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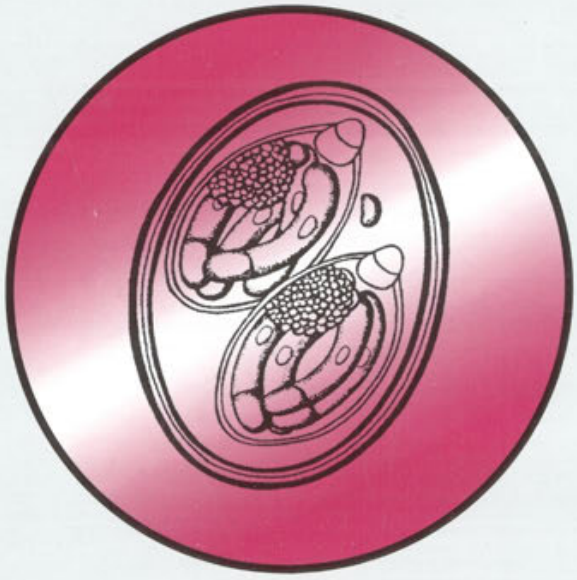

NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY
WARSAW, POLAND

2000

VOLUME 39 NUMBER 4
ISSN 0065-1583

Polish Academy of Sciences
Nencki Institute of Experimental Biology
and
Polish Society of Cell Biology

ACTA PROTOZOOLOGICA
International Journal on Protistology

ACTA PROTOZOOLOGICA appears quarterly.

The price (including Air Mail postage) of subscription to ACTA PROTOZOOLOGICA at 2001 is: US $ 200.- by institutions and US $ 120.- by individual subscribers. Limited numbers of back volumes at reduced rate are available. TERMS OF PAYMENT: check, money oder or payment to be made to the Nencki Institute of Experimental Biology account: 111-01053-401050001074 at Państwowy Bank Kredytowy XIII Oddz. Warszawa, Poland. For matters regarding ACTA PROTOZOOLOGICA, contact Editor, Nencki Institute of Experimental Biology, ul. Pasteura 3, 02-093 Warszawa, Poland; Fax: (4822) 822 53 42; E-mail: jurek@ameba.nencki.gov.pl For more information see Web page **http://www.nencki.gov.pl/ap.htm**).

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This publication is supported by the State Committee for Scientific Research

Desktop processing: Justyna Osmulska, Data Processing Laboratory of the Nencki Institute
Printed at the MARBIS, ul. Kombatantów 60, 05-070 Sulejówek, Poland

Acta Protozool. (2000) 39: 257 - 273

ACTA
PROTOZOOLOGICA

The *Centropyxis aerophila* Complex (Protozoa: Testacea)

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Summary. We show that the varieties *Centropyxis aerophila aerophila*, *C. aerophila sphagnicola*, and *C. aerophila sylvatica*, described by Deflandre in 1929 and later recorded by many workers worldwide, cannot be distinguished with the features provided in the original descriptions, and even not with refined morphometrical data. Very likely, this applies also to most varieties and forms described later. Some of these taxa are obviously extremes of variability clines, while others are distinct but morphometrically inseparable (sibling) species or subspecies, as indicated by concrete morphological traits and/or different ecologies. A representative example is the variety *sylvatica*, which is a distinct species because it has a unique inner pseudostome (ventral lip perforation) produced by material agglutinated on the dorsal and lateral shell wall. This peculiar feature, first recognised by Bonnet and Thomas (1955), is shown by clear light and scanning electron micrographs in the present paper. However, the inner pseudostome is recognisable only on refined investigation. Thus, we suggest to lump taxa, which are morphologically and/or morphometrically difficult to distinguish, into "complexes", as it has been done in sibling ciliate species. This would make testacean alpha-taxonomy more reliable and elegant, especially for field ecologists. Nomenclaturally, the varieties described by Deflandre (1929) are subspecies because he did not unambiguously indicate that the names were proposed for infrasubspecific entities.

Key words: *Centropyxis* spp., *Centropyxis sylvatica*, *Centropyxis aerophila sphagnicola*, morphology, morphometry, nomenclature, sibling species, varieties.

INTRODUCTION

Testate amoebae are common in terrestrial habitats and some authors consider them as the most important soil protozoa for their large biomass and production reaching that of earthworms (Lousier and Parkinson 1984; Foissner 1987, 1999; Schönborn 1992b). Accordingly, they are valuable bioindicators in a variety of terrestrial (Foissner 1987, 1999) and limnetic (Schönborn 1973) habitats. However, testacean ecology has been notoriously plagued by nomenclatural and taxonomical problems because many taxa are superficially described and taxonomy is almost entirely based on shell morphologies (Medioli and Scott 1985, Foissner and Korganova 1995). Recent studies show that shell features are valuable, if supported by sufficient morphometric data and used cautiously. Nonetheless, there is accumulating

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evidence that many of the subspecies, varieties, and forms described fall into the range of natural variability of a species (Wanner 1991, Schönborn 1992a, Wanner and Meisterfeld 1994, Bobrov *et al.* 1995, Foissner and Korganova 1995).

So far, several hundred testacean taxa have been recorded from terrestrial habitats worldwide, and some of these are very likely restricted to such biotopes and/or certain biogeographical regions (Bonnet 1975, Chardez and Lambert 1981, Foissner 1987). One of the most abundant and frequent species, contained in most species lists worldwide, is *Centropyxis aerophila* Deflandre, 1929 and its varieties, *C. aerophila sylvatica* Deflandre, 1929 and *C. aerophila sphagnicola* Deflandre, 1929. The variety *sylvatica* was later classified as a distinct species by Bonnet and Thomas (1955, 1960). They observed that it has an enlarged ventral pseudostome lip extending to the shell's dorsal wall, dividing it in an anterior and posterior compartment connected by a roundish opening in the lip through which the pseudopods can extend. The observations of Bonnet and Thomas (1955, 1960) were confirmed by Lüftenegger *et al.* (1988) and Rauenbusch (1987). Thus, if classical morphological traits are used, *C. sylvatica* must be considered as a distinct species, although the lip perforation is often difficult to recognise. In the fifties and sixties further varieties were described (see Discussion).

Problems in separating the varieties of *C. aerophila* were mentioned by many authors, for instance, Jung (1936), Schönborn (1966, 1975), and Chardez (1979). They became obvious also during our work, and several redescribers could not find better features for distinguishing these varieties, but usually emphasised their high similarity (Schönborn 1966, 1975; Rauenbusch 1987; Lüftenegger *et al.* 1988). On the other hand, the three taxa were mentioned in hundreds of ecological and faunistic studies, including our own (Foissner and Peer 1985, Korganova 1988, Todorov 1993, Aescht and Foissner 1994), and ecologists even provided different autecologies for *C. aerophila aerophila* and *C. aerophila sphagnicola* (Bonnet 1989). Finally, an examination of the original descriptions revealed that they contain very few, if any, features justifying the establishment of distinct taxa.

The purpose of our study was: (i) to investigate whether three taxa can be distinguished with the features provided by Deflandre (1929); (ii) to investigate how previous authors separated Deflandre's taxa; (iii) to confirm *C. aerophila* var. *sylvatica* as a distinct species with the features provided by Bonnet and Thomas (1955, 1960); and (iv) to suggest a practicable solution of the problem acceptable for both taxonomists and ecologists.

MATERIALS AND METHODS

Material

The specimens were isolated from the upper litter and soil layer of an about 110 years old secondary spruce forest (mixed with some *Corylus* and *Tilia*; ground covered mainly by *Carex* spp.) circa 50 km out of Moscow, that is, in the territory of the biogeocoenological experimental station "Malinki" of the Institute of Ecology and Evolution of the Russian Academy of Sciences. Soil was a "dern-podsol", that is, a podsol with a rather distinct, about 10 cm deep, greyish humus layer having 7% total organic matter and pH 5.2. It was covered by an up to 6 cm thick litter layer, depending on microhabitat and season. The sample was taken in August 1999, when soil water content was circa 40%, from the "F-horizon", that is, the upper 2 cm of the dern-podsol and the overlying 2cm of the rather strongly decomposed litter. The sample, which consisted of 5 subsamples taken from an area of about 25 m^2, was air-dried for four weeks and then stored in a paper bag.

Isolation of taxa and sampling protocol

In October 1999, the air-dried sample was rewetted with distilled water and gently shaken some minutes to separate shells from soil particles. *Centropyxis aerophila*-like shells were selected from the soil suspension with a micropipette under a dissecting microscope (x70), using "classical" features, such as shape, shell structure, size, and length:width ratio. Fortunately, the sample contained few other *Centropyxis* species, which could be easily distinguished by size (*C. orbicularis*) or size and shape (*C. constricta*). There was no selection for full (alive), empty (dead), and cystic specimens. The shells were put into glycerol drops to make them clearer and easier to handle while measuring. The following sample program was performed to meet the objectives mentioned in the introduction: (a) 127 specimens were taken at random for basic statistics and considerations; (b) Selected specimens, that is, shells identified with Deflandre's (1929) features either as *C. aerophila aerophila*, *C. aerophila sphagnicola* or *C. aerophila sylvatica*. We did not look for the perforation in the ventral pseudostome lip, which unequivocally separates *C. sylvatica* from *C. aerophila aerophila* and *C. aerophila sphagnicola* (Bonnet and Thomas 1955) in selecting these tests. 30 specimens from each "species" were collected and measured. All specimens (about 50%), which could not unequivocally be assigned to one of the three taxa, were discarded. Selected specimens were also used for scanning electron microscopy, using the technique described by Schönborn *et al.* (1983); (c) With respect to Deflandre's remark on *C. aerophila* var. *sylvatica* "Cette varieté differe de l'espèce type par ses dimensions généralement supérieurs" and because the inner pseudostome is difficult to recognise, we made the following experiment: 30 large (length ≥ 85 µm) *C. aerophila sylvatica*-

like looking shells were compared with 30 small (length 60-75 µm) shells more similar to either *C. aerophila aerophila* or *C. aerophila sphagnicola*.

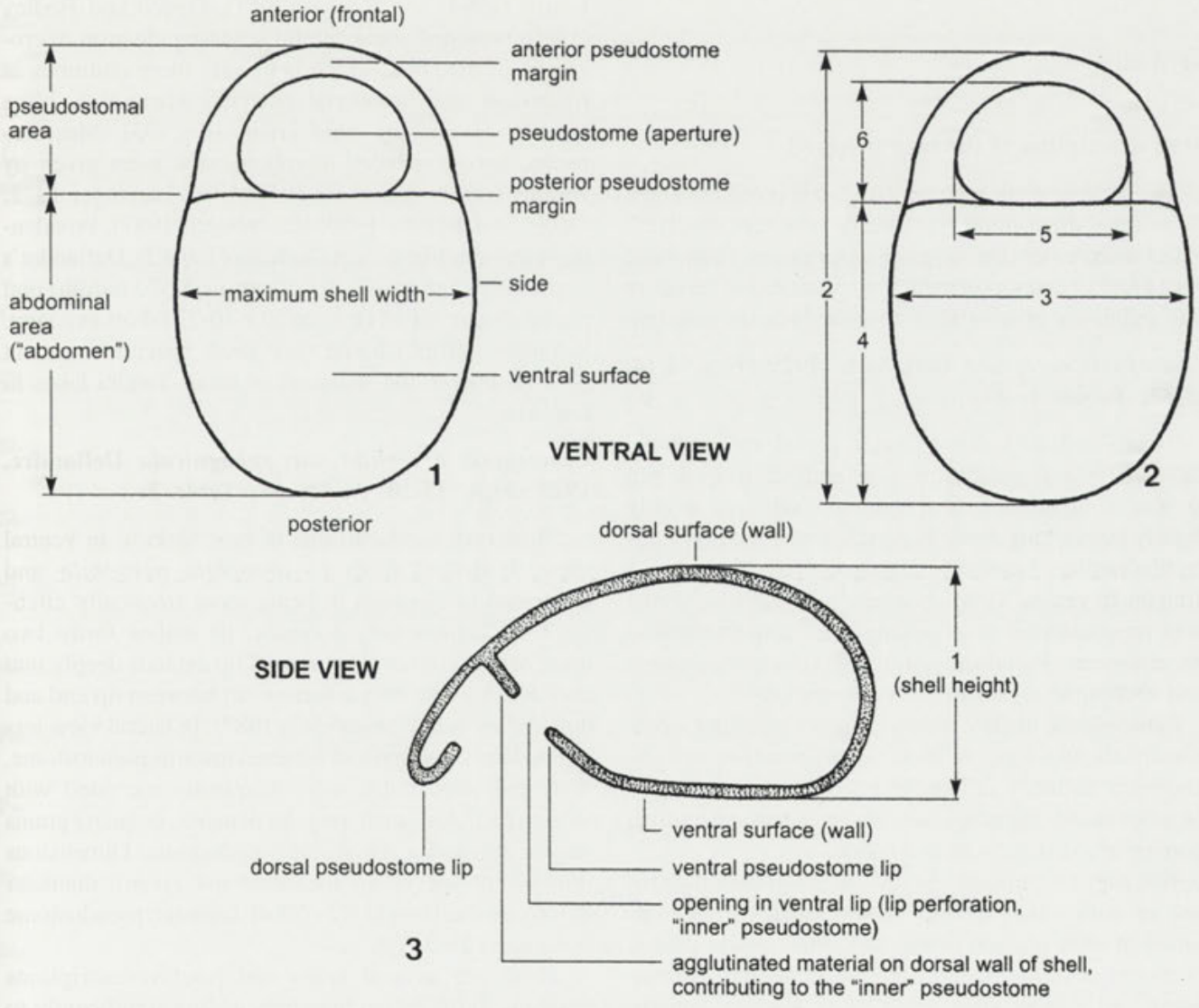
Measurements and statistics

Six classical features were measured in series (a) and (b), namely shell height (character 1 in Figure 3); shell length (character 2 in Figure 2); maximum shell width (3); "abdomen length" (4), that is, the distance between posterior margin of the pseudostome and posterior shell end; long axis of pseudostome (5), and short axis of pseudostome (6). Furthermore, ratios between characters 2 and 4 and 3 and 4 were calculated because they provide some overall measure for shell shape. All measurements were made in glycerinated specimens at middle magnification (x400), where a micrometer unit was 5 µm; accordingly, values were usually rounded up in 5 µm steps.

Statistics and diagrams were prepared according to textbooks using the computer program BIOMstat, version 3.3. As most variables were not normally distributed, the parameter-free analysis of variance (ANOVA) of Kruskal-Wallis was applied. To increase n (sample number) in some tests, we put together measurements from series (a) and (b).

Figures and terminology

Line drawings were taken from the literature because our investigations did not show significant new details. All micrographs were taken from the Moscow material under bright field illumination or in



Figs. 1-3. Terminology and morphometrics (numbers 1 - 6 in Figs. 2 and 3) used in this study.

the scanning electron microscope. Specimens usually must be strongly tilted and very carefully orientated to show the inner pseudostome of *C. sylvatica*.

Terminology of testate amoebae is rather confused and many terms, for instance, the so-called visor, vestibulum, and pseudostome are used in different ways by different or even the same authors (Bonnet 1964, 1975). Thus, we use "simple" terms, shown in Figures 1 and 3, for the scope of the present paper.

Permanent slides

The specimens used, and several others, were embedded in a drop of glycerol sealed by a rather thick ring of artificial resin (Histofluid, Merck Company, Germany) to prevent shells from desiccation and distortion by the cover glass pressure. All slides and the SEM-preparations have been stored in the Oberösterreichische Landesmuseum in Linz (LI).

RESULTS

Brief description of the taxa involved

To understand our argumentation it is crucial to know the original description and status of the taxa involved. Thus, we provide the original descriptions (translated from French) and supplement them with recent literature data and/or our observations from the Moscow material.

Centropyxis aerophila Deflandre, 1929 (Figs. 4-14, 28-33; Tables 1, 2)

"Test small, abdomen globular, dorsal wall strongly flattened towards pseudostome. Ventral outline oval with circular or slightly elliptical abdomen, sides not or only slightly converging towards pseudostome having semicircular outline. Sides only slightly curved, often almost straight. In ventral view, abdomen seemingly separated from pseudostomal area, looking like being attached to the abdomen. Pseudostomal region more transparent than abdominal region in resin preparations.

Pseudostome usually semicircular with straight, occasionally slightly concave (Figs. 7, 10) posterior margin. Abdomen distinctly inflated in lateral view, but steeply flattens towards pseudostome, whose anterior margin is more or less distinctly turned inside.

Test entirely chitinous, finely and irregularly punctuated or with rather distinct flakes, carries sometimes brown or dark organic debris and small quartz grains, colourless or yellowish, occasionally rather dark brownyellow. At certain sites, shell often appears entirely covered with foreign particles.

Pseudopods and nucleus not yet observed. Dimensions (number of specimens measured not given): overall size 53 - 85 x 42 - 66 µm, shell height about 2/3 of length; pseudostome 21 - 28 x 15 - 21 µm".

There are several notes and brief redescriptions available in the recent literature, adding significantly to the morphology of the shell and its inhabitant, but leaving untouched the basic features described by Deflandre (1929). Our selected material from Russia also matches Deflandre's description (Figs. 28-33; Table 2). No cement structures are recognisable in the scanning electron microscope.

Lobose pseudopods and their movements, as well as two contractile vacuoles, were described in detail by Bartoš (1954) and Bonnet (1961). Ogden and Hedley (1980) provided some helpful scanning electron micrographs of the shell, which is usually more chitinous in freshwater than terrestrial habitats, where it is often entirely covered by sand grains (Fig. 33). Measurements, but no detailed morphometrics, were given by various authors (Decloitre 1954, 1956; Laminger 1972; Ogden and Hedley 1980; Rauenbusch 1987), broadening, however, like our detailed data (Table 2), Deflandre's limits only slightly: 47 - 93 (length) x 32-77 (width) µm; pseudostome: 19-34 (long axis) x 10-22 (short axis) µm. Schönborn (1966) found very small specimens (about 30 x 30 µm) in the sediment of small Tundra lakes in Lapland.

Centropyxis aerophila var. *sphagnicola* Deflandre, 1929 (Figs. 15-20, 34-40, 58; Table 2)

"Test very similar to that of type species. In ventral view, it differs from *C. aerophila aerophila* and *C. aerophila sylvatica* in being more frequently circular. Pseudostome very eccentric, its outline forms two more or less convex arcs, ventral lip extends deeply into shell leaving free only a narrow slit between lip end and dorsal shell wall (Rauenbusch 1987). In lateral view less inflated than type and less flattened towards pseudostome. Wall chitinous, flaky, more frequently incrusted with foreign particles, small irregular platelets, or quartz grains mostly at anterior margin of pseudostome. Dimensions (number of specimens measured not given): diameter 49-66 µm, shell height 1/2 - 3/5 of diameter; pseudostome (long axis) 25-37 µm".

There are several notes and brief redescriptions available in the recent literature, adding significantly to the morphology of the shell and its inhabitant, but leaving

Table 1. Morphometric data on *Centropyxis aerophila*. Upper line: 127 randomly selected specimens (including varieties and *C. sylvatica*) identified with the features given by Deflandre (1929). Lower line: the 127 specimens mentioned above and the 90 specimens from Table 2 pooled

Characteristics[1]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Shell, height (1)	42.3	43.0	7.8	0.7	18.5	15.0	65.0	127
	43.5	44.0	8.3	0.6	19.0	15.0	65.0	217
Shell, length (2)	70.8	70.0	10.2	0.9	14.4	51.0	110.0	127
	71.2	70.0	9.7	0.7	13.6	51.0	110.0	217
Shell, width (3)	66.1	65.0	9.9	0.9	14.9	47.0	95.0	127
	66.6	65.0	10.2	0.7	15.3	47.0	95.0	217
Abdomen, length (4)	45.0	45.0	6.6	0.6	14.7	30.0	65.0	127
	45.4	45.0	6.5	0.4	14.3	30.0	65.0	217
Pseudostome, long axis (5)	29.6	30.0	5.6	0.5	18.8	16.0	47.0	127
	29.6	30.0	5.6	0.4	19.0	16.0	47.0	217
Pseudostome, short axis (6)	16.3	15.0	3.8	0.3	23.5	10.0	27.0	127
	16.2	15.0	3.8	0.3	23.4	10.0	27.0	217
Shell length:abdomen length, ratio	1.6	1.6	0.1	0.0	7.1	1.3	1.9	127
	1.6	1.6	0.1	0.0	6.5	1.3	1.9	217
Shell width:abdomen length, ratio	1.5	1.5	0.2	0.0	11.5	1.0	2.0	127
	1.5	1.5	0.2	0.0	11.0	1.0	2.0	217

[1]Numbers in parenthesis designate features as shown in Figs. 2 and 3. Measurements in µm. CV- coefficient of variation in %; M - median; Max - maximum; Min - minimum; n - number of specimens investigated; SD - standard deviation; SE - standard error of mean; $\bar{x}$ - arithmetic mean

Table 2. Morphometric data on 30 " selected specimen " each (see Materials and Methods section) of *Centropyxis aerophila aerophila* (CAA), *C. aerophila sphagnicola* (CAS), and *C. aerophila sylvatica* (CS), identified with the features given by Deflandre (1929)

Characteristics[1]	Species	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Shell, height (1)	CAA	36.6	36.0	5.7	1.0	15.6	25.0	45.0	30
	CAS	44.9	45.0	3.8	0.7	8.4	37.0	51.0	30
	CS	54.1	55.0	4.5	0.8	8.3	45.0	62.0	30
Shell, length (2)	CAA	66.6	65.0	4.5	0.8	6.8	55.0	80.0	30
	CAS	66.2	65.0	4.5	0.8	6.7	60.0	75.0	30
	CS	82.8	82.0	4.4	0.8	5.3	75.0	90.0	30
Shell, width (3)	CAA	56.4	55.0	4.5	0.8	8.1	50.0	70.0	30
	CAS	65.6	65.0	5.1	0.9	7.8	57.0	76.0	30
	CS	79.5	79.5	4.8	0.9	6.0	74.0	90.0	30
Abdomen, length (4)	CAA	42.2	42.0	3.9	0.7	9.3	35.0	50.0	30
	CAS	42.8	41.5	4.1	0.8	9.7	35.0	54.0	30
	CS	53.2	52.0	3.4	0.6	6.5	50.0	60.0	30
Pseudostome, long axis (5)	CAA	24.9	25.0	3.3	0.6	13.2	20.0	30.0	30
	CAS	28.9	26.5	4.6	0.8	15.7	25.0	37.0	30
	CS	35.2	35.0	3.6	0.7	10.3	27.0	42.0	30
Pseudostome, short axis (6)	CAA	14.3	15.0	2.6	0.5	18.3	10.0	20.0	30
	CAS	13.7	14.5	2.4	0.4	17.4	10.0	19.0	30
	CS	19.8	20.0	2.6	0.5	13.2	16.0	25.0	30
Shell length:abdomen length, ratio	CAA	1.6	1.6	0.1	0.0	6.5	1.4	1.9	30
	CAS	1.6	1.6	0.1	0.0	4.5	1.4	1.7	30
	CS	1.6	1.5	0.1	0.0	5.0	1.5	1.8	30
Shell width:abdomen length, ratio	CAA	1.3	1.3	0.1	0.0	11.0	1.1	1.6	30
	CAS	1.5	1.5	0.1	0.0	7.9	1.3	1.9	30
	CS	1.5	1.5	0.1	0.0	7.2	1.3	1.7	30

[1]Numbers in parenthesis designate features as shown in Figs. 2 and 3. Measurements in µm. CV - coefficient of variation in %; M - median; Max - maximum; Min - minimum; n - number of specimens investigated; SD - standard deviation; SE - standard error of mean; $\bar{x}$ - arithmetic mean

untouched the basic features described by Deflandre (1929). Our selected material from Russia also matches Deflandre's description (Figs. 34-40; Table 2). No cement structures are recognisable in the scanning electron microscope.

Lobose pseudopodia, which form rapidly and eruptively, were described by Bonnet (1963), who provided also detailed ecological data (Bonnet 1989). Detailed morphometrics and helpful scanning electron micrographs were published by Lüftenegger *et al.* (1988) and Rauenbusch (1987). Scattered measurements were provided by other authors (Laminger 1972, Chardez 1979), broadening, however, Deflandre's limits only slightly: shell diameter 47-75 µm, shell height 30-45 µm; pseudostome 18-44 x 9-23 µm. Decloitre (1956) and Rosa (1971) observed very small specimens with a length of 30-36 µm.

Centropyxis aerophila var. *sylvatica* Deflandre, 1929 (Figs. 21-27, 41-61; Table 2)

"This variety differs from the type species in that it is usually larger and more robust and the anterior pseudostome margin has a well-recognisable double contour.[1] The pseudostome is elliptical, more often it is semicircular (again, this is unclear and thus we provide the original text: "la bouche elle-même est elliptique plus souvent que semicirculaire"). In ventral view, the shell is usually exactly ("régulière") elliptical or subcircular. The sides are more strongly curved than in the type.

The shell is chitinous as in the type; usually, it carries a certain amount of small, angular quartz grains towards the posterior end. It appears more punctuated and flaky than the type and is reinforced by fine, transparent, irregular platelets and very small quartz grains. Dimensions (number of specimens measured not given): 68-102 x 63-85 µm; pseudostome 32-53 x 17-30 µm".

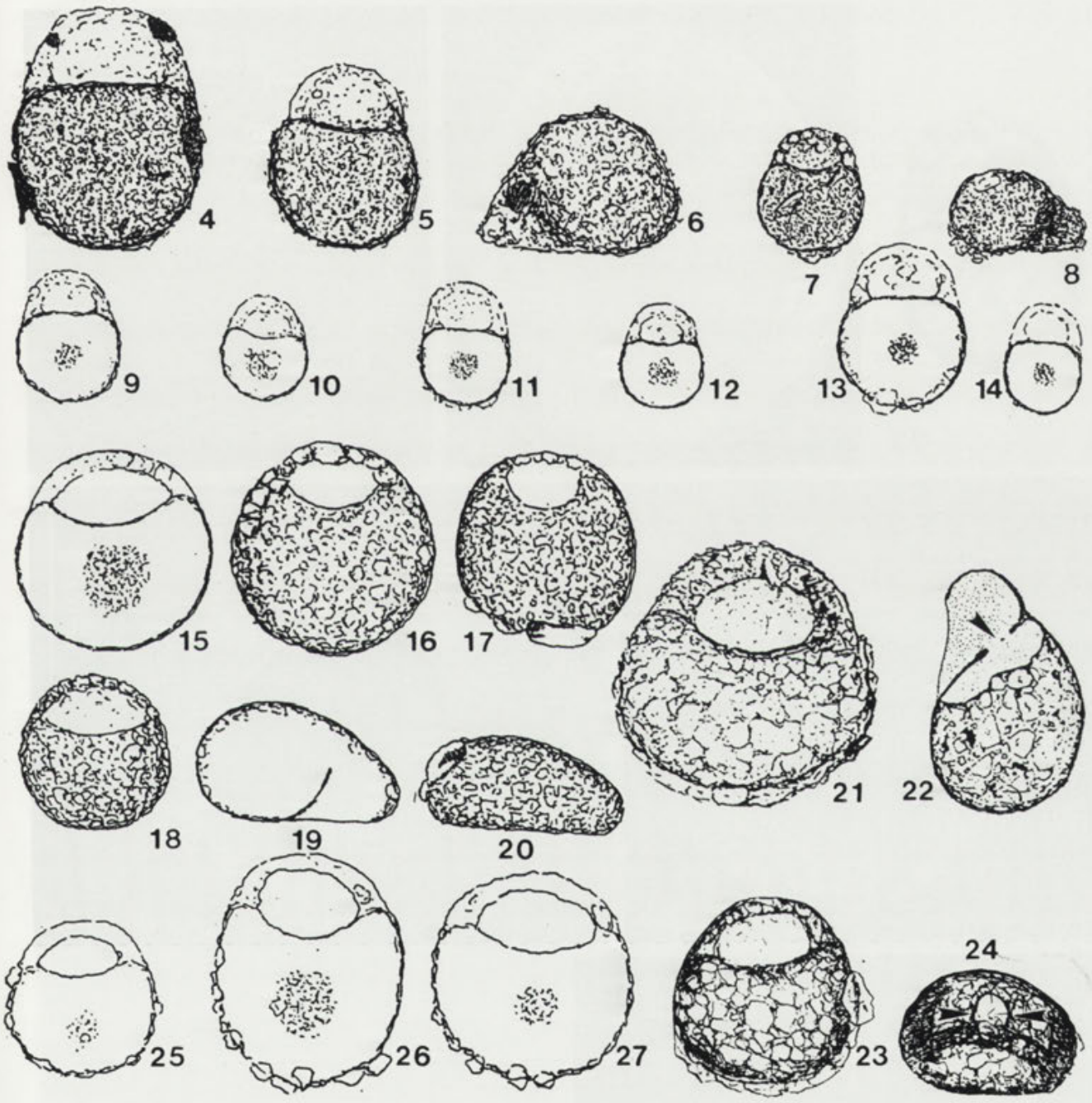
There are several notes and redescriptions available in the recent literature, significantly modifying Deflandre's original description. Most important is the observation by Bonnet and Thomas (1955) that the ventral pseudostome lip [called "membrane ventrale" by Bonnet and Thomas (1955) and "diaphragma" by Lüftenegger *et al.* (1988) and Rauenbusch (1987)] extends to the dorsal shell wall, dividing the test in a small "pseudostomal chamber" and a large "abdominal chamber" connected with each other by a more or less large opening (lip perforation) through which the pseudopods can extend. The observations of Bonnet and Thomas (1955) were confirmed by Lüftenegger *et al.* (1988) and Rauenbusch (1987). We put much effort in observing and documenting these details in our material and thus can, for the first time, clearly show the lip perforation which, in fact, is an inner pseudostome (Figs. 41-61).

The 30 large shells, which should have been *C. aerophila sylvatica* according to Deflandre's criteria (see "isolation of taxa and sampling protocol"), were very difficult to investigate because they were composed of rather large quartz grains making them opaque and refractive. Thus, nothing definite could be seen; only in five to six out of the 30 specimens there was a bright spot, possibly the lip perforation, at the right place. In contrast, we could see the lip perforation in 22 out of the 30 small, transparent shells, when they were optimally orientated, that is, viewed frontally; if debris adhered to the pseudostome, visibility of the lip perforation decreased. The transparent specimens showed that the site where the lip internally abuts to the dorsal shell wall is often marked by an inconspicuous groove (Figs. 52, 61), less distinct than those shown by Lüftenegger *et al.* (1988).

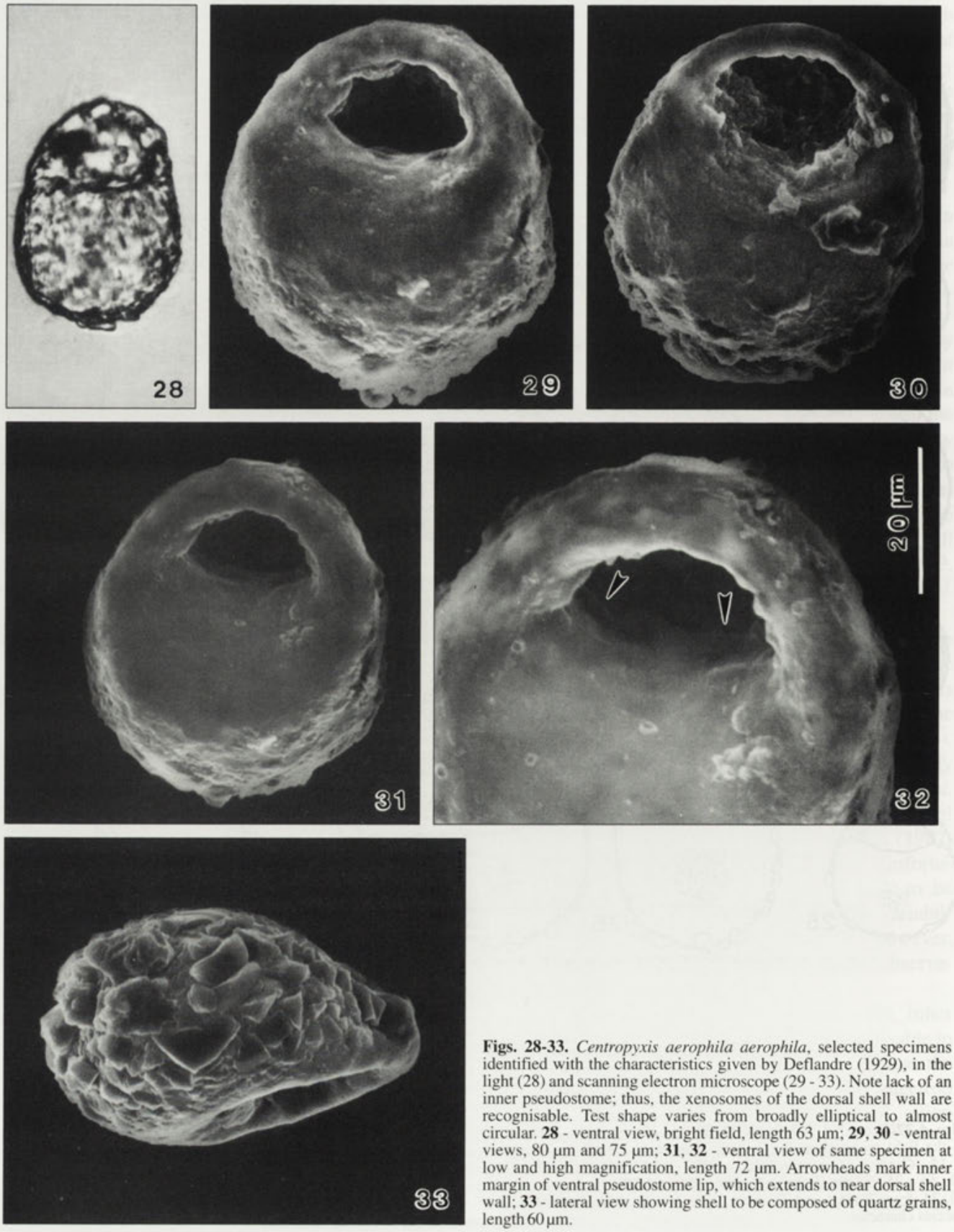
The lip perforation has a mean size of 17 x 11 µm (10-25 x 8-15 µm, n 22) in specimens with a pseudostome size of 28 x 15 µm (20-38 x 10-25 µm). Thus, it is elliptical and rather large; the margin is uneven. In the population investigated by Bonnet and Thomas (1955), the perforation is roundish with a diameter of only 5-8 µm. In the specimens studied by Lüftenegger *et al.* (1988), the lip perforation is very similar to our material having a mean size of 18 µm. In Rauenbusch's (1987) specimens the perforation is sickle-shaped (unfortunately, the scanning micrographs are too dark to be entirely convincing). Thus, there is considerable variability in the size and shape of the lip perforation; however, some of the variability might be caused by the observation problems described above.

In the scanning electron microscope, the inner pseudostome (lip perforation) was better recognisable in the large than in the small specimens (in contrast to the light microscope, see above). The scanning electron microscope investigations suggest that the lip perforation

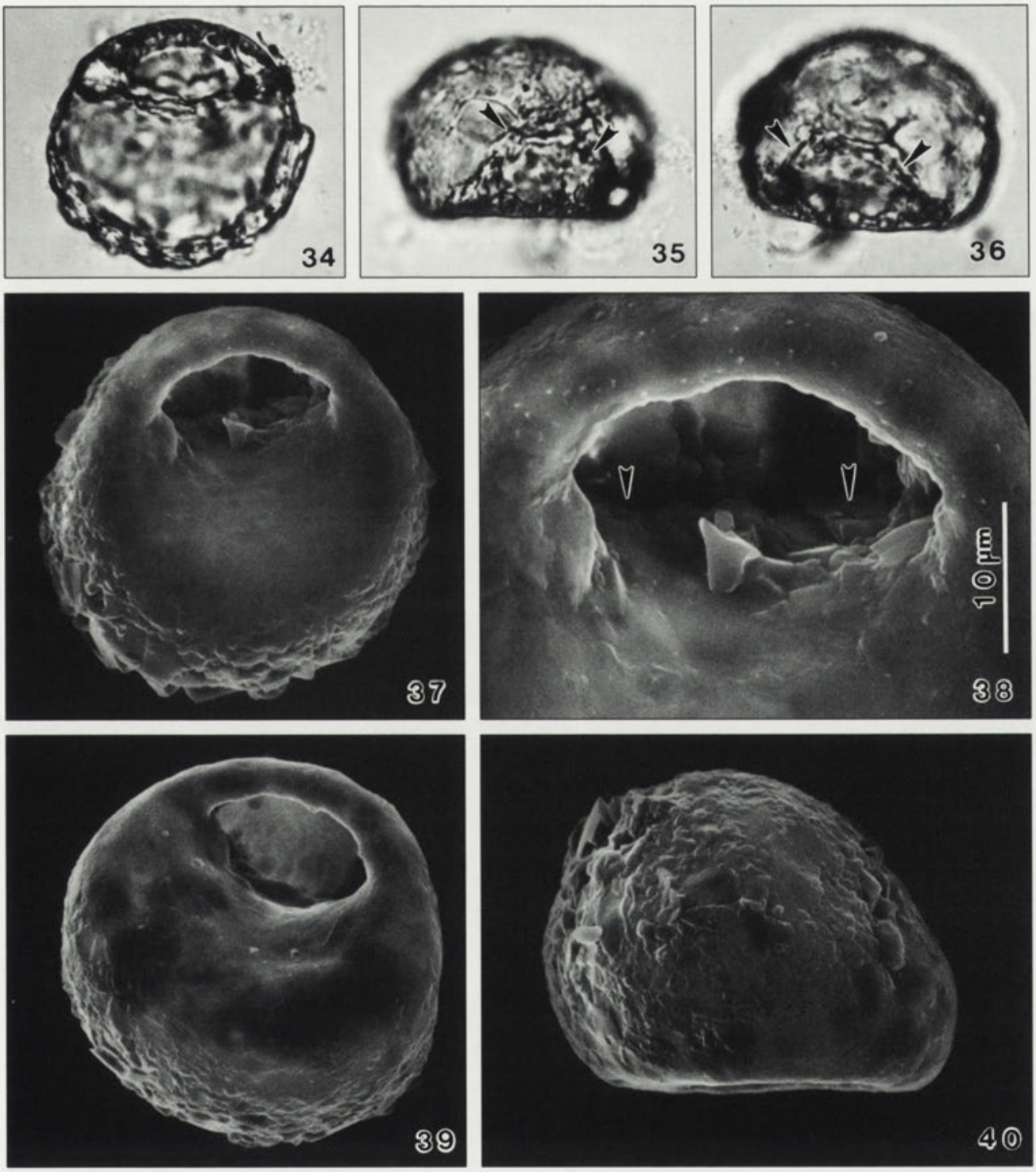
[1] "... vers la bouche dont le bord extérieur montre un double contour tres net". Very likely, Deflandre (1929) describes a dorsal pseudostome lip as shown in Figure 3. However, such a lip has not yet been convincingly shown and we could not find it in our material.



