoites. Again, sporocyst residuum was granular in *D. bengalensis* but it was finely globular in the present form.

Hence the parasite under report never corresponded to any known species dealt with in this connection and declared new to science. The species has been named *Dorisa indica* sp. n. as the parasite is being reported for the first time from the Indian rat.

ACKNOWLEDGEMENT

The authors are indebted to Dr. B. K. Tikader, Director, Zoological Survey of India, Calcutta for kindly allowing to undertake this work. Thanks are due to the staff members of the Mammal and Osteology Section of the Zoological Survey of India, Calcutta for identifying the mammalian specimen. Thanks are also due to Dr. B. Dasgupta, Principal, Darjeeling Government College, Darjeeling, Dr. A. K. Mandal, Superintending Zoologist and Dr. A. K. Das, Zoologist, Zoological Survey of India, Calcutta for their valuable suggestions from time to time.

REFERENCES

Bandyopadhyay S. and Ray R. 1982: On a new coccidium, *Dorisiella bengalensis*, from an Indian palm squirrel, *Funambulus pennanti* Wroughton. Indian J. Parasitol., 6, 265 - 266.

Levine N. D. 1980: *Dorisa* n. gen. (*Protozoa, Apicomplexa, Eimeriidae*). J. Parasitol., 66, 11.

Levine N. D., Ivens V. and Kruidenier F. J. 1955: *Dorisiella arizonensis* n. sp., a coccidium from the desert woodrat, *Neotoma lepida*. J. Protozool., 2, 52 - 53.

Ray H. N. 1930: Studies on some *Sporozoa* in polychaete worms. II. *Dorisiella scolelepidis* n. gen., n. sp. Parasitology, 22, 471 - 480.

Sinha C. K. 1979: *Dorisiella harpia* n. sp. from hairy-winged bat. Indian J. Parasitol., 3, 173 - 175.

Sinha C. K. and Dasgupta B. 1978: On the occurrence of *Dorisiella* in a Himalayan bat, *Harpiocephalus harpia lasyurus* (Hodgson). Abstr. Asian Congr. Parasitol., p. 188.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 119 - 122 (1986)

Sphaerospora anguillae sp. n. (*Myxospora, Bivalvulida*), a Parasite of Eel, *Anguilla anguilla* (L.)

Jadwiga WIERZBICKA

Institute of Ichthyology, Faculty of Marine Fisheries, Academy of Agriculture
4 Kazimierza Królewicza Str., 71 - 550 Szczecin, Poland

Received on 22 December 1984

Synopsis. A new protozoan species, *Sphaerospora anguillae* sp. n. is described, the protozoans were found in the urinary bladder of eel, *Anguilla anguilla* (L.). The generic identity of some species classified as *Sphaerospora* Thélohan, 1892 is discussed.

Protozoans of the genus *Sphaerospora* Thélohan, 1892 were described by many authors. Kudo (1933) listed 14 species parasitizing marine and freshwater fish assigned to that genus. Some of them (*S. divergens* Thélohan, 1895 and *S. polymorpha* Davis, 1917) were by Šulman (1966) transferred to the new created genus *Ortholinea* Schulman, 1962 as well as species *S. orientalis* described by Šulman and Šulman-Albova (1953). In his 1984 paper, Šulman described in detail 21 *Sphaerospora* species parasitizing the fish. Besides, Evlanov (1981) found in the *Tinca tinca* kidney canaliculi a new species which he described as *S. galinae*. Meglitsch (1970) described *S. undulans* sp. n. from some fishes off New Zealand. He also gives the key for taxonomic characters of 24 species recorded in the literature and belonging, in his opinion, to the genus *Sphaerospora*. That list includes *S. irregularis*, described by Kabata (1962) from the urinary bladder of *Drepanopsetta platessoides* from the North Sea, it also includes the species transferred earlier to the genus *Ortholinea*.