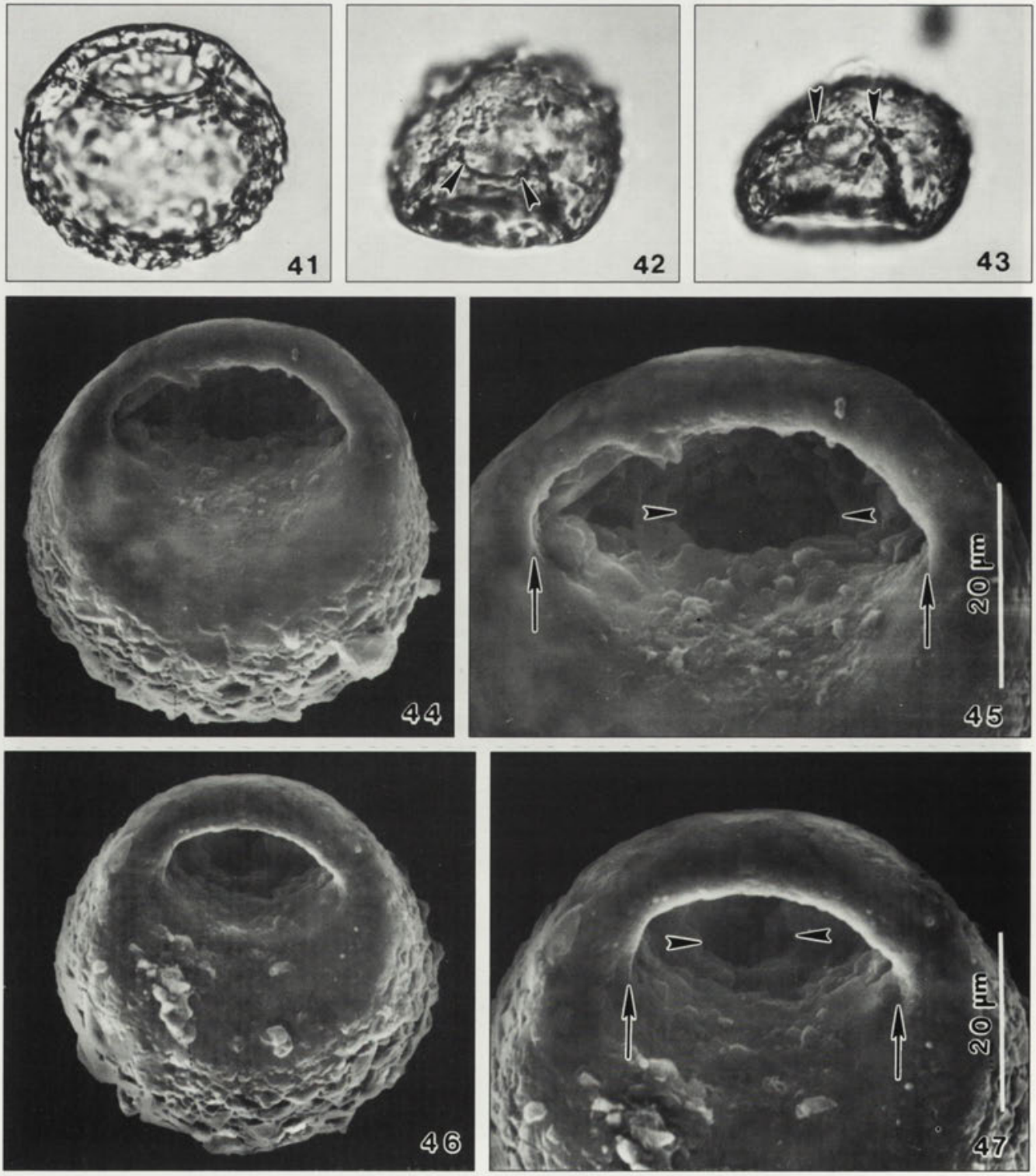
Figs. 4-27. Ventral (4, 5, 7, 9 - 18, 21, 23, 25 - 27), lateral (6, 8, 19, 20, 22), and frontal (24) views of shells of the *Centropyxis aerophila* complex in the light microscope (bright field illumination). All figures are reproductions (Xerox copies) from the original literature cited below. **4- 14** - *Centropyxis aerophila aerophila*, size 53 - 85 x 42 - 66 µm (from Deflandre 1929); **15-20** - *Centropyxis aerophila sphagnicola*, size 49 - 66 x 25 - 37 µm (from Deflandre 1929); **21-27** - *Centropyxis sylvatica*, size 65 - 105 x 60 - 87 µm (21, 22, from Bonnet and Thomas 1960; 23, 24, from Bonnet and Thomas 1955; 25 - 27, from Deflandre 1929). Arrowheads in Figs. 22 and 24 mark inner pseudostome, the main species character.



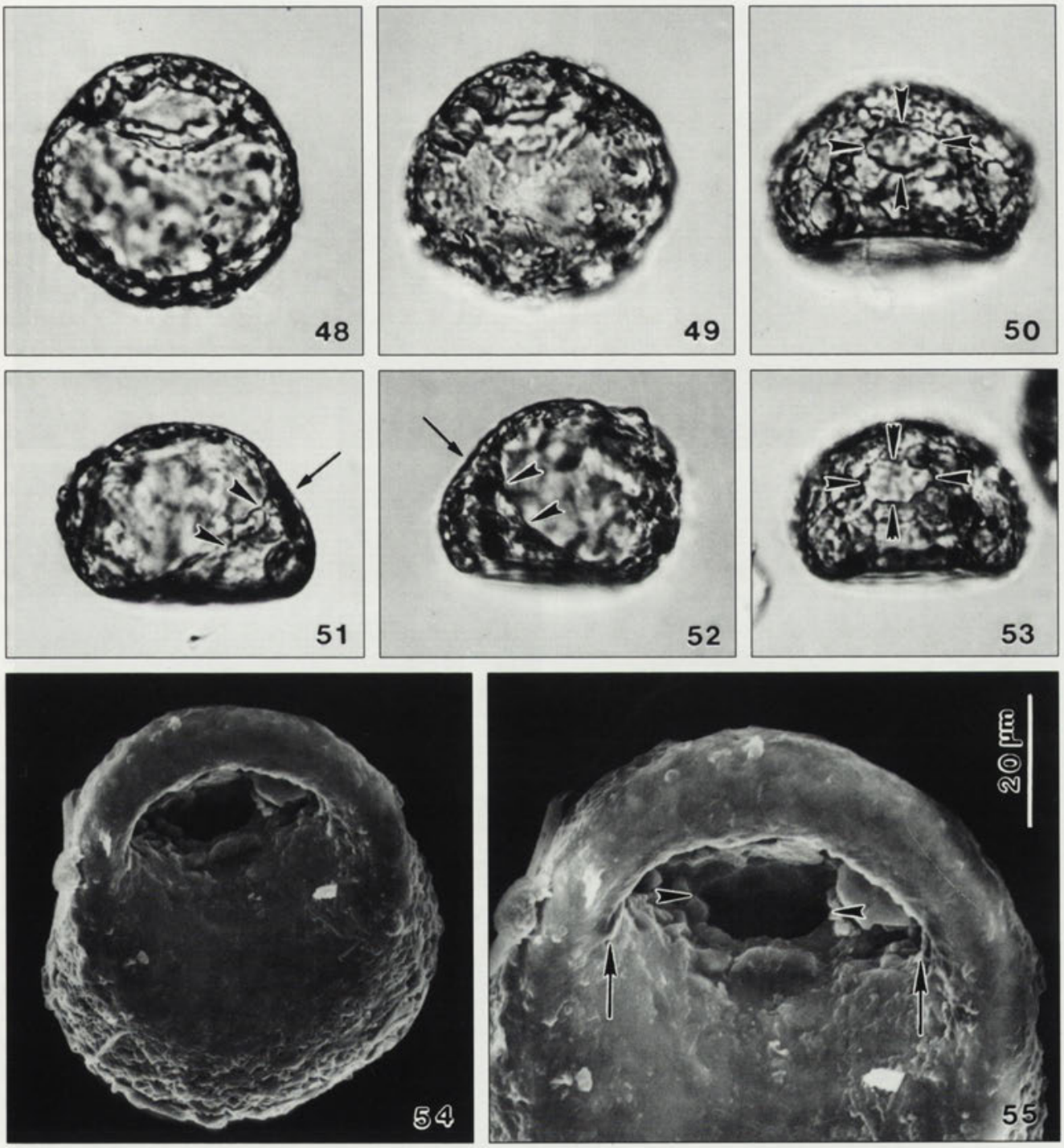
Figs. 28-33. *Centropyxis aerophila aerophila*, selected specimens identified with the characteristics given by Deflandre (1929), in the light (28) and scanning electron microscope (29 - 33). Note lack of an inner pseudostome; thus, the xenosomes of the dorsal shell wall are recognisable. Test shape varies from broadly elliptical to almost circular. **28** - ventral view, bright field, length 63 µm; **29**, **30** - ventral views, 80 µm and 75 µm; **31**, **32** - ventral view of same specimen at low and high magnification, length 72 µm. Arrowheads mark inner margin of ventral pseudostome lip, which extends to near dorsal shell wall; **33** - lateral view showing shell to be composed of quartz grains, length 60 µm.



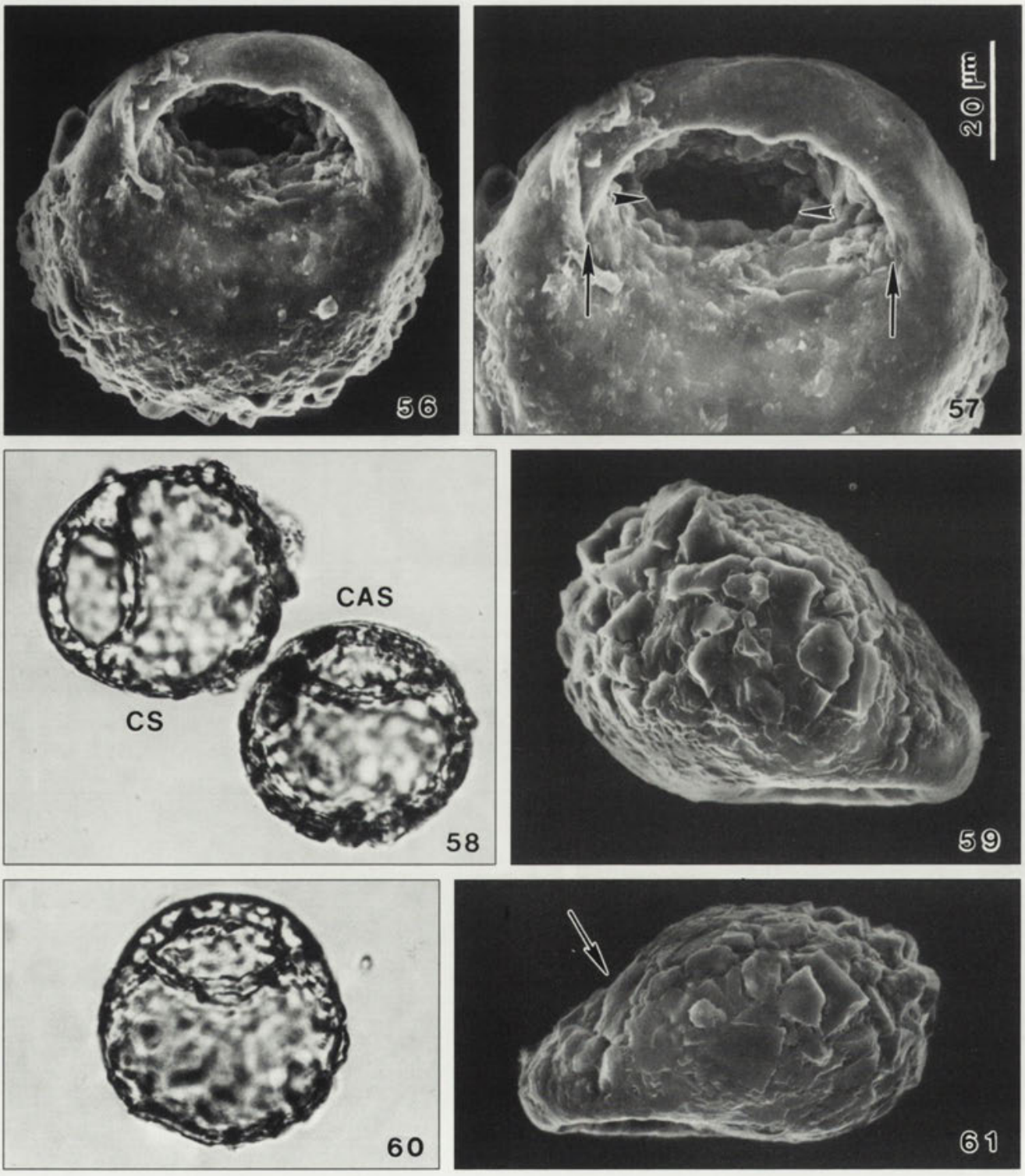
Figs. 34-40. *Centropyxis aerophila sphagnicola*, selected specimens identified with the characteristics given by Deflandre (1929), in the light (34 - 36) and scanning electron microscope (37 - 40). Note lack of an inner pseudostome; thus, the xenosomes of the dorsal shell wall are recognisable. The shells are circular or slightly broader than long and have agglutinated small quartz grains mainly in posterior portion. **34-36** - same specimen in ventral and frontal views, size 68 x 68 x 46 µm. Arrowheads mark ventral pseudostome lip; **37**, **38** - ventral view of same specimen at low and high magnification, length 61 µm. Arrowheads mark inner margin of ventral pseudostome lip, which extends to near dorsal shell wall; **39** - ventral view, length 63 µm; **40** - frontolateral view, length 58 µm.



Figs. 41-47. *Centropyxis sylvatica*, selected small ("transparent") specimens identified with the characteristics given by Deflandre (1929), in the light (41-43) and scanning electron microscope (44-47). Note that specimens are circular or slightly broader than long and have agglutinated small quartz grains mainly in posterior portion. **41-43** - same specimen in ventral view and two frontal focal plans showing lower and upper margin of inner pseudostome (arrowheads), size 68 x 74 x 53 µm; **44-47** - ventral views at low and high magnification showing the outer (arrow) and inner (arrowheads) pseudostome, length 61 µm and 66 µm. The inner pseudostome is formed by agglutinated material on the dorsal and lateral shell wall



Figs. 48-55. *Centropyxis sylvatica*, selected small ("transparent") specimens (48-53) and a large opaque specimen (54, 55) identified with the characteristics given by Deflandre (1929), in the light (48-53) and scanning electron microscope (54, 55). Arrow in Figs. 51 and 52 marks minute groove, where the inner pseudostome abuts to the dorsal shell wall. **48-51** - same specimen (size 68 x 74 x 46 µm) in ventral view, where the inner pseudostome is not recognisable; in oblique ventral view, where the pseudostome becomes minute; in frontal view, where the broadly elliptical inner pseudostome is well recognisable (arrowheads); and in lateral view, where the lip perforation (inner pseudostome, arrowheads) is difficult to recognise; **52**, **53** - lateral and frontal view of another specimen showing the inner pseudostome (arrowheads); **54**, **55** - ventral view of same specimen at low and high magnification showing impressively the outer (arrow) and inner (arrowheads) pseudostome, length 94 µm. The inner pseudostome is obviously made of small xenosomes attached to the dorsal wall of the shell and the lateral walls of the outer pseudostome



Figs. 56-61. *Centropyxis sylvatica*, selected small ("transparent") specimens (58-60) and large opaque specimens (56, 57, 61) identified with the characteristics given by Deflandre (1929), in the light (58, 60) and scanning electron microscope (56, 57, 59, 61). **56**, **57** - ventral view of same specimen (length 87 µm) at low and high magnification showing the outer (arrow) and inner (arrowheads) pseudostome; **58**, **60** - ventral views of *C. sylvatica* (CS) and *C. aerophila sphagnicola* (CAS), size of specimen shown in Fig. **60**, 61 x 65 x 43 µm; **59**, **61** - lateral views showing shells to be composed mainly of quartz grains, length 73 µm and 88 µm. Arrow marks minute depression, where the inner pseudostome attaches to the dorsal shell wall (cp. Figs. 51, 52)

is mainly brought about by accumulation of agglutinated material on the dorsal and lateral shell wall. Thus, the ventral lip is possibly not "perforated" in the strict sense of the word; this is also indicated by its general appearance, which is as in the other varieties (Figs. 32, 38). In the scanning electron microscope, a lip perforation was seen in several small (transparent; Figs. 41-47, 48-53) and large ("typical") *C. sylvatica* specimens (Figs. 54, 55, 56, 57), showing that the species cannot be recognised by size.

Lobose pseudopods and their movements, as well as two contractile vacuoles, were described by Bonnet (1961) in *C. sylvatica* var. *minor*. Rauenbusch (1987) and Lüftenegger *et al.* (1988) provided some helpful scanning electron micrographs showing that shell wall structure highly depends on the substrate the organisms live. Lüftenegger *et al.* (1988) provided also detailed morphometrics showing that pseudostome features are more variable than the length and width of the shell. Scattered measurements were given by other authors (Bonnet and Thomas 1955, Rosa 1971), broadening, however, Deflandre's limits only slightly: 56-113 (length) x 47-100 (width) x 45-68 (height) µm; pseudostome 23-55 (long axis) x 20-32 (short axis) µm.

Morphometry

Basic statistics show that most variables have usual coefficients of variation and the number of specimens investigated is sufficient because mean and median hardly change if 127 or 217 specimens are analysed (Table 1). Of course, variation is distinctly lower in the selected specimens (Table 2). Only a few of the variables measured are normally distributed, viz. shell width, ratio shell length: abdomen length, and ratio shell width: abdomen length. All other features are slightly skewed to the left.

Analysis of variance: If the 30 selected specimens of each are compared (all intermediate shells removed, see Method section!), all variables tested (length, width...) are significantly different ($p \leq 0.001$), except the ratio shell length: abdomen length, that is, the three taxa can be clearly distinguished. If the 127 randomly chosen specimens are compared with the 30 selected specimens of either *C. aerophila aerophila* or *C. aerophila sylvatica*, highly significant differences ($p \leq 0.001$ to $p \leq 0.05$) still occur in most variables, except for the ratios; in contrast, only shell height, shell length, short pseudostome axis, and the length: width ratio are different ($p \leq 0.05$) in *C. aerophila sphagnicola*, indicating that this variety is intermediate between the two others. Finally, when the 30 selected specimens of each are pooled (= 90 specimens) and compared with the 127 randomly chosen specimens, all variables become indistinguishable ($p \geq 0.05$).

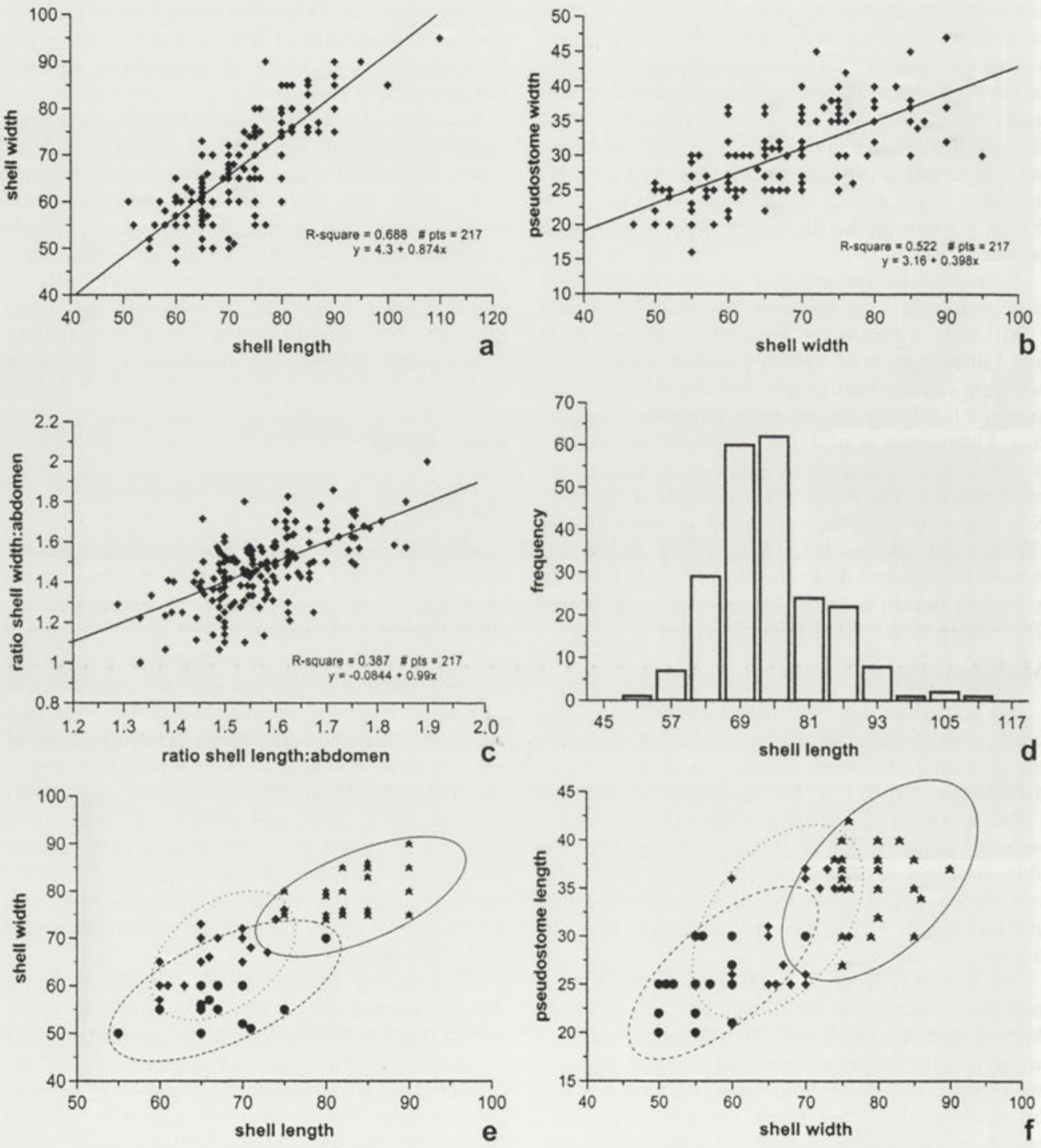
Frequency distributions and relationships between variables (only some representative examples each are shown, Figs. 62a-f): Frequency distributions show curves with a single peak (Fig. 62d). Likewise, rather homogenous clusters are formed in the randomly chosen specimens, when variables are plotted against each other (Figs. 62a-c). In contrast, two distinct clusters are usually formed, if the selected specimens are plotted (Figs. 62e, f): one contains *C. aerophila aerophila* and *C. aerophila sphagnicola*, the other *C. aerophila sylvatica*.

DISCUSSION

Morphometry

Although species cannot be proven mathematically, some basic statistics are often useful to distinguish them more properly. As concerns the present material, neither the coefficients of variation (Table 1) nor frequency distributions (Fig. 62d) and relationships between the variables tested (Figs. 62a - c) give any indication that the randomly chosen specimens consist of more than one species. Variation coefficients (Tables 1, 2) are of the same order of magnitude as in multicellular organisms (Mayr 1975) and other protozoans, e.g. ciliates (Foissner 1984, 1993), and as in testate amoebae in general (Lüftenegger *et al.* 1988, Wanner 1991, Foissner and Korganova 1995). Only the pseudostome variables have coefficients higher than 20%; however, this seems to be a general feature of testacean shells (Lüftenegger *et al.* 1988, Wanner 1991).

However, the situation changes drastically in the selected material, that is, when all intermediate specimens, which could not unequivocally assigned to one of Deflandre's varieties, are excluded (Table 2). Then, three taxa can be distinguished by analysis of variance and at least two in scatter diagrams (Figs. 62e, f), just as different species were compared (Lüftenegger *et al.* 1988). *Centropyxis aerophila sylvatica* is separated by its larger dimensions, while *C. aerophila aerophila* and *C. aerophila sphagnicola* are distinguished mainly by the shell proportions (Table 2): the former is broadly elliptical (66.6 x 56.4 µm), the latter almost circular (66.2 x 65.6 µm); furthermore, the long pseudostome axis is distinctly larger in *C. aerophila sphagnicola*



Figs. 62a - f. Representative examples from the measurements (for details, see chapter on morphometry). **62a - c** - when all (217) specimens of *Centropyxis aerophila aerophila*, *C. aerophila sphagnicola* and *C. sylvatica* are pooled and the main characteristics plotted, rather homogenous clouds of dots are formed, indicating that the taxa are morphometrically inseparable; **62d** - likewise, frequency distributions of the 217 specimens do not provide any indication that several taxa are mixed; **62e, f** - when the 30 selected (all intermediates discarded, see Materials and Methods) specimens each are plotted, at least two distinct clouds are formed, one contains *C. sylvatica* (*), the other *C. aerophila aerophila* (•) and *C. aerophila sphagnicola* (♦). We hypothesise that most authors distinguished Deflandres varieties by a similar (mental) selection process

than in *C. aerophila aerophila* (28.9 vs. 24.9 µm). However, such result is expected and can be obtained with most living things, even with man, when intermediate specimens are removed. In fact, the experiment was performed specifically with the goal of obtaining information as to how previous authors probably distinguished Deflandre's varieties (see next but one chapter).

Centropyxis sylvatica is a distinct morphospecies

We agree with Bonnet and Thomas (1955) that the lip perforation of *C. sylvatica* is difficult to observe and usually recognisable only in optimally orientated and transparent specimens. Indeed, when looking at an unorientated shell assemblage one gets doubts whether the lip perforation exists at all! And this doubt is strengthened by the fact that, contrary to Deflandre's (1929) claim, *C. sylvatica* is obviously not larger than *C. aerophila aerophila* and *C. aerophila sphagnicola* (Figs. 62a - c), this being also evident from the size (69-72 x 67-73 µm) of the specimens studied by Bonnet and Thomas (1955). Nonetheless, the lip perforation exists and distinguishes *C. sylvatica* from the other varieties (Figs. 41 - 61). This conclusion is supported by the investigations of Lüftenegger *et al.* (1988) and Rauenbusch (1987). Furthermore, the lip perforation has been clearly shown, i.e. by scanning electron microscopy, in a larger, related species, *Centropyxis matthesi* Rauenbusch, 1987; and a similar, probably homologous opening occurs in *Paracentropyxis mimetica* Bonnet, 1960. Thus, we agree with Bonnet and Thomas (1955, 1960) that *C. aerophila* var. *sylvatica* should obtain species rank. It is well defined by its extraordinary lip perforation, forming a second, inner pseudostome.

Much more difficult is the question whether *C. sylvatica* is a member of the genus *Centropyxis* at all! There are at least two other, well-defined species with a distinct lip perforation, viz., *Centropyxis matthesi* and *C. deflandriana*. We cannot exclude that these three taxa represent a specific (homologous) evolutionary line different from that of *Centropyxis*, that is, it could happen that the similarities in shape and size of *C. aerophila* and *C. sylvatica* are an analogy. The solution of this question will require genetic and molecular methods.

How did previous authors distinguish Deflandre's *Centropyxis aerophila* varieties?

As mentioned in the introduction, Deflandre's varieties of *C. aerophila* have been reported by many testacean researchers from terrestrial and freshwater habitats worldwide, usually even from the same sample. Obviously, all used Deflandre's traits to distinguish the varieties, at least none mentioned to have applied other and/or additional features. Furthermore, none mentioned having looked for the lip perforation, even after the pioneering paper of Bonnet and Thomas (1955), although it is the sole feature unequivocally separating *C. aerophila sylvatica* from *C. aerophila aerophila* and *C. aerophila sphagnicola*.

We showed that it is impossible to distinguish three varieties in *C. aerophila* with the morphological and morphometrical features given by Deflandre (1929); and our data give no indication that other, as yet undescribed reliable characteristics exist (Figs. 62a-d; Table 1). How then, could so many authors distinguish Deflandre's varieties, although some mentioned problems (Jung 1936; Schönborn 1966, 1975; Chardez 1979)? In our opinion it is because they considered mainly the extremes of a variability cline, which fit to Deflandre's descriptions, obviously assigning intermediate specimens more or less arbitrarily to one of the varieties. We could also distinguish three taxa when we sorted out all intermediates, that is, about half of the shells (Figs. 62e, f; Table 2).

A practicable solution for the problem: the "*Centropyxis aerophila* complex"

The species problem in general and of testate amoebae in particular has been extensively investigated and discussed recently (Schönborn and Peschke 1988, Medioli *et al.* 1990, Schönborn 1992a, Foissner and Korganova 1995, Bobrov *et al.* 1995, Wanner 1999). These studies showed the lack of a simple answer and emphasised the need for thorough species descriptions and avoidance of infrasubspecific taxa, unless they can be proven by reliable morphological and/or morphometrical features. They also showed that testacean shells are not extraordinarily variable, in contrast to the widespread believe, because the variation coefficients are hardly greater than in other organisms. This is emphasised by the present results (Tables 1, 2).

We showed that it is impossible to distinguish varieties in *C. aerophila* with the features used by Deflandre (1929). The situation became even worse when the varieties described later were taken into account (for reviews, see Decloitre 1978, 1979).

Basically, there are two ways to solve the problem. First, one might recognise only a single species, namely, *Centropyxis aerophila*, and classify all varieties and forms as falling into the species natural range of variability. Actually, this has been done by the describers of the

varieties and forms. This, however, resulted in an extraordinary variability of a single species casting doubts on the decision because such a broad range indicates the inclusion of several species or, at least, several cryptic (sibling) species (Mayr 1975), which is emphasised by the varying proportions the varieties were found in field samples (Chardez 1979, Bonnet 1989, Aescht and Foissner 1994). On the other hand, a reliable separation and identification of the varieties is often impossible and will be difficult also with modern molecular techniques (Wanner *et al.* 1997), if they exist at all. Accordingly, we prefer the second way at the present state of knowledge, that is, to unite all taxa in a "*Centropyxis aerophila* complex", as has been done in several "difficult" (cryptic) ciliate species [*Paramecium aurelia*, *Tetrahymena pyriformis*, *Sterkiella histriomuscorum*; see Foissner and Berger (1999) for a brief review]. This will at least relieve ecologists and practitioners of work which can hardly be done during field investigation, e.g., counting work. The theoretical background of this suggestion is the assumption that the phenospectrum *aerophila-sphagnicola* is an adaptive polymorphism to meet wetter (*aerophila*) and drier (*sphagnicola*) environmental conditions by selective shifting of certain genes.

We suggest that *C. sylvatica* is also included in the *C. aerophila* complex, although it is a distinct species, because its overall appearance is so similar and the species character, the lip perforation, is too difficult to recognise in routine counting work. Certainly, taxonomists and biogeographers (faunists) must look for this feature to get a reliable species list. Very likely, further taxa need to be put into the complex, for instance, *C. cassis* and *C. constricta*. However, these and other candidates, especially the subspecies and varieties of *C. aerophila aerophila* and *C. aerophila sphagnicola* (for reviews, see Decloitre 1978, 1979), are still poorly known and thus a final decision must await further investigations.

Nomenclature

There is great uncertainty whether Deflandre's "varieties" should be considered as infrasubspecific taxa or as subspecies. Indeed, many authors cited the varieties like subspecies or species, but none formally raised *C. aerophila sphagnicola* to subspecies or species rank. Fortunately, the matter can be unambiguously decided by the International Code of Zoological Nomenclature (1999), article 45.6. Deflandre (1929) described *C. aerophila sphagnicola* and *C. aerophila sylvatica* as "var. n."; however, his work does not **unambiguously** reveal that the names were proposed for infrasubspecific entities, which are not regulated by the code, and accordingly *sphagnicola* and *sylvatica* have subspecific rank from their original publication. Thus, Deflandre (1929) is author of the subspecies *C. aerophila sylvatica*, raised to species rank by Bonnet and Thomas (1955). Accordingly, the species formally must be cited as "*Centropyxis sylvatica* Deflandre, 1929". The same applies to *C. aerophila* var. *sphagnicola*, if it is not considered as synonym of *C. aerophila*, and to most other varieties and forms described before 1961 (see previous chapter). All varieties and forms described after 1961 have infrasubspecific rank, unless they were adopted as valid name of a species or subspecies before 1985 (e.g. *C. aerophila* var. *globulosa* Bonnet and Thomas, 1955).

Acknowledgements. Dr. Galina A. Korganova was supported by a grant of the Wilhelm and Ilse Foissner Stiftung. The technical assistance of Dr. H. Berger, Dr. E. Herzog, Dr. B. Moser, and Mag. E. Strobl is greatly acknowledged.

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Received on 14th March, 2000; accepted on 15th May, 2000

Acta Protozool. (2000) 39: 275 - 280

ACTA
PROTOZOOLOGICA

Budding and Asymmetric Reproduction of a Trichomonad with as many as 1000 Nuclei in Karyomastigonts: *Metacoronympha* from *Incisitermes*

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Summary. *Metacoronympha senta* Kirby (Trichomonadida: Calonymphidae), an exclusive symbiont of *Incisitermes* (Kalotermitidae) divides by budding and unequal fission, so that large cells can divide to produce both large and small cells. In contrast to *M. senta* in *Incisitermes snyderi* from Florida, which has a unimodal population distribution and a maximum cell length of 90 μm, *M. senta* in *I.* nr. *incisus* from Trinidad has a bimodal population distribution with cells as long as 210 μm and with as many as 1000 nuclei, each associated with a mastigont organelle system (karyomastigont). A densely packed parabasal body (Golgi complex) is located on the cell membrane-side of each nucleus, which has a typical oval shape. Kirby's report of "polygonal compartments, formed by contiguous nuclear membranes" prove by electron microscopy to be microtubules of the axostyles arrayed as a polygon around each nucleus. *Metacoronympha* and other parabasalid symbionts (*Coronympha*, *Trichonympha*) of *I. snyderi* and *I.* nr. *incisus* are reported in this second paper ever written on this genus.

Key words: Calonymphidae, Parabasalia, parabasal body, termite symbiont, Trinidad.

INTRODUCTION

The Calonymphidae is the family of multinucleate trichomonads found exclusively in dry-wood-eating termites (Kalotermitidae). To date these protists have been reported as hindgut symbionts in *Calcaritermes*, *Cryptotermes*, *Glyptotermes*, *Incisitermes*, *Kalotermes*, *Neotermes*, *Procryptotermes*, *Proglyptotermes*, *Proneotermes* and *Rugitermes* (Yamin 1979). The trichomonads, like other early branching lineages of eukaryotes, contain the major cytoskeletal unit called the karyomastigont. A nucleus, several undulipodia (eukaryotic flagella, Margulis 1993), a parabasal body or Golgi complex, a microtubular axostyle-pelta complex, and other proteinaceous structures such as a costa or cresta typically comprise the trichomonad karyomastigont. In the Calonymphidae this cytoskeletal unit, the karyomastigont, has been multiplied many times as in *Coronympha*, with a ring of eight or sixteen karyomastigonts, *Metacoronympha*, with five or six spiral arms, each with 25 or more karyomastigonts, and *Stephanonympha*, with several rings of karyomastigonts. Two genera are thought to be derived from these basal calonymphids through disassociation of nuclei from the mastigont complex, producing akaryomastigonts.

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Calonympha has numerous anterior akaryomastigonts and several, more posterior rings of karyomastigonts. In *Snyderella* all nuclei are detached from mastigonts and are suspended in the cytoplasm while the surface of the cell is covered with hundreds of akaryomastigonts (Kirby 1929, Dolan 1999). The evolution of this family apparently occurred by Kirby's "mastigont multiplicity" (Kirby and Margulis 1994). Two processes are involved: karyokinesis without cytokinesis which generated the multinucleated state, and replication of the mastigonts without cytokinesis and/or karyokinesis that generated the multimastigont (karyomastigonts and/or akaryomastigonts) state.

In describing three genera and numerous species of calonymphids Kirby (1929, 1939; Kirby and Margulis 1994) recorded minute details of karyokinesis, and karyomastigont reproduction, but had little to say about cytokinesis. He confirmed for *Coronympha* and *Metacoronympha* (1939), as first observed by Janicki (1915) for *Stephanonympha silvestrii* and *Calonympha grassii*, that cells lose their polarity and become spherical, that the nuclei as karyomastigonts distribute randomly at the cell surface. Karyokinesis then occurs simultaneously in all nuclei. The same process was recently reported in *Snyderella* (Dolan *et al.* 2000). Because dividing cells of these termite gut symbionts are rare, the pattern of cell division, if any, has not been reported.

We have examined two species of *Incisitermes* for population distribution and cell division patterns of *Metacoronympha senta*. We report for the first time the symbionts found in these two termites, and make additional observations to clarify Kirby's description of *M. senta*.

MATERIALS AND METHODS

The termites examined here were collected from the roots and stumps of red mangrove (*Rhizophora mangle* L.) Caroni Swamp, Trinidad in the summer 1997. They were identified as *Incisitermes* nr. *incisus* based on morphology by Dr. Rudolf Scheffrahn of the University of Florida's Fort Lauderdale Education Center. An authoritative description of this species has not yet been published in part because the termites of the Caribbean have not been fully described. Specimens of *Incisitermes snyderi* (Light) from central Florida (Lake Placid) and their gut contents were examined for comparison with the Trinidad termite.

Five pseudergates from a single colony of each species were removed from their native wood and sacrificed. Their hindgut contents were broken open in 0.6% NaCl. The protists were fixed for 15 min in 1% glutaraldehyde in phosphate buffered saline (PBS), washed with PBS and stained for 30 min with 2 μM of the DNA stains DAPI or SYTOX (Molecular Probes, Eugene, OR), which stain DNA, or 1 μM $DIOC_7$, which stains membranes (Liu *et al.* 1987). Wet mounts were prepared, sealed with Vaseline and examined within one day of preparation. The cells were measured under a phase contrast microscope with an ocular micrometer and examined using epifluorescence microscopy. Smears were prepared on poly-L-lysine-coated coverslips, fixed in 1% glutaraldehyde, and stained with Heidenhain's hematoxylin (Conn and Darrow 1946).

To sample the population, the hindgut contents from two pseudergates of each species were fixed separately as above, centrifuged at 9 g for two min, washed once with PBS and allowed to settle to the bottom of the tube. Wet mounts, each containing the gut contents of a termite, were scanned under 9 x g magnification. The length and width of 120 cells from each of two termites from each species were measured using an eyepiece micrometer.

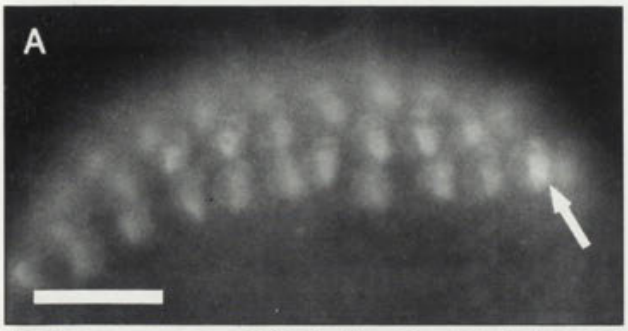
Hindgut contents were removed and fixed in glutaraldehyde (2.5% for 48 h at room temperature) in cacodylate buffer pH 7.2. Specimens were post fixed in 1% OsO_4 for 1 h and washed 15 min twice in cacodylate buffer. Dehydration in an alcohol series (50%, 70%, 80%, 90%, 95%, 100% three times, 15 min) and immersion three times in propylene oxide followed. The specimen was left over night to embed in a mix of 1:1 propylene oxide and Hepler's Epon-Araldite in a 60°C oven, mounted and sectioned with a glass knife using an Ultracut ultramicrotome. Sections collected on 200 mesh hex copper grids, were stained in uranyl acetate (5 min), lead citrate (5 min) and viewed with a JEM-100s electron microscope at 80 kV.

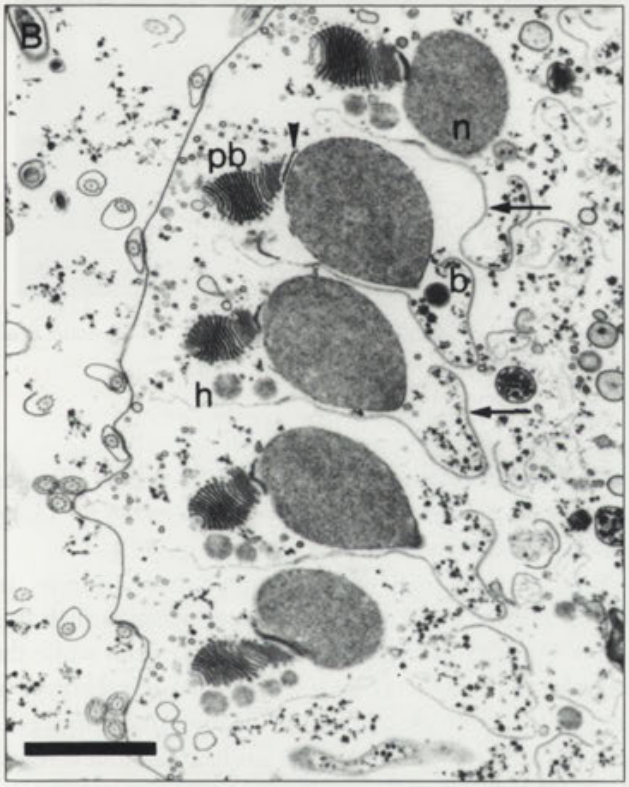
RESULTS

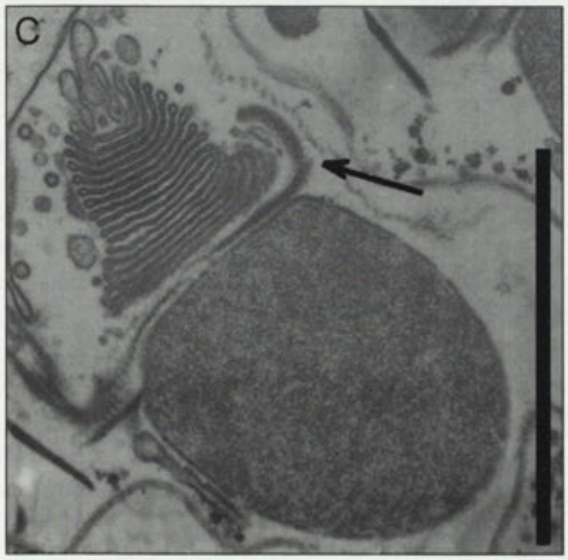
Incisitermes nr. *incisus* from Trinidad harbors *Metacoronympha senta*, *Coronympha octonaria*, *Trichonympha chattoni* and a small unidentified monocercomonad. *M. senta* and *C. octonaria* but no *Trichonympha* were in *I. snyderi* from central Florida.

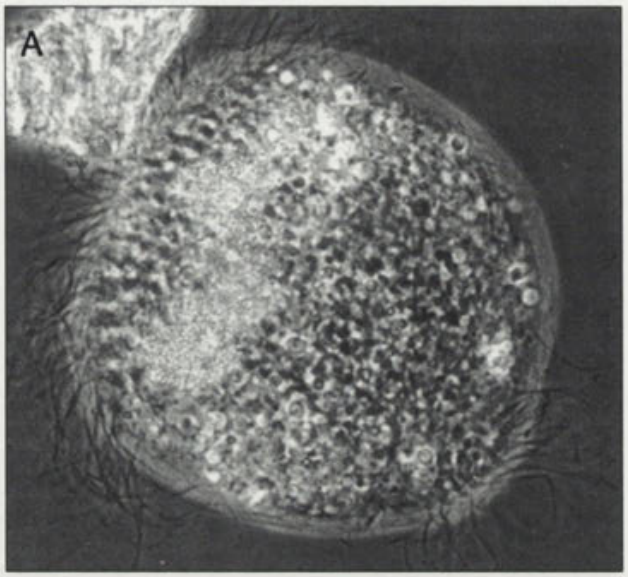
M. senta interphase cells contain five or six spiralling arms of karyomastigonts. The karyomastigont of *M. senta* bears a 3 μm diameter nucleus, and a small rod-shaped parabasal body 2 μm long located at the cell-periphery side of the nucleus. The axostyles are not fused into a central axostyle bundle. Preparations stained with $DIOC_7$ revealed typical, oval nuclear membranes (Fig. 1A). No polygonal array of membranes pressed against each other from adjacent karyomastigont nuclei were seen as described by Kirby (1939). However, hematoxylin staining revealed polygons as Kirby reported (not shown). The polygonal array surrounding each nucleus is made of axostylar microtubules as revealed by electron microscopy (Fig. 1B).

Five of the dozens to hundreds of ovoid to pyriform nuclei are seen surrounded by their microtubule arrays and their peripherally oriented parabasal bodies in









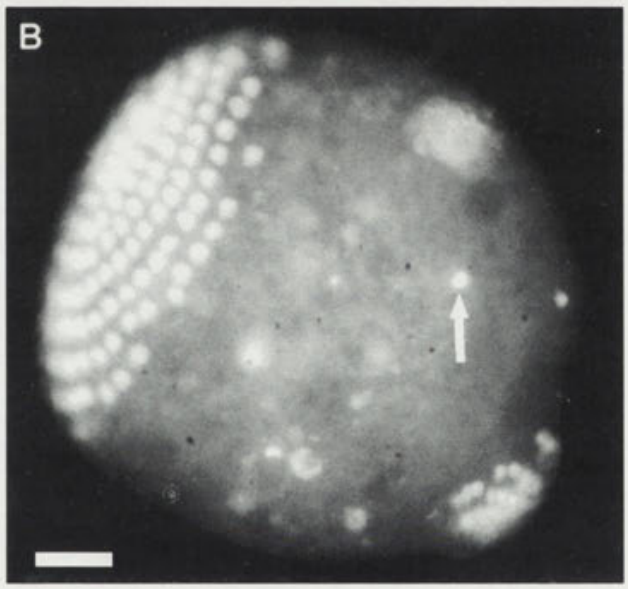


Fig. 2. *Metacoronympha senta* undergoing unequal cell division. **A** - phase contrast, **B** - SYTOX stain. Arrow indicates nucleus not incorporated into spiral arms. Scale bar - 5 μm

Fig. 1. *Metacoronympha senta* from *Incisitermes snyderi*. **A** - the spherical to oval nuclear envelopes and parabasal bodies (arrow) are apparent. $DIOC_7$ - stained for epifluorescence microscopy to highlight lipid-rich structures. Scale bar - 10 μm. **B** - nuclei of *M. senta* from *I. snyderi*. Nuclei (n) are spherical to pyriform. Each is associated with a parabasal body (pb) and its parabasal filament (arrowhead) on its side toward the cell periphery. The axostylar microtubules (arrows) are adjacent to the nuclei in a distinct pattern. Spherical structures that may be hydrogenosomes (h) are associated with each karyomastigont. Endocytic bacteria (b) are also seen. **C** - nucleus with smoothly distributed chromatin, parabasal filament (arrow) underlying stacked Golgi cisternae (parabasal body) at higher magnification. Transmission electron micrographs scale bars - 2 μm

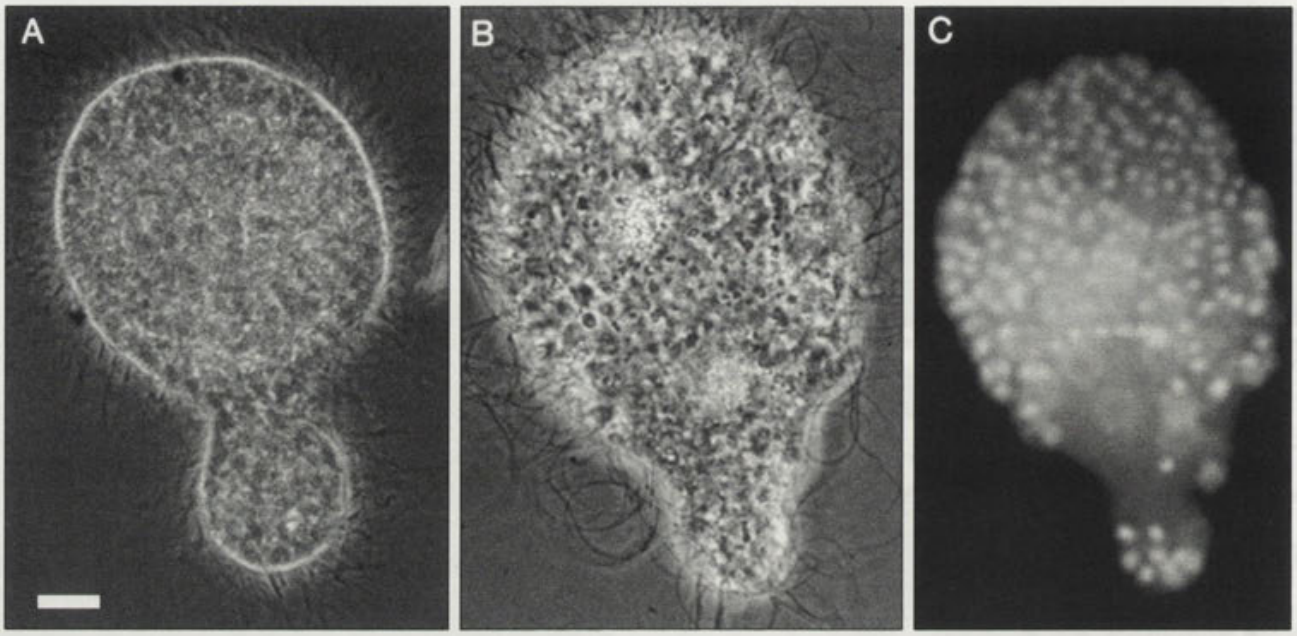


Fig. 3. *Metacoronympha senta* budding. **A** - budding cell, **B** - another budding cell after karyokinesis, **C** - same cell as B, SYTOX stain. Scale bar - 5 μm

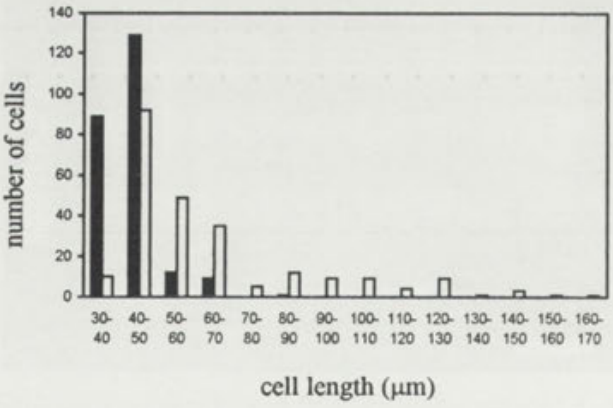


Fig. 4. *Metacoronympha senta* population distribution by cell length in *Incisitermes snyderi* (black columns) and *Incisitermes* nr. *incisus* (white columns). N = 240 cells of each termite species combined from the hindguts of two termites

Fig. 1B. A parabasal filament runs laterally along the nucleus. At regular intervals lateral to the parabasal bodies are several spherical, unwalled bodies that may or may not be membrane bounded. By extrapolation of similar sized and shaped structures in other parabasalids they may be hydrogenosomes. Endobiotic bacteria with walls are also noted in the cytoplasm of *Metacoronympha* (b in Fig. 1B). In none of the karyomastigont nuclei does the chromatin appear clumped and all display the parabasal filament between the Golgi and distal surface of each nucleus (Fig. 1C).

Metacoronympha senta displays two patterns of division. Prior to cytokinesis, cells reorganize such that one offspring cell may have a hundred nuclei while the other has only 20-30 (Figs. 2A, B). Two spiral arrays of nuclei form with one containing the majority of the cell's karyomastigonts and the other containing much fewer (Fig. 2B). Additional fluorescent structures are nuclei (arrow) that have not yet been incorporated into a spiral array. This cell is interpreted to have completed karyokinesis, and to have organized its spiral arrays prior to cytokinesis. It is inferred that it will divide into two cells of unequal size and karyomastigont number. Similarly, portions bud off disorganized cells immediately after karyokinesis, prior to rearrangement of the spiral arrays. These processes generate small offspring cells with many fewer nuclei than the parent (Figs. 3A, B, C).

Incisitermes nr. *incisus* contained a distinct subpopulation of *M. senta* with huge cells, roughly twice the size of those Kirby (1939) described (Fig. 4). This larger-sized *Metacoronympha* subpopulation, cells 118 ± 40 μm long and 114 ± 30 μm wide (n=35), comprised 15 percent of the entire sample of *M. senta* population from two termites (n=240). They were absent in *I. snyderi*. The large cells often had several hundred

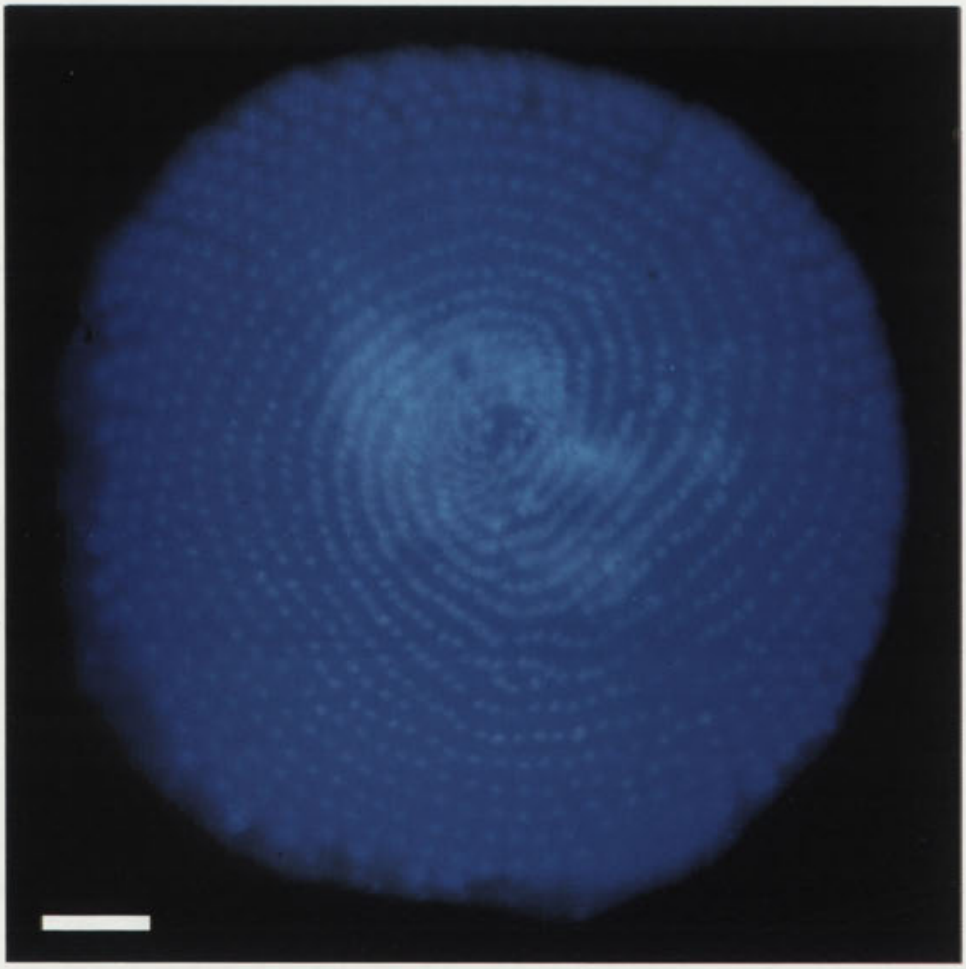


Fig. 5. Large *Metacoronympha senta* with over 1000 nuclei from *Incisitermes* nr. *incisus*. DAPI stain. Scale bar - 20 μm

karyomastigonts in excess of those described by Kirby (1939). Ten cells at least contained more than 1000 karyomastigont nuclei (Fig. 5). Huge multinucleate *M. senta* cells, maximum length of 210 μm, were observed as well in six other *I.* nr. *incisus* termites. Although the larger cells were more rounded, besides size and nuclei number, no other differences were noticed between the two subpopulations.

DISCUSSION

Incisitermes snyderi most resembles *I. banksi* and *I. platycephalus* based on hindgut symbionts: all three species harbor the two calonymphids *Coronympha octonaria* and *Metacoronympha senta* but lack the hypermastigid *Trichonympha* (Yamin 1979). While no *Incisitermes* species has the same symbiont complement as *I.* nr. *incisus* (the two calonymphids and *Trichonympha chattoni*), two species, *I. milleri* and *I. schwarzi*, are reported to harbor *T. chattoni*.

Metacoronympha senta from five species of *Incisitermes*, (*I. emersoni*, *I. lighti*, *I. pacificus*, *I. platycephalus*, and *I. tabogae*), was described by Kirby (1939) in the only paper ever written on this genus. The population distribution within different species of the termite was not mentioned. Kirby described *M. senta's* mean length as 45 μm (range: 22-92 μm) with 150 nuclei per cell (range: 66-345). The huge cells with up to 1000 nuclei apparently he never saw, perhaps because he never examined *I.* nr. *incisus*, a Trinidad termite.

The budding and unequal cell divisions observed here were not reported in previous accounts of calonymphid cytokinesis. Kirby (Kirby and Margulis 1994) wrote of

Stephanonympha cytokinesis, "the nuclei become organized at opposite ends of the cell, and the cell divides to form two mastigotes". The same was observed by Das and Choudhury (1972). Janicki (1915) reported *Calonympha* dividing by a longitudinal fission, producing two cells with equal numbers of nuclei and mastigonts. We have observed similar equal division in *Snyderella*, but have also seen budding in that genus (Dolan *et al.* 2000). Since few cells in division are seen in termites, and since removal from the termite hindgut disrupts their physiology and soon leads to death, the most frequent pattern of karyo- and cytokinesis remains unclear. Yet from the budding and unequal cell division we infer that a large *Metacoronympha* may produce small offspring cells. Many nuclei per cell (300-1000) were present in all large cells whereas the small cells studied had relatively fewer (60-100). Apparently the reorganization of mastigonts after karyokinesis is not tightly controlled so that distinct, separate groups of mastigonts can organize independently within the same cytoplasm, like a colony of mastigotes (Kirby 1949).

Unmeasured variables in the gut may induce the formation and persistence of the large forms of *M. senta* (such as properties of the mangrove wood, nitrogen or salt concentrations). Yet one may not attribute the presence of huge *M. senta* to selection pressure by *Trichonympha chattoni* because other species of *Incisitermes* that also harbor *Trichonympha* lack the large-sized *M. senta* seen in *I.* nr. *incisus*. *Metacoronympha senta*, like all calonymphids, undergoes synchronous karyokinesis where its karyomastigont reproduces as an integrated unit. Thus large cells of this genus must result from karyokineses followed by delayed cytokinesis. This developmental sequence at the cell level corresponds to the evolutionary scenario thought to have produced the multinucleate, multimastigont Calonymphidae from its monomastigont trichomonad ancestor (Kirby 1949, Margulis *et al.* 2000).

The bimodal population distribution in the size of *Metacoronympha senta* (Fig. 4) may indicate extant in-progress speciation in these protists. No sexual processes have ever been reported for any of the five Calonymphid genera. The smaller *M. senta* may be simply a bud cloned from the large form, consistent with the budding pattern seen here (Fig. 3). That, by contrast, it is an incipient new species is testable by comparison of gene sequences (ssrRNA for example), and ultrastructure between cells of the two size classes.

Acknowledgments. We thank Mark Deyrup of the Lake Placid (Florida) Biological Station for providing *I. snyderi*, and Dr. Rudolf Scheffrahn for identifying the Trinidad termite. This work was supported by the University of Massachusetts Graduate School, the Richard Lounsbery Foundation and a NASA Office of Space Sciences Exobiology Program grant to Lynn Margulis.

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Received on 19th June, 2000; accepted on 14th August, 2000

Acta Protozool. (2000) 39: 281 - 288

ACTA
PROTOZOOLOGICA

On the Labeling of Malaria Parasites *In Vivo* with ^{3}H-hipoxanthine While Developing an Infection in the Mouse

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Summary. After previous experiments on oral transmission of malaria and babesiosis in rodents, it was decided to follow the malaria parasites inoculated by the oral route through the alimentary canal, to look for the site where they cross to reach blood and establish an infection. To trace the parasites along the canal they had to be labeled preserving its viability as much as possible to maintain full infectivity. *In vivo* labeling was tried during the normal development of infection in the mouse, to avoid *in vitro* handling of the parasite. The purpose of this communication is to describe the *in vivo* labeling experiments of the rodent malaria parasite *Plasmodium yoelii yoelii* and labeling characterization. Labeling was performed with ^{3}H -hypoxanthine at single doses of 1, 2 or 3μCi per mouse g. Detection of labeled parasites was done by autoradiography at different post-labeling times from 2 or 10min to 96h. The grains resulting from individual parasites ^{3}H-hypoxanthine uptake were detected and counted at light microscopy. As observed by autoradiography, *in vivo* labeling probed to be very selective to the malaria parasites, no leukocytes nor either platelets or non-infected erythrocytes were observed labeled. *In vivo* labeling of malaria parasites may be useful for tracing the parasite population or individual stages during development of its life cycle into the host.

Key words: autoradiography, ^{3}H-hypoxanthine, *in vivo* labeling, *Plasmodium yoelii yoelii.*

Abbreviations: bgg - background grains, ^{3}H-h - ^{3}H-hypoxanthine, mf - microscopic field, Pyy - *Plasmodium yoelii yoelii.*

INTRODUCTION

Rodent malaria and babesiosis can be transmitted by mouth through the blood forms of *Plasmodium Berghei* and *Babesia microti* as we previously observed (Malagon *et al.* 1993,1994; Malagon and Tapia 1994). To follow this line of experiments we now try to make some light on how the parasite do its entry from the lumen of the digestive tract to blood. It was decided to trace the parasites along the lumen to look for the place or region where they cross the epithelium to reach blood, which would probably give us the opportunity to see the stage of the parasite that do the crossing, and perhaps to observe if the travel to blood is done directly through the cells or making a stop in epithelial cells. To trace the parasites they had to be previously labeled, and the labeling procedure to be used, had to conserve the parasites vitality at its maximum, so the invasiveness of the parasite remained the same as that from the untouched parasites directly obtained from the blood.

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To fulfill these requirements the labeling procedure applied had to avoid as much as possible the handling of the parasite outside its host. It was decided, for this reason, that the best labeling procedure would be one that labels the parasite *in vivo*, i.e. while it is developing a normal infection inside its host, so parasites would not be disturbed nor handle during labeling as it is in an *in vitro* assay. Search in current literature for an *in vivo* labeling procedure for malaria parasites was unsuccessful. Considering that malaria parasites used hypoxanthine as substrate for the synthesis of all purine nucleotides (Yamada and Sherman 1981, Berman and Human 1991, Werner *et al.* 1991), and that assays for malaria parasites tritiated hypoxanthine uptake *in vitro* are currently in use to follow the growth of parasite cultures (Chulay *et al.* 1983), to asses viability of parasites (Izumo *et al.* 1987), and to measure antimalarial activity of drugs (Strath *et al.* 1993, Ittarati *et al.* 1994, Wang *et al.* 1997), the ^{3}H-hypoxanthine (^{3}H-h) was selected as the labeler for the *in vivo* experiments. These experiments were done to characterize the uptake of ^{3}H-h by parasites and blood cell components, during the development of a blood infection in the mouse at 3 different single doses of the ^{3}H-h, as measured by autoradiography. To comment our findings on characterization of this *in vivo* labeling of the malaria parasites with ^{3}H-h is the purpose of this communication.

MATERIALS AND METHODS

The model

These experiments were performed using *Plasmodium yoelii yoelii*, (Pyy) obtained from the London School of Hygiene and Tropical Medicine thanks to the courtesy of the late Professor P.C.C. Garnham and Dr. D.E. Davidson in 1969 and kept by mouse to mouse passages since then, and male mice weighing 20 to 25g of the strain CD1 Charles River.

Experiments

All experiments consisted of two mice, one experimental and one control. In a typical experiment, the experimental mouse was inoculated by intraperitoneal route with 10µl of Pyy infected blood (parasitemia range of blood donors was 8.7-14.5) suspended into 1ml of saline, allowing the parasitemia for 3 days to progress, while control mouse remained uninfected until labeling. Experimental and control mice were labeled with a single doses of Amersham (G-^{3}H) hypoxanthine 1-5Ci/mM (37-185 Gbg/mmol) solubilized in saline. Doses of 1, 2 and 3µCi (37, 74, 111 KBg) per mouse g were applied by intravenous route. Blood smears were taken at different time intervals from 2min to 96h after ^{3}H-h labeling. Smears were dried, fixed, processed for autoradiography, stained with Giemsa and observed at light microscope. The labeled parasites and grains labeling each individual parasite were counted. Parasites were grouped into four stages during counting: young trophozoites, mature trophozoites, schizonts and gametocytes. Experiments were replicated per each dose, at least once.

Autoradiography

Blood samples were fixed with methanol and coated in darkness with NTB3 Nuclear Track Emulsion (Eastman Kodak), previously diluted at 50% with distilled water and stirred for about 30 min at 40°C. Coating was performed in a plastic container specially designed to coat only the smear side of the slides. The slides, after coating and drying, were exposed in black boxes for two weeks at 4°C. Exposed smears were developed in a bath of 1:1 Kodak Dektol solution in distilled water, at room temperature (16-20°C) for 2 min, and washed twice for 10 seconds each in distilled water. Once developed, slides were kept for 5 min in a bath of Kodak Rapid Fixer (prepared according to manufacturer specifications for films and plates applications), washed once with distilled water and once with 0.2M Sörensen phosphate buffer pH 7 for 5 min each. Slides were air dried and stained with Giemsa (manufactured in our laboratory) for 30 min, and observed in light microscope at magnification x 100.

Microscopic observation

Experimental samples: 500 parasites, at least, per sampling time were observed. Intracellular ring forms, very small trophozoites and free merozoites just released from the schizont, were grouped as young trophozoites, big trophozoites proximal to render into schizonts were the mature trophozoites, schizonts included all the spectrum of multinucleated parasites from the younger binucleated through the older segmenter, and gametocytes. Registrations made on each parasite: developmental stage, presence of grains, number of grains (on the nucleus, on the cytoplasm or closely associated to it). Grains and grain patterns were searched in non-infected erythrocytes, and leukocytes. Background grains (bgg), defined as grains laying in free spaces, or as single grains randomly distributed on cells other than parasites, were also counted in each microscopic field (mf).

Control samples, leukocytes: grain counts were performed only on microscopic fields where leukocytes were present until 50 leukocytes were observed per sampling time. The total numbers of grains counted per mf were registered, as well as the number of grains on leukocytes.

Control samples, erythrocytes: grain counts were performed on 2000 erythrocytes per sampling time. The total numbers of grains counted per mf were registered, as well as the number of grains on erythrocytes.

RESULTS

Experimental samples

Early uptake. The ^{3}H-h parasite uptake had already began 2 min after labeling. Parasite population from experiments with readings at the 2 min was labeled in a

range of 2 to 13% and a mean of 6.5% (Table 1, 2 min column). By the 10 min post-labeling the uptake had increased substantially, 19 to 52% of the circulating parasites were labeled (Table 1, 10 min column) and the mean for all experiments was of 30.6%. At the 10 min readings, however, the mean value per doses was 29.5% for 1 μCi, 22.7% for 2 μCi and 42% for 3 μCi.

Maximum labeling. Short-term experiments 3 and 4 are excluded, because it is not certain that the 4 h readings represented the maximum labeling values. The maximum proportion of labeled parasites (Table 1, figures in bold) was reached between 1 to 8h after labeling. The maximum parasite population labeled ranged from 44 to 90%, while its mean value was 67.1%. However, the mean of maximum values from labeled population per doses was 51% with 1 μCi, 53% labeling with 2 μCi and 87.3% with 3 μCi.

For practical purposes of collecting labeled parasites, it was desirable to us to estimate a band of time at which to harvest the parasites, when they were labeled near its maximum, so that, it was defined as "band of maximum labeled population", the band of reading times reaching at least 85% of the maximum value, before and after of it, in any given experiment (Table 1, horizontal rows). The reading times of these bands that intersected with reading times of all experiments of the same doses were regarded as the "harvesting time bands". This harvesting time band for 1 μCi would be from 3 to 4h after labeling, for 2 μCi from 3 to 6h and for 3 μCi from 3 to 8h (Table 1, vertical boxes, at the three doses).

Individual ^{3}H-h uptake. The individual ^{3}H-hypoxanthine uptake was measure by the amount of grains display by each parasite. The amount of grains labeling each parasite at the two min after labeling ranged from 1 to 2 (Table 2, 2 min column), with a mean of 1.4. At the 10 min, it ranged from 1.8 to 4.6 grains (Table 2, 10 min column), with a mean value for the nine experiments of 3 grains per parasite. However, the mean individual value per doses at the 10 min was of 3.2 grains per parasite when labeled with 1 μCi, 2.8 grains when labeled with 2 μCi and 3.0 grains with 3 μCi.

The maximum individual load of grains was reached between the 60 min and the 8h (excluding the short term experiments 3 and 4), with a range per parasite of 4.4 to 9.6 (Table 2, figures in bold), and a mean of 7.6 grains per parasite, while the mean of maximum values per doses was of 6.2 grains per parasite in 1 μCi, 7.4 grains in 2 μCi, and 8.6 grains per parasite when doses was 3 μCi. The parasites were found individually labeled by 1.2 to 4 grains with a mean of 2.3 at 96 h post-labeling. However, individual parasites showed a mean number of grains per doses of 1.9 for 1 μCi, 1.4 for 2 μCi, and 3.0 for 3 μCi. For the individual load of grains, the "time band of maximum labeling", appear in the horizontal rows of Table 2, and from them the "harvesting time band" related to grain load was of 3 to 4 for 1 μCi, 3 to 4 h for 2 μCi and 6 to 7 h for 3 μCi.

Labeled population by stage. Of all parasites found labeled, young trophozoites with a ranged from 59 to 91%, and a mean of 77% predominated. The mature trophozoite follows with a range of 2 to 28%, and a mean of 11%. Schizonts were found labeled in a proportion between 7 to 19%, and a mean of 11%. The labeled population of gametocytes never reached values higher than 0.5% (Table 3).

Labeling of leukocytes. The number of leukocytes found with grains in experimental samples ranged from 13.1 to 14.8%, corresponding to a mean of 13.8% of all leukocytes observed in all experiments. However, 86 to 92.4% of those leukocytes found with grains, exhibited just one grain, which represented a mean value of 90% in all experiments. Therefore, few leukocytes with grains showed more than one grain (with 2 grains, range 4.0-8.0%, mean 6.2%, with 3 grains, range 0.7-6.3%, mean 3.3%).

Labeling of erythrocytes. In experimental samples the population of erythrocytes found with grains on them ranged from 2.5 to 4.7%, and the mean value was 3.6% regarding all experiments. Of all erythrocytes with grains on them, 94.6 to 97.7% exhibited one grain, giving a mean of 96.3 in all experiments. The range of erythrocytes population with two grains was from 1.9 to 4.9% and the mean was of 3.2%. The erythrocytes population with three grains had a range from 0.2 to 0.5% and a mean of 0.3%.

Background correction factor. To estimate the number of parasites randomly labeled by background radiation, the following procedure was designed: the background grains (bgg) in each microscopic field (mf) were counted, and from this, the total number of bgg and the mean number of bgg per mf per experiment were calculated. The mean number of parasites per mf per experiment was also calculated.

The surface of a microscopic field at 100 x objective and 10x ocular was calculated, resulting 15,393.84 μm^{2} per mf. The mean surface of a parasite was estimated, measuring the diameter of 500 parasites of all stages and calculating the mean diameter that was of 4μm, and its

Table 1. Autoradiography. Proportion (%) of malaria parasite population found labeled at different reading times, in all experiments. Values adjusted to the unit

exp	reading times after labeling											
	2'	4'	6'	8'	10'	20'	30'	45'	60'	2h	3h	4h
1					40	48	51	51	**56**	52	48	48
2					19	26	30	35	33	35	42	39
3	2	5	3	3	21	25	25	27	34	19	39	**44**
4	13	12	15	16	24	23	29	36	34	29	35	**38**
5	6	9	19	24	26	30	34	31	33	50	**62**	**62**
6	6	12	16	7	20	22	24	18	32	29	42	43
7					52	67	76	84	77	66	75	75
8					34	33	52	44	62	73	80	80
9					40	41	47	43	75	78	81	77

exp - experiment number; ^{3}H-h doses: 1μCi experiments 1 and 2; 2μCi experiments 3,4,5 and 6; 3μCi experiments 7,8 and 9; gray rows with no divisions: "harvesting time bands"; figures in bold: maximum proportion of labeled polulation,

early readings at 2 and 10 min after labeling, | band of maximum parasite population labeled

Table 2. Autoradiography. Mean number of grains per individual malaria parasite at any given stage in the different reading times, in all experiments. Values adjusted to decimals

exp	reading times after labeling											
	2'	4'	6'	8'	10'	20'	30'	45'	60'	2h	3h	4h
1					4.6	5.2	7.2	7.4	**8.0**	5.7	5.4	5.8
2					1.9	3.0	2.0	2.6	2.6	2.0	3.8	**4.4**
3	1.2	1.6	2.8	4.0	2.8	2.4	2.8	2.8	3.0	**5.0**	4.6	4.2
4	1.3	2.3	2.0	2.2	2.7	2.6	3.5	3.7	4.3	4.2	**5.9**	**5.4**
5	2.0	2.8	4.0	3.0	3.8	3.2	4.4	4.4	6.6	**7.0**	6.2	6.8
6	1.0	1.7	1.8	1.0	2.0	2.0	2.8	1.8	3.4	3.0	5.1	5.0
7					4.2	4.6	4.4	4.4	4.2	**7.6**	6.1	5.2
8					1.8	3.2	4.6	5.6	5.4	6.2	7.6	8.2
9					3.2	4.4	6.6	5.8	5.4	6.8	8.2	6.4

exp - experiment number; ^{3}H-h doses: 1μCi experiments 1 and 2; 2μCi experiments 3,4,5 and 6; 3μCi experiments 7,8 and 9; figures in bold - maximum individual labeling,

early readings at 2 and 10 min after labeling, | band of maximum individual labeling

surface 12.5μm. The maximum number of parasites to be contained in mf was calculated and it was 1,231.5 parasites.

Fundamentals: if a mf were cover with a layer of parasites in such a way that no space were left among them, the number of parasites filling the mf would be

Table 1. (contd)

reading times after labeling											
5h	6h	7h	8h	9h	10h	11h	12h	24h	48h	72h	96h
47	43	44	38	23	42	33	31	26	17	9	7
39	35	**46**	36	29	23	24	20	12	11	12	16
57	56	40	43	40	36	34	28	15	14	7	1
43	**44**	39	39	30	18	18	20	13	8	8	5
80	62	80	**87**				74	82	66	86	50
88	**90**	86	82				63	40	31	23	8
85	84	**85**	81	77	81	84	78	59	55	44	34

Table 2. (contd)

reading times after labeling											
5h	6h	7h	8h	9h	10h	11h	12h	24h	48h	72h	96h
7.2	5.5	5.8	4.9	3.9	5.8	4.2	4.3	2.5	2.4	1.8	1.4
2.8	3.6	3.6	2.8	2.4	3.0	2.6	2.4	2.0	2.2	2.0	2.4
6.0	5.1	4.3	4.6	4.8	4.3	4.2	3.8	2.4	1.4	1.8	1.2
5.0	6.8	6.1	**7.8**	3.6	2.4	2.7	3.6	2.0	1.8	1.9	1.7
6.7	7.0	6.8	6.9				4.3	6.1	4.4	3.7	4.0
8.4	6.2	**8.6**	7.2				4.2	3.4	2.2	2.4	1.5
7.4	**9.6**	8.4	9.2	8.0	7.2	2.4	4.6	2.5	2.2	2.4	3.6

constant. Therefore, each bgg found in that mf would label at random one parasite, at most. So, the number of bgg found in that mf is equal to the number of at random labeled parasites. Then, the number of bgg found in mf may be converted into number of parasites labeled at random, per each 1,231.5 parasites (the constant). So, if the number of parasites seen on mf were not constant (as it is the case), the number of parasites, whatever it is,

Table 3. Autoradiography. Proportional mean distribution of labeled population by malaria parasite stages in all experiments including all reading times.Values adjusted to the unit

Exp	% of labeled parasites by stage			
	young t.	mature t.	schizonts	gametocytes
1	79	7	14	0
2	74	13	13	0
3	73	7	19	0
4	75	15	10	0
5	82	7	10	0
6	73	17	10	0
7	59	28	13	0
8	89	4	7	0
9	91	2	8	0

exp - experiment number
^{3}H-h doses: 1μCi experiments 1 and 2
2μCi experiments 3,4,5 and 6
3μCi experiments 7,8 and 9
t - trophozoites
zero may be a value between 0 to 0.5

would be proportional to the relation between the constant number of parasites per mf and the number of bgg per mf (number of parasites labeled at random). For instance, in experiment 1 the mean number of bgg per mf was 30 and the mean number of parasites per mf was 25.8, Therefore, in a mf with constant number of parasites, 30 bgg would label at random 30 parasites of the 1231.5 available (the constant), now, if the mf with the 30 bgg has only 25.8 parasites instead of 1231.5, how many of those parasites (X) would be at random labeled? So, 1231.5 is to 30 as the number of parasites found (25.8) in mf is to X. i.e.:

$$X = \frac{\text{number of parasites in a mf X number of bgg,}}{\text{constant}}$$

$$\text{i.e. } X = \frac{28.5 \times 30}{1231.5} \qquad \text{so, } X = 0.6$$

Which means that 30 bgg spread in mf containing 28.5 parasites, would at random label 0.6 of those parasites, at most. In experiment one 551 mf were observed, so, the total number of parasites labeled at random in this experiment was 330.6. In the 551 mf observed in experiment one, 14, 240 parasites were seen, so the proportional amount of parasites labeled at random was 2.3%, which finally conforms the error factor of the experiment one (2.3% of the labeled parasites have to be regarded as labeled by bgg). The error factor in all experiments ranged from 0.9 to 2.3%, with a mean of 1.7%.

Control samples

Leukocytes. Of all leukocytes observed in labeled noninfected animals blood, 8.5 to 14.5% of them were found with grains, and their mean value corresponded to 11.2%. However, between 77.2 and 92.5% of those leukocytes with grains, presented just one grain (mean 87.7%), 3.7 to 20.9% showed 2 grains (mean 9%) and 0.9 to 4.7% exhibited 3 grains (mean 3.1%).

Erythrocytes. The range of control mice erythrocytes with no grains on them was 96.9 to 97.9% with a mean value of 97.2%, while the range of erythrocytes with grains was from 2 to 3% with a mean value for all experiments of 2.6%. However, erythrocytes with one grain ranged from 94.2 to 95.6% with a mean of 95.1%, with two grains 3.5 to 5.1% with a mean of 4.1% and, with three grains 0.3 to 1.0% with a mean of 0.5%.

DISCUSSION

Labeling of malaria parasites with radioactive precursors for DNA synthesis started in 1967-68 when Bungener and Nielsen demonstrated incorporation of purine nucleotides into DNA, and RNA of *Plasmodium berghei* by film autoradiography of the pellet of *in vitro* labeled parasites (Bungener and Nielsen 1967). Van Dyke *et al.* (1970) confirmed the findings of the German workers and started the development of a new antimalarial drug screening system using the quantitative uptake estimation of tritiated adenosine by scintillation, in an *in vitro* model (Van Dyke *et al.* 1970). Further studies established that malaria parasites used hypoxanthine as substrate for the synthesis of all purine nucleotides (Yamada and Sherman 1981, Berman and Human 1991, Werner *et al.* 1991) and since then radioactive hypoxanthine incorporation assays are being used for labeling malaria parasites for different purposes. However, whatever the purpose all assays are performed *in vitro* and in current literature no experiences are to be found on malaria parasites labeling *in vivo*, nor either, on measuring the labeling by autoradiography.

Incorporation time of ^{3}H-hypoxanthine

In these experiments, the time the circulating blood parasites needed to uptake ^{3}H-h was smaller than 2 min, because up to 13% of the parasite population

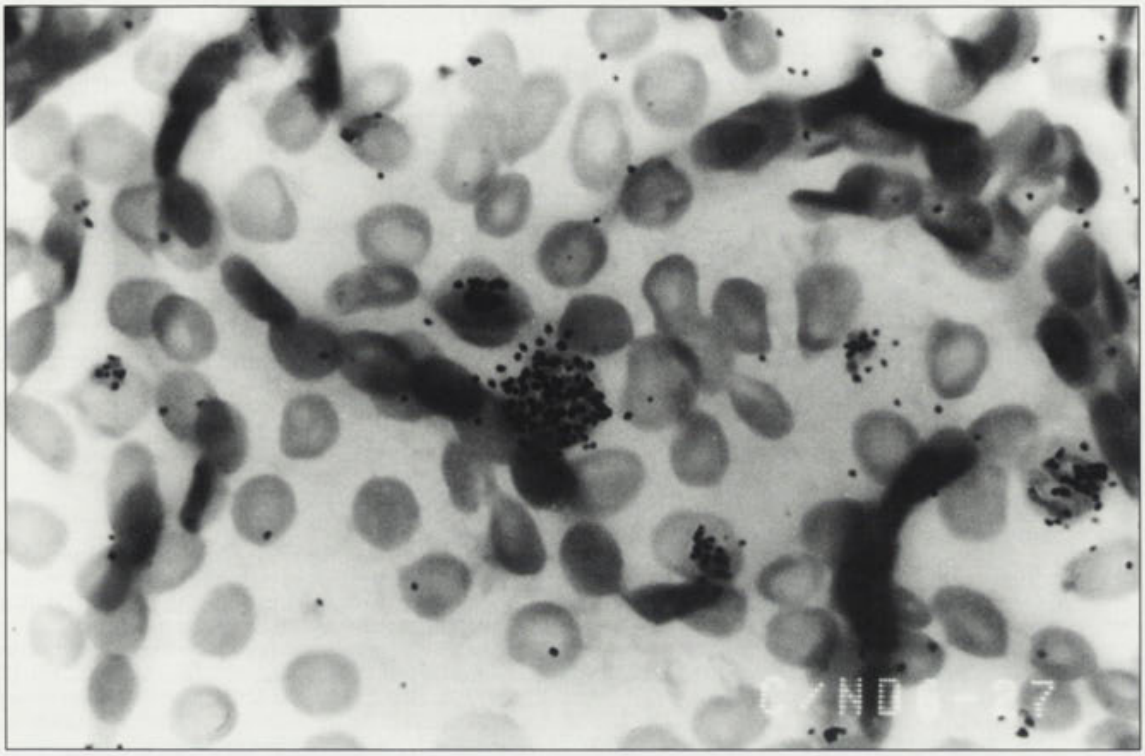

Fig. 1. Central parasitized erythrocyte showing more than 50 grains and several peripheral parasites also labeled. Sample from an infected mouse receiving a dose of 3 μCi ^{3}H-hypoxanthine. Magnification x 100

was labeled at that time. The starting point for ^{3}H-h incorporation was probably instantaneous. Ting and Sherman (1981) found, in an *in vitro* assay, that the hypoxanthine uptake process is very rapid in outdate normal and *P. falciparum* infected human erythrocytes with a maximum labeling reached within 30 seconds, suggesting that the hypoxanthine entry process is by simple diffusion. The starting time of parasite incorporation of ^{3}H-h *in vivo*, seem to be the same as *in vitro*. It is so fast, that appears to occur instantly, as if the hypoxanthine present in plasma could enter in direct contact with the parasite, as in the model of Povell's parasitophorous ducts, (Pouvell and Spiegel 1991). However, the time of maximum uptake in both conditions differs, while in Sherma's *in vitro* assay was very fast (30sec), *in vivo* the uptake was gradual reaching its maximum in 4 to 7 h (in most cases).