The sporozoans found in *Anguilla anguilla* (L.) differ in their morphology from the *Sphaerospora* species described so far. These differences prompted the author to describe the organisms found as a new species *Sphaerospora anguillae* sp. n.

Materials and Methods

The fishes examined were caught in the Szczecin Lagoon (Stara Odra) in August 1983. A total of 24 individuals of *Anguilla anguilla* (L.) were examined, their total length and weight ranged within 39 - 60 cm and 100 - 450 g, respectively. Live fish were transported to the laboratory and kept in containers. Immediately after killing a fish, the material scraped off from its urinary bladder was examined under the microscope. Additionally, smears were made on glass slides, dried and stained by means of Pappenheim's technique (May-Grünwald and Giemsa). Drawings of spores were made from fresh materials, the measurements were taken from stained mounts. A total of 20 specimens were measured.

Results

Description of *Sphaerospora anguillae* sp. n. (Fig. 1)

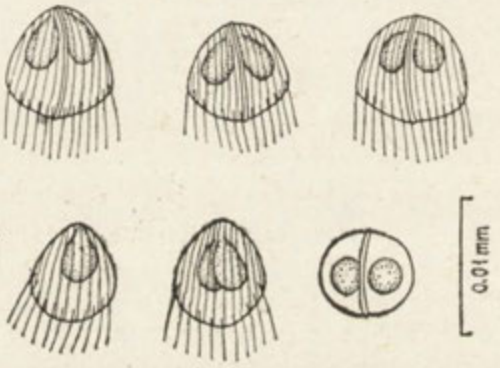


Fig. 1. *Sphaerospora anguillae* sp. n. (drawn from fresh specimens)

Host species: *Anguilla anguilla* (L.)
Location in host: urinary bladder
Site: Szczecin Lagoon
Invasion incidence and intensity: the parasites were found in 21 out of 24 eels examined (87.5% incidence), invasion intensity ranged from single to very numerous protozoans.

The shape of spores deviates substantially from spherical, the anterior part is somewhat sharpened, while the posterior part, in the plane perpendicular to the seam, is somewhat flattened. The width of spores is slightly smaller than their thickness. A thin test of a spore consists of two parts (valves) connected by a poorly visible seam. The spore surface is covered by delicate, longitudinal stripes parallel to the seam, they are visible on fresh individuals only. Two pear-shaped polar capsules, equal in size, are located in the anterior part, they extend to

the mid-region of the spore. The posterior part gives rise to very thin delicate processes (filaments) placed in a single row around the spore, they are clearly seen on fresh individuals only.

The dimension of spores (stained individuals): length 5.6-6.8 μm, thickness 5.6-6.8 μm, filament length about 3.2-3.6 μm, polar capsules length 2.6-3.2 μm, width 1.6-2.4 μm, spiral filament length 26.0-58.0 μm. The size of spores in stained mounts was slightly reduced (by about 1.5 μm). No spore arranged in the seam plane was found in those mounts and therefore the width cannot be given.

Vegetative stages and development of the species will be described in a separate paper.

Discussion

One of the most characteristic features in the myxosporidians *Sphaerospora anguillae* sp. n. is the presence of delicate, very thin processes beginning in the posterior part of the spore. Filamentous processes are observed also in four other species of the genus *Sphaerospora*. The protozoans *S. anguillae* sp. n., however, differ clearly from those species. *S. donecae* Gasimagomedov, 1970 and *S. caudata* Parisi, 1910 have much longer processes, their length exceeding that of the spore (about 2 and 3 times), and the shape of spores is different (Šulman 1984). The processes in *S. dubinini* Schulman, 1962 are very short (about 1 μm), while in *S. anguillae* sp. n. they are about three times longer and equal to about half the spore length. Besides, the *S. dubinini* spores are almost spherical. The posterior filament length in *S. cyprini* Fujita 1912 and *S. anguilla*, sp. n. is almost the same, the shape of spores, however, being completely different (Šulman 1966, 1984). Some of the protozoans (*S. caudata* and *S. cyprini*) are placed by Kudo (1919) in the genus *Mitraspora* Fujita, 1912. Meglitsch (1970) did not place species possessing the filamentous processes in the *Sphaerospora*, either, instead, he suggested they belong to the genus *Mitraspora*.