Incorporation of hypoxanthine in erythrocytes

In vivo labeling also differs in that infected erythrocytes seem not to uptake hypoxanthine. There are two views about the incorporation of ^{3}H-h by non-infected erythrocytes. Ting and Sherman (1981) found in an *in vitro* experiment that when incubated at 24°C, human and duck erythrocytes, (normal and infected by *P. falciparum, P. lophurae)* uptake similar amounts of ^{3}H-h, but when at 37°C, infected erythrocytes incorporated 5 times more than the normal. However, Chulay *et al.* (1983) in an *in vitro* experiment, also with *P. falciparum,* observed that hypoxanthine uptake by non-infected erythrocytes was very low and that incorporation of ^{3}H-h by *P. falciparum* was directly proportional to the number of parasitized erythrocytes in culture, suggesting that only the infected erythrocytes incorporated hypoxanthine. Autoradiography in these *in vivo* experiments gave us the opportunity to directly observe cell by cell the label in the microscope, and we were unable to see the number and grouping patterns of grains on uninfected erythrocytes to consider them as labeled. More than 97% of erythrocytes from control mice did not show grains, and 95% of those presenting grains had just one grain. Even more, in experiment 1 only 7 erythrocytes out of 40,000 observed presented 3 grains on its surface and with no typical clustering, making difficult to think

that those erythrocytes really had uptaken ^{3}H -h. Results are similar in control and experimental animals of the other experiments. These findings seems to indicate that perhaps the outdatedness of erythrocytes might account for abnormal incorporation of ^{3}H-h in the Sherman's experiment, or because ^{3}H-h erythrocytes uptake do not take place *in vivo*.

Leukocytes hypoxanthine incorporation

Leukocytes have a DNA mass hundreds of times bigger than that of the parasite. If leukocytes consume hypoxanthine for DNA synthesis, the amount they might use would be far more than the used by the parasites, which would result in a richer load of grains in leukocytes than in parasites. Although, proportionally more leukocytes than erythrocytes presented grains (about 11% in control mice), about 88% of those leukocytes showed just one grain, and from the total population of leukocytes observed, just 0.3% present 3 grains, so it is very difficult to say that those leukocytes were really labeled.

It is very common to see parasites with 30 or more grains at maximum uptake (Fig. 1), while in leukocytes exceptionally 4 grains can be seen and with no cluster pattern. Our results seem to be in accordance with Petersen (1986) who state that "it is well known that white cells do not incorporate ^{3}H-h during cultivation of malaria parasites nor either during *in vitro* drug resistance assays", although our observations are *in vivo*.

Individual parasites uptake

The mean number of grains contained by the individual parasite including all experiments and all stages at its maximum uptake varied in a range of 4.4 to 9.6 grains, although in some specimens it is possible to find 30 or more grains in a single parasite (Fig. 1).

In general, it can be expected for experimental purposes that maximum labeling can be achieved between 4 and 7 h post-labeling and that the label is present in the parasites for at least 72 h while circulating in the blood (three reproductive cycles in Pyy) after the application of a single doses of ^{3}H-h.

One could expect to label about 50% of the circulating parasites when applying a doses of 1μCi per mouse g, about 55% when a doses of 2μCi are given and about 85 to 90% when 3μCi are dispensed (these figures have to be fine tune with more experiments).

The error factor on parasites grain counting in autoradiography is expected to be of 1.7% (mean value). However, we were not always sure if the background grains corresponded to background radiation or it was coming from residual chromatin of parasites in advanced process of cytolysis, phenomenon that is very common during certain moments of the infection.

The results of these experiments seem to indicate that the *in vivo* ^{3}H-h labeling is a very selective way to label the intraerythrocyte forms of the malaria parasites, at least in the rodent malaria model. Labeling of malaria parasites during their daily life activities into their hosts seems very attractive to follow up the parasite movements in the host body, and other biological processes.

Acknowledgement: This work was funded by CONACyT México (5386-M)

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Received on 27th April, 2000; accepted on 12th July, 2000

Acta Protozool. (2000) 39: 289 - 294

ACTA
PROTOZOOLOGICA

Characterization of a New Species of the Ciliate *Tetrahymena* (Ciliophora: Oligohymenophorea) Isolated from the Urine of a Dog: First Report of *Tetrahymena* from a Mammal

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Summary. An approximately 10 yr-old male Dalmation was admitted to the Exeter Animal Hospital presenting symptoms of continuous urination, polyuria/polydypsia, and regurgitation. Urinalysis showed glucosuria, pyuria, proteinuria, casts, and white blood cells. Microscopic examination of urine revealed considerable numbers of a ciliated protozoan. The ciliate was isolated and aseptically cultured in proteose peptone medium. Cytological staining of cells with the Chatton-Lwoff silver nitrate and silver proteinate procedures demonstrated that the ciliate was a species of the genus *Tetrahymena*, measuring about 50 x 25 µm, placing it within the "*pyriformis*" species complex. Polymerase chain reaction amplification of the small subunit rRNA (SSrRNA) gene followed by DNA sequence analysis confirmed this identification. Analysis of the complete SSrRNA gene demonstrated significant differences in primary sequence from all other members of the "*pyriformis*" species complex and justified the designation of a new species, *Tetrahymena farleyi* sp. n.

Key words: polymerase chain reaction, small subunit rRNA sequence, *Tetrahymena farleyi* sp. n., urinary infection.

INTRODUCTION

Ciliated protozoans are symbionts of a wide variety of animals, both invertebrate and vertebrate (Corliss 1979). Typically, they are commensal organisms, neither helping nor harming their host. There is some evidence that the ruminant ciliates may be mutualistic, enhancing the growth and weight gain of their ruminant hosts while themselves benefiting from the rumen environment (Hungate 1966). A minority of the species has been designated as parasitic or facultatively parasitic (Corliss 1979). *Balantidium*, a member of the Class Litostomatea (Subclass Trichostomatia), has long been considered the only parasitic ciliate of vertebrates (Corliss 1979), typically invading the intestinal tissues of human beings and their domestic and other animals (Zaman 1978). However, a recent report has recorded *Balantidium* in the urinary bladder of a human being (Maleky 1998). Facultative parasites are common in the Class Oligohymenophorea, especially the Subclasses Scuticociliatia and Hymenostomatia (Hoffman 1978). Several genera of scuticociliates have been reported to infect mostly marine organisms, including oysters (Elston *et al.* 1999), crabs (Morado and Small 1995, Messick and

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Small 1996), lobsters (Cawthorn *et al.* 1996), and fishes (Hoffman 1978, Cheung *et al.* 1980). The hymenostome ciliates *Tetrahymena*, *Ophryoglena*, and *Ichthyophthirius* are reported to parasitize a wide variety of freshwater organisms. *Ichthyophthirius* is an obligate parasite of fishes often resulting in the death of the host fish (Hoffman 1978, Corliss 1979) while *Ophryoglena* is mainly an opportunistic histophage on moribund or dead invertebrates (Corliss 1979).

Species in the genus *Tetrahymena* and its phylogenetically close relative *Lambornella* are also typically characterized as facultative parasites. They have been reported to infect and cause the death of invertebrates, such as black flies (Corliss *et al.* 1979, Lynn *et al.* 1981, Batson 1983), mosquitoes (Washburn *et al.* 1988, Jerome *et al.* 1996), and vertebrates, especially fishes (Hoffman *et al.* 1975, Hoffman 1978, Ferguson *et al.* 1987). To our knowledge, there has never been a report of a *Tetrahymena* infection of a mammal.

The present study was undertaken when a Dalmation dog was admitted to the Exeter Animal Hospital with an infection of the urinary system. Microscopic observation of the urine revealed numerous ciliated protozoa swimming freely in the fluid. These ciliates were tentatively identified as members of the genus *Tetrahymena* on initial examination. To confirm this preliminary identification, the ciliates were cultured in sterile medium, stained for cytological examination, and the small subunit rRNA (SSrRNA) gene was amplified by the polymerase chain reaction (PCR). These further characterizations corroborated the ciliate as a *Tetrahymena* and moreover indicated that this was a new species, *Tetrahymena farleyi* sp. n., which is described in detail herein.

MATERIALS AND METHODS

The agent

This isolate of *Tetrahymena* was obtained from the urine of a Dalmation dog admitted for euthanasia on 25 September, 1996 to the care of Dr. Ellen Shapiro, Exeter Animal Hospital, 660 Main Street South, Exeter, ON N0M 1S1, Canada. No information is available about how the dog acquired the infection.

Culture techniques

The ciliates were isolated in 0.05% Cerophyl culture medium inoculated with *Enterobacter aerogenes*. Clonal isolates were established in proteose peptone-yeast extract (PPYE) medium (0.5% proteose peptone, 0.5% yeast extract, 0.125% dextrose anhydrate) on 15 April 1998 and have been submitted to the American Type Culture Collection with Accession No. 50748. Isolates have been propagated by biweekly transfers since that time.

Cytological techniques

Ciliates were pelleted from the Cerophyl medium by centrifugation. They were then fixed in Champy's Fluid followed by Da Fano's Fluid, and stained by the Chatton-Lwoff silver nitrate technique (Foissner 1992) or fixed in Bouin's Fluid and stained using a silver proteinate stain (Montagnes and Lynn 1993).

Polymerase chain reaction

Isolation of DNA and PCR amplification of the SSrRNA genes followed procedures described in Jerome and Lynn (1996).

DNA sequencing and phylogenetic analysis

PCR-amplified DNA was purified on 1.0% agarose gels using the GeneClean® Kit. The SSrRNA was sequenced directly in both directions with an ABI Prism 377 Automated DNA Sequencer (Applied Biosystems Inc.) using dye terminator and Taq FS with three forward and three reverse internal universal 18S primers (Elwood *et al.* 1985).

Sequence availability and systematic analysis

The nucleotide sequences in this paper are available from the GenBank/EMBL databases under the following accession numbers: *Tetrahymena australis*, M98015, X56167; *Tetrahymena borealis*, M26359, M98020; *Tetrahymena canadensis*, M26359, M98022, X56170; *Tetrahymena capricornis*, M98018, X56172; *Tetrahymena hegewischi*, M98019, X56166; *Tetrahymena hyperangularis*, M98014, X56173; *Tetrahymena malaccensis*, M26360; *Tetrahymena nanneyi*, M98016, X56169; *Tetrahymena patula*, M98017, X56174; *Tetrahymena pigmentosa*, M26358; *Tetrahymena pyriformis*, M98021, X56171; *Tetrahymena tropicalis*, M98023, X56168 (Sogin *et al.* 1986); *Tetrahymena thermophila*, M10932 (Spangler and Blackburn 1985); *Tetrahymena corlissi*, U17356 (Wright and Lynn 1995); *Tetrahymena empidokyrea*, U36222 (Jerome *et al.* 1996). All sequences were globally aligned using the Dedicated Comparative Sequence Editor (DCSE) program (De Rijk and De Wachter 1993) and further refined by considering secondary structural features of the 18S molecule. Nucleotide differences between species were then tabulated.

RESULTS

Evaluation of the dog

An approximately 10 yr-old, neutered male Dalmation, named "Farley", was admitted presenting symptoms of continuous urination, polyuria/polydypsia, and regurgitation. The dog weighed about 27 kg and had been losing weight for about one month: the owner reported the normal weight to have been around 36 kg. Blood glucose was > 500 mg/dl, suggesting diabetes mellitus. The

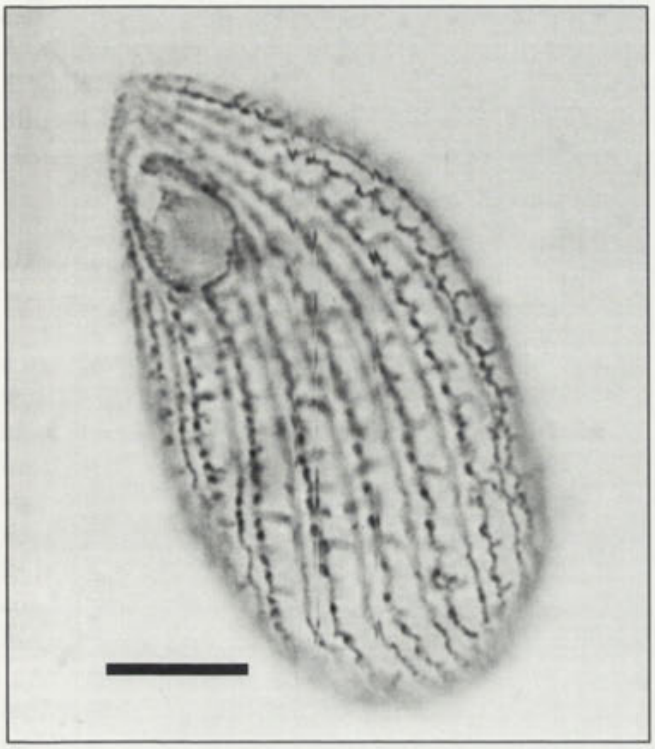

Fig. 1. Ventral view of the ciliate *Tetrahymena farleyi* sp. n., which was found in a urinary system infection of a Dalmation dog. Chatton-Lwoff silver stain. Scale bar - 10 μm

bladder was thickened but there were no palpable stones. Urine was collected midstream. It was deep yellow in colour, aromatic, and turbid. Urinalysis showed a specific gravity of 1.023, glucosuria, pyuria, proteinuria, casts, white blood cells, and ciliates.

Immediately after the dog was euthanized, the urine sample was collected aseptically via aspiration through the bladder and abdominal wall. The urine sample was then used as the source to establish the clonal cultures of the ciliate.

Cytology of ciliate

The ciliate was ovoid to pyriform in cell shape. Both silver stains revealed an oral apparatus typical of the genus *Tetrahymena*: there were three oral membranelles on the left side of the oral cavity and an undulating membrane on the right side. The cell body was covered by ciliary rows evenly spaced over the cell surface (Fig.1). After Chatton-Lwoff staining, the following measurements characterized the ciliate: body length - 48.0 (42-54.3) ± 3.5 μm (mean - range ± standard deviation) (n = 31); body width - 19.4 (14-24.5) ± 2.4 μm (n = 31); oral cavity length - 10.4 (8.8-12.3) ± 0.54 μm (n = 31); length of oral membranelle 1- 5.7 (5.3-7) ± 0.65 μm (n = 26); length of oral membranelle 2 - 4.9 (3.5-5.3) ± 0.60 μm (n = 26); length of oral membranelle 3 - 2.5 (1.8-3.5) ± 0.53 μm (n = 26); number of somatic kineties or ciliary rows - 16 (15-17) ± 0.58 (n = 28); and two postoral kineties (n=31). After QPS staining, the following measurements characterized the ciliate: body length-50 (43.8-56) ± 3.1 μm (n = 30); body width - 21.8 (17.5-26.3) ± 2.29 μm (n = 30); macronuclear length - 11.4 (8.8-14.9) ± 1.27 μm (n = 30); and macronuclear width - 10.5 (8.8-14) ± 1.01 μm (n = 30). The ciliate did not appear to have a micronucleus nor a caudal cilium.

PCR and sequence analysis of ciliate

PCR amplification yielded a PCR fragment of approximately 1,700 bp in length. Complete sequence of the SSrRNA from this ciliate was 1749 nucleotides and is deposited under Accession Number AF184665. It differed at least one of a total of 64 positions from the other species of the "*pyriformis*" species complex. It differed from all other species at two positions: it had a T rather than a C at nucleotide positions 700 and 1663 of *Tetrahymena thermophila* (Table 1). Phylogenetic analysis (data not shown) indicated that it was most closely related to *Tetrahymena tropicalis* with which it showed the fewest differences in sequence (Table 1).

Description of new species

On the basis of these sequence differences, we justify the establishment of a new species in the genus *Tetrahymena*.

Tetrahymena farleyi sp. n. (Fig. 1, Table 1)

Etymology: named after "Farley", the Dalmation dog from which the isolate was derived.

Type locality: near Exeter, ON, Canada (48° 21'N, 81° 29'W)

Description: *Tetrahymena farleyi* sp. n. is ovoid to pyriform in cell shape, and morphologically indistinguishable from previously described species in the "*pyriformis*" species complex. *Tetrahymena farleyi* sp. n. cultured in PPYE ranged in body length from 42 - 56 μm and in body width from 14 - 26 μm, with 15 - 17 somatic kineties with two postoral kineties. This species is amicronucleate and lacks a caudal cilium.

Type specimens: a type culture of *T. farleyi* sp. n. (Accession No. 50748) has been submitted to the Ameri-

Table 1. Basepair differences between *T. farleyi* and some other *Tetrahymena* species

Species	Sequence Position 5' → 3'																																		
	129	189	216	228	262	267	268	273	270	275	276	349	423	479	480	484	485	487	491	492	512	518	642	643	645	648	649	653	660	663	666	671	679
T. farleyi	G	T	G	T	A	A	G	C	G	T	T	T	G	G	A	C	T	-	T	C	A	T	G	T	T	A	A	C	T	A	A	A	T
T. australis	.	.	.	.	.	T	A	.	.	.	A	.	.	.	.	T	A	.	.	.	.	.	.	.	C	T	T	.	A	G	.	G	C
T. borealis[1]	.	C	.	C	.	.	A	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	C	T	T	.	.	G	.	.	C
T. capricornis	.	C	.	C	.	T	A	.	.	.	A	.	.	.	.	T	A	-	.	.	.	.	.	.	C	T	T	.	A	G	.	G	C
T. corlissi	A	C	.	.	.	C	A	.	.	.	G	.	.	.	.	T	.	-	.	.	.	.	A	C	C	.	.	.	.	G	G	.	C
T. hegewischi	.	C	.	C	.	T	A	.	.	.	A	.	.	A	C	T	A	-	G	T	.	.	.	.	C	T	T	.	A	G	.	G	C
T. malaccensis	.	C	.	C	G	.	.	T	A	C	.	.	.	.	.	A	.	C	.	.	C	A	.	.	C	T	T	T	.	G	.	.	.
T. empidokyrea	.	.	A	C	.	T	A	.	.	.	A	Y	A	.	.	T	A	-	.	.	.	.	.	C	C	T	T	.	A	G	.	.	C
T. nanneyi[2]	.	.	.	C	.	T	A	.	.	.	A	.	.	.	.	T	A	-	.	.	.	.	.	.	C	T	T	.	A	G	.	G	C
T. patula	.	.	.	C	.	T	A	.	.	.	A	.	.	.	.	T	A	-	.	.	.	.	.	.	C	T	T	.	A	G	.	G	C
T. pyriformis	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	T	.	-	.	.	.	A	.	.	C	T	T	.	A	G	.	.	C
T. tropicalis	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	A	.	.	.	.	.	.	.	.	.	.	C
T. thermophila	.	C	.	C	.	.	.	T	A	C	.	.	.	.	.	T	.	C	.	.	C	A	.	.	C	T	T	.	.	G	.	.	.

Species	Sequence Position 5' → 3'																																		
	700	722	723	752	771	804	953	1035	1059	1326	1327	1328	1332	1335	1340	1341	1342	1371	1456	1461	1464	1481	1482	1532	1657	1659	1660	1663	1667	1668	1670	1674	1745
T. farleyi	T	A	A	C	A	A	-	A	A	G	C	T	G	C	G	G	T	G	G	T	T	T	A	G	C	A	T	T	A	T	G	A	T
T. australis	C	G	G	T	.	.	-	T	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	T	T	.	C	C	G	.	A	.	.
T. borealis[1]	C	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	C	A	.	.
T. capricornis	C	G	G	T	.	.	-	T	.	.	T	C	.	.	.	A	.	.	.	.	.	.	.	T	T	.	C	C	G	.	A	.	.
T. corlissi	C	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	C	.	A	.	.	.	.	.	C	.	.	.	.	A
T. hegewischi	C	G	G	T	.	.	-	T	.	A	.	C	.	.	.	.	.	.	.	.	.	.	.	T	T	.	C	C	G	.	A	.	.
T. malaccensis	C	.	.	.	.	.	-	.	.	.	.	G	.	T	T	.	.	T	A	.	.	.	.	.	.	.	C	C	G	.	.	.	.
T. empidokyrea	C	G	.	T	G	G	-	T	R	.	T	C	.	.	.	A	.	.	.	.	C	A	T	T	.	G	C	C	G	C	.	.	A
T. nanneyi[2]	C	G	G	T	.	.	-	T	.	.	T	C	.	T	.	A	.	.	.	.	.	.	.	T	T	.	C	C	G	.	A	.	.
T. patula	C	G	G	T	.	.	-	T	.	.	T	C	.	.	.	A	.	.	.	.	.	.	.	T	T	.	C	C	G	.	A	.	.
T. pyriformis	C	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	C	A	G	.
T. tropicalis	C	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	C	.	.	A	.	.
T. thermophila	C	.	.	.	.	.	-	.	.	.	.	.	A	T	.	.	.	T	A	.	.	.	.	.	.	.	C	C	G	.	.	.	.

[1]*T. borealis* and *T. canadensis* have the same sequence; [2]*T. nanneyi, T. hyperangularis,* and *T. pigmentosa* have the same sequence.

can Type Culture Collection (Manassas, VA, USA). A Chatton-Lwoff stained type slide (USNM) of *T. farleyi* sp. n. has been submitted to the Ciliate Type Slide Collection of the National Museum of Natural History (Smithsonian Institution, Washington, D.C., USA).

DISCUSSION

This is the first report of a *Tetrahymena* species from a mammal. It is not known whether the ciliate infection was a primary or secondary one, responsible for the bladder infection of this dog. *Tetrahymena* infections of invertebrates (Corliss *et al.* 1979; Batson 1983, 1985) and vertebrates (Thompson 1958, Hoffman *et al.* 1975, Ferguson *et al.* 1987) are known to be fatal. Therefore, it is not unreasonable to suppose that the extreme condition of this dog was caused by the ciliate infection in the bladder.

Not only is this the first report of a *Tetrahymena* infection in a mammal, the isolate also appears to be a new species of *Tetrahymena*, which we have named *Tetrahymena farleyi* sp. n. Since this species was maintained for several months on a bacterized culture medium, it must be classified as a facultative parasite. It is not known how this ciliate came to infect the dog nor whether this ciliate species has a preference for mammalian hosts.

Acknowledgements. We thank Wilma Lagerwerf, Animal Health Technician, for collecting the urine sample.

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Received on 4th June, 2000; accepted on 18th July, 2000

Acta Protozool. (2000) 39: 295 - 322

ACTA
PROTOZOOLOGICA

Morphological and Taxonomical Studies on Some Marine Scuticociliates from China Sea, with Description of Two New Species, *Philasterides armatalis* sp. n. and *Cyclidium varibonneti* sp. n. (Protozoa: Ciliophora: Scuticociliatida)

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Summary. The morphology, infraciliature and silverline system of 7 marine scuticociliates including two new species are described: *Pleuronema coronatum* Kent, 1881, *Cohnilembus verminus* Müller, 1786, *Philasterides armatalis* sp. n., *Pseudocohnilembus persalinus* Evans & Thompson, 1964, *Porpostoma notatum* Möbius, 1888, *Cyclidium varibonneti* sp. n. and *Ancistrum crassum* Fenchel, 1965. The new species, *Philasterides armatalis* is identified by the following characteristics: *in vivo* 50-80 x 18-25 µm with roundly pointed apical end and one prolonged caudal cilium; oral field about 1/3 of cell length; buccal apparatus with distinctly bipartite paroral membrane, 16-18 somatic kinety, one macro- and one micronucleus; single contractile vacuole caudally located, marine form. The diagnosis of the new species, *Cyclidium varibonneti*: small marine *Cyclidium in vivo* 15-25 x 10-15 µm with two macronuclei; buccal field about 3/4 of cell length; contractile vacuole caudally located near ventral margin; 11-13 bipolar somatic kineties, of which the posterior end of SKn is conspicuously shortened and that of SKn-1 is composed of densely spaced kinetosomes. Based on the data obtained, the following species are considered to be junior synonyms: *Pseudocohnilembus longisetus* Evans & Thompson, 1964 and *P. marinus* Thompson, 1966 (= *P. persalinus* Evans & Thompson, 1964); *Lembus punctatus* Kahl, 1928 and *Lembus reesi* Kahl, 1931 [= *Cohnilembus verminus* (Müller, 1786)].

Key words: *Ancistrum crassum, Cohnilembus verminus, Cyclidium varibonneti* sp. n., marine ciliates, morphology, new species, *Philasterides armatalis* sp. n., *Pleuronema coronatum, Porpostoma notatum, Pseudocohnilembus persalinus,* Scuticociliatida.

INTRODUCTION

Ciliates assigned to the order Scuticociliatida are found in all kinds of habitats with great biodiversity regarding their life styles, structure, behaviour and many other biological characters (Buddenbrock 1920; Kahl 1931; Czapik 1963; Borror 1965; Agamaliev 1968; Fenchel 1968; Thompson 1968; Grolière 1974, 1980; Coats and Small 1976; Wilbert and Kahan 1981; Foissner *et al.* 1982, 1994; Aescht and Foissner 1992; Fernandez-Leborans and Novillo 1994b). In marine biotopes, scuticociliates occur usually abundantly in coastal area, especially in eutrophic mariculture waters (Raabe 1959, Fenchel 1965, Puytorac *et al.* 1974, Hu *et al.* 1996, Song *et al.* 1999). History of taxonomic studies on those small ciliates might be traced back to two hundred years ago though regular "modern" researches have been conducted

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only since 1960's with application of silver impregnation methods to reveal the infraciliature and silverline system (Dragesco 1963; Even and Corliss 1964; Borror 1965; Thompson 1966; Small 1967; Foissner 1971, 1985; Grolière 1974; Grolière and Detcheva 1974; Czapik and Jordan 1976; Foissner and Wilbert 1981; Wilbert 1986; Fernandez-Leborans and Novillo 1994a).

Many studies, including some of those performed recently, concerning these organisms are often, however, carried out more or less deficiently and some considerable potential value for precise identification of taxa has been overlooked. A common event involved is that many species described previously are usually studied either only based on impregnated specimens or just after living observations (Kahl 1931, Evans and Thompson 1964, Fernandez-Leborans and Zaldumbide 1984, Small and Lynn 1985, Fernandez-Leborans and Novillo 1994a, Morade and Small 1994). This leads, furthermore, the following problems: all species described in past time (using "classical" methods) are thus difficult or virtually impossible to reidentify if investigations are merely based on infraciliature and the features of silverline system. Most work conducted before or around Kahl's time gave basically only information obtained from living observations while modern identification is based largely on the structure of silvered specimens, especially the structure of buccal apparatus, which are completely lacking in classical work.

Since some important diagnostic features seen only in living cells (e.g. behaviour or movement, location of contractile vacuole, distribution of extrusomes, body shape, cell colour *et al.*) will be lost after fixation, modern techniques combined with detailed living observations - e.g. "all-side descriptions"- are hence significant for taxonomic determinations. In another word, it is always extremely necessary to make critical comparison before report new taxa.

Since 1989, a long-term taxonomic project on ciliate fauna has been being carried out in coastal waters off the Yellow and Bohai Sea, north China, in which many scuticociliates were isolated, observed and taxonomically investigated, and approximately 18 species from this order had been reported (Song 1993; Hu *et al.* 1996; Song and Packroff 1997; Xu *et al.* 1997; Song and Wei 1998; Xu and Song 1998, 1999a,b; Song and Wilbert 1998, 2000; Song *et al.* 1999). As a new contribution, the present paper gives descriptions of 5 known forms and 2 new species with emphasis on their living morphology and diagnostic characters of infraciliature and silverline system.

MATERIALS AND METHODS

Ciliates are collected from the coast off Qingdao (Tsingtao, 36 08' N; 120 43' E), China and some off-shore maricultural ponds for farming molluscs or shrimp near Qingdao. After isolation, all specimens were maintained as pure or raw cultures in Petri dishes in the laboratory for days to weeks with wheat/ rice grains as food source to enrich bacteria. Observations on living cells were carried out with a microscope equipped with Normarski differential interference optics. Protargol (Wilbert 1975) and Chatton-Lwoff method (Corliss 1953) were used for revealing the infraciliature and silverline system.

Drawings of impregnated specimens were conducted with the help of camera lucida; measurement was performed under the x 1250 magnification. Systematic and terminology are mainly based on Corliss (1979) and Thompson (1964).

RESULTS AND DISCUSSION

Order Scuticociliatida Small, 1967

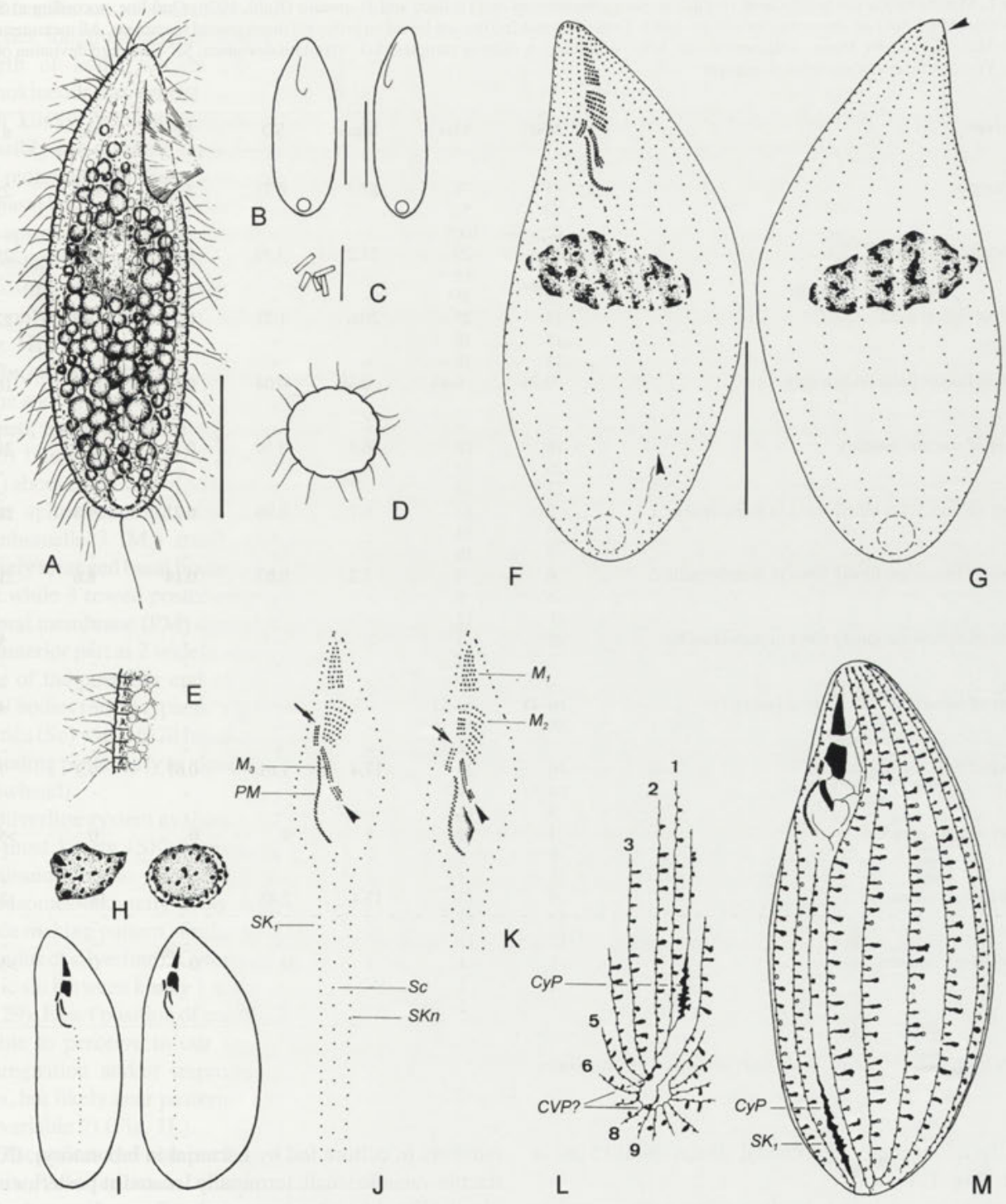
Genus *Philasterides* Kahl, 1926, *Philasterides armatalis* sp. n. (Figs. 1 A-M, 28, 29; Table 1)

Diagnosis. Medium-sized marine *Philasterides in vivo* 50-80 x 18-25 μm; body usually cylinder-shaped with roundly pointed apical end, one macro- and one micronucleus; oral field about 1/3 of cell length; buccal apparatus genus typical with distinctly bipartite paroral membrane; 16-18 somatic kinety; single contractile vacuole terminally located; one prolonged caudal cilium.

Type location and ecological characters. This species was found for several times in low numbers in mariculture waters (ponds for mollusc farming) near Qingdao. Salinity was about 31‰, pH 7.9-8.0, water temperature 7-12°C.

Type specimens. One holotype as a slide of protargol and 1 paratype of Chatton-Lwoff silver nitrate stained specimens have been deposited in the Laboratory of Protozoology, Ocean University of Qingdao, Qingdao, China.

Descriptions. Cells *in vivo* mostly 60-70 x 20-25 μm, body shape cylinder-like to slender bag-shaped, circular in cross-section with anterior portion often bending backwards; apical end slightly to distinctly pointed depending on thickness of cells while posterior generally rounded (Figs. 1 A, B). Ventral surface gently indented around buccal area, dorsally convex. Buccal field with shallow depression, about 1/3 of cell length. Pellicle rigid and slightly notched, thereunder arranged densely spaced extrusomes (*ca* 2 μm in length) (Fig. 1 E). Cilia usually



Figs. 1 A-M. *Philasterides armatalis* sp. n. from life (**A-E**), after protargol (**F-K**) and silver nitrate impregnations (**L, M**). **A** - side view, a typical individual; **B** - to show different body shape and size; **C** - crystals; **D** - to demonstrate the cross section at about middle body; **E** - detail of cortex, to exhibit the arrangement of extrusomes; **F, G** - infraciliature of ventral and dorsal side, arrowhead in **F** indicates the posterior end of the scutica, while in **G** marks the small bold apex; **H** - macronuclei; **I** - position of the buccal field in different individuals; **J, K** - buccal apparatus, note the prominent scutica, the bipartite paroral membrane (arrow) and the uniquely arranged membranelle 3 (arrowhead); **L** - caudal view of silverline system; **M** - ventral left-side view. Abbreviation: CVP - contractile vacuole pore, CyP - cytopyge, M_{1-3} - membranelle 1-3, PM - paroral membrane, Sc - scutica, SK1, n - the first and last somatic kinety. Scale bars - 20 μm (in **A, B**), 5 μm (in **C**)

Table 1. Morphometric characterisation of *Philasterides armatalis* sp. n. (1st line) and *P. armata* (Kahl, 1926) (2nd line, according to Song and Wilbert 1989; 3rd line, according to Grolière 1980). Data in 1st and 2nd line are based on protargol impregnated specimens. All measurements in μm. Max - maximum; Mean - arithmetic mean; Min - minimum; n - size of samples; SD - standard deviation; SE - standard deviation of the mean, Vr - coefficient of variation in percent

Character	Min	Max	Mean	SD	SE	Vr	n
Body length	45	74	57.7	6.71	1.34	11.6	25
	42	53	-	-	-	-	-
	65*	100*	-	-	-	-	-
Body width	16	29	21.2	3.39	0.68	16.0	25
	11	15	-	-	-	-	-
	20*	35*	-	-	-	-	-
Length of buccal field	18	25	20.6	1.71	0.37	8.3	22
	15	18	-	-	-	-	-
	14	18	-	-	-	-	-
Length of buccal field: body length	0.30	0.44	0.36	0.04	0.01	11.8	16
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
Number of somatic kineties	16	18	16.8	0.54	0.18	3.2	21
	26	32	-	-	-	-	-
	27	32	-	-	-	-	-
Number of transverse kinety rows in membranelle 1	7	12	9.7	0.96	0.21	9.8	21
	10	14	-	-	-	-	-
	9	10	-	-	-	-	-
Number of transverse kinety rows in membranelle 2	6	9	7.2	0.63	0.14	8.6	21
	6	8	-	-	-	-	-
	11	12	-	-	-	-	-
Number of transverse kinety rows in membranelle	22	3	-	-	-	-	5
	2	2	-	-	-	-	-
	-	-	-	-	-	-	-
Number of basal bodies in somatic kinety 1**	*ca* 45	*ca* 51	-	-	-	-	4
	46	55	-	-	-	-	-
	-	-	-	-	-	-	-
Number of basal bodies in scutica	16	20	17.4	1.61	0.61	9.3	7
	ca 10	-	-	-	-	-	-
	9	12	-	-	-	-	-
Number of macronucleus	1	1	1	0	0	0	>50
	1	1	-	-	-	-	-
	1	1	-	-	-	-	-
Length of macronucleus	9	18	13.4	2.45	0.59	18.3	17
	12	17	-	-	-	-	-
	12	17	-	-	-	-	-
Number of micronuclei	1	1	1	0	0	0	>50
	1	1	-	-	-	-	-
	1	1	-	-	-	-	-

* - data from live, ** - basal body pairs accounted as single ones

8 to 10 μm long, one single caudal cilium about 15 μm in length (Fig. 1 A).

Cytoplasm colourless, hyaline, often with several to many sparsely distributed bar-shaped crystals (Fig. 1C). In thick forms (well-fed cells) always with numerous light-reflecting granules (2-5 μm in cross, possibly inactive food vacuoles), which often render specimens rather dark (Fig. 1A). No food vacuoles observed, but likely to be bacteria-feeder in nature (quickly reaching tremendous numbers in culture fed by bacterial in laboratory). Contractile vacuole small, terminally located at posterior end of cell (Fig. 1A). One large oval macronucleus centrally located, which is often irregularly shaped after protargol impregnation (Figs. 1F, G); micronucleus undetermined, possibly adjacent to macronucleus.

Locomotion generally hastily. As swimming always straight ahead or motionless for a long while when feeding on surface of debris.

Infraciliature as shown in Figs. 1F, G and J. Somatic kineties longitudinally arranged, extending over entire length of body, which are basically composed of monokinetids throughout except the anterior-most end of each kinety, yet not seldom to see some large cells (possibly pre-dividing ones ?) with "normal", mixed di- and monokinetids. Each row with about 50 basal bodies (or basal body pairs). Anteriorly one small, rounded cilia-free apical area formed by kineties. Caudal cilium complex at posterior end, as seen in most other scuticociliates, consisting of one basal body and 2 parasomal sacs, which are only revealed after silver nitrate impregnation (Fig. 1L).

Buccal apparatus consisting of bipartite paroral membrane and 3 membranelles; membranelle 1 (M_1) triangle-shaped, about 6-8 μm long, consisting of 7-12 (mostly 9-10) transverse rows of kinetosomes; membranelle 2 (M_2) about equal size of M1, 6-9 (usually 7) rowed, which often splits longitudinally into two parts (Fig. 1K); membranelle 3 (M_3) small, obliquely positioned with densely arranged basal bodies, often two rowed in anterior part while 3 rowed posteriorly (Figs. 1J, K, 13, 14, 28). Paroral membrane (PM) considerably bipartite, of which the anterior part as 2 widely separated rows, begins on the right of the posterior end of M2, while in the 2nd part, basal bodies form a typical "zig-zag" pattern (Figs. 1 J, K). Scutica (Sc) with 16-20 basal bodies, arranged in one line, extending posteriorly to almost 4/5 of cell length (Fig. 1F, arrowhead).

Silverline system as shown in Figs. 1L, M, 15 and 29, left-most kinety (SKn) crossing over caudal area and continuing on to caudal cilium complex (Fig. 1L). Extrusomes distinctly away from direct silverlines and hence making pattern similar to that of *Tetrahymena* (yet no indirect silverline). Cytopyge (CyP) as short argentophilic slit between kinety 1 and n, irregularly shaped (Figs. 1L, 29). Exact position of contractile vacuole pore (CVP) unable to perceive in our specimens because of over-impregnation and/or improper embedding position of cells, but likely near posterior end of either kinety 3 or 9 (or variable ?) (Fig. 1L).

Discussion and comparison. By now, the monotypic genus *Philasterides* is characterized by the bipartite paroral membrane, *Tetrahymena*-like silverline system and three well-defined membranelles which are arranged in a *Paranophrys*-pattern (Mugard 1949, Grolière 1980, Song and Wilbert 1989).

The only known species in this genus, *P. armata* Kahl, 1926, is a freshwater form, which has been already described for several times up to date (Kahl 1931, Mugard 1949, Grolière 1980, Song and Wilbert 1989). It differs from the new species mainly in markedly higher number of somatic kinety (26-32 *vs.* 16-18), the position of contractile vacuole (about posterior 2/5 of cell length *vs.* entirely terminal) as well as the different habitats (fresh water *vs.* marine) (Table 1).

Genus *Cyclidium* O. F. Müller, 1786, *Cyclidium varibonneti* sp. n. (Figs. 2A-K, 12; Tables 2, 3)

Diagnosis. Small marine *Cyclidium in vivo* 15-25 x 10-15 μm, consistently with two macronuclei; buccal field about 3/4 of cell length; contractile vacuole caudally located on ventral side; 11-13 bipolar somatic kineties, of which the posterior end of SKn is conspicuously shortened and that of SKn-1 is composed of densely spaced monokinetids.

Type location and ecological characters. Once occurred as pelagic form in low numbers in coastal water off Qingdao (23 April 1995). Salinity was about 31‰, pH 7.9-8.0, water temperature 7-8°C.

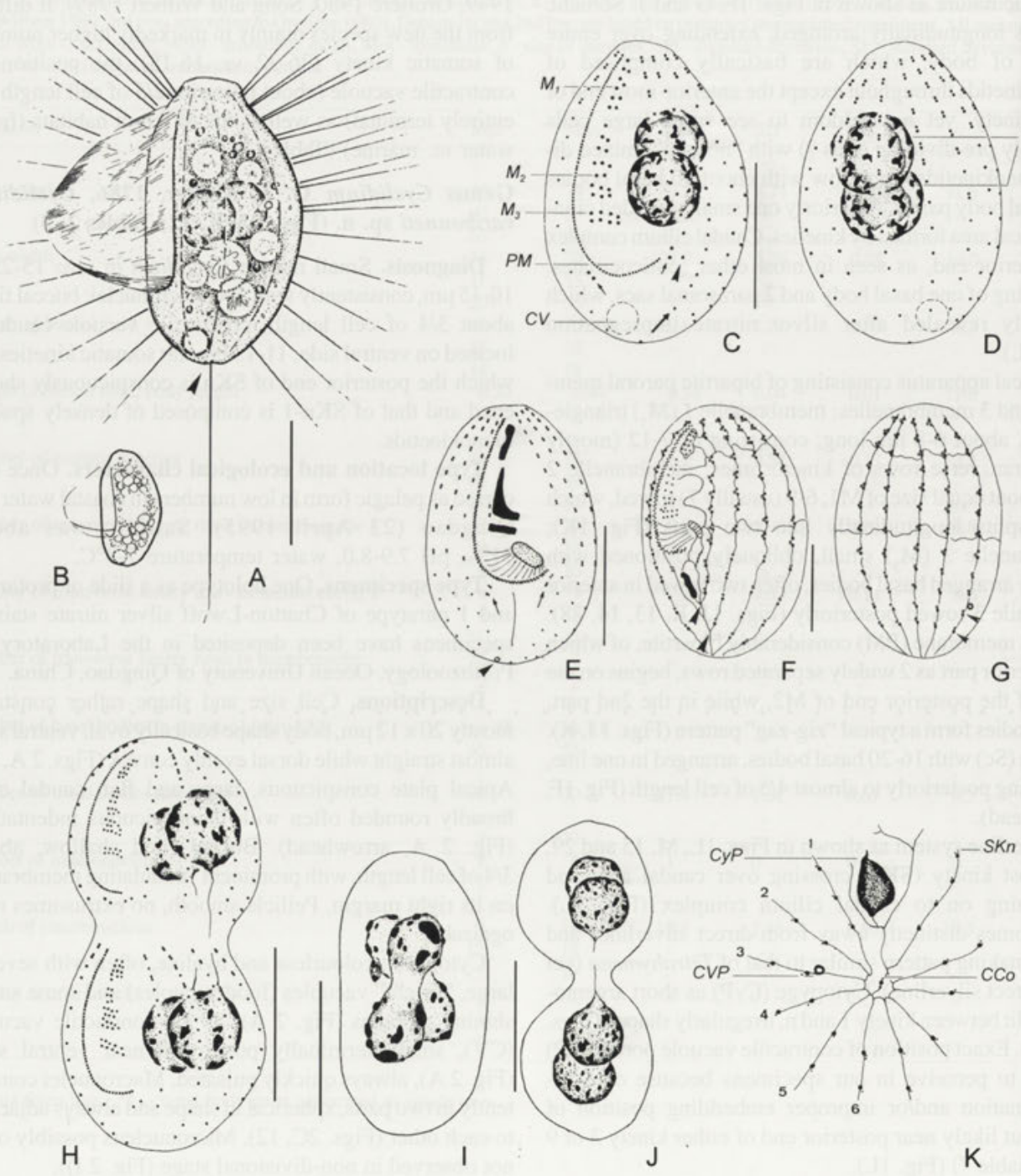
Type specimens. One holotype as a slide of protargol and 1 paratype of Chatton-Lwoff silver nitrate stained specimens have been deposited in the Laboratory of Protozoology, Ocean University of Qingdao, China.

Descriptions. Cell size and shape rather constant, mostly 20 x 12 μm, body shape basically oval, ventral side almost straight while dorsal evenly convex (Figs. 2 A, B). Apical plate conspicuous, large and flat, caudal area broadly rounded often with inconspicuous indentation (Fig. 2 A, arrowhead). Buccal filed shallow, about 3/4 of cell length, with prominent "undulating membrane" on its right margin. Pellicle smooth, no extrusomes recognizable.

Cytoplasm colourless and hyaline, often with several large, "bright" vacuoles (food vacuoles) and some small shining globules (Fig. 2 A). Single contractile vacuole (CV), small, terminally positioned near ventral side (Fig. 2 A), always quickly pulsated. Macronuclei consistently in two parts, spherical in shape and always adjacent to each other (Figs. 2C, 12). Micronucleus possibly one, not observed in non-divisional stage (Fig. 2 I)).

Cilia of somatic region about 6-8 μm in length, like in most other congeners, stiff and motionless during feeding (or pause); single caudal cilium *ca* 20 μm long. Locomotion genus typical: quickly jumping, then remaining on same spot (or suspended in water) for long periods.

Mostly 11-12 (only one individual with 13 meridians among specimens observed) somatic kineties arranged longitudinally, which are usually dikinetids in anterior 1/5-2/5 of each row. All kineties more or less shortened



Figs. 2 A-K. *Cyclidium varibonnensi* sp. n. (**A-D, H-K**), a morphotype from Australia (**F, G**, after Niessen 1984, master thesis) and *Cyclidium bonnensi* Grolière, 1980 (**E**, after Grolière, 1980) from living observations (**A, B**), after silver nitrate (**F, G, K**) and protargol impregnations (**C-E, H-J**). **A, B** - side view of typical cells, arrowhead indicates the indentation at the caudal portion; **C, D** - infraciliature of ventral and dorsal view. Small arrows mark the scutica; large arrow indicates the densely ciliated somatic kinety n-1, arrowhead demonstrates the strongly shortened SKn; **E** - ventral view of infraciliature, arrowhead marks the pore of contractile vacuole (CVP); arrows indicate the scutica; **F, G** - ventral and dorsal view of silverline system, arrowheads indicate the SKn, SKn-1 and basal bodies posterior to the CVP; **H-J** - cells in different divisional stages, note the nuclear division is about to complete and each dividing part with two macronuclei; **K** - caudal view of silverline system. Abbreviation: CCo - caudal cilium complex, CV - contractile vacuole, CVP - pore of contractile vacuole, CyP - cytopyge, M_{1-3} - membranelle 1-3, Ma - macronucleus, PM - paroral membrane, Sc - scutica, SKn - the last somatic kinety (No. n). Scale bars - 10 μm

Table 2. Morphometric characterisation of *Cyclidium varibonnesi* sp. n. Data based on protargol impregnated specimens. All measurements in µm. Max - maximum; Mean - arithmetic mean; Min - minimum; n - size of samples; SD - standard deviation; SE - standard deviation of the mean, Vr - coefficient of variation in percent

Character	Min	Max	Mean	SD	SE	Vr	n
Body length	12	19	13.5	1.46	0.36	10.9	17
Body width	8	13	9.8	0.90	0.22	9.2	17
Length of buccal field	9	13	9.7	0.66	0.16	6.8	17
Length of buccal field: body length	0.67	0.81	0.73	0.04	0.01	5.7	17
Number of somatic kineties	11	13	11.7	0.60	0.15	5.1	16
Number of basal bodies in somatic kinety 1*	14	16	15.2	0.58	0.16	3.9	13
Number of basal bodies in somatic kinety n*	10	11	10.4	0.51	0.15	4.9	11
Number of basal bodies in somatic kinety n-1*	14	17	14.5	3.59	1.08	24.8	11
Number of macronucleus	2	2	2	0	0	0	>100
Length of macronucleus	3	5	3.9	0.72	0.18	18.5	16

* - basal body pairs accounted as single ones

in posterior portion making one large cilia-free caudal region (Figs. 2 C, D). Pattern of infraciliature generally *glaucoma*-like, left-most kinety (SKn) terminating highly at about cytostome level (Fig. 2 C, small arrows), while SKn-1 (one left to SKn) extending posteriorly to about 90% of cell length, with posterior portion evidently densely ciliated (Fig. 2 C, arrow).

Buccal apparatus as shown in Fig. 2 C: membranelle 1-3 (M1-3) lined up close to each other along shallow buccal cavity, each with *ca* 6, 7 and 2 transverse rows of basal bodies respectively. Paroral membrane (PM) gently curved, extending posteriorly to about 3/4 of cell length, consisting of single-rowed basal bodies.

Silverline system genus typical, silverline making small opened circle around caudal pole (polar circle); line from kinety n (SKn) continuing to caudal cilium complex (CCo) and crossing over onto polar circle (dorsally) near meridian 5 and 6 (Fig. 2 K). Cytopyge (CyP) subterminally as broad argentophilic patch between SK1 and SKn. Contractile vacuole pore (CVP) positioned at end of 2nd somatic kinety, somewhat away from polar circle (Fig. 2K).

Discussion and comparison. Among scuticociliates, many species of the genus *Cyclidium* remain confused with reference to their identification. This is, above all, because most of them exhibit similar behavior, body size and appearance, as well as the basic pattern of silverline system and infraciliature. Some widely used, significant criteria in other related groups (e.g. the structure of the oral apparatus) are likely non-species-specific for these organisms and usually, therefore, unable to be used for species separation. The other problems seem to derive form the form variation, for many diagnostic features seem quite population-dependent and thus overlap each other among strains/ populations and species (Kahl 1931, Berger and Thompson 1960, Didier and Wilbert 1981, Wilbert 1986, Foissner *et al.* 1994). All those yield, in author's opinion, great difficulties to define species, even in some "well-described" forms.

As to the authors' knowledge, *Cyclidium bonneti* Grolière 1980 is the only taxon in this genus consistently with two macronuclear nodules, while all other congeners have basically only one macronucleus (though the number may vary in some species from 1 to several in some cases).

Compared with *Cyclidium bonneti* Grolière 1980, the new species described in the present paper is identified by: (1) lower number of somatic kineties (mostly 11-12 *vs.* 14-16 in *C. bonneti*); (2) relatively smaller size (12-19 *vs* 18-28 µm in length after impregnation); (3) appearance of some ciliature features (e.g. somatic kinety No. n not shortened posteriorly in *C. bonneti*) and (4) different habitats (marine *vs.* fresh water) (Table 3). All those indicate that the two taxa should be separated at species level.

In 1984, Niessen isolated also a 2-Ma-possessing organism from saline soil samples in Australia (master thesis, unpublished), which was identified as *Cyclidium bonneti*. It corresponds to our form in many characteristics, e.g. shortened SKn, related biotope (saline environment) and similar number of somatic kineties (Table 3, Figs. F, G). It differs only in the larger size (28-31 *vs.* 16-20 µm) and likely the position of CVP

Table 3. Morphometric comparison of three 2-Ma-having morphotypes of *Cyclidium*. Measurements in μm

Character	*Cyclidium varibonneti*	*"C. bonneti"* (misidentification)	*C. bonneti*
Body size *in vivo*	15-25 x 10-15	-	-
Body size after protargol impregnation	12-19 x 8-13	28-31 x 16-20	18-28 x 11-18
Length of buccal field : cell length	3/4-4/5	*ca* 2/3	*ca* 3/4
Number of macronucleus	2	2	2
Number of somatic kineties (SK)	11-13	11	14-16
Number of basal bodies in SKn*	10-11	?	*ca* 16**
Position of contractile vacuole pore	terminal of SK3	subterminal of SK2 (*ca* posterior.2/5 of cell length)	terminal of SK2 ?
Characters of habitats	marine, coastal water	saline soil	fresh water
Geographical location	Yellow Sea, China	Australia	France
Data sources	original	Niessen (1984)	Grolère (1980)

? - data not available; * - basal body pairs accounted as single ones; ** - accounted from original drawings, data not given

Table 4. Comparison of some other closely related *Cyclidium*-species. Measurements in μm

Character	*C. varibonneti*	*C. bonneti*	*C. glaucoma*	*C. glaucoma*	*C. glaucoma*	*C. citrullus*	*C. setiger*
Body size *in vivo*	15-25 x 10-15	?	?	14-30 x 6-16	?	12-16 x 7-11	?
Body size (based on impregnated specimens)	12-19 x 8-13	18-28 x 11-18	15-27 x 8-16	?	*ca* 17 x 8	?	15-20 x 9-12
Length of buccal field : cell length	3/4-4/5	*ca* 3/4	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 2/3	*ca* 3/4
Number of macronucleus	2	2	1	1	1	1	1
Number of somatic kineties (SK)	11-13	14-16	*ca.* 10	10-11	11	10-11	11-13
Position of CV	terminal	terminal	terminal	terminal	terminal	terminal	terminal
CVP located	end of SK3	end of SK2 ?	end of SK2	end of SK2	end of SK2	end of SK2	end of SK5
Posterior end of somatic kinety No. n	shortened	not shortened	not shortened	not shortened	not shortened	shortened	shortened
Habitat	marine	fresh water	fresh water	fresh water	fresh water	marine	fresh water
Geographical location	Qingdao, China	Puy-de-Dome, France	Virginia, USA	Europe	Europe	Qingdao, China	Ontario Lake, Canada
Data sources	original	Grolière, 1980	Berger & Thompson, 1960	Foissner *et al.*, 1994	Didier & Wilbert, 1981	Song & Wei, 1998	Wilbert, 1986

CV - contractile vacuole, CVP - contractile vacuole pore, ? - data not available

Table 5. Morphometric characterisation of 2 populations of *Pseudocohnilembus persalinus*. Data based on protargol impregnated specimens. All measurements in µm. Max - maximum; Mean - arithmetic mean; Min - minimum; n - size of samples; SD - standard deviation; SE - standard deviation of the mean, Vr - coefficient of variation in percent

Character	Min	Max	Mean	SD	SE	Vr	n
Body length	25	37	29.9	3.81	0.87	12.7	19
	29	43	33.7	3.66	0.92	10.7	16
Body width	15	26	20.3	3.32	0.76	16.3	19
	16	23	18.9	2.60	0.65	13.8	16
Length of buccal field	12	21	17.3	2.84	0.82	16.4	14
	14	20	16.5	1.57	0.45	9.5	12
Length of buccal field: body length	0.46	0.59	0.51	0.02	0.01	4.1	14
	-	-	-	-	-	-	-
Number of somatic kineties	9	11	10.2	0.75	0.19	7.4	16
	10	11	10.3	0.46	0.11	4.5	16
Number of basal bodies in somatic kinety 1*	17	21	18.2	1.30	0.33	7.1	9
	-	-	-	-	-	-	-
Number of macronucleus	1	1	1	0	0	0	>50
	1	1	1	0	0	0	>50
Length of macronucleus	7	12	8.9	1.48	0.37	16.6	16
	10	15	11.6	1.43	0.36	12.3	16

* - Basal body pairs accounted as single ones

(end of SK2 *vs.* SK3) (Table 3). These minor differences suggest that it is possibly conspecific with this species.

According to the infraciliature, at least 2 other *Cyclidium* species should be compared, which exhibit equally the small size and similar number of somatic kineties: *C. glaucoma* and *C. setiger* Wilbert 1986 (Table 4). The former is, as accepted by most taxonomists, a typical freshwater form with (normally) one macronucleus and has relatively shorter buccal field (*ca* 1/2 *vs.* 3/4). In addition, it has a different ciliary pattern (without shortened SKn) (Berger and Thompson 1960, Didier and Wilbert 1981, Song and Wilbert 1989, Foissner *et al.* 1994). *C. setiger* is also a freshwater form, which can be recognized by the position of contractile vacuole pore (end of SK5 *vs.* SK2-3), a single macronucleus and the non-densely ciliated SKn-1 at the posterior end (Table 4) (Wilbert 1986).

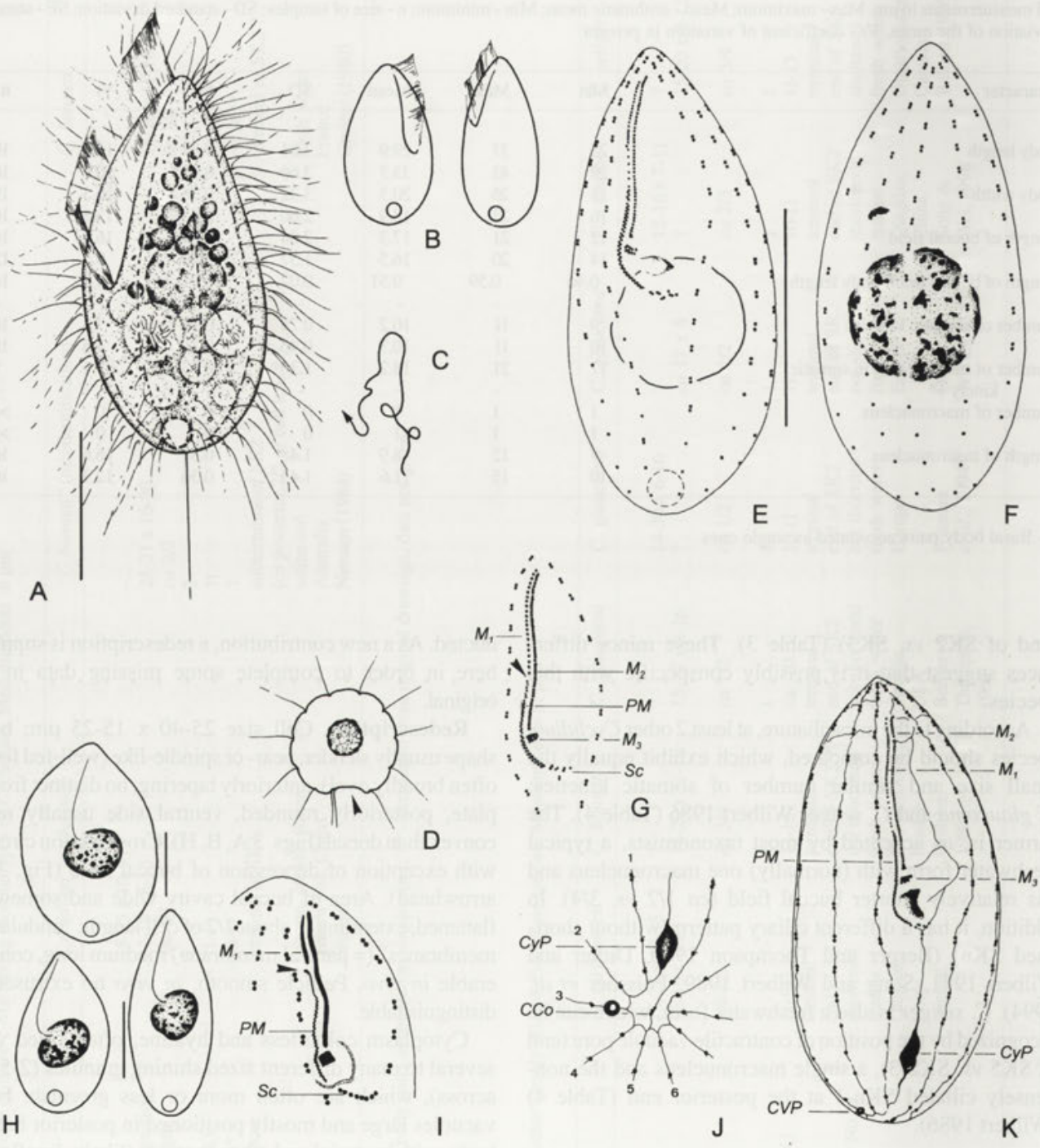
Genus *Pseudocohnilembus* Evans & Thompson, 1964; *Pseudocohnilembus persalinus* Evans & Thompson, 1964 (Figs. 3 A-K, 9-11, 13-15; Table 5); syn. *Pseudocohnilembus longisetus* Evans & Thompson, 1964; *Pseudocohnilembus marinus* Thompson, 1966

This species was originally described by Evan and Thompson (1964) based on silver nitrate impregnated specimens, and then no further studies have been conducted. As a new contribution, a redescription is supplied here in order to complete some missing data in the original.

Redescription. Cell size 25-40 x 15-25 µm; body shape usually slender, pear- or spindle-like (well-fed forms often broadly oval), anteriorly tapering, no distinct frontal plate, posteriorly rounded, ventral side usually more convex than dorsal (Figs. 3 A, B, H). Cross section circular with exception of depression of buccal field (Fig. 3 D, arrowhead). Area of buccal cavity wide and somewhat flattened, extending to about 1/2 of cell length; "undulating membranes" (= paroral membrane) medium long, considerable *in vivo*. Pellicle smooth, *in vivo* no extrusomes distinguishable.

Cytoplasm colourless and hyaline, often filled with several to many different sized shining granules (2-5 µm across), which are often more or less greenish. Food vacuoles large and mostly positioned in posterior half of body, with bacterial and other contents (likely dinoflagellates) (Fig. 3 A). Contractile vacuole small, located caudally near ventral margin of cell (Fig. 3 A), pulsated frequently (with about several seconds intervals). One large spherical macronucleus centrally located with many irregular-shaped nucleoli; micronucleus not observed.

Cilia densely arranged, 7 to 10 µm long, one single caudal cilium about 15 µm. Movement moderately fast, no speciality, rotating about long axis of body (Fig. 3 C).



Figs. 3 A-K. *Pseudocohnilembus persalinus* from living observations (**A-D, H**), after silver nitrate (**I-K**) and protargol impregnations (**E-G**). **A** - side view of a typical cell; **B, H** - to show different body shape; **C** - scheme of movement; **D** - cross section, to show the buccal depression (arrowhead); **E, F** - infraciliature of ventral (**E**) and dorsal (**F**) view, note that the scutica composed of several densely spaced basal bodies; **G** - buccal apparatus, arrowhead indicates the anterior end of paroral membrane; **I** - portion of buccal area, arrowhead marks the anterior end of paroral membrane (after Pomp and Wilbert 1988); **J** - caudal view, to show the silverline system; **K** - ventral view of silverline system. Abbreviation: CCo - caudal cilium complex, CVP - pore of contractile vacuole, CyP - cytopyge, M_{1-3} - membranelle 1-3, PM - paroral membrane, Sc - scutica. Scale bars - 15 μm (in **A, H**), 20 μm (in **E, F**)

Table 6. Morphometric comparison of some *Pseudocohnilembus persalinus*-populations reported by different authors. All measurements in μm

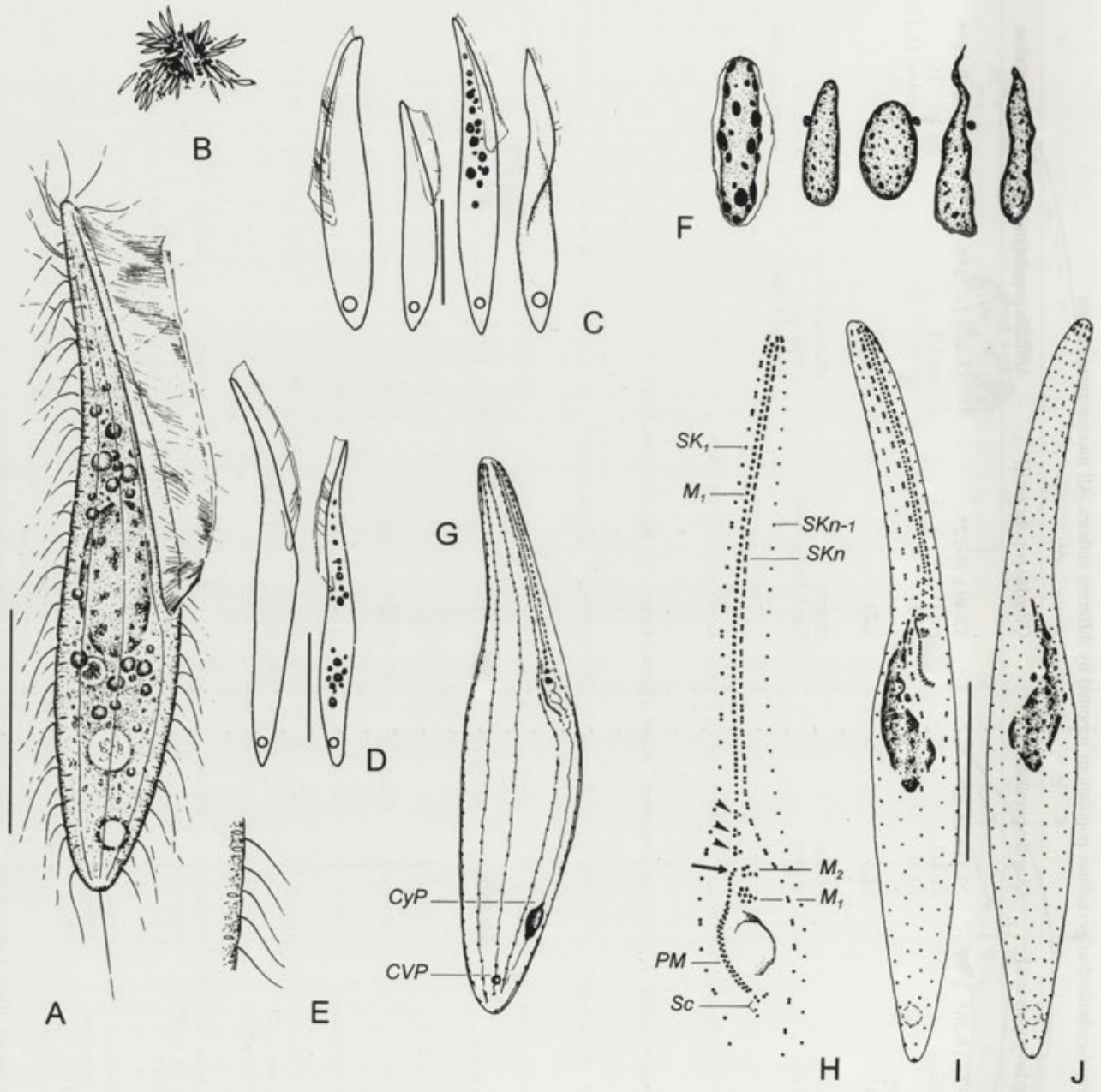
Character	Qingdao population I	Qingdao population II	Qingdao population III	Australia population	USA population
Body size (after impregnation)	25-37 x 15-26*	29-43 x 16-23*	32-49 x 16-22*	33-41 x 21-25**	20-37 x 10-18**
Length of buccal field : cell length	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2 1/2
Number of somatic kineties (SK)	9-11	10-11	10-11	9-10	8-9 (seldom 11-12)
Number of basal bodies in SK1	17-21	?	?	?	? ?
Position of CVP, located at the end of somatic kinety (SK)	SK3	SK3	SK3	SK3	SK3
Habitats	marine	marine	marine	alkaline soil	salt lake
Data sources	original	original	Xu & Song, 1999	Pomp & Wilbert, 1988	Evans & Thompson, 1964

? - data not available, * - data based on protargol impregnated specimens, ** - data based on Chatton-Lwoff silvered specimens

Table 7. Morphological comparison of some closely-related "*P. persalinus*-congeners" under different names. All measurements in μm

Character	*P. persalinus***	*P. persalinus**	*P. hargisi**	*P. hargisi***	*P. longisetus**	*P. longisetus**	*P. marinus**	*P. marinus***	*P. marinus**
Body size (after impregnation)	25-43 x 15-26	20-38 x 10-18	*ca* 44 x 18	37-55 x 17-23	*ca* 27 x 12	*ca* 29 x 14	27-40 x 9-18	34-47 x 14-20	32-36 x 20-22
Length of buccal field: cell length	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2	*ca* 1/2
Number, basal bodies in dorsal kineties	15-18	*ca* 20	*ca* 27	?	*ca* 16	*ca* 18	*ca* 18	*ca* 30	14-17
Number, somatic kineties (SK)	9-11	8-9	14 (13-15)	13-14	11 (10-11)	10 (9-11)	10	*ca* 10	8-9
Number, contractile vacuole pores, (CVP)	1	1 (seldom 2)	2 (1-3)	2 (1-2)	1	2 (1-3)	1	1	1
Position of CVP, located near the end of somatic kinety	SK3	SK3 (when two, then near SK3 & 4	SK4 & 5	SK3 & 4	between SK2 & 3	mostly SK3 & 4 (SK3-5)	SK4	SK1 or 2	SK3
Habitat	marine	Great Salt Lake (salt water)	brackish water	marine	marine	marine	marine	marine	Solar Lake (salt water)
Geographical location	China	USA	USA	USA	USA	Nelly Island, Antarctica	USA	USA	Israel
Data sources	original	Evans & Thompson, 1964	Evans & Thompson, 1964	Borror, 1972	Evans & Thompson, 1964	Thompson, 1965	Thompson, 1966	Borror, 1972	Foissner & Wilbert, 1981

? - data not available, * - data based on Chatton-Lwoff silvered specimens, ** - data based on protargol impregnated specimens



Figs. 4 A-J. *Cohnilembus verminus* from life (**A-E**), after protargol (**F, H-J**) and silver nitrate impregnations (**G**). **A** - side view, a typical individual; **B** - to show cells "piled up" on debris; **C** - to show different body shape, note the cell size (the same proportion); **D** - two slender forms; **E** - detail of cortex, to exhibit the arrangement of extrusomes; **F** - different shape of macronucleus (after impregnation); **G** - right side of silverline system; **H** - ventral view, to show the buccal apparatus, arrowheads mark the last portion of the membranelle 1, arrow indicates the anterior end of paroral membrane; **I, J** - ventral and dorsal view of infraciliature. Abbreviation: CVP - contractile vacuole pore, CyP - cytopyge, M_{1-2} - membranelle 1, 2, PM - paroral membrane, Sc - scutica, SK1, SKn, SKn-1 - the first, last and the last-but-one somatic kinety. Scale bars - 25 μm (in **A, C, D**), 20 μm (in **I, J**)

When feeding, usually motionless and crowing with its ventral side on the bottom of Petri dish.

Infraciliature as shown in Figs. 3 E, F, mostly 10 bipolar somatic kineties arranged sparsely, which are usually dikinetids in the anterior 2/3 of the body.

Buccal apparatus consisting of 3 highly specialised membranelles (M1-3), M1 single-rowed and parallel to M2, positioned immediately anterior to paroral membrane (PM) (thus only detectable in well-impregnated specimens); M2 also one-rowed (basal bodies arranged

likely more densely than in M1), almost 4/5 of length of buccal field, close to M1 and PM on right margin of oral cavity (Figs. 3 E, G); M3 very small, consisting of *ca* 3 rows of kinetids (each containing about 3-4 basal bodies) and positioned at about half way of paroral membrane (Fig. 9). Paroral membrane gently curved, composed of zig-zag row of basal bodies; scutica (Sc) composed of about 4-5 paired kinetosomes (Figs. 3 E, G).

Silverlines as shown in Figs. 3 J, K, extrusomes not observed. Line along kinety No. n (first kinety to left of buccal field) continuing to caudal cilium complex, crossing through polar circle and adjacent near meridian 5 dorsally (Fig. 3 J). Cytopyge (CyP) as thick argentophilic patch, located subcaudally (Figs. 3 J, K). Contractile vacuole pore (CVP) positioned at end of 3rd somatic kinety (Figs. 3 J, K).

Ecological features. Two populations were found in low number from a farming pond for shrimp culture in Qingdao (9 November 1993) and coastal water off Jiaonan, near Qingdao (22 April 1995). Salinity was about 30-32‰, pH 8.0-8.2.

Discussion and comparison. The genus *Pseudocohnilembus* is characterized by "a buccal cavity containing 2 large membranes whose infraciliature consisted of parallel rows of kinetosomes nearly equal in length" as described originally (Evans and Thompson 1964). Since then, more than 10 species have been assigned to this genus (Thompson 1965, 1966; Small 1967; Czapik 1968; Borror 1972; Foissner and Wilbert 1981; Fernandez-Leborans and Zaldumbide 1984; Foissner 1985; Fernandez-Leborans and Novillo 1994b).

From the previous studies the following conclusions can be approached: (1) the structure of the buccal apparatus of all known members is extremely similar; (2) many diagnostic characters (e.g. the body size, the number and position of contractile vacuole pores) appear to be population-dependent and overlapped among species and (3) most known species are described based mainly on silver impregnated specimens with little information about the living morphology (Evans and Corliss 1964, Evans and Thompson 1964, Thompson 1965, Czapik 1968, Borror 1972, Foissner and Wilbert 1981, Foissner 1985, Pomp and Wilbert 1988, Xu and Song 1999a) (see also Table 7). All these points mentioned above indicate the difficult and confusing (even contradictory) state for the taxonomy of this genus and suggest that the species separation/ identification must be based on combined features.

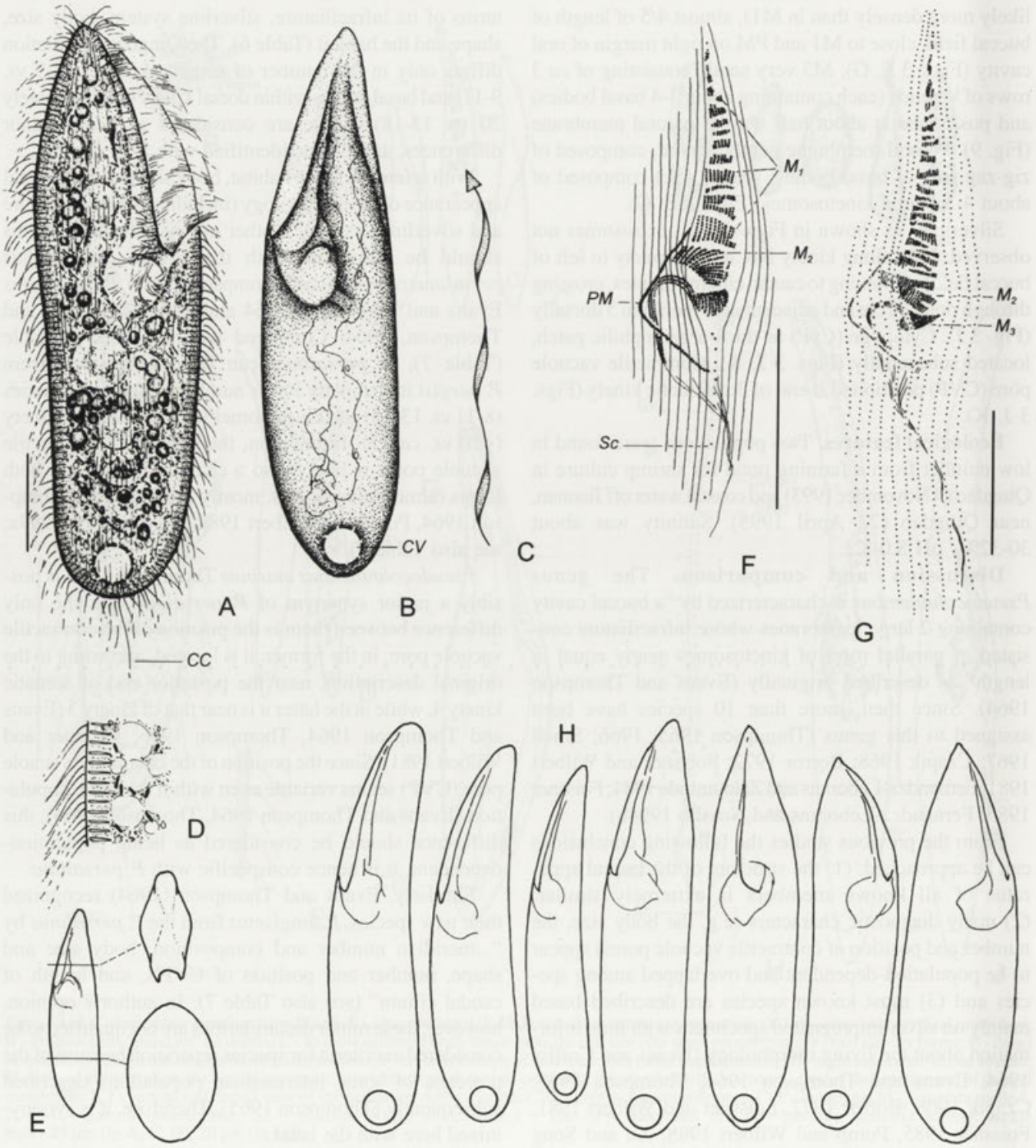
The original descriptions of *Pseudocohnilembus persalinus* is very similar to the form found in Qingdao in terms of its infraciliature, silverline system, body size, shape and the habitat (Table 6). The Qingdao-population differs only in the number of somatic kineties (8-9 *vs.* 9-11) and basal bodies within dorsal kinety (approximately 20 *vs.* 15-18). These are considered as being minor differences, and is thus identified with *P. persalinus.*

With reference to the habitat, body size and the general appearance of the morphology (including the infraciliature and silverline system), 3 other marine-inhabiting forms should be compared with the *Pseudocohnilembus persalinus: P. marinus* Thompson, 1966, *P. longisetus* Evans and Thompson, 1964 and *P. hargisi* Evans and Thompson, 1964. Compared with the data available (Table 7), *P. persalinus* can be distinguished from *P. hargisi* in distinctly lower number of somatic kineties (8-11 *vs.* 13-15) and kinetosomes within the dorsal kinety (<20 *vs. ca* 27). In addition, the number of contractile vacuole pores is likely also a charater to separate both forms (almost always 1 *vs.* mostly 2) (Evan and Thompson 1964, Pomp and Wilbert 1988, Xu and Song 1999a; see also Table 7).