Apart from morphological differences between spores, the host species of *S. caudata*, *S. cyprini*, *S. donecae*, and *S. dubinini* are different, too (Kudo 1919, Šulman 1966, 1984). No *Sphaerospora* parasite has been so far described from eel.

Having read the description of *S. undulans* Meglitsch, 1970 from marine fish (*Caulopsetta scapha* and *Peltorhamphus novaezelandiae*) off New Zealand one can see that the diagnostic characters of the species are not compatible with the *Sphaerospora* generic characteristics as given by Šulman (1966, 1984). Pear-shaped polar capsules are

not placed in a plane perpendicular to the seam, their outlets being far apart. The spore valves are connected with a slightly undulating seam (Meglitsch 1970). These features place the species closer to the genus *Ortholinea* Schulman, 1962. The definite transfer of *S. undulans* to that genus is prevented by the slightly undulating seam line. This character, much stronger expressed, is typical of the genus *Sinuolinea* Davis, 1917. Besides, the *Sinuolinea* species show spherical polar capsules (Kudo 1919, Šulman 1966).

Moser and Noble (1977), when studying the *Myxosporidia* from Macrourid fish, *Coelorhynchus coelorhynchus carminatus*, found some protozoans identified as *Sphaerospora divergens* Thélohan, 1958, this species was transferred to the genus *Ortholinea* by Šulman (1966). The authors mentioned above did not, however, follow Šulman's (1962, 1966) classification which is convincing and clear. It is, anyway, interesting that the sporozoans were found in the gall bladder, they had been recorded in the kidney canaliculi and the urinary bladder before.

ACKNOWLEDGEMENTS

I thank Professor Eugeniusz Grabda, Ph. D. for his support during the work.

REFERENCES

Evlanov I. A. 1981: Novye vidy miksosporidij iz ryb Kaliningradskoj oblasti. Parazitologija, 15, 168 - 170.

Kabata Z. 1962: Five new species of *Myxosporidia* from marine fishes. Parasitology, 52, 177 - 186.

Kudo R. 1919: Studies on *Myxosporidia*. A synopsis of genera and species of *Myxosporidia*. Ill. Biol. Monogr., 5, 245 - 503.

Kudo R. 1933: A taxonomic consideration of *Myxosporidia*. Trans. Am. Microsc. Soc., 52, 195 - 216.

Meglitsch P. A. 1970: Some coelozoic *Myxosporidia* from New Zealand fishes: family *Sphaerosporidae*. J. Protozool., 17, 112 - 115.

Moser M. and Noble E. R. 1977: Myxosporidan genera *Auerbachia*, *Sphaerospora*, *Davisia* and *Chloromyxum* in macrourid fishes and the sablefish, *Anoplopoma fimbria*. Z. Parasitenk., 51, 159 - 163.

Šulman S. S. 1962: *Myxosporidia*. In: Opredelitel Parazitov Presnovodnyh Ryb SSSR (ed. Byhovskij B. E.) Izd. AN SSSR, Moskva - Leningrad, p. 47 - 130.

Šulman S. S. 1966: Miksosporidii fauny SSSR, Izd. "Nauka", Moskva - Leningrad.

Šulman S. S. (ed.) 1984: Opredelitel parazitov presnovodnyh ryb fauny SSSR. I. Parazitičeske prostejše. Izd. AN SSSR, Leningrad.

Šulman S. S. and Šulman-Albova R. E. 1953: Parazity ryb Belogo Morja. Izd. AN SSSR, Moskva - Leningrad, p. 1 - 199.