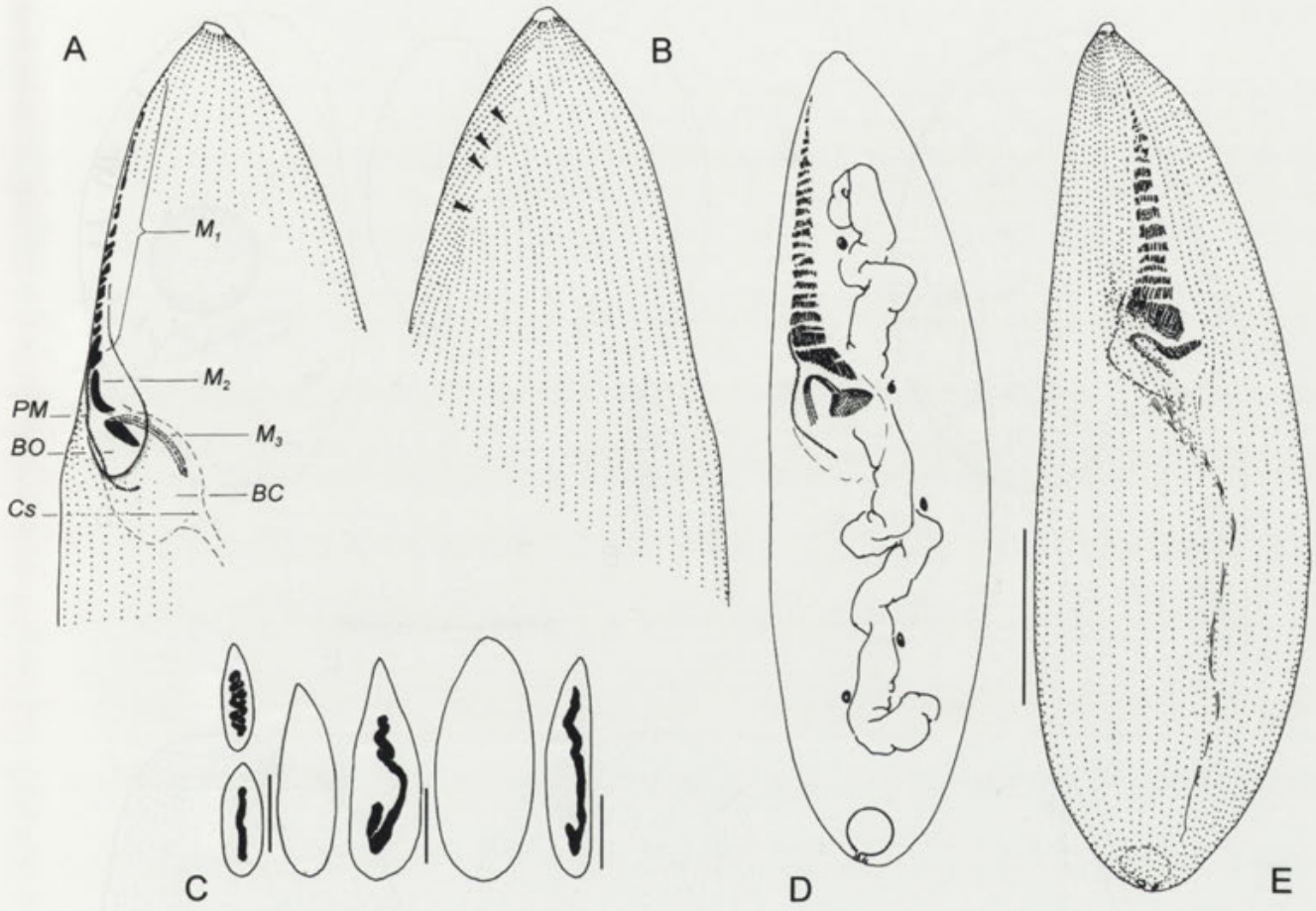
Pseudocohnilembus marinus Thompson, 1966 is possibly a junior synonym of *P. persalinus*, for the only difference between them is the position of the contractile vacuole pore: in the former, it is located, according to the original description, near the posterior end of somatic kinety 4, while in the latter it is near that of kinety 3 (Evans and Thompson 1964, Thompson 1966, Foissner and Wilbert 1981). Since the position of the contractile vacuole pore (CVP) seems variable even within the same population (Evans and Thompson 1964, Thompson 1966), this difference should be considered as being population-dependent, it is hence conspecific with *P. persalinus.*

Similarly, Evans and Thompson (1964) recognized their new species, *P. longisetus* from the *P. persalinus* by "...meridian number and composition, body size and shape, number and position of CVP's, and length of caudal cilium" (see also Table 7). In author's opinion, however, these minor dissimilarities are not qualified to be considered as criteria for species separation because of the presence of some intermediate populations described subsequently (Thompson 1965). Therefore, it is synonymized here with the latter.

It is still questionable whether the "*Pseudocohnilembus marinus*" reported by Borror (1972) is really a population of *P. persalinus* because the contractile vacuole pore in Borror's population is located near the end of somatic kinety 1 or 2 and there are "...approximately 30 cilia per meridian..." as he described (misinterpreted ?), which differs clearly from the original description by



Figs. 5 A-H. *Porpostoma notatum* from living observations (**A-E, H**), and after protargol impregnation (**F, G**). **A** - side view of a thick cell; **B** - to show an individual with narrowed anterior part, note the deeply sunk buccal cavity, which is complicatedly "engraved"; **C** - scheme of movement; **D** - detail of cortex, to show the arrangement of extrusomes and crystles; **E** - to show the cross sections in anterior (upper) and posterior (lower) part; **E, G** - buccal apparatus, note that the posterior portion sinks deeply within the buccal cavity; H - to illustrate different body shape (not keeping the same proportion !). Abbreviation: M_{1-3} - membranelle 1-3, PM - paroral membrane, Sc - scutica. Scale bars - 20 μm (in **A**), 30 μm (in **F, G**)



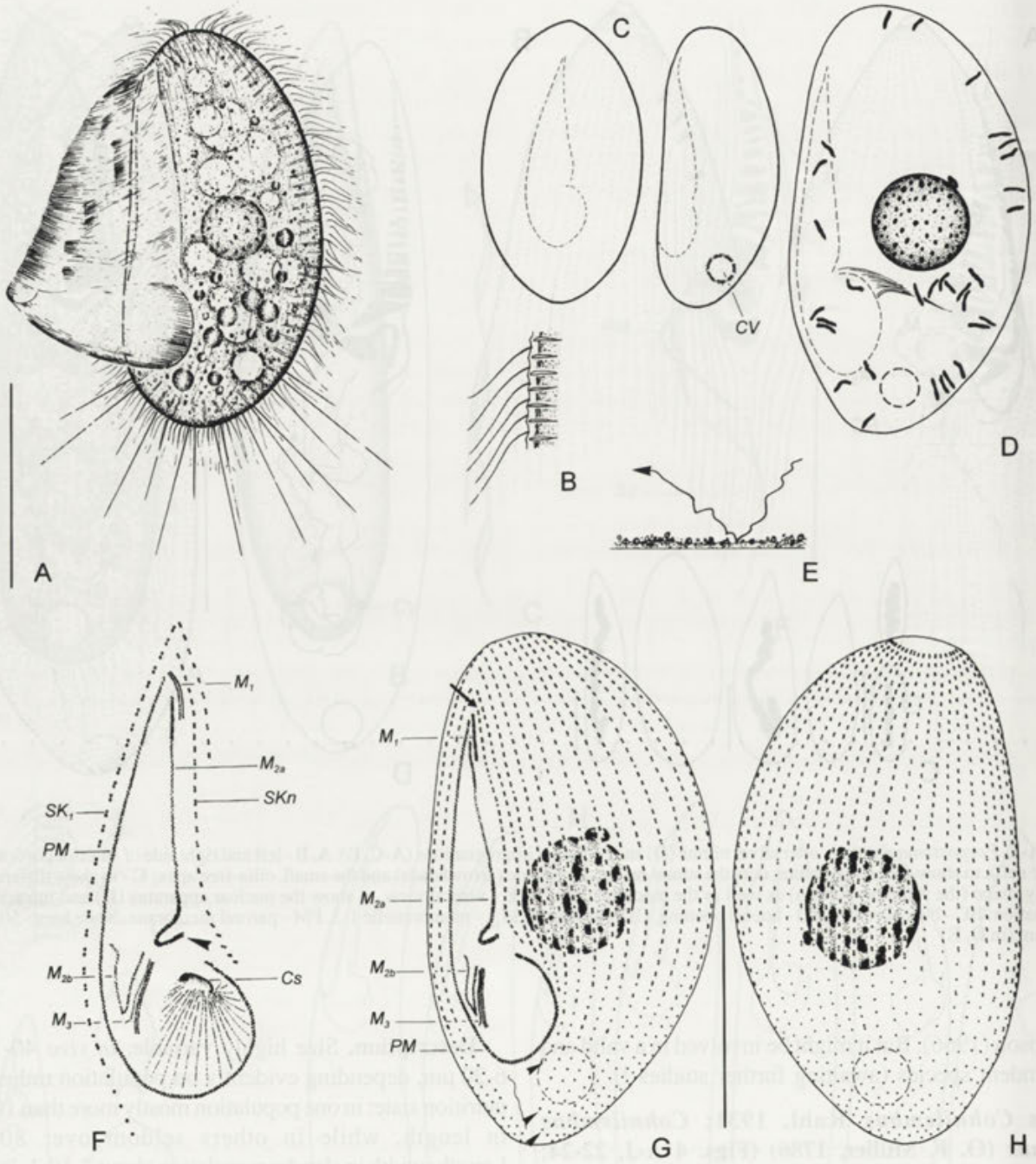
Figs. 6 A-E. *Porpostoma notatum*, after silver nitrate (**D**) and protargol impregnations (**A-C, E**). **A, B** - left and right side of anterior portion (from the same cell), to show the infraciliature, note the suture on the dorsal side (arrowheads) and the small, cilia-free apex; **C** - to show different size and body shape (the same proportion) as well as the macronucleus; **D, E** - ventral view, to show the nuclear apparatus (**D**) and infraciliature. Abbreviation: BC - buccal cavity, BO - buccal opening, Cs - cytostome, M_{1-3} - membranelle 1-3, PM - paroral membrane. Scale bars - 50 μm (in **C**), 30 μm (in **D, E**)

Thompson (1966). But it might be involved in a valid and independent species (awaiting further studies !).

Genus *Cohnilembus* Kahl, 1931; *Cohnilembus verminus* (O. F. Müller, 1786) (Figs. 4 A-J, 22-24; Table 8); Syn. *Lembus punctatus* Kahl, 1928; *Lembus reesi* Kahl, 1931

Many descriptions about this "well-known" species have been reported in last decades using modern methods (Borror 1963, 1972; Evans and Thompson 1964; Thompson 1964, 1968; Small 1967; Didier and Detcheva 1974; Jones 1974; Hu *et al.* 1996). Since its general morphology *in vivo* is highly variable and few recent studies concern its detailed living morphology, a reinvestigation is nevertheless still necessary.

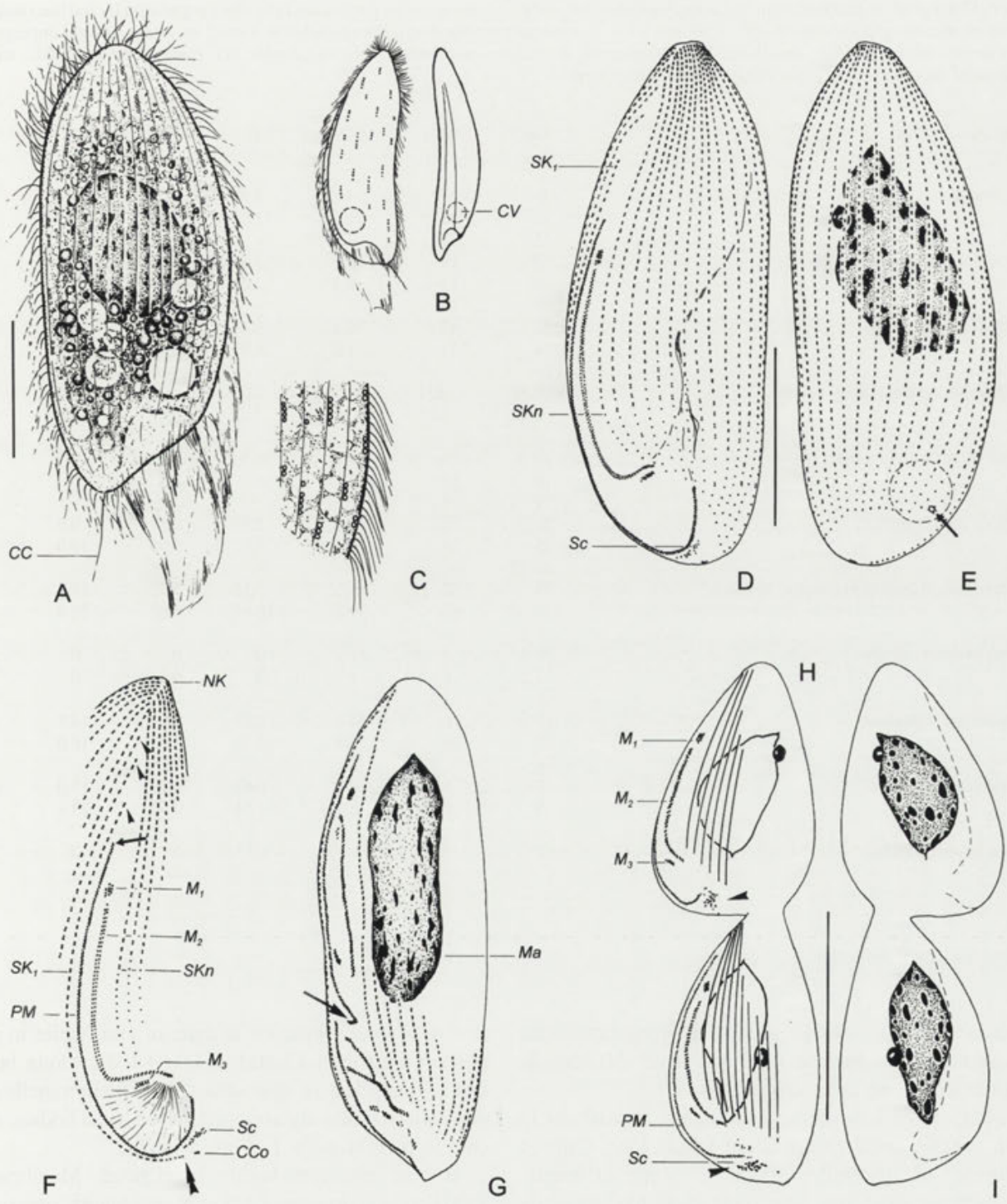
Description. Size highly variable, *in vivo* 40-130 x 6-20 μm, depending evidently on population rather than nutrition state: in one population mostly more than 100 μm in length, while in others seldom over 80 μm. Length: width in slender population about 7-10:1, in thick populations *ca* 5-6:1 (Figs. 4 C, D). Outline basically very slender, spindle-shaped or fusiform with anterior portion considerably narrowed and tapering, both ends pointed (Figs. 4 A, 22). Cross section circular, yet in starved (?) specimens much flattened (likely bilaterally) so that the cells sometimes make a fold-up or twisted appearance (Figs. 4 C, 23). Buccal field narrow, about 2/5 to over 1/2 of body length. Pellicle smooth. Extrusomes spindle-like, about 1 μm long, sparsely arranged beneath pellicle (Fig. 4 E), which are recognised from life only at high



Figs. 7 A-H. *Pleuronema coronatum* from living observations (**A-C, E**), and after protargol impregnation (**D, F-H**). **A** - left side view, a typical individual; **B** - detail of cortex, to show the arrangement of extrusomes; **C** - ventral and side view, to show the dorsoventrally flattened body shape; **D** - showing the extrusomes; **E** - scheme of movement; **F** - ventral view, to show the buccal apparatus, arrowhead indicates the hook-like end of the M2a; **G, H** - ventral and dorsal view of the infraciliature, arrow in **G** marks the anterior end of SKn, arrowheads indicate the shortened somatic kineties. Abbreviation: CV - contractile vacuole, Cs - cytostome; M_{1-3} - membranelle 1-3, PM - paroral membrane, SK1, n - somatic kinety 1 and n. Scale bars - 40 μm (in **A**), 30 μm (in **G, H**)

magnification (after protargol or silver nitrate impregnation no more to spot).

Cytoplasm colourless and hyaline, always containing several to many large (2-4 μm across), shining greenish granules. Food vacuoles large and few in number, containing possibly bacterial (Fig. 4 A). Contractile vacuole small, located caudally or slightly subcaudally near ventral margin of cell (Figs. 4 A, 23) with frequent pulsation (about several seconds intervals). Macronucleus large, elongate to oval in shape, centrally located with many to



Figs. 8 A-I. *Ancistrum crassum* from living observations (**A-C**), and after protargol impregnation (**D-I**). **A** - left side view, showing a typical individual; **B** - right lateral and ventral view of same specimen, to show the flattened body shape and the distribution of cortical granules; **C** - detail of cortex to show the cortical granules; **D, E** - ventral and dorsal view, note that almost all kineties terminate subcaudally and make a large bald field around caudal pole; arrow in **E** indicates the contractile vacuole pore; **F** - buccal apparatus, arrowheads indicate the shortened kineties, arrow demonstrates the anterior end of paroral membrane, double-arrowheads mark the posterior end of somatic kinety No. 1 (the rightmost one); **G-I** - cells in division; arrow in **G** indicates the hook-like structure of UM-anlage which will generate the scutica; arrowheads in **H** mark the on-going formed scutica. Abbreviation: CC - caudal cilium, CCo - caudal cilium complex, CV - contractile vacuole, M_{1-3} - membranelle 1-3, Ma - macronucleus, PM - paroral membrane, Sc - scutica, SK1, n - somatic kinety 1 and n. Scale bars - 30 μm (in **A, D**), 40 μm (in **I**)

Table 8. Morphometric characterisation of three populations of *Cohnilembus verminus*, population I (1st line), population II (2nd line) and USA-population according to Thompson, 1968 (3rd line, after silver nitrate impregnation). Both population 1 and 2 are after protargol impregnation. All measurements in µm. Max - maximum; Mean - arithmetic mean; Min - minimum; n - size of samples; SD - standard deviation; SE - standard deviation of the mean, Vr - coefficient of variation in percent

Character	Min	Max	Mean	SD	SE	Vr	n
Body length	39	67	54.8	8.98	2.59	16.4	12
	49	98	71.6	11.82	3.94	16.5	9
	55	75	-	-	-	-	-
Body width	7	14	10.1	2.34	0.96	33.2	12
	6	13	8.4	2.07	0.69	24.5	9
	10	15	-	-	-	-	-
Length of buccal field	32	42	36.8	3.35	0.97	9.1	12
	24	47	38.8	6.91	2.30	17.8	9
	-	-	-	-	-	-	-
Length of buccal field: body length	0.52	0.71	0.59	0.06	0.02	10.2	12
	0.47	0.65	0.55	0.08	0.03	14.0	9
	-	-	-	-	-	-	-
Number of somatic kineties	10	11	10.6	0.50	0.13	4.7	16
	10	10	10	0	0	0	9
	9	9	-	-	-	-	-
Length of membranelle 1	26	32	28.9	2.47	0.82	9.6	9
	25	34	30.3	3.02	0.96	10.0	9
	ca 33	-	-	-	-	-	-
Number of basal bodies in somatic kinety 1*	39	52	44.6	5.15	1.72	11.6	9
	37	49	39.2	15.07	5.02	38.4	9
	-	-	-	-	-	-	-
Number of macronucleus	1	1	1	0	0	0	>30
	1	1	1	0	0	0	9
	1	1	-	-	-	-	-
Length of macronucleus	13	18	14.9	2.11	0.61	14.1	12
	15	24	18.8	3.38	1.13	18.0	9
	-	-	-	-	-	-	-
Width of macronucleus	3	5	4.3	0.65	0.19	15.0	12
	5	9	6.4	1.51	0.50	23.4	9
	-	-	-	-	-	-	-
Number of micronuclei	1	1	1	0	0	0	>30
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-

* - basal body pairs accounted as single ones

numerous nucleoli, which are often irregular-shaped after protargol impregnation (Figs. 4 F, 24). Single micronucleus close to macronucleus.

Cilia more or less sparsely arranged, 7 to 10 µm in length, single caudal cilium about 15 µm long. Cilia of membrane/ membranelles about 12-15 µm in length. Movement not very active, swimming snake-like or slightly zig-zag, moderately fast; in culture usually motionless for a long time or gathered together on sediment (food) as shown in Fig. 4 B. When disturbed, swimming for a short distance and then motionless again.

Somatic kineties longitudinal, extending over entire length of body. Unlike in most other scuticociliates, among these "mixed" kineties each composed of often only a few to several dikinetids at anterior end, while in most portion with monokinetids (Figs. 4 I, J). Along buccal field, kinety left of oral area (SKn) membranelle-like, consisting of densely arranged paired basal bodies, adjacent to membranelle 1 (Figs. 4 H, 24)

Buccal apparatus highly specialized. Membranelle 1 (M1) occupying about 4/5 of buccal length, one-rowed in most portion; its posterior portion splitting into 3 groups, each with 3 basal bodies (Fig. 4H). Most part of M1 adjacent to densely-ciliated kinety n (SKn), both of which form together the prominent "undulating membranes". Membranelle 2 and 3 close to M1, relatively small and each with about 5-8 basal bodies (Fig. 4 H). Paroral membrane (PM) on right of shallow buccal

cavity, short and gently curved, extending anteriorly to about level of M2; its anterior half one-rowed, posterior with zig-zag structure. Scutica (Sc) Y-shaped, consisting of about 3 pairs of basal bodies (Fig. 4 H).

Silverline system as demonstrated in Fig. 4 G. No extrusomes recognised; line from kinety n crossing over polar circle. Cytopyge (CyP) between kinety 1 and n (Fig. 4 G). Contractile vacuole pore (CVP) located near end of 2nd somatic kinety (Fig. 4 G).

Ecological characters. Four populations encountered in coastal water off Qingdao and from a mollusc farming pond in Haiyang (near Qingdao) were isolated and observed. It seems to occur throughout seasons of the year. Salinity was about 30-33‰, pH 7.9-8.1.

Discussion and comparison. Until now, *Cohnilembus verminus* is the only one within the genus, which has been described with modern methods since Kahl established this genus (Borror 1963; Thompson 1964, 1968; Small 1967; Didier and Detcheva 1974; Jones 1974; Hu *et al.* 1996). As revealed in previous work, this species appear very much size- and shape-variable and may exhibit rather different appearances *in vivo*, but after impregnation, all share the same patterns of the silverline system and infraciliature (Kahl 1931; Borror 1963; Thompson 1964, 1968; Jones 1974; Foissner 1976; Foissner *et al.* 1982; Ströder 1991, master thesis; Hu *et al.* 1996).

Based on the body shape and the general appearance from life, two marine slender *Lembus*-species described by Kahl, *L. punctatus* Kahl, 1928 and *L. reesi* Kahl, 1931 should be compared. The former has a narrowed posterior end (similar to the thicker form of the Qingdao population) with relatively shorter buccal field. Considering the fact that the body shape of *Cohnilembus verminus* is highly population-dependent, it is reasonable to come to the conclusion that Kahl's *L. punctatus* represents only a form-variation and should be thus conspecific with the former.

Lembus reesi is characterized by the distinctly smaller cell size (*ca* 30 μm long) as described originally (Kahl 1931). However, the minimum size of some starving cells of the Qingdao-population could be-during culture-only 30-40 μm long, therefore, *Lembus reesi* should be possibly synonymized with *Cohnilembus verminus*.

Genus *Porpostoma* Möbius, 1888; *Porpostoma rotatum* Möbius, 1888 (Figs. 5 A-H, 6 A-E, 16-21; Table 9)

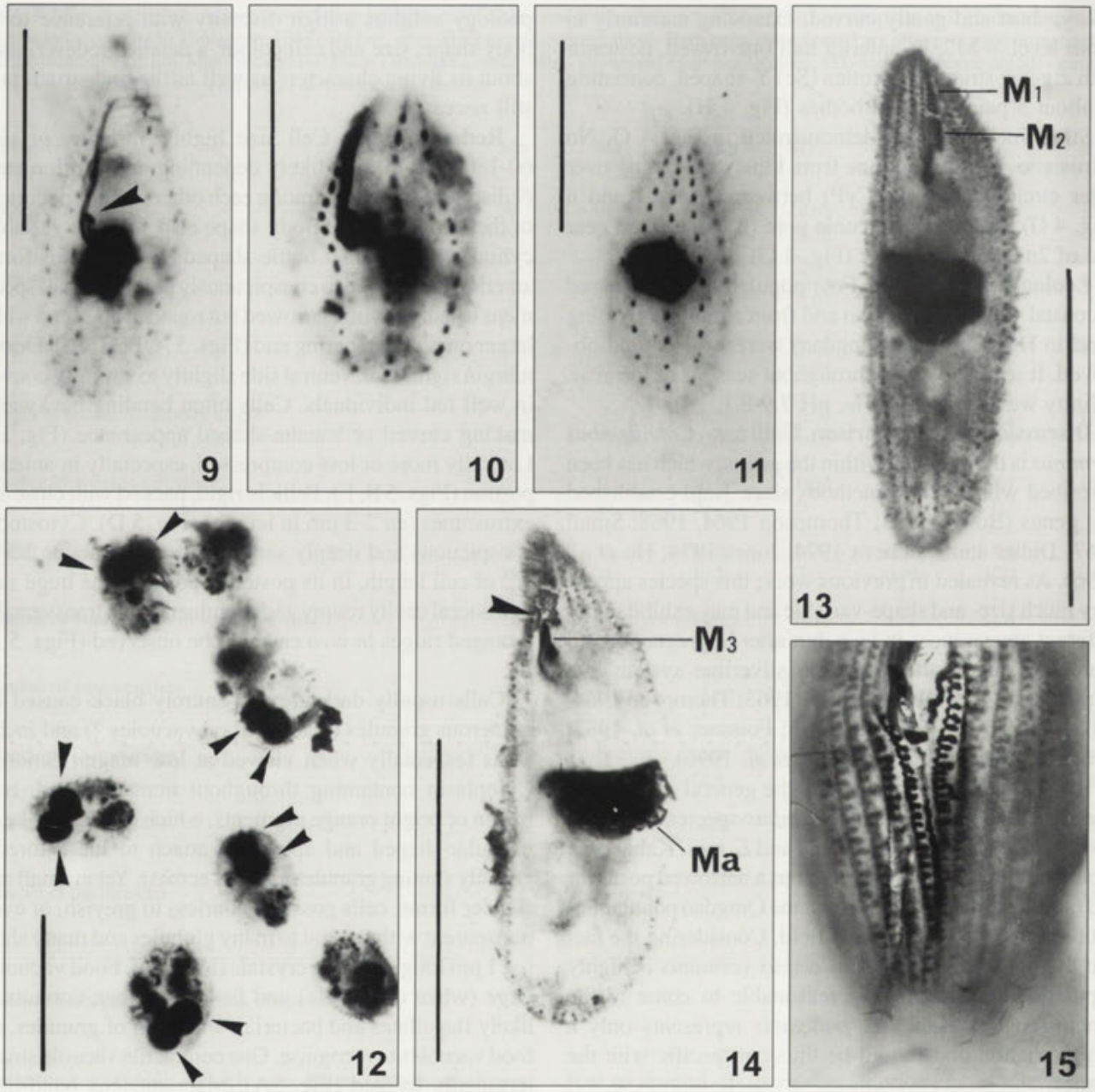
This well-known species, as the genus type, had been already described for a couple of times (Kahl 1931, Mugard 1949, Czapik and Jordan 1977). Since its morphology exhibits a high diversity with reference to its body shape, size and cell colour, a detailed redescription about its living characters as well as the oral structure is still necessary.

Redescriptions. Cell size highly variable, *in vivo* 60-180 x 20-60 μm, likely depending on nutrition state (? distinctly different among each other within specimens of the same sample). Body shape also strongly variable, cylinder-like, slender bottle-shaped to slightly fusiform; anteriorly narrowed to conspicuously pointed, small specimens usually having narrowed but rounded apex end while larger ones with tapering end (Figs. 5 A, B, H; 16). Dorsal margin sigmoidal, ventral side slightly to strongly convex in well fed individuals. Cells often bending backwards making curved or banana-shaped appearance (Fig. H). Laterally more or less compressed, especially in anterior portion (Figs. 5 B, E). Pellicle rigid, packed with close-set extrusomes (*ca* 2-3 μm in length) (Fig. 5 D). Cytostome conspicuous and deeply sank; buccal field about 2/5 to 1/2 of cell length, in its posterior portion, one huge sac-like buccal cavity roomy and prominent, with transversally arranged ridges *in vivo* easily to be observed (Figs. 5 A, B).

Cells usually dark grey to entirely black caused by numerous granules (inactive food vacuoles ?) and inclusions (especially when viewed at low magnifications). Cytoplasm containing throughout numerous red, red-brawn or bright orange pigments, which are patch-like or irregular-shaped and appear to attach to the "stored", greasily shining granules (2-5 μm across). Yet in small and slender forms, cells possibly courless to greyish, or even transparent with several to many globules and many short (*ca* 1 μm long) bar-like crystals (Fig. 5 D). Food vacuoles large (when detectable) and few in number, containing likely flagellates and bacterial, when full of granules, no food vacuoles to recognise. One contractile vacuole small, terminally located (Fig. 5A). Macronucleus band-like, slightly to highly twisted, positioned centrally along main body axis with several oval micronuclei closely attached to it (Figs. 5 B; 6 C, D; 20).

Somatic cilia *ca* 7-10 μm long, one caudal cilium 12-15 μm; in oral area, cilia about 10-15 μm long, forming conspicuous membranes/ membranelles. Movement fast, spirally and wobbly, rotating about its main body axis (Fig. 5 C), with resting for short periods when feeding.

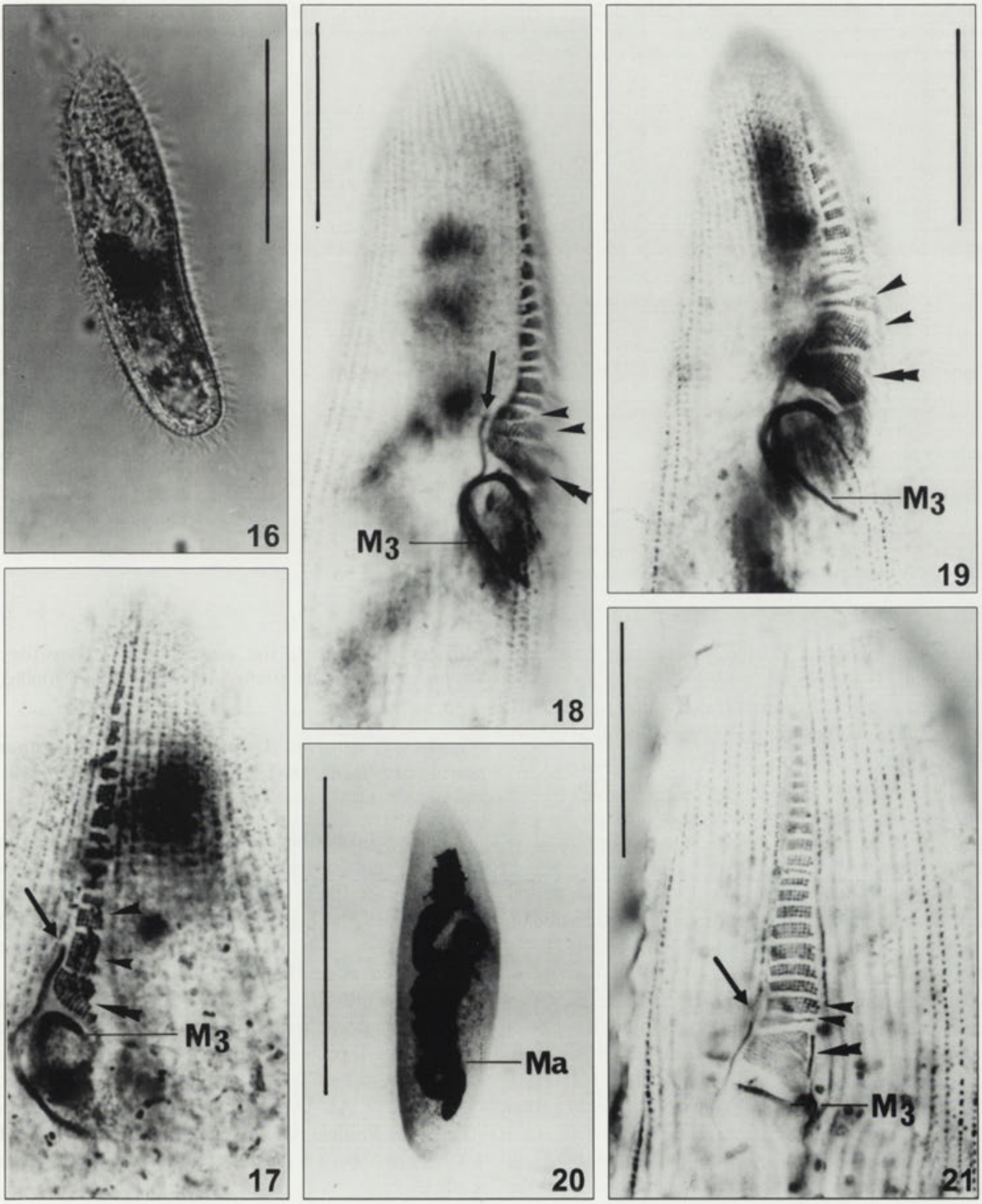
On average 50 somatic kineties composed of densely arranged basal bodies (monokinetids, but possibly depending on physiological state, some individuals at the beginning of division with almost completely dikinetids) (Figs. 6 A, B). On ventral side, left and right kineties



Figs. 9-15. Photomicrographs of *Pseudocohnilembus persalinus* (**9-11**), *Cyclidium varibonneti* sp. n. (**12**) and *Philasterides armatalis* sp. n. (**13-15**) after protargol (**9-14**) and silver nitrate impregnation (**15**). **9** - ventral view, to show the buccal apparatus, arrowhead indicates the membranelle 3; **10** - a "thick" individual, ventral view, note the somatic kineties arranged widely spaced from each other; **11** - dorsal view; **12** - to show macronuclei, note all cells with two nodules (arrowheads); **13, 14** - ventral view, to show the buccal apparatus, note the anterior part of paroral membrane clearly separated from the posterior part (arrowhead); **15** - posterior portion of ventral view, to show the *Tetrahymena*-similar silverline system. Abbreviation: M_1, M_2 - membranelle 1, 2. Scale bars - 25 μm (in **Figs. 9-12**), 50 μm (in **Figs. 13, 14**)

terminating at both cell poles (Figs. 5 E, 6 A), while on dorsal side, kineties shortened anteriorly and making one conspicuous suture (Fig. 6 B, arrowheads). In caudal region, no remarkable bold area to spot (likely very small, if present), where the contractile vacuole pore is located (Fig. 6 E).

Buccal apparatus as shown in Figs. 5 F, G; 6 D, E; 17-19. Adoral membranelle 1 (M1) extending to apex, composed of 10-20 irregular shaped parts which gradually widen posteriad (1 to 8 μm in width), each part containing 1 to *ca* 12 longitudinal rows of basal bodies (Figs. 5 F, G); membranelle 2 (M2) with about 15-20



Figs. 16-21. Photomicrographs of *Porpostoma notatum*, from life (**16**), after protargol (**18-20**) and silver nitrate impregnated specimens (**17, 21**). **16** - a slender individual from life; **17-19, 21** - buccal apparatus, arrow marks the anterior end of paroral membrane, arrowheads indicate the posterior parts of membranelle 1, double-arrowheads exhibit the membranelle 2. Note the M3 extending deeply into the buccal cavity (**Fig. 19**); **20** - to show the highly twisted macronucleus. Abbreviations: CyP - cytopyge, M3 - membranelle 3, Ma - macronucleus. Scale bars - 60 μm (in **Fig. 16**), 30 μm (in **Figs. 17, 19, 21**)

Table 9. Morphometric characterisation of 2 populations of *Portostoma notatum*. Data based on protargol impregnated specimens. All measurements in μm. Max - maximum; Mean - arithmetic mean; Min - minimum; n - size of samples; SD - standard deviation; SE - standard deviation of the mean, Vr - coefficient of variation in percent

Character	Min	Max	Mean	SD	SE	Vr	n
Body length	115	167	140	18.7	6.61	13.4	8
	59	103	83.9	16.63	6.29	19.8	7
Body width	32	74	40.8	13.38	4.46	32.8	9
	22	48	29.6	8.81	3.33	29.8	7
Length of buccal field	38	74	57.6	9.30	2.94	16.2	10
	32	46	39.7	5.62	2.12	14.2	7
Length of buccal field : body length	0.38	0.48	0.43	0.04	0.01	9.2	8
	0.41	0.47	-	-	-	-	5
Number of somatic kineties	53	59	55.6	2.00	0.67	3.6	9
	ca 50-55	-	-	-	-	-	-
Length of undulating membrane	14	23	18.5	2.73	0.96	14.7	8
	13	17	-	-	-	-	4
Distance from apex to anterior end of membranelle 2	32	49	39.1	6.30	1.99	16.1	10
	22	28	-	-	-	-	4
Number of macronucleus	1	1	1	0	0	0	10
	1	1	1	0	0	0	7
Length of macronucleus	64	135	101	23.75	8.98	23.5	7
	35	74	51.9	17.0	6.43	32.8	7

* - basal body pairs accounted as single ones

longitudinal rows, not distinctly separated from M1; membranelle 3 (M3) having bipartite structure, which connect each other: its left part consisting of about 10 rows of basal bodies, its right part likely 3 rowed, extending deeply into buccal cavity (cytopharynx) (Fig. 5 F). Paroral membrane (PM) L-shaped on right of oral cavity, slightly oblique to main body axis (Figs. 5 F, G). Scutica with *ca* 15 basal bodies, sparsely arranged as one line (Figs. 5 G, 6 E).

Ecological characters. Frequently occurred in coastal water near Qingdao, the present descriptions are based on 2 populations collected (11 Nov. 1993; 14 Oct. 1995). Salinity was about 31‰, pH 7.9-8.0.

Discussion and comparison. Up to date, only two species in this genus are identified and both have been described using modern techniques (Mugard 1949, Czapik and Jordan 1977, Corliss and Snyder 1986, Petz *et al.* 1995, Song and Wilbert 2000b), hence the identification is clear and undoubted.

For species definition and separation, the infraciliature, especially the buccal apparatus must be revealed for the living appearance of these organisms is highly variable.

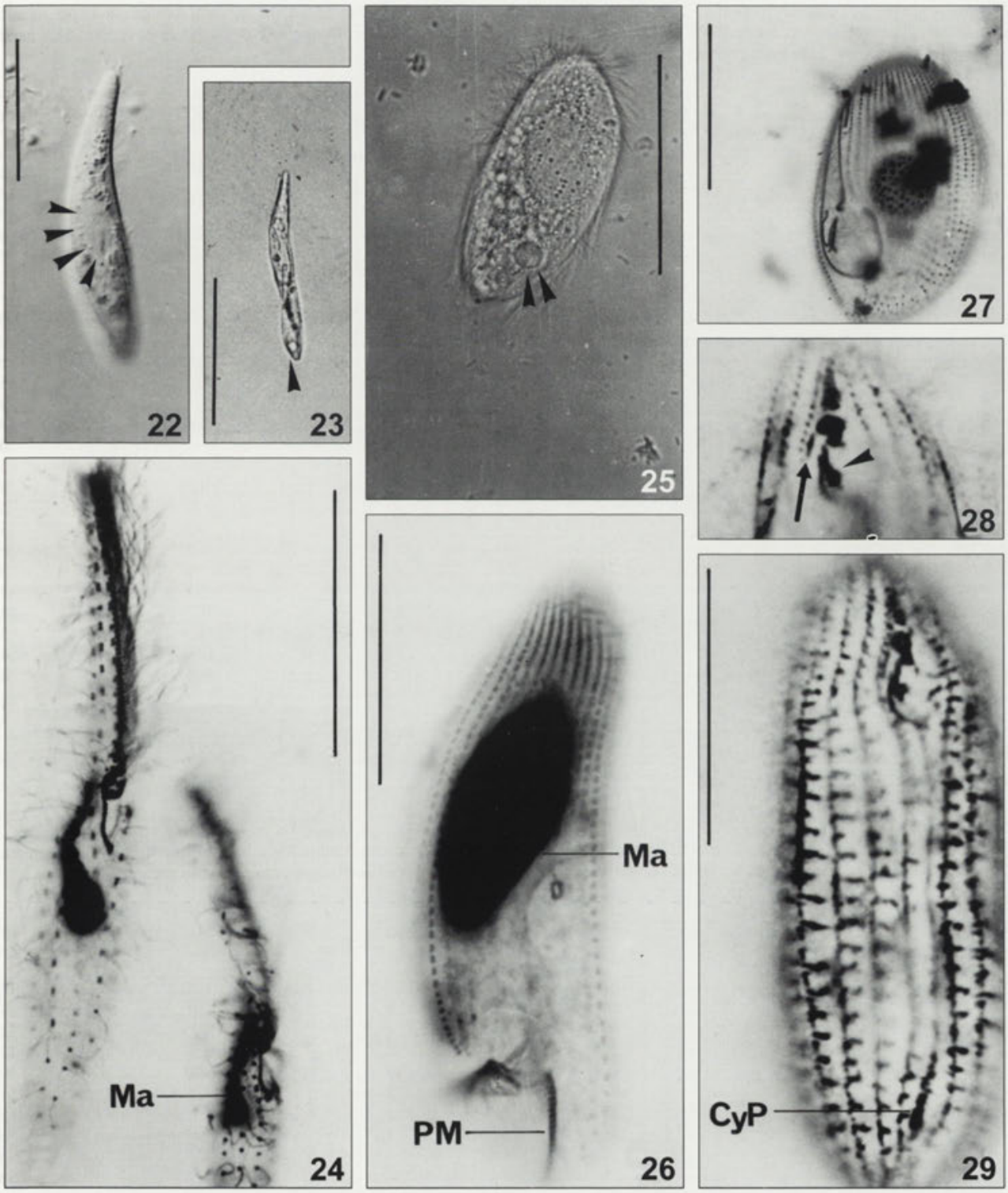
Compared with *Porpostoma grassei* (Corliss & Snyder, 1986) Petz, Song & Wilbert, 1995, which was found in the Antarctica area, *P. notatum* can be identified by the shape of macronuclei (band-like *vs.* oval) and different structure of the membranelle 3 (bipartite *vs.* solitary) (Petz *et al.* 1995, Song and Wilbert 2000b).