ACTA PROTOZOOLOGICA
Vol. 25, No. 1, pp. 123 - 129 (1986)

Several Photomicrographic Methods for Measuring Ciliates

Henry TAMAR

Department of Life Sciences, Indiana State University, Terre Haute, Indiana 47809, USA

Received on 12 July 1985

Synopsis. Three methods for the photomicrographic measurement of live ciliates are described and evaluated. These are: a method in which a specimen negative is superimposed over a scale negative and both are viewed through a magnifier, a method in which an enlargement of a stage micrometer scale is used to obtain measurements on enlargements of specimens, and an adjunct method in which negatives are only projected onto ordinary paper and the desired distances are marked. Additional methods for measuring ciliates are pointed out.

In the last decade we have seen an increasing application of quantitative methods in the area of ciliate systematics. Quantitative study of the characteristics of a population (the actual unit sampled) or of a species results in a more complete description which includes the limits of variation. Further, as Gates (1978) points out, after the variations in ciliate populations have been measured quantitatively, the mathematical analysis of variance can be applied to these variations to get objective measures of the distinctness of populations. Thus a better basis for differentiating species and making advances in ciliate systematics becomes available.

Also, the variations in populations are the basis of the evolutionary processes in ciliates, and therefore the measurement of these variations lays the foundation for evolutionary investigations. Berger (1978) calls attention to the suitability of ciliates for microevolutionary studies, and Poljansky (1982) underlines the importance of analyzing intraspecific variation and polymorphism in protozoans in connection to their evolution and speciation.

Studies of variation and application of the techniques of numerical taxonomy both usually require that a series of measurements be made on each of numerous specimens. Thus Berger (1978) emphasizes the importance of examining as many attributes as feasible in large samples of individuals. If the species selected for investigation is distorted by fixing, or a species is rare and individuals must take a certain position to make a particular measurement possible, it may be preferable to make direct measurements on living specimens with a phase-contrast microscope. This would require the use of an ocular micrometer and the later transformation of the ocular units to μms. However, the organisms may move too rapidly to permit effective measurement of a number of morphological characters in each of many individuals. In such a case recourse can be taken to photomicrography. This makes methods for measuring ciliates through photomicrographs of increasing interest.

Methods of Photomicrographic Measurement

A photograph of a stage micrometer scale at a magnification identical to that of a specimen photograph has been used in the past to indicate specimen dimensions. Thus at the poster sessions of scientific meetings it was not uncommon to see a small portion of a photographic enlargement of a micrometer scale mounted in one corner of a photographic enlargement of a specimen.

However, Kazubski (1971) first gave a complete description of a photomicrographic technique for specimen measurement. He used photographic prints (enlargements) in which specimens were magnified 1000, 2000 or 5000 times. It was a simple matter to measure with a millimeter ruler parts of specimens magnified 1000 times, since 1 μm magnified 1000 times is equal to 1 mm. Similarly, at a magnification of 2000 × a length of 2 mm on a ruler represents 1 μm, and at 5000 × a 5 mm distance indicates a 1 μm length in a specimen. Kazubski (1971) suggested photographing a stage micrometer scale at the start of a film and explained how to use the resulting negative to obtain enlargements at 1000, 2000 or 5000 ×.

In the present paper three additional methods of photomicrographic measurement are fully described. All three methods require that a stage micrometer scale (I use a Zeiss stage micrometer with 10 μm divisions) be photographed in exactly the same way as the specimens. The photomicrographic measurement of living ciliates also necessitates photographing them through a phase-contrast microscope. The film exposures should be 1/100 s or less to eliminate the effects of specimen motion.

Such short exposures are possible if one mounts a microflash unit beneath the phase-contrast condenser and stage of the microscope and synchronizes this unit with the camera shutter. I employ a Zeiss Microflash Device. When I take black and white dark phase-contrast photographs on Kodak Tri-X 35 mm film at 590×, I use a flash intensity of 60Ws and an exposure of 1/125 s. The film is developed in D-76 for about 30% more time than is normal.