Genus *Pleuronema* Dujardin, 1836; *Pleuronema coronatum* Kent, 1881 (Figs. 7 A-H, 27; Table 10)

This well-described species has been studied for many times by previous researchers (Kahl 1931; Dragesco 1960; Borror 1963, 1972; Agamaliev 1968; Didier and Detcheva 1974; Dragesco and Dragesco-Kernéis 1986; Foissner *et al.* 1994). Here thus only a brief mention is given based on the Qingdao population.

Redescriptions. *In vivo* 60-90 x 30-50 μm, outline elongate oval to elliptical, widest at or behind mid-body; anteriorly and posteriorly broadly rounded; ventral margin almost straight while dorsal side conspicuously convex (Fig. 7 A). Dorso-ventrally about 3:2 flattened (Fig. 7 C). Pellicle rigid and slightly notched (Fig. 7 B); extrusomes about 4 μm long, closely arranged beneath pellicle.

Cytoplasm colourless and hyaline, often containing several to many greasily shining globules which are mostly 3-5 μm across and located in the posterior half of the cell (Fig. 7 A). Contractile vacuole small, located slightly dorsally in posterior 1/5-1/6 of cell length (at about level



Figs. 22-29. Photomicrographs of *Cohnilembus verminus* (**22-24**), *Ancistrum crassum* (**25, 26**), *Pleuronema coronatum* (**27**) and *Philasterides armatalis* sp. n. (**28, 29**) after living observations (**22, 23, 25**), protargol (**24, 26, 27**) and silver nitrate impregnations (**28, 29**). **22, 23** - to show a short and a slender specimen respectively, arrowheads in **Fig. 22** mark the macronucleus, while arrowhead in **Fig. 23** indicates the contractile vacuole; **24** - ventral view, to show the buccal and nuclear apparatus, note the macronucleus (Ma) is of an irregular shape; **25** - a typical cell, arrowheads indicate the contractile vacuole; **26** - left side, to show the macronucleus and the somatic infraciliature; **27** - left-ventral view; **28** - ventral view of anterior portion, to show the buccal apparatus, arrowhead indicates the membranelle 3, arrow marks the anterior part of the paroral membrane; **29** - ventral view of silverline system. Abbreviations: CyP - cytopyge, Ma - macronucleus, PM - paroral membrane. Scale bars - 20 μm (in **Fig. 22**), 40 μm (in **Figs. 23, 26, 27**), 60 μm (in **Fig. 25**), 30 μm (in **Figs. 26, 29**)

Table 10. Morphometric characterisation of 3 populations of *Pleuronema coronatum*. Data based on protargol impregnated specimens. All measurements in μm. Max - maximum; Mean - arithmetic mean; Min - minimum; n - size of samples; SD - standard deviation; SE - standard deviation of the mean, Vr - coefficient of variation in percent

Character	Min	Max	Mean	SD	SE	Vr	n
Body length	56	74	65.5	6.66	2.35	10.2	8
	77	84	-	-	-	-	5
	78	85	-	-	-	-	3
Body width	37	50	42.0	4.90	1.73	11.7	8
	28	44	-	-	-	-	5
	43	48	-	-	-	-	3
Length of buccal field	38	49	45.8	3.62	1.28	7.9	8
	47	58	-	-	-	-	5
	64	68	-	-	-	-	3
Number of somatic kineties	42	45	43.1	1.36	0.48	3.1	8
	39	40	-	-	-	-	5
	46	48	-	-	-	-	3
Distance between apex and anterior end of undulating membrane	5	12	9.0	2.27	0.80	25.2	8
	9	12	-	-	-	-	5
	12	13	-	-	-	-	3
Number of preoral kineties	4	6	4.9	0.64	0.23	13.1	8
	4	4	-	-	-	-	5
	5	5	-	-	-	-	3
Number of basal bodies in somatic kinety 1*	25	30	27.8	1.72	0.70	6.2	6
	21	25	-	-	-	-	5
	25	27	-	-	-	-	3
Number of macronucleus	1	1	1	0	0	0	14
	1	1	1	0	0	0	5
	1	1	1	0	0	0	4
Length of macronucleus	12	19	15.3	2.25	0.80	14.8	8
	20	27	-	-	-	-	5
	20	28	-	-	-	-	3

* - basal body pairs accounted as single ones

Table 11 Morphometric characterisation of two populations of *Ancistrum crassum*. Data based on protargol impregnated specimens. All measurements in μm (data according to Xu *et al.* 1997 and Xu and Song 1999). Max - maximum; Mean - arithmetic mean; Min - minimum; n - size of samples; SD - standard deviation; SE - standard deviation of the mean, Vr - coefficient of variation in percent

Character	Min	Max	Mean	SD	SE	Vr	n
Body length	63	77	70.7	4.48	1.29	6.3	12
	54	72	62.6	5.46	1.46	8.7	14
Body width	20	28	22.1	2.43	0.70	11.0	12
	20	33	22.7	3.38	0.90	14.9	14
Length of buccal field	40	48	43.8	2.62	0.83	6.0	10
	32	45	39.2	3.98	0.99	10.2	14
Number of somatic kineties	26	38	31.3	4.42	1.14	14.1	15
	27	33	29.1	1.54	0.41	6.5	10
Number of macronucleus	1	1	1	0	0	0	>50
	1	1	1	0	0	0	>50
Length of macronucleus	20	32	25.6	3.59	0.96	14.0	14
	20	30	23.2	2.69	0.72	11.6	14
Width of macronucleus	8	12	9.7	1.27	0.34	13.1	14
	7	16	10.4	3.50	0.94	33.8	14
Number of micronuclei	1	1	1	0	0	0	>50
	1	1	1	0	0	0	>50
Length of micronucleus	3	5	-	-	-	-	-
	-	-	-	-	-	-	-

Table 12. Morphometric comparison of some related *Ancistrum*-species found from the mantle cavity of marine molluscs (data according to Xu and Song 1999). All measurements in μm

Character	*A. crassum*	*A. haliotis*	*A. japonicum*	*A. acutum*
Body length *in vivo*	50-80 x 20-30	45-75 x 20-30	70-100 x 20-35	80-100 x 25-30
Body size*	54-77 x 20-33	65-75 x 18-31	71-95 x 18-20	79-93
Length of buccal field*	32-48	59-64	60-73	60-67
Number of somatic kineties*	26-38	28-32	18-21	21-25
Morphological features	with narrowed apex,	broadly rounded apex,	slender, with narrowed apex	conspicuously pointed apex and tapering anterior portion
Caudal "bald" (cilia-free) area (viewed from lateral)	distinctively large	distinctively large	distinctively large	small, inconspicuous
Anterior terminating position of paroral membrane	extending to *ca* 2/3 of cell length	extending to *ca* 90% of cell length	extending to *ca* 4/5 of cell length	extending to *ca* 3/4 of cell length
Host (molluscs)	*Ruditapes philippinarum, Protothaca jedoensis, Saxidomus purpuratus, Cyclina sinensis*	*Haliotis discus hannai*	*Dosinia japonica, Caecella chinensis*	*Mactra chinensis, Mactra veneriformis, Meretrix meretrix*
Author	Fenchel, 1965	Xu & Song, 1999	Uyemura, 1937	Xu & Song, 1999

* - data based on protargol impregnated specimens

of cytostome). Food vacuoles usually large, with indefinable contents (possibly bacterial, Fig. 7 A). Macronucleus roundish, usually with many globular nucleoli. In some specimens only one large, centrally located nucleolus to be observed. Single oval to spherical micronucleus closely adjacent to macronucleus (Figs. 7 D, G).

Somatic cilia about 10 μm long, *ca* 10-15 caudal cilia about 3 times as long as somatic ones, stretching always in radial manner (Fig. 7A); cilia of buccal apparatus 20-40 μm in length. Movement moderately fast, somewhat drifting and wobbly, and then motionless for short periods on detritus (Fig. 7 E).

About 40 somatic kineties extending over entire length of cell, which are shortened anteriorly and form thus an inconspicuous suture, while others terminate at the apical end and compose one large bold apical plate (Figs. 7 G, H). Caudally one cilia-free area to recognise posterior to buccal field (Fig. 7 G). All kineties in anterior 2/3 of body composed of (mainly) paired basal bodies, monokinetids posteriorly. Left of buccal field mostly 4-5 shortened kineties (Table 10).

Adoral membranelle 1 (M1) short, anteriorly with two rows of basal bodies; membranelle 2 (M2) bipartite -as in all other congeners- M2a posteriorly hook-like, anteriorly and posteriorly distinctly 2-rowed, middle portion zig-zag shaped; M2b V-shaped, separated form M2a; membranelle 3 (M3) consisting of 3 rows of basal bodies, which are arranged densely (Figs. 7 F, G). Paroral membrane prominent and genus characteristic, about 4/5 of cell length with its posterior end strongly curved (Figs. 7 F, 27).

Ecological characters. As remarked recently by Foissner *et al.* (1994), this species seems to be cosmopolitan inhabiting both freshwater and marine biotopes. The population studied here was frequently encountered (in low number) in coastal water near Qingdao, descriptions based on 4 populations collected. Salinity was about 31‰, pH 7.9-8.0.

Discussion and comparison. The Qingdao population corresponds very well to the previous descriptions (Kahl 1931, Dragesco 1960, Borror 1963, Agamaliev 1968, Didier and Detcheva 1974, Dragesco and Dragesco-Kernéis 1986, Foissner *et al.* 1994) and hence the identification is pretty curtain.

Sometimes, *Pleuronema coronatum* might be similar to another marine congener, *P. smalli* Dragesco, 1968, the latter is, however, smaller (80 *vs.* 50 μm in length on average) with lower number of somatic kineties (*ca* 40

vs. ca 30). Different from the *P. marina* Dujardin, 1841, *P. coronatum* is much plumper in outline when viewed ventrally (vs. *ca* 1:2 slender), smaller (less than 90 *vs.* generally over 110 μm long) and has different structure of membranelle 2a (posterior end with a hook *vs.* straight) (Borror 1963).

Genus *Ancistrum* Maupas, 1883; *Ancistrum crassum* Fenchel, 1965 (Figs. 8 A-J, 25, 26; Table 11)

This species was originally found by Fenchel (1965) within the mantle cavity of a marine shellfish, *Venerupis aurea* from the Scandinavian water, Denmark. Very recently, Xu and Song reported this species from the lamellibranch mollusc, *Haliotis discus hannai* (Xu and Song 1999b). Since this reinvestigation is written in Chinese, an additional description is supplied based on the Qingdao population.

Description. Body shape slender oval to banana-like with both anterior and posterior end rounded to broadly pointed; *in vivo* about 60-80 x 20-35 μm. Bilaterally strongly flattened (about 2-3:1), dorsal margin straight, ventral evenly convex (Figs. 8 A, B). Buccal field as long, narrow and shallow grove along ventral margin extending from caudal area to anteriorly about 4/5 of cell length. Cytostome positioned in subterminal region, deeply in spacious and conspicuous depression (buccal cavity), which appears *in vivo* always to be transparent and hyaline when viewed from lateral side (Figs. 8 A, B). Pellicle smooth, cortical granules *ca* 2 μm across, typically 3-5 adjacent and lined up along longitudinal grooves where somatic kineties are associated (Fig. 8 C).

Cytoplasm colourless and hyaline, often filled with numerous different-sized granules, which are arranged mostly in central or posterior part of cell (Fig. 8 A). Food vacuoles usually large, several in number containing possibly bacterial. One large, oval macronucleus about 20-30 μm long from life, centrally located with many large, irregularly shaped nucleoli as revealed with protargol impregnation. One micronucleus in shallow indentation of macronucleus (Fig. 8 E). No extrusomes recognizable. Single contractile vacuole, *ca* 10 μm across, positioned anterior to buccal cavity (Figs. 8 A, 25), with its pulsation pore located at end of somatic kinety No. 2 (Fig. 8 E, arrow).

Somatic cilia about 10 μm long, slightly specialized: cilia in anterior-dorsal portion more densely arranged than in other region, forming one thigmotactic area and stretching anteriorly; one caudal cilium (CC) on caudal pole, about 15 μm long, but often difficult to discern (Fig. 8 A). Cilia of buccal apparatus considerably long (10-30 μm in length), especially in rear part, "drifting" posteriorly as swimming (Figs. 8A, 25).

Movement slowly crawling on detritus or sometimes jerking after isolated from host, then motionless for short whiles; when swimming slowly (even like floating), rotating about main body axis forward.

Usually 30-35 longitudinal somatic kineties densely ciliated, extending to about 90% of body length (with exception of SK1, which is parallel to buccal field and terminates at about caudal pole, Fig. 8 F, double-arrowheads) and terminating subcaudally around posterior part of buccal field, while epically forming one inconspicuous suture (Figs. 8 D, F). Right preoral kineties anteriorly shortened (Fig. 8 F, arrowheads). Some kineties left of buccal filed shortened anterior to cytostome. In anterior 3/4 of body, kineties consisting of usually paired basal bodies, while monokinetids posteriorly.

Buccal apparatus genus typical (Figs. 8 D, F). Membranelle 1 (M1) short with about 3 longitudinal rows of kinetosomes; membranelle 2 (M2) highly developed, L-shaped with posterior end curved to left and distinctly 2-rowed; membranelle 3 (M3) immediately beneath short arm of M2, about as long as M1, consisting of possibly 2 rows of kinetosomes. Paroral membrane (PM) prominent, about 3/4 of cell length, gently curved around buccal field, with densely arranged (zig-zag structure) kinetosomes. Scutica (Sc) patch-like, consisting of *ca* 10 basal bodies, close to caudal cilium complex (CCo) (Fig. 8 F).

Ecological characters. Isolated from the gills of cultured mollusc, *Haliotis discus hannai* in Jiaonan, near Qingdao (23 April 1995). Water salinity was about 31‰, pH *ca* 8.0.

Discussion and comparison. As endocommensal ciliates from marine animal hosts, many members of the genus *Ancistrum* have been reported during the past half of century (Chatton and Lwoff 1926, 1949; Raabe 1936, 1959, 1970; Fenchel 1964, 1965; Hatzidimitriou and Berger 1977; Xu and Song 1999b). Among them, available diagnostic characteristics are relatively incomplete, while the buccal structure in most species had been only -more or less- insufficiently described (Raabe 1936, 1970; Chatton and Lwoff 1949).

According to the data obtained, i.e. the general appearance of buccal filed, the arrangement of somatic kineties, the body size and shape. The form studied in the present paper corresponds well to *Ancistrum crassum* described

originally by Fenchel (1965) though the number of somatic kineties is slightly higher (26-38 *vs.* 26 on average).

Differs from the morphologically similar form, *Ancistrum japonicum* Uyemura, 1937, the paroral membrane in *A. crassum* is much shorter, which extends anteriorly to about 2/3 of cell length (vs. to about 80% of cell length in *A. japonicum*) (Xu *et al.* 1997, Xu and Song 1999b). In addition, *A. crassum* has considerably higher umber of kineties (26-38 *vs.* 18-21) and broader body shape (Table 12). Compared with other closely related congeners which occur from the same habitat, *A. acutum* and *A. haliotis* (Xu and Song 1999b), *A. crassum* can be recognized by the combination of the body shape, relative length of buccal field and the number of somatic kineties (Table 12).

Acknowledgements. This work was supported by research grants of "The State 973-Program (project number: G-1999012001), "The Natural Science Foundation of China" (project number: 39970098, 39425002) and "The Cheung Kong Scholars Programme". My thanks are also due to Dr. Xu Kuidong, Mr. Chen Zigui, Mr. Hu Xiaozhong and Mr. Ma Honggang, Laboratory of Protozoology, OUQ, for collecting samples, laboratory help and computer treatment for some illustrations.

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Received on 6th April, 2000; accepted on 16th May, 2000

Acta Protozool. (2000) 39: 323 - 329

ACTA
PROTOZOOLOGICA

Two New Coccidia, a *Goussia* and an *Eimeria* spp. from the Gut of Kessler's Goby (*Gobius kessleri* Günther) in the River Danube

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Summary. Kessler's goby, a recently introduced fish species in the Budapest region of the river Danube, proved to be infected with two previously undescribed coccidian species. The species having a smaller oocyst (7-8.5 µm) proved to be a *Goussia* and was named *G. kessleri* sp. n. The species having larger oocysts measuring 13-14 µm showed characteristics typical of an *Eimeria* and was designated as *E. daviesae* sp. n. Oocyst of *G. kessleri* were spherical, contained four ellipsoidal sporocysts lacking Stieda body. Sporocysts measured 5.8 (5-6) x 3.9 (3.8-4) µm. Oocysts of *E. kessleri* were spherical, contained four short ellipsoidal sporocysts which tapered at one end and formed at that end a cap-like Stieda body. Sporocysts measured 8 (7.5-8.2) x 4.9 (4.8-5.2) µm. Both species formed oocysts in the intestinal epithelium. The oocysts of the *Goussia* were released sporulated, while those of the *Eimeria* passed in unsporulated or semisporulated condition.

Key words: Coccidia, *Eimeria daviesae* sp. n., *Goussia kessleri* sp. n., Pisces, *Gobius*, new species.

INTRODUCTION

Relatively little is known about the coccidian parasites of gobiid fishes. Known species include *Eimeria gobii* from *Gobius medius* and *E. glossogobii* from *Glossogobius giuris*, but the species *E. variabilis* (Thélohan, 1893) common in cottid fishes has also been recorded in *Gobius bicolor* and *G. paganellus* (Dyková and Lom 1983). Recently Abollo *et al.* (1998) reported the occurrence of the latter species in *G. paganellus* from the coastal waters of Spain.

Coccidian infection of freshwater gobies was reported by Moshu (1992), who described the species *E. credintsi* from the gut of the tube-nosed goby (*Proterorhinus marmoratus*) in natural waters of Moldavia. In specimens of the tube-nosed goby occurring in the river Danube, Molnár (1996) found, in addition to *E. credintsi*, another coccidian species and described it as *E. marmorata*. The occurrence of coccidia in Kessler's goby, a species common in both brackish waters and freshwaters, has not been known until now.

The present paper reports the occurrence of two new coccidia species from Kessler's gobies that have become numerous in the Budapest region of the river Danube.

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MATERIALS AND METHODS

Forty-two Kessler's goby (*Gobius kessleri* Günther, 1861) specimens measuring 3-10 cm were collected with the help of an electrofishery

device from reaches of the river Danube north and south of Budapest on several occasions in the period between June and October in 1998 and 1999. The fish were kept in aquarium for 1-2 days, killed by decapitation and subjected to parasitological examination. Fresh mucus from the intestinal tract was examined for the presence of oocysts and parts of the foregut were fixed for histological examination in Bouin's solution. Drawings of sporulated oocysts were made, and measurements taken using an Olympus interference contrast microscope immediately after the oocysts had been detected or when sporulation had been completed. Unsporulated oocysts were placed in small Petri dishes with tap water at room temperature and allowed to sporulate. To prevent excess proliferation of bacteria, a few micrograms of penicillin were added. For histological examination, guts showing mixed infection with the two coccidian species were fixed in addition to guts in which the oocysts of only one of the two coccidia species could be detected by the examination of fresh preparations. Fixed material was embedded in paraffin and serially sectioned at 4 µm; sections were stained with haematoxylin and eosin. Histological preparations were photographed using a photographic attachment mounted on a Jenaval microscope. Fresh preparations and other histological preparations were photographed with an Olympus Dp 10 digital camera mounted on an Olympus BH-2 microscope. All measurements are in µm.

RESULTS

Two types of coccidia were found in the gut of the examined gobies. The species with smaller oocysts proved to be a *Goussia* sp. while that with the larger oocysts was an *Eimeria* sp. In the majority of cases a mixed infection with the two species was recorded. However, *Eimeria*-type oocysts were found only in fish collected in June and July, while *Goussia* oocysts were found from June through October. Mixed infection was characterised by the dominance of one or the other species. The smaller oocysts of the *Goussia* species occurred mostly in the sporulated stage in the mucus, and unsporulated oocysts were obtained only from the mucosal scrapings. The larger oocysts of the *Eimeria* species were mostly found in unsporulated stage in the mucus covering the epithelium and in the mucosal scrapings but semisporulated oocysts and less frequently sporulated ones were found as well. By the morphological characteristics of the sporulated oocysts both species proved to be new for science and are described below.

Description of the species

Goussia kessleri sp. n. (Figs. 1, 3)

Type host: *Gobius kessleri* Günther, 1861, Kessler's goby (Perciformes: Gobiidae).

Type locality: River Danube, near Budapest.

Site: mucus and epithelium of the foregut.

Prevalence of infection: 41/42 infected specimens, 98%.

Type material: syntypes in paraffin blocks and video images are in the author's possession. Histological preparations and photosyntypes (Coll. No. HNHM-67394) have been deposited in the parasitological collection of the Natural History Museum, Budapest, Hungary.

Etymology: the *nomen triviale* is derived from the name of the fish host.

Oocysts spherical, 8.1 (7-8.5) (n=25). Wall of the oocyst smooth, colourless and thin. Micropyle, polar granule and oocyst residuum absent. Sporocysts ellipsoidal. Stieda-body absent, an indistinct suture along the longitudinal axis present. Size of sporocysts 5.8 (5-6) x 3.9 (3.8-4); sporocyst wall very thin. Sporocysts compact, tightly filling the space of the oocyst. Four sporocysts arranged in oocyst either in pairs or three oocysts in one plane. Sporozoites vermiform, with reflexed end in the sporocyst. Size of the sporozoites 7 x 1.5. Sporocyst residuum finely granulated and globular.

Oocysts passed the gut sporulated.

Goussia kessleri sp. n. resembles *G. carpelli* (Léger et Stankovitch, 1921) in size and by its compact sporocysts, but differs from that species by the more elongated shape of sporocysts. *Goussia kessleri* sp. n. somewhat resembles *Goussia* spp. of the percid fishes *Goussia laureleus* (Molnár et Fernando, 1973) and *G. acerinae* (Pellérdy et Molnár, 1971) but the sporocysts of the latter two species have triangular shape in the transversal axis, while *G. kessleri* has a regular round shape.

Eimeria daviesae sp. n. (Figs. 2, 4)

Type host: *Gobius kessleri* Günther, 1861, Kessler's goby (Perciformes: Gobiidae).

Type locality: River Danube, near Budapest.

Site: mucus and epithelium of the foregut.

Prevalence of infection: 17/42 infected specimens, 40%.

Type material: video images are in the author's possession. Histological preparations and photosyntypes (Coll. No. HNHM-67395) have been deposited in the parasitological collection of the Natural History Museum, Budapest, Hungary.

Etymology: the *nomen triviale* is given in honour of Dr. Angela J. Davies, the well-known specialist of fish apicomplexans.

Oocysts spherical 13.4 (13-14) (n=25). Wall of the oocyst smooth, colourless and thin. Micropyle and oocyst residuum absent, an amorphous small polar granule

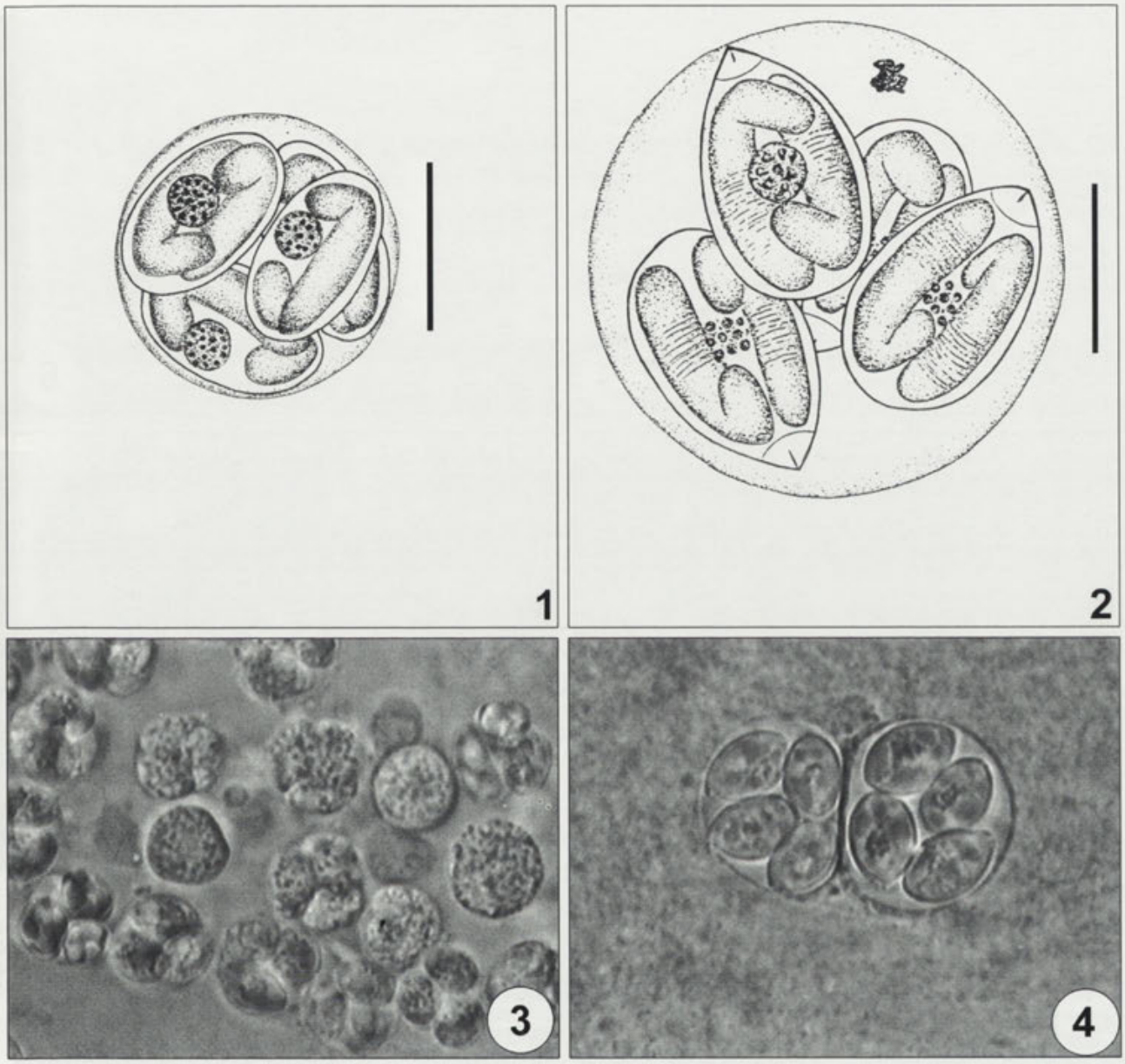

Fig. 1. Schematic illustration of the sporulated oocysts of *Goussia kessleri*. Scale bar - 5 μm
Fig. 2. Schematic illustration of the sporulated oocyst of *Eimeria daviesae*. Scale bar - 5 μm
Fig. 3. Sporulated and unsporulated oocysts of *Goussia kessleri* in a mucosal scraping of the intestine. x 1500
Fig. 4. Sporulated oocysts of *Eimeria daviesae* after 48 h of sporulation. x 2000

with a diameter of 2 present. Sporocyst short ellipsoidal but tapered at one end and forms at that end a cap-like Stieda-body; no substieda body found. Size of sporocysts 8 (7.5-8.2) x 4.9 (4.8-5.2); sporocyst wall very thin. Sporocysts moderately compact with a narrow space separating them from oocyst wall. Four sporocysts arranged randomly in oocyst. Sporozoites vermiform, with reflexed end in the sporocyst. Size of the mechanically excysted sporozoites 9.6 (9.5-10) x 2 (1.8-2.3). Sporocyst residuum globular, compact in mature oocysts, but composed of some rough granules in more mature oocysts.

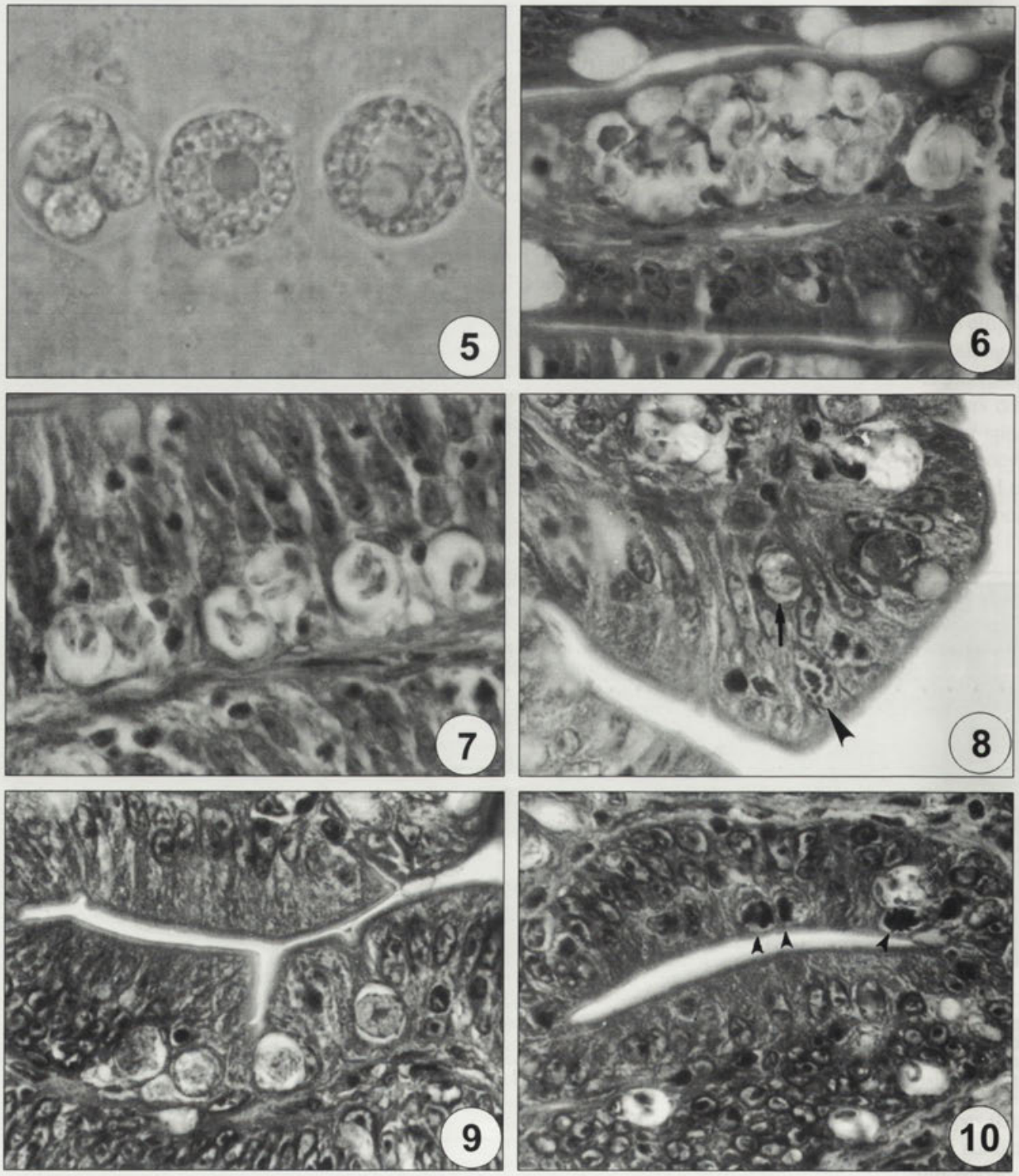

Fig. 5. Unsporulated and semisporulated oocysts of *Eimeria daviesae* in the intestinal mucus. x 2000
Fig. 6. Histological section of the anterior part of the intestine of a Kessler's goby. A group of sporulated oocysts of *Goussia kessleri* in the intestinal epithelium. H & E, x 1000
Fig. 7. Histological section of the anterior part of the intestine of a Kessler's goby. Solitary oocysts of *Eimeria daviesae* in the basal part of the epithelium. H & E, x 1000
Fig. 8. Histological section of the anterior part of the intestine of a Kessler's goby. Unsporulated oocyst (arrow) and a meront with cross-sectioned merozoites (arrow head) of *Goussia kessleri* in the epithelium. H & E, x 800
Fig. 9. Unsporulated oocysts of *Goussia kessleri* in the basal cytoplasm of epithelial cells. H & E, x 1000
Fig. 10. *Goussia kessleri* microgamonts (arrow heads) in the apical cytoplasm of epithelial cells. H & E, x 1000

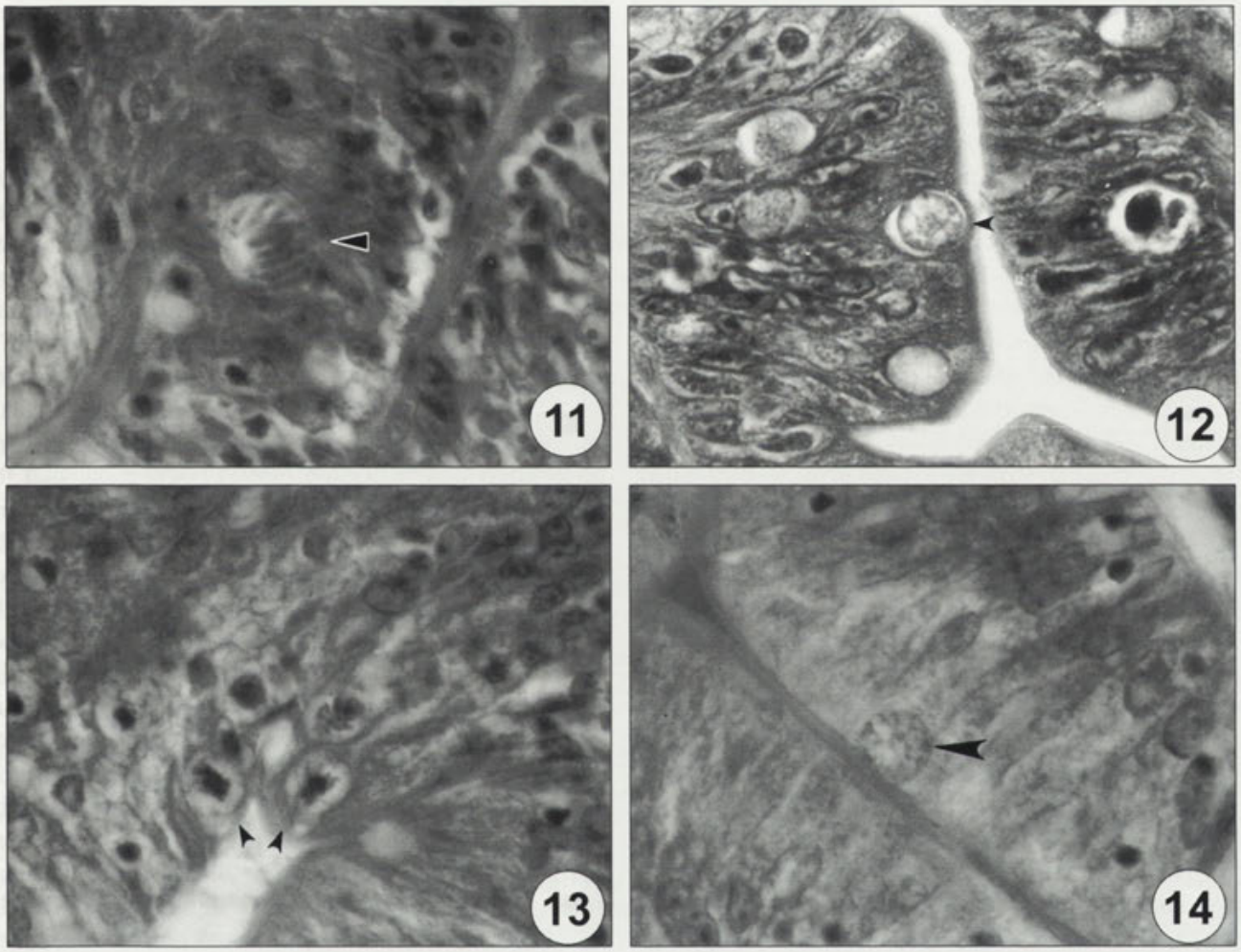

Fig. 11. *Eimeria daviesae* meront with merozoites (arrowhead) in the epithelial cell. H & E, x 1000
Fig. 12. Unsporulated oocyst of *Eimeria daviesae* (arrowhead) just leaving the apical part of the epithelial cell. H & E, x 1000
Fig. 13. Young *Eimeria daviesae* microgamonts (arrowheads) in the epithelium. H & E, x 1000
Fig. 14. *Eimeria daviesae* microgamont with microgametes (arrowhead) in the apical part of the epithelial cell. H & E, x 1000

Oocysts passed the gut unsporulated or semisporulated, less frequently sporulated (Fig. 5). Unsporulated oocysts proved to be 12-13 globules with coarse granules. Sporocysts filled by rough granules of the residual body were formed 24 hours after sporulation, fully sporulated oocysts were obtained after 48 hours sporulation in room temperature.

Of the coccidia of gobiid fishes *E. daviesae* resembles *E. variabilis* (Thélohan, 1893) but differs from it by the more tapered end of the oocyst and by having polar granule in the oocyst. *E. daviesae* also resembles *E. marmorata* Molnár, 1996, a parasite of the tubenosed goby, but differs from it by the larger size of the oocysts, by the less compact sporocysts and by having polar granule.

Histology

The histological picture was dominated by mixed infection in the majority of cases. However, even in these cases the smaller sporulated oocysts of *Goussia kessleri,* aggregated in the epithelium in larger groups (Fig. 6) were well distinguishable from the larger solitary sporulated oocysts of *Eimeria daviesae* located in the basal part of the epithelium (Fig. 7). Besides the oocysts, meronts, macrogamonts and microgamonts were also frequently seen. The developmental stages of the two

species, which were less easily distinguishable by morphological characteristics and size, were studied in preparations in which only one or the other species was found by the examination of fresh preparations.

Infection with merogonic, gamogonic and sporogonic stages of *G. kessleri* was mostly found in the foregut and in the middle portion of the intestine. Ellipsoidal or round meronts 10.2-12 x 7-10 in size were located in the apical part of the epithelial cells and contained 16 merozoites (Fig. 8). Macrogamonts and young oocysts measuring 8.0-8.5 µm could not be distinguished with certainty. Those located in the apical cytoplasm were considered macrogamonts, while others lying in the basal region of the epithelial cells were regarded as young oocysts (Fig. 9). Microgamonts (Fig. 10) were located in the apical zone of the epithelial cells; the younger ones had dark colour and were smaller in size (7-8 x 4-5) than the macrogamonts. The larger microgamonts stained less intensively, contained a large number of microgametes, and measured 10 x 7. Sporulated oocysts 8-9 in diameter were in most cases aggregated in small batches (Fig. 6); however, unsporulated solitary oocysts scattered in the epithelial cells were also found in the same preparations (Figs. 8, 10). Local damage of the intestinal epithelium was evident, but a general pathogenic effect of the infection could not be demonstrated.