The Negative — Magnifier Method

In this simple method the negative of a specimen is superimposed over the negative of the micrometer scale. Each negative should be protected by a cellophane sleeve. The two negatives are viewed through a strong magnifying lens against an illuminated background, preferably a light source behind ground glass. The scale negative is moved in relation to the specimen negative until the outer edge of a scale line lies behind the outer edge of either the organism or a body component. The observer, knowing the equivalent in μms of the distance between every two scale lines, then makes an estimate of the dimension in question.

I use an old slide viewer made by the Bausch and Lomb Optical Company of Rochester, New York (U.S. des. patent 109, 397) for this method. This viewer provides high magnification and has a sufficiently large open space through which to insert and manipulate the two negatives behind the lens.

The Negative — Magnifier Method yields only general size estimates. However, it also requires the least effort. It can provide preliminary measurements for deciding the direction of future research, or it can be used to check results obtained by other methods.

The Measured Scale — Print Method

In this technique the micrometer scale negative is enlarged to precisely the same degree, perhaps with the exception of achieving fine focus, as the specimen negatives. The a v e r a g e distance between the scale lines on the paper enlargement of the scale is now determined by making, under a magnifying lens on a stand, a number of measurements between every two of a series of scale lines on the paper. Averaging many measurements produces greater accuracy because the distances between the etched lines on a stage micrometer scale are not uniform and the etched lines have uneven and ragged edges. The average distance between the scale lines on the enlargement, in whatever

linear units it is expressed, represents, and is mathematically equated to, the distance which should separate the lines on the scale itself. This equation is used to determine the length in μms which one linear unit represents. Now the desired specimen dimensions are measured on each specimen paper enlargement, under the magnifying lens, in the same linear units. The values obtained are multiplied by the equivalent length in μms of one linear unit to get the length in μms of each specimen dimension.

I measure with the 50 marks-per-inch scale on a triangular pearwood Henschel Nr. 625 U.S. st'd. engineer ruler. On the micrometer scale paper enlargement I find the distance between the right edges of adjacent scale lines. I do not use a dark line which is visible within each scale line because these dark lines progressively shift their position within the scale lines as one approaches either end of the enlargement. I measure at points where the right edges of both adjacent scale lines neither jut out or indent. I insure with a protractor that the ruler lies at right angles to the scale lines. I make up to six measurements between every two of the 5 - 11 scale lines on an enlargement. Since I position the enlarging paper at an angle to the scale lines to include more of them, the scale lines at each end of an enlargement are much shorter. I therefore make fewer measurements (perhaps only three) between the scale lines toward the ends. However, the values from each set of two scale lines are averaged separately. These last averages are averaged in turn to get the average distance on the enlargement, in 50 marks/inch engineer ruler units, which represents 10 μm on my Zeiss stage micrometer. Dividing 10 μm by this average distance yields the μms represented by a single ruler unit.

I sometimes use a transparent plastic ruler to make certain that the greatest extent of a subject or the distance between desired points will be measured, and then place the engineer ruler over it. Occasionally a second transparent ruler arranged at right angles to the first is helpful.

Curved distances on the specimen paper enlargements, such as the lengths of curved membranelles, can be measured with a digitizer. I make use of a Numonics 1224 Electronic Digitizer (Numonics Corporation) which is normally utilized to measure nonlinear distances on maps. I transform the digitizer units to μms by employing the scale paper enlargement, much as the 50 marks/inch engineer ruler units are transformed to μms.

Gates and Berger (1976) describe how they use a digitizer directly with slides of fixed and stained specimens under a light microscope. Promising for the future are image display systems, which can view a specimen enlargement through a television camera and digitize the picture to supply measurements. Such a system, by means of its computer, can enhance or highlight certain areas of a picture, or break a picture down into its detailed components. It should be possible to connect the television camera of an image display system to a microscope and "freeze", i.e., inactivate, an image of a living organism on

the system's screen to permit measurement. Books about digital image processing and two recent articles (Cannon and Hunt 1981, Nagy 1983) cover the potential of image display systems.