In preparations in which only *E. daviesae* oocysts had been detected by the examination of fresh preparations, meronts, macrogamonts and microgamonts were found in the same locations as in the case of *G. kessleri* infection. On morphological basis these stages could not be clearly differentiated from similar stages of the above species. Round or short ellipsoidal meronts contained 16 merozoites and measured 11-13 x 10-11 (Fig. 11). Macrogamonts and young oocysts 10 (9-11) in diameter occupied mostly the apical region of the cells, but were found also in the median region (Fig. 12). On the other hand, microgamonts were always found in the apical cytoplasm of the cells. Developing microgametes in young stages of 7-10 were located in the centre of the microgamonts (Fig.13), while in more developed microgamonts 10-13 in diameter the microgametes were scattered in the cytoplasm (Fig. 14). Young unsporulated oocysts measuring 13 (12-14) were found in both the median and the basal regions, but sporulated oocysts 12-14 in diameter were always located in basal position, just above the propria layer, without gathering into groups (Fig. 7). In contrast to what was found by the examination of fresh preparations, the majority of *E. daviesae* oocysts were found in sporulated state.

DISCUSSION

Either as a result of active, intentional fish introductions (Holcik 1991) or as a passive consequence of human activity (Leach 1995), the appearance of different fish species in new habitats has become increasingly common. In the wake of ill-considered fish translocations, the fish host is accompanied to the new habitat by its pathogens, which sometimes cause major diseases among the introduced, and the indigenous fishes alike (Bauer and Hoffman 1976, Molnár 1984). The passive transfer of fish is facilitated primarily by the movement of ships, which occasionally carry spawn and fish specimens to distant regions. Some parasites of these fish species may also get to the new habitat. Leach (1995) pointed out that the percid ruffe (*Gymnocephalus cernuus*) and the gobiid round goby (*Neogobius melanostomus*) and tube-nosed goby (*Proterorhinus marmoratus*) had probably been carried to the American Great Lakes with the ballast of ships. Supposedly Kessler's goby, first recorded in Hungary by Erös and Guti (1997), had also gotten in a similar manner from its original habitat in the Lower Danube to reaches of the river near Budapest, where its population became very numerous (Molnár and Baska 1998).

Studies on the coccidian fauna of Kessler's goby indicate that this fish species, not recorded in the Hungarian reaches of the river Danube until 1997, shows infection of high prevalence and high intensity with the *Goussia* and *Eimeria* species found during the survey. Almost 100% of the Kessler's goby specimens examined during the five months of the survey were infected with *G. kessleri*, and in June and July the oocysts of *E. daviesae* could also be detected frequently. This species seems to have brought along its coccidian infection from its original habitat, and that infection has become stabilised during the short time spent in the new habitat. Few reliable data exist on the host specificity of coccidian species. Doubts regarding host specificity may arise especially in the case of the morphologically similar *Goussia* species of small size. The same coccidian species can probably parasitise different closely related fish species. Paterson and Desser (1982) demonstrated that the species *G. iroquoina* could parasitise both the fathead minnow and the common shiner. At the same time, it is unquestionable that several morphologically distinct *Goussia* species (*G. carpelli, G. sinensis, G. legeri, G. iroquoina*) occur also in cyprinids. It is unlikely that Kessler's goby could have obtained its coccidia from these taxonomically distant fish species. Even the parasites of the perch and the ruffe, which are

percid fishes common in the river Danube though taxonomically distant from Kessler's goby, are morphologically well distinguishable from the species *E. daviesae* and *G. kessleri*. The tube-nosed goby, which is taxonomically closer to Kessler's goby and lives in the same habitat (Molnár and Baska 1998), is also infected by well-distinguishable *Eimeria* species. The notion that the described *Eimeria* is a distinct species could most of all be queried by the example of the species *Eimeria variabilis*, which is a common parasite of marine cottid fish species (Davies 1978, 1990) but has also been recorded, probably erroneously, from gobiid fishes (see Dyková and Lom 1983, Abollo *et al.* 1998). On the basis of the morphologically differences found, the occurrence of this, presumably collective, species in Kessler's gobies examined from the Hungarian reaches of the river Danube can also be excluded.

It seems to be contradictory that in the case of *E. daviesae*, a species characterised by oocysts passed unsporulated on the basis of the examination of fresh preparations, masses of sporulated oocysts were detectable in the epithelium histologically. This contradiction is virtual; namely, from the work of Marincek (1973) it is known that in *E. subepithelialis* infection a certain proportion of the oocysts are passed unsporulated, while other oocysts sink to deep layers of the epithelium, undergo sporulation, and leave the fish gut only later. Probably a similar phenomenon occurs in the case of *E. daviesae*, and the numerous oocysts found by fresh examination obviously represent oocyst stages passed earlier.

Both coccidian species are typical epithelial parasites that complete their entire life cycle in the columnar epithelial cells of the gut. While, however, the merogonic stages and the early gamonts are located in the apical cytoplasm of the epithelial cells, unsporulated and sporulated oocysts are gradually pushed to deep layers of the epithelium. According to Molnár (1995), this is a consequence of epithelial regeneration, since infected cells of impaired function are being gradually replaced by proliferating intact epithelial cells. Oocysts driven to deep layers of the epithelium show a different location in the case of the two species. While the smaller oocysts of the *Goussia* species are aggregated into smaller or larger groups, the larger *E. daviesae* oocysts are isolated separately by the host reaction.

Acknowledgements. The author is indebted to Dr. Ferenc Baska, Dr. Csaba Székely and Miss Edit Eszterbauer for their help in the collection of host fish and in preparing digital pictures. The histological sections were made by Zsuzsa Kis. This study was rendered possible by grants from the Hungarian Scientific Research Fund (project no. T 014648 and T 029200).

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Received on 11th April, 2000; accepted on 4th July, 2000

Acta Protozool. (2000) 39: 331 - 335

ACTA
PROTOZOOLOGICA

Licnophora bassoni sp. n. (Ciliophora: Heterotrichea) from South African Turban Shells (Gastropoda: Prosobranchia)

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Summary. During surveys on the symbionts of intertidal invertebrates, turban shells, *Turbo sarmaticus* Linnaeus, 1758 and *Turbo cidaris cidaris* Gmelin, 1791, were found to host heterotrichous ciliophorans on their gills. The ciliophoran conform to the morphology of the genus *Licnophora* Claparéde, 1867, but differ from all the known species based on body morphology, details of the nuclear apparatus and host preference. Based on light and scanning electron microscopy this species is described as a new species, *Licnophora bassoni* sp. n.

Key words: heterotrichous ciliophoran, *Licnophora bassoni* sp. n., turban shells.

INTRODUCTION

The results presented below was obtained during surveys carried out as part of a comprehensive study on the biodiversity of intertidal symbionts, along the coast of South Africa by the Aquatic Parasitology group of the University of the Free State. This study already resulted in the descriptions of scyphidiid peritrichs (Van As *et al.* 1998, Basson et *al.* 1999), a heterotrichous ciliophoran (Van As *et al.* 1999), blood parasites (Smit and Davies 1999) and isopod symbionts (Smit *et al.* 1999, 2000) found associated with a variety of invertebrate hosts and tidal pool fishes. During these surveys another heterotrichous ciliophoran was found on the gills of two *Turbo* Linnaeus, 1758 species. This licnophorid differs from the known *Licnophora* species based on general body morphology, characteristics of the nuclear apparatus and host preference and is described as a new species. The description is based on Bouin's fixed specimens stained with hematoxylin, specimens impregnated with protargol as well as scanning electron microscopy.

MATERIALS AND METHODS

Specimens of *Turbo sarmaticus* Linnaeus, 1758 and *T. cidaris cidaris* Gmelin, 1791 (Mollusca: Archaeogastropoda: Trochacea: Turbinidae) were collected from the De Hoop Nature Reserve and Jeffreys Bay, South Africa and taken to a field laboratory where wet

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smears were prepared and examined. Positive smears were fixed in Bouin's and transferred to 70 % ethanol. Some smears were stained with Mayer's hematoxylin (Humason 1979) for studying the nuclear apparatus and for obtaining body measurements. Other smears were impregnated with protargol, using the method described by Lom and Dyková (1992).

For scanning electron microscopy, licnophorids were fixed in 2.5 % glutaraldehyde, transferred to 5 µm nuclearpore filters, washed with phosphate buffer, dehydrated through a series of ethanol concentrations and critical point dried. Filters were mounted on stubs, sputter coated with gold and studied at 5 kV, using a JEOL WINSEM JSM 6400 scanning electron microscope (SEM).

Body and micronucleus measurements and the number of macronuclear segments were obtained from microscope projection drawings, using fixed material (Table 1). Measurements of specimens are presented in the following way: minimum and maximum values are given, followed in parentheses by the arithmetic mean (mode in the case of the number of macronuclei and micronucleus diameter), standard deviation (only in n>9) and number of specimens measured. The type material is deposited in the collection of the National Museum, Bloemfontein, South Africa.

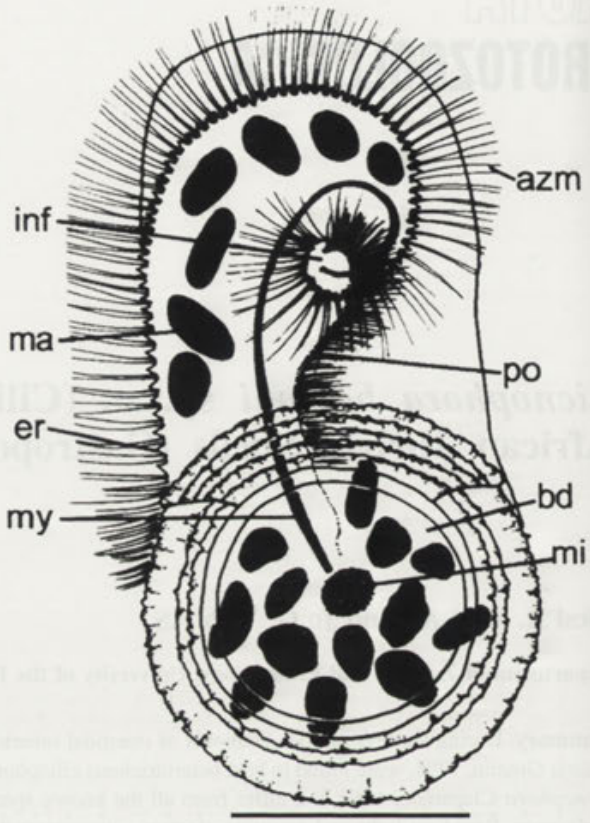


Fig. 1. Microscope projection drawing, based on hematoxylin stained and protargol impregnated specimens, of *Licnophora bassoni* sp. n. occurring on the gill filaments of *Turbo sarmaticus* Linnaeus, 1758 collected from the De Hoop Nature Reserve, South Africa. azm - adoral zone of membranelles, bd - basal disc, er - endoplasmic ribs, inf - infundibulum, ma - macronucleus, mi - micronucleus, my - myoneme, po - paroral organelle. Scale bar - 20 µm

RESULTS AND DISCUSSION

Licnophora bassoni sp. n. (Figs. 1-7)

Hosts: *Turbo sarmaticus* Linnaeus, 1758, *Turbo cidaris cidaris* Gmelin, 1791.

Position on host: gills.

Localities: De Hoop Nature Reserves and Jeffreys Bay on the south coast of South Africa.

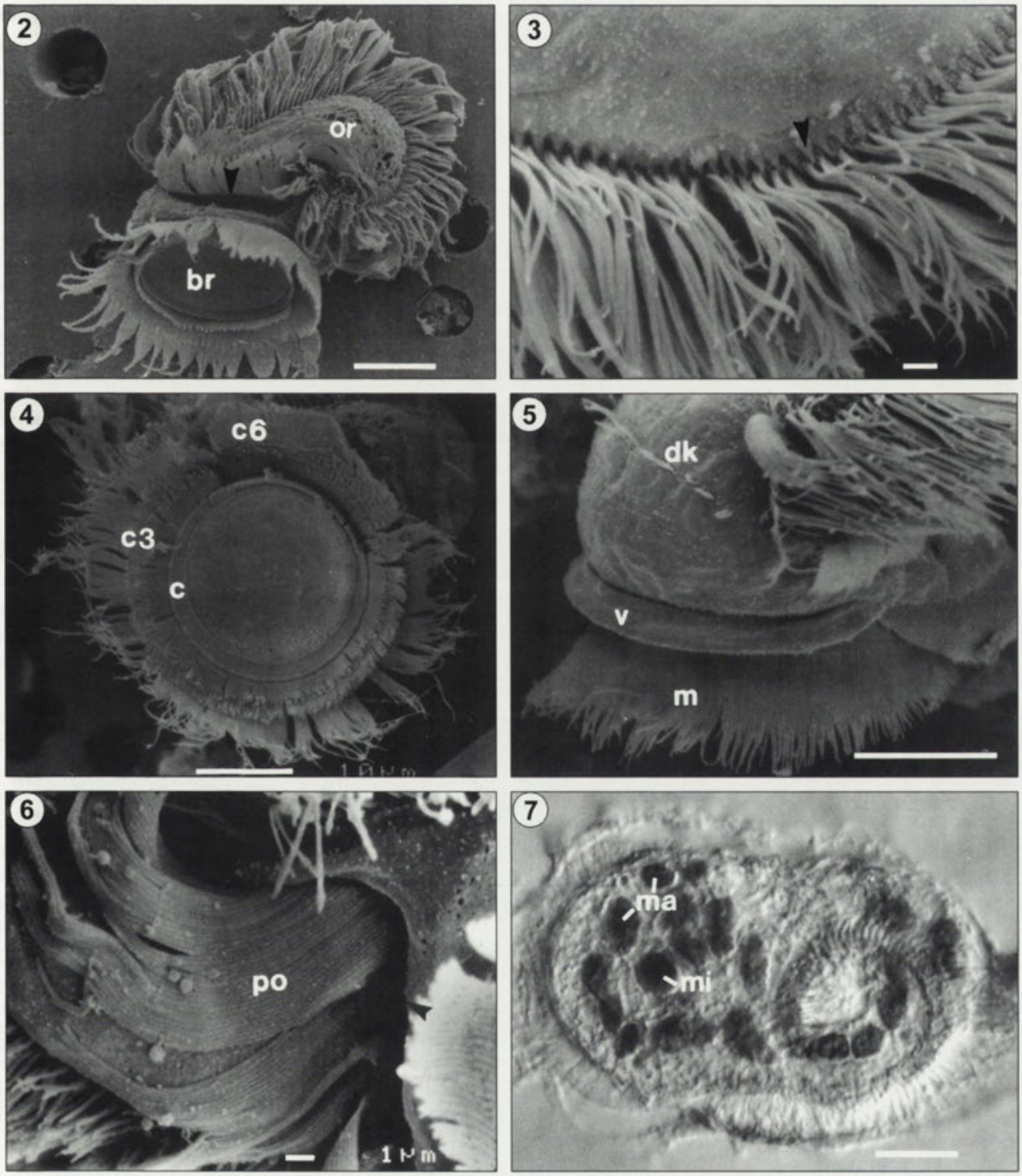
Type-specimens: holotype slide S98/3/30-1 (NMBP 252), paratype slides, S96/4/8-10 (NMBP 253), S98/3/30-2 (NMBP 254) in the collection of the National Museum, Bloemfontein, South Africa, other material in the collection of the authors.

Type host and locality: *T. sarmaticus* De Hoop Nature Reserve, South Africa (34° 28'S; 20° 30'E).

Etymology: named after Professor Linda Basson in recognition of her contribution to the knowledge of ciliophorans.

Description

Body squat, total length 40-70 µm (56.5 ± 6.7, 71), consists of two distinct regions; oral region and basal region (Figs. 1, 2, 7). Oral region diameter at broadest part 20-41 µm (28.4 ± 5.1, 71). Adoral side of oral region fringed by broad band of adoral zone of membranelles (AZM) describing spiral of 270°, before plunging into infundibulum. AZM comprising 71-129 (96.8 ± 16.5, 14) rows of membranelles (Figs. 1, 2), between 16 and 20 kinetids wide. Rows of membranelles separated by sharply pointed endoplasmic ribs (Fig. 3). First two thirds of AZM follows periphery of body, last third of AZM deviates from body periphery spiralling inward towards centre of oral region (Fig. 1). Spiral diameter at widest point in oral region 10-29 µm (17.94 ± 3.4, 71). Centre of aboral surface smooth without cilia, fringed by AZM. Neck short, not clearly distinguishable from adjacent oral and basal regions. Basal region (Fig. 4) round, surface slightly concave, diameter 22-41 µm (30.3 ± 3.9, 71). Basal disc diameter 15-30 µm (19.9 ± 3.2, 44), disc surrounded by a single circular ring of short, densely packed cilia of uniform length. Three additional rings of cilia extend around two thirds of anterior basal disc, proximal row shortest, distal row longest. Posterior third of disc with six rows of cilia, inner periphery shortest, outer longer (Fig. 4). Anterior part of basal region separated from membranelles by velum (Fig. 5). Single row of 20-25 kinetids and cilia on dorsal side of basal



Figs. 2-7. Scanning electron micrographs (**2-6**) and photomicrograph of hematoxylin stained specimen (**7**) of *Licnophora bassoni* sp. n. collected from turban shells from De Hoop Nature Reserve. **2** - adoral view: oral region (or), basal (br) region and ectoplasmic furrow (arrow); **3** - endoplasmic ribs (arrow) and part of azm; **4** - basal region with membranelles: single, circular ring of short cilia (c), three additional cilia rings (c3) and six posterior cilia rows (c6); **5** - basal region with dorsal kinetids (dk), velum (v) and membranelles (m); **6** - paroral organelle cilia (po), plunging into infundibulum (arrow); **7** - aboral view: macronuclear segments (ma) and micronucleus (mi). Scale bars - 10 µm (**2**, **4**, **5**, **7**); 1 µm (**3**, **6**)

Table 1. Morphometric measurements (μm) and numbers of macronuclear segments and membranelles of specimens of *Licnophora bassoni* sp. n. collected from the gills of *Turbo* spp. Linnaeus, 1758, from the south coast of South Africa

Measurements	*Turbo sarmaticus* De Hoop Nature Reserve	*Turbo sarmaticus* Jeffreys Bay	*Turbo cidaris cidaris* De HoopNature Reserve
TBL	40-70 (56.5 ± 6.7, 71)	45-70 (53.5 ± 6.1, 19)	44-76 (55.2 ± 7.5, 33)
ORD	20-41 (28.4 ± 5.1, 71)	21-34 (27.5 ± 3.7, 19)	10-34 (23.4 ± 4.5, 33)
SD	10-29 (17.9 ± 3.4, 71)	20-25 (21.9 ± 2.2, 19)	12-21 (17.5 ± 2.3, 27)
BRD	22-41 (30.3 ± 3.9, 71)	20-31 (27.6 ± 2.5, 19)	20-31 (26.3 ± 3.4, 33)
BDD	15-30 (19.9 ± 3.2, 44)	16-24 (20.3 ± 1.9, 18)	15-26 (19.4 ± 3.0, 18)
MaOR	3-13 (7, 71)	5-9 (5, 19)	3-9 (4, 33)
MaBR	9-21 (13, 71)	8-17 (14, 19)	6-14 (9, 33)
TMA	14-32 (21, 71)	13-24 (19, 19)	9-18 (18, 33)
MiD	3-7 (5, 30)	4-6 (4, 6)	4-9 (5, 31)
AZM	71-129 (96.8 ± 16.5, 14)	80-123 (104.5 ± 12.7, 14)	70-102 (87.3 ± 8.7, 25)

TBL - total body length, ORD - oral region diameter, SD - spiral diameter, BRD - basal region diameter, BDD - basal disc diameter, MaOR - number of macronuclei in oral region, MaBR - number of macronuclei in basal region, TMA - total number of macronuclei, MiD - micronuclei diameter, AZM - adoral zone of membranelles

Table 2. Body length (BL), number of macronuclear segments (No. MaS) and hosts of all the species of the genus *Licnophora* Claparéde, 1867, including *L. bassoni* sp. n.

Species	BL	No. MaS	Hosts	Reference
L. auerbachii (Cohn, 1866)	80-120	10-25	Echinoderm Bivalve Nudibranch	Cohn (1866)
L. bassoni sp. n.	40-70	14-32	Gastropod	Present study
L. biecheleri Villeneuve-Brachon, 1940	90-100	40-50	Cnidarian	Villeneuve-Brachon (1940)
L. bullae Dustin, 1915	123-130	15-20	Gastropod	Dustin (1915)
L. chattoni Villeneuve-Brachon, 1939	70-90	10-20	Ascidian	Villeneuve-Brachon (1939)
L. cohnii Claparéde, 1867	55-60	unknown	Polychaete	Claparéde (1867)
L. conklini Stevens, 1904	100-135	5-6	Gastropod	Stevens (1904)
L. hippocampi Meng and Yu, 1985	50-87	70-79	Seahorse	Meng and Yu (1985)
L. limpetae Van As, Van As and Basson, 1999	50-95	13-25	Gastropod	Van As *et al.* (1999)
L. lyngbycola Fauré-Fremiet, 1937	100	13	Algae	Fauré-Fremiet (1937)
L. macfarlandi Stevens, 1901	67-96	25-30	Echinoderm	Stevens (1901)

disc (Fig. 5). Myoneme extends from centre of basal disc, stretching directly upwards before following curve of AZM, plunging into infundibulum (Fig.1). Myoneme in basal region, broader than rest. Paroral organelle extends from inner periphery of basal disc close to micronucleus (Fig. 1), following myoneme curving upwards before extending down to plunge into infundibulum (Fig. 6). Paroral organelle consisting of single row of densely packed kinetosomes from which a single row of long cilia originates, aborally visible in an ectoplasmic furrow (Fig. 2).

Macronucleus consists of round, sometimes elongated, separate nuclei, varying in number between 14 and 32 (21, 71), distributed throughout body (Figs. 1, 7). Number of macronuclear segments in oral region 3-13 (7, 71), in basal region 9-21 (13, 71). Micronucleus round, diameter 3-7 (5, 30) situated in the centre of basal disc (Figs. 1, 7). No food vacuoles observed, endoplasm with granular appearance. No contractile vacuole found.

Remarks

Both *Turbo sarmaticus* and *T. cidaris cidaris* from De Hoop Nature Reserve and Jeffreys Bay were infested with *Licnophora bassoni*. No significant difference between the body measurements of these heterotrich populations could be found (Table 1). A very

consistent feature of *L. bassoni* is the micronucleus, which is distinct in its round shape situated in the center of the basal region.

Licnophora auerbachii (Cohn, 1866); *L. biecheleri* Villeneuve-Brachon, 1940; *L. bullae* Dustin, 1915; *L. chattoni* Villeneuve-Brachon, 1939; *L. conklini* Stevens, 1904; *L. lyncbycola* Fauré-Fremiet, 1937 and *L. macfarlandi* Stevens, 1901 are larger species than *L. bassoni.* All of these species except, *L. bullae* and *L. conklini*, are found on non-gastropod hosts (see Table 2). The number of macronuclear segments of *L. bullae* ranges between 15 and 20, which falls within the range of *L. bassoni*. Nevertheless, *L. bullae,* is a very large licnophorid with an elongated body and long neck found in the pallial cavity of the bubble snail *Bulla* Linnaeus, 1758 (Dustin 1915). *L. conklini* has 5-6 macronuclear segments and was described from the egg capsules of the slipper snail *Crepidula* Lamark, 1799 found in Woods Hole, Massachusetts (Stevens 1904). *L. bassoni* can be distinguished from the remaining three species (*L. cohnii* Claparède, 1867; *L. hippocampi* Meng and Yu, 1985 and *L. limpetae*) in the following ways: *L. cohnii* was found on the gills of an Italian polychaete and according to the drawings provided, this species has a more circular oral region and a very thin neck (Kahl 1932). *L. hippocampi* is a small species with 70-79 macronuclear segments and is so far the only licnophorid known from a vertebrate host (Meng and Yu 1985). *L. bassoni* differs from *L. limpetae*, the other South African species, not only in body length and number of nuclei (Table 2), but also in the arrangement of the nuclei. In the case of *L. limpetae* the segments are in the shape of an eight (Van As *et al.* 1999), whereas those of *L. bassoni* are scattered throughout the body. Another prominent difference between these two species is that *L. limpetae* has a distinct neck region, which is indistinguishable in *L. bassoni.*

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Received on 20th April, 2000; accepted on 5th July, 2000

Acta Protozool. (2000) 39: 337 - 344

ACTA
PROTOZOOLOGICA

Testate Amoebae (Protozoa: Rhizopoda) from Thailand

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Summary. A total of 91 taxa belonging to 30 genera of testate amoebae were established in 10 moss, soil and aquatic samples, collected in the park of the Chulalongkorn University, Bangkok and in the "Khao Yai" National Park, Thailand. Four genera and 18 species of them were announced for the first time to the testacean fauna of Thailand. The morphological and biometrical characterisation of some rare testaceans like *Cyclopyxis intermedia* Kufferath, 1932, *Cyclopyxis lithostoma* Bonnet, 1974, *Ellipsopyxis lamottei* Bonnet, 1974, and *Lamtopyxis cassagnaui* Bonnet, 1977, were made using a light and scanning electron microscope. The following synonyms for *C. intermedia* have been adopted: *C. gigantea* Bartoš, 1963; *C. kahli grandis* Chibisova, 1967 and *C. bathystoma* Chibisova, 1967.

Key words: biometry, faunistic, morphology, taxonomy, testate amoebae, Thailand.

INTRODUCTION

The testacean fauna of Thailand has as yet been poorly studied. Only the publications of Bonnet (1981, 1987) and of Todorov and Golemansky (1999) concern the testaceans of this country. On the basis of many investigated soil samples, Bonnet established rich soil testacean fauna composed of more than 150 species and 37 genera of testate amoebae. He observed many cosmopolitan and eurybiotic species, including some genera and species of Gondwanan origin. Among them are *Lamtopyxis*, *Distomatopyxis*, *Deharvengia*, *Planhoogenraadia* etc., which are interesting from a taxonomical and evolutional point of view, and are typical soil inhabitants of the South-eastern Asiatic-North Oceanic region (Bonnet 1987).

In July 1995 one of us (V. Golemansky) visited Thailand and collected samples from freshwater biotopes, epiphytic and terrestrial mosses and soils, in which we established rich testacean fauna. The faunistic data resulted in our study and the morphological and biometrical characterization of some rare and interesting species, observed in this region, are the subject of the present publication.

MATERIALS AND METHODS

The samples for the present study were collected in July 1995 in the park of Chulalongkorn University, Bangkok and in the "Khao Yai" National Park, Thailand. The "Khao Yai" National Park is located 180 km northeast of Bangkok in the Dongrak Mountains. Its altitude ranges from 250 to 1351 m a.s.l. and more than 85% of its

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surface is covered with typical tropical forests. The annual rainfall is about 2270 mm and the very high humidity supports an abundant growth of soil and epiphytic mosses. The materials were collected especially from the following localities and stations:

I. "Khao Yai" National Park: (1) forest stream (06.07.1995); (2) submerged aquatic mosses (06.07.1995); (3) humid soil mosses (06-07.07.1995); (4) humid epiphytic mosses on tree trunks (06.07.1995); (5) humid soil in a tropical forest (06.07.1995).

II. Bangkok, Park of the Chulalongkorn University: (6) humid soil under banana trees (11.07.1995); (7) a small artificial lake with aquatic vegetation (*Potamogeton* sp., *Myriophyllum* sp., *Chara* sp.) (10.07.1995).

The morphology of some shells was examined with a scanning electron microscope, JEOL Superprobe-733 operating at 15 kV.

RESULTS AND DISCUSSION

Faunistical and biogeographical aspects

A total of 4745 specimens belonging to 91 species and 30 genera of testate amoebae were established as a result of this study. Two species of them (*Planhoogenraadia bonneti* and *Centropyxis thailandica*) proved to be unknown and were described as new species in our previous paper (Todorov and Golemansky 1999). In addition, four genera and 18 species were announced for the first time to the testacean fauna of Thailand. Thus

Table 1. List of taxa and their presence (number of individuals) in the studied biotopes

Taxa	Biotopes						
	1	2	3	4	5	6	7
Arcella arenaria Greeff	-	-	-	3	-	-	-
* *A. discoides* Ehr.	-	-	-	-	-	-	2
Assulina muscorum Greeff	-	-	-	15	-	-	-
Awerintzewia cyclostoma (Pen.) Schout.	-	-	15	12	-	-	-
Bullinularia indica Pen.	2	-	15	-	-	-	-
Centropyxis aculeata (Ehr.) Stein	-	-	-	-	-	-	8
C. aerophila Defl.	-	-	11	45	-	1	-
C. cassis (Wall.) Defl.	-	3	-	51	-	-	-
C. constricta (Ehr.) Pen.	-	-	-	-	2	-	-
C. ecornis (Ehr.) Leidy	-	2	-	-	-	-	3
C. elongata (Pen.) Thomas	-	1	24	15	-	-	-
C. minuta Defl.	-	-	15	4	-	-	-
C. orbicularis Defl.	-	-	32	-	-	-	-
* *C. plagiostoma* Bonnet & Thomas	-	-	27	-	-	-	-
C. plagiostoma terricola Bonnet & Thomas	-	-	3	-	-	-	-
C. platystoma (Pen.) Defl.	5	-	39	18	1	-	-
C. stenodeflandriana Bonnet	-	-	64	21	1	-	-
C. sylvatica (Defl.) Thomas	2	-	246	4	-	-	-
C. sylvatica minor Bonnet & Thomas	-	-	7	-	-	-	-
C. thailandica Todorov & Golemansky	-	-	-	24	-	-	-
Corythion dubium Taranek	-	-	120	-	-	-	-
Cyclopyxis arceloides Pen.	-	-	9	-	-	-	-
C. eurystoma Defl.	-	1	114	34	-	-	-
C. eurystoma parvula Bonnet & Thomas	-	-	9	-	-	-	-
C. intermedia Kufferath	-	-	90	-	-	-	-
C. kahli Defl.	4	2	288	-	-	-	-
* *C. kahli cyclostoma* Bonnet & Thomas	-	-	5	7	-	-	-
C. lithostoma Bonnet	-	-	-	84	-	-	-
C. puteus Thomas	1	-	24	-	-	-	-
** *Cryptodifflugia compressa* Pen.	-	-	3	-	-	-	-
** *Cucurbitella mespiliformis* Pen.	-	-	-	-	-	-	3
* *Difflugia corona* Wallich	-	5	-	-	-	-	-
* *D. gramen* Pen.	-	-	-	-	-	-	4
D. lucida Pen.	-	-	8	-	-	-	-
* *Ellipsopyxis lamottei* Bonnet	-	10	2	-	-	-	-
Euglypha bryophila Brown	-	-	-	2	-	-	-

Table 1. (contd)

E. ciliata Leidy		-	3	7	3	-	-	-
* *E. compressa* Carter		-	2	-	4	-	-	-
E. cristata Leidy		-	-	4	-	-	-	-
E. denticulata Brown		-	-	9	-	-	-	-
E. filifera Pen.		-	-	24	-	-	-	-
E. laevis Perty		2	5	178	86	7	-	-
E. polylepis (Bonnet) Bonnet & Thomas		-	-	13	-	-	-	-
E. rotunda Wailes		-	5	75	65	4	-	-
E. strigosa (Ehr.) Leidy		-	-	-	3	-	-	-
E. tuberculata Dujardin		-	2	-	-	-	-	-
Heleopera petricola Leidy		-	-	-	6	-	-	-
H. rosea Pen.		-	-	7	-	-	-	-
H. sylvatica Pen.		-	-	16	11	-	-	-
Hyalosphenia subflava Cash		-	-	12	-	-	-	-
Lamtopyxis cassagnaui Bonnet		-	-	15	9	-	-	-
** *Microcorycia flava* (Greeff) Pen.		-	1	-	-	-	-	-
Nebela bigibbosa Pen.		-	-	5	-	-	-	-
N. bohemica Taranek		-	-	-	11	-	-	-
* *N. caudata* Leidy		-	-	5	-	-	-	-
N. collaris (Ehr.) Leidy		-	-	36	18	-	-	-
N. dentistoma Pen.		-	-	-	8	-	-	-
N. lageniformis Pen.		-	-	21	-	-	-	-
N. militaris Pen.		-	-	30	61	-	-	-
N. minor Pen.		-	-	12	-	-	-	-
N. parvula Cash		-	-	-	6	-	-	-
N. tincta (Leidy) Awerintzew		-	-	-	18	-	-	-
N. tubulata Brown		-	-	13	-	-	-	-
** *Netzelia oviformis* (Cash & Hopkinson)		-	-	-	-	-	-	6
Phryganella acropodia (Hertw. & Les.) Hopk.		1	2	165	78	-	1	-
Plagiopyxis callida Pen.		-	-	126	14	15	-	-
P. declivis Thomas		3	5	285	114	5	-	-
P. intermedia Bonnet		-	-	9	-	-	-	-
P. minuta Bonnet		1	-	21	9	3	-	-
P. oblonga (Bonnet & Thomas)		-	2	-	-	2	-	-
P. pusilla Bonnet		1	-	-	-	-	-	-
Playfairina valkanovi Golemansky		-	-	12	-	-	-	-
Planhoogenraadia bonneti Tod & Gol.		-	-	-	27	-	-	-
Pseudodifflugia gracilis terricola Bon. & Th.		-	-	4	-	-	-	-
Quadrulella quadrigera (Wall.) Defl.		-	-	-	11	-	-	-
Q. symmetrica (Wall.) Defl.		-	1	-	-	-	-	-
Schwabia terricola thomasi Bonnet		-	-	2	-	-	-	-
Sphenoderia fissirostris Pen.		-	4	-	-	-	-	-
* *S. lenta* Schlumberger		-	-	9	5	-	-	-
* *S. macrolepis* Leidy		-	-	12	-	-	-	-
Tracheleuglypha acolla Bonnet & Thomas		-	-	57	-	-	-	-
* *T. dentata* (Vejdovsky) Defl.		-	-	8	-	-	-	-
Trachelocorythion pulchellum (Pen.) Bonnet		-	-	26	15	-	-	-
Trigonopyxis arcula (Leidy) Pen.		-	36	129	75	-	-	-
* *T. arcula major* Chardez		-	-	45	-	-	-	-
* *T. bathystoma* Bartoš		-	-	57	-	-	-	-
* *T. microstoma* Hoogenraad & de Groot		-	6	-	34	-	-	-
Trinema complanatum Pen.		-	1	156	45	3	-	-
T. enchelys (Ehr.) Leidy		-	3	45	-	-	2	1
T. grandis (Chardez) Golemansky		-	-	-	2	-	-	-
T. lineare Pen.		3	4	468	156	16	3	-
Total taxa / individuals:	91	11	23	60	42	11	4	7
	4745	25	98	3295	1234	59	7	27

* new species to the testacean fauna of Thailand
** new genera to the testacean fauna of Thailand

the total number of the established testate amoebae in this country amounts to 168 species of 41 genera. The list of the testate amoebae observed and their distribution in different biotopes are given in Table 1.

The present study shows that the testacean fauna of Thailand is very rich and diverse. It is composed of an ensemble of cosmopolitan species and of species with restricted geographical distribution. According to Bonnet (1987) more of the testate amoebae with a restricted geographical distribution are typical of the regions with Gondwanan origin (Africa, South America, Southeast Asia, North Oceania) and possibly represent Gondwanan relicts. The species *Centropyxis stenodeflandriana, C. lithostoma, Ellipsopyxis lamottei, Lamtopyxis cassagnaui, Nebela caudata, Plagiopyxis pusilla, Planhoogenraadia bonneti, Quadrulella quadrigera* and *Trigonopyxis bathistoma* were some of these rare and characteristic species observed also by us in Thailand. The morphological and the biometrical characteristics of some of them, observed in large numbers in our samples, are given bellow.

Systematics and synonymy

***Cyclopyxis intermedia* Kufferath, 1932 (Figs. 1, 2)**

Syn.: *Cyclopyxis gigantea* Bartoš, 1963, p. 150; *C. bathystoma* Chibisova, 1967, p. 184; *C. kahli grandis* Chibisova, 1967, p. 185.

This species was observed and described for the first time by Kufferath (1932) in moss samples from Congo. According to Kufferath the shell dimensions of the species are: diameter - 110-220 µm, aperture - 50 µm, H/D - 0.6-0.62. Later Bartoš (1963) described *C. gigantea* in moss samples from Java, which were similar to *C. intermedia* and we accepted the opinion of Foissner and Korganova (1995) that it is a junior synonym for this species. According to Bartoš the shell dimensions of *C. gigantea* are: diameter - more than 200 µm, height - 100 µm, aperture - 50 µm, invagination of aperture - 29-30 µm. Foissner and Korganova (1995) redescribed *C. intermedia* on the basis of material from a deciduous forest in the East Caucasus and indicated the following shell dimensions: diameter - 162-187 µm, height - 118-137 µm, aperture - 33-62 µm and invagination of aperture - 37 µm.

In our study we established a relatively rich population of *C. intermedia* in the humid soil mosses of the "Khao Yai" National Park (Station 3). The shell form and the structure of the observed specimens are similar to the original description. The differences between our specimens and those observed by Kufferath, Bartoš and Foissner and Korganova are mainly in the shell dimensions. As a whole, all our specimens have bigger shell dimensions: diameter - 190-232 µm, height - 131-142 µm and diameter of aperture - 57-70 µm (Table 2). Our population is nearest in dimensions to the Caucasian population of *C. intermedia*.

Foissner and Korganova (1995) considered three other taxa (*C. lithostoma* Bonnet, 1974, *C. bathystoma* Chibisova, 1967 and *C. kahli grandis* Chibisova, 1967) also as junior synonyms for *C. intermedia* Kufferath, 1932. In our opinion, the suggested synonymy is only correct for *C. bathystoma* Chibisova and for *C. kahli grandis* Chibisova.

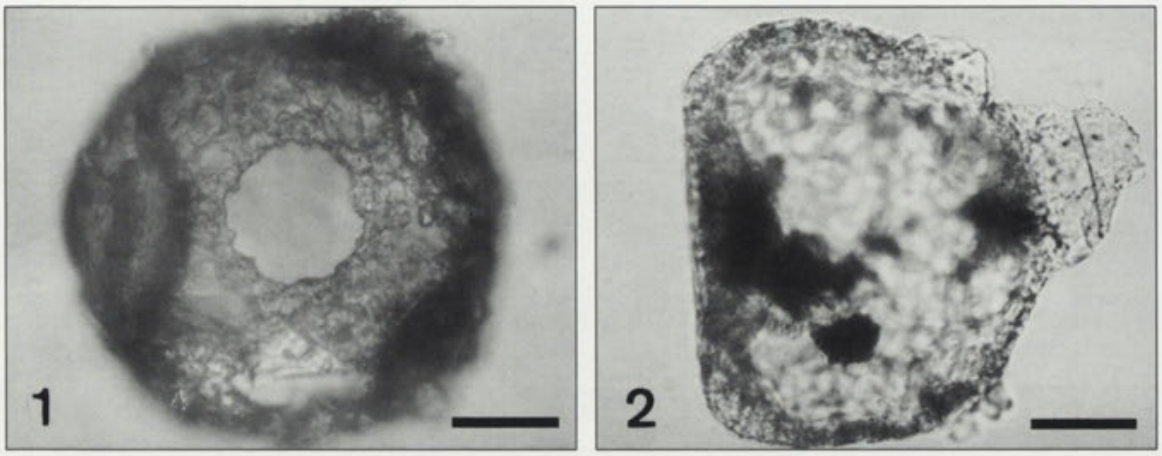
We consider that *C. lithostoma* Bonnet, as well as *C. pirini* Golemansky, 1974, mentioned by Foissner and Korganova as a possible synonym for *C. intermedia*, were described in detail. In our opinion the original description of these two species was more detailed and informative than that of *C. intermedia*. Furthermore, *C. lithostoma* Bonnet and *C. pirini* Golemansky possess many morphometrical features like shell structure, apertural construction and shell size, which clearly distinguishes them from *C. intermedia*. Furthermore, their ecology is also different. *C. lithostoma* Bonnet is a soil inhabitant and *C. pirini* Golemansky is an aquatic or *Sphagnum* dwelling species. We think, therefore, that their synonymy is not acceptable.

According to us, of the five taxa suggested by Foissner and Korganova (1995) there is reason to synonymize only *C. gigantea, C. bathystoma* and *C. kahli grandis*. Their original descriptions were not detailed and were based on a small number of observed specimens. Remember that *C. bathystoma* was described on the basis of only one specimen (Chibisova 1967). Furthermore, these taxa have similar morphometrical characteristics to *C. intermedia*.

***Cyclopyxis lithostoma* Bonnet, 1974 (Figs. 3-9)**

Bonnet (1974) described *Cyclopyxis lithostoma* from soil samples of gramineous rhizosphere (*Loudetia, Hyparrhenia*) from the Lamto region of the Ivory Coast, Africa. During our investigations, this species was frequently