A morphometric study of a variety of *Mesodinium pulex* (Tamar 1984), a ciliate species referred to as Species II by Tamar, provided an opportunity to check the accuracy of the Measured Scale — Print Method. In this study a number of living specimens were both measured directly with a Wild-Heerbrugg 15 × SK ocular micrometer and photographed in side view for measurement by the Measured Scale — Print Method. Table 1 gives the range of variation, between the values obtained by each of the two measuring techniques, for the group of specimens measured for each attribute. Only four clearly-delimited cell body dimensions are covered in the Table (*Mesodinium pulex* has a body constriction that divides it into an anterior portion, the cone, and a posterior portion, the flask). The mean of the variation is given, as is the standard deviation of the variation within each sample (the sum of the squares about the mean was divided by n, not by the corrective $n - 1$). The mean of the direct measurements obtained for the same group of specimens also is included for each attribute. This permits evaluation of the variation in results in relation to each dimension's extent. That the variations are not consistently greater for the longer dimensions indicates that it is not the measurements made on the scale paper enlargement, but rather difficulties in determining where certain specimen body parts end, which contribute the most to inaccuracy.

Table 1

Variations between direct and Measured Scale — Print Method results

Attribute	Number of specimens	Range of the variation (μms)	Mean of the variation (μms)	Standard deviation of the variation	Mean of the direct measurements (μms)
Body length	11	0.3–1.6	0.58	0.37	22.7
Cone length	8	0.3–2.1	0.96	0.64	9.0
Flask length	8	0.1–1.0	0.43	0.25	14.7
Width of cone base	8	0.1–1.2	0.74	0.32	12.2

The accuracy of the Measured Scale — Print Method could be increased by shooting some negatives at the level of focus of each of the attributes to be measured in a cell. This would be particularly important in the case of larger species. Also, more accurate length values would result if one photographed only specimens oriented at a 90°

angle to the focal axis of the microscope's objective lens. Structures will be more clearly delimited in living cells if exposures are made when the organisms are inactive or show the least motion.

The Measured Scale — Print Method is the most accurate of the three fully-described methods. It offers the advantage that a degree of enlargement most suitable for publication can be chosen. The best enlargements of selected negatives can then be set aside for reproduction while the others are measured. However, in this method excessive time and effort must be devoted to making numerous satisfactory enlargements.

The easier direct measurement of living specimens, if practical, is to be preferred to the above procedure because the investigator can focus up and down to better ascertain the limits of organisms and organelles. In addition, black and white phase-contrast photographs have a gray background which makes it harder to see many organelles.

The Enlarged Negative Method

This technique differs from the previous one only in that paper enlargements are not actually made. Instead, a separate sheet of ordinary white paper is placed under the enlarger for each negative, and the distances to be measured are carefully marked on the paper with a sharp pencil and are labeled.

The Enlarged Negative Method was developed to eliminate the expenditure of time and effort needed to produce many paper enlargements. While this method is somewhat less accurate in each instance than the previous one, it is suited to the exploitation of large numbers of negatives. It should not be used with organelles whose boundaries are more difficult to establish in a projection from a negative.

In the future I will make photomicrographic measurements mainly by the Enlarged Negative Method, but I will employ the Measured Scale — Print Method when this appears to be of value.

Gates and Curds (1979) describe a technique in which points marked on acetate sheets by means of a drawing tube are projected with a projector onto graph paper.

ACKNOWLEDGEMENTS

The three methods extensively described in this paper were developed during research supported in part by Grant 1532 from the Johnson Fund of the American Philosophical Society and by a stipend from the Indiana State University Research Committee.

REFERENCES

Berger J. 1978: Quantification of ciliophoran species descriptions: an appeal to reason. Trans. Am. Microsc. Soc., 97, 121 - 126.

Cannon T. M. and Hunt B. R. 1981: Image processing by computer. Sci. Am., 245, 214 - 225.

Gates M. A. 1978: An essay on the principles of ciliate systematics. Trans. Am. Microsc. Soc., 97, 221 - 235.

Gates M. A. and Berger J. 1976: Morphometric inseparability of *Paramecium primaurelia* and *P. pentaurelia*. Trans. Am. Microsc. Soc., 95, 507 - 514.

Gates M. A. and Curds C. R. 1979: The dargyrome of the genus *Euplotes* (*Hypotrichida, Ciliophora*). Bull. Br. Mus. nat. Hist. (Zool.), 35, 127 - 134.

Kazubski S. L. 1971: Some aspects of applying the photo method for investigating the morphology of ciliates. Acta Protozool., 9, 113 - 114.

Nagy G. 1983: Optical scanning digitizers. Computer, 16, 13 - 24.

Poljansky G. I. 1982: The problem of species and intraspecific variation in Protozoology. Progress in Protozoology, Proc. VIth int. Congr. Protozool., Warsaw 1981, Special Congr. Vol. of Acta Protozool., Part I, 23 - 30.

Tamar H. 1984: *Mesodinium* Species II at Santa Catalina Island, CA. (Abstr.) J. Protozool., 31 (Suppl.), 21A.

NOTICE TO AUTHORS

ACTA PROTOZOOLOGICA is intended as a journal serving for the publication of original papers embodying the results of experimental or theoretical research in all fields of protozoology with the exception of faunistic notices of the local character and purely clinical reports. The papers should be concise and will not be accepted if they have been previously published elsewhere. After acceptance by the Editors papers will be printed in the possibly shortest time.

Papers are accepted in English, French, German and Russian. Every paper should begin with the name and postal address of the laboratory, name and the surname of the author and title in the language of the text. The titles in German and French should be translated into English according to the demands of Current Contents. The paper should be accompanied by synopsis in the language of the text not exceeding 100 words and by short summary in one of 4 languages accepted in the Journal. In the Russian text also the name and the postal address of the laboratory, legends of tables, plates and text illustrations must be translated, the translation of the summary may be somewhat more extensive, and the name of the author should be given additionally also in the Latin characters.

Manuscripts should be doublespaced typescript (30 lines on one side of a sheet) with a normal margin. No elements of the text should be fully typed in capitals nor in spaced set (only underlining with pencil is admittable). In decimal fractions points not commas should be used. The generally accepted abbreviations and symbols are recomended. Nomenclature must agree with the International Code of Zoological Nomenclature, London 1961. The original and one carbon copy of the whole text material should be supplied.

References must be cited in the text indicating only the author and year thus: "Kinosita (1954) found that, etc."

Only all references cited in the text should be listed. The list must be arranged as follows:

Ehret C. F. and Powers E. L. 1959: The cell surface of *Paramecium*. Int. Rev. Cytol., 8, 97 - 133.

Gelei J. von 1939: Das aussere Stützgerustsystem des Parameciumkörpers. Arch. Protistenk., 92, 245 - 272.

Titles of references are given in their original language (not translated). In papers written in English, French or German, the Cyrillic type of the Russian is transliterated according to the international system (ISO Recommendation R 9 September). This regulation is not applied to names if there exists in their traditional spelling. Also author may freely choose in transliteration of his own name. In Russian papers, the Russian references are cited in Cyrillic, the others in the Latin characters, but they must be listed all together in the Latin alphabetical order.

The following material should be supplied on separate sheets 1. the running tittle for the page headlines, 2. tables, 3. legends for text figures, 4. legends for plates. Line-drawings will be published in the text, photographs and raster figures on separate plates. No coloured photographs can be published presently. Lettering on photographs and drawings should be marked in pencil. With no regard to the language of the text, only the Latin lettering, arabic numerals or generally accepted symbols are admittable for marking on illustrations. Numbering of text-figures, plates and tables must be also marked in pencil, as well in the legends as in the text.

Galley proofs are sent to the authors. Authors receive 75 reprints without covers.

In preparation:

S. Serrano, M. T. Fernandez-Galiano and D. Fernandez-Galiano: Morphology of the Anterior Fibrillar System in Two Species of the Genus *Entodinium* (*Ciliophora, Entodiniomorphida*), and its Formation during the Bipartition Process — M. Wolska: *Pseudoentodinium elaphantis* gen. nov., sp. n. from the Order *Entodiniomorphida*. Proposition of the New Family *Pseudoentodiniidae* — D. C. Lennartz: A Preliminary Study of Induction of Macrostomal Development in *Tetrahymena vorax*, V_2S Treated with d-alpha-tocopheryl Succinate — R. Swydan, Z. Darvas and G. Csaba: Stimulation of Phagocytosis by Diazepam in Several Subsequent Generations of the *Tetrahymena* — A. Grębecki: Recovery of Cytoskeletal and Motor Capacities by the Hyaline Blebs Produced by *Amoeba proteus* — E. Wyroba: Effect of β-receptor Antagonist — Dichloroisoproterenol on *Paramecium* Endocytosis — E. Mikołajczyk, W. Nultsch and D.-P. Häder: Chemoaccumulations of the Colorless Flagellate, *Astasia longa*, in the Presence of the Photosensitizer Methylene Blue — W. Lueken and B. Oelgemoller: Graded Morphogenetic Response to Prepairing Cell-Cell Interactions in *Euplotes vannus* — G. Fernandez-Leborans and M. T. Antonio-Garcia: Interaction Lead-zinc in a Natural Community of Protozoans — C. C. Patil and S. D. Amoji — Eugregarine Parasites of *Coleoptera* from North-East Region of Karnataka. IV. *Steinina rodgii* sp. n., Found in the Gut of *Scleron reitteri* Gb. — J. J. Lipa and R. Wohlgemuth: A New Neogregarine Infection of *Prostephanus truncatus* (Horn) (*Coleoptera, Bostrychidae*) Caused by *Mattesia* sp. (*Ophryocystinae, Neogregarinida*) — N. C. Nandi: *Leucocytozoon squamatus* sp. n. from Scaly-bellied Green Woodpecker, *Picus squamatus squamatus* Vigors — N. K. Sarkar: On Two New Species of *Myxobolus* Butschli, 1882 (*Myxozoa: Myxosporea*) from the Fresh Water Fishes of West Bengal, India

Warunki prenumeraty

Cena prenumeraty krajowej: rocznie 560 zł, półrocznie 280 zł

Terminy przyjmowania prenumerat na kraj i za granicę:

— do dnia 10 listopada na I półrocze roku następnego i na cały rok następny,
— do dnia 1 czerwca na II półrocze roku bieżącego.

Prenumeratę na kraj przyjmują i informacji udzielają urzędy pocztowe i doręczyciele na wsi oraz Oddziały RSW „Prasa-Książka-Ruch" w miastach.
Prenumeratę ze zleceniem wysyłki za granicę przyjmuje RSW „Prasa-Książka-Ruch", Centrala Kolportażu Prasy i Wydawnictw, ul. Towarowa 28, 00-958 Warszawa, konto NBP XV Oddział w Warszawie Nr 1153-201045-139-11. Wysyłka za granicę pocztą zwykłą jest droższa od prenumeraty krajowej o 50% dla zleceniodawców indywidualnych i o 100% dla zlecających instytucji i zakładów pracy.

Bieżące i archiwalne numery można nabyć lub zamówić we Wzorcowni Ośrodka Rozpowszechniania Wydawnictw Naukowych PAN, Pałac Kultury i Nauki, 00-901 Warszawa.

Cena zł 140.—

CONTENTS

Państwowe Wydawnictwo Naukowe — Oddział Wrocławski

Wrocławska Drukarnia Naukowa

Indeks 351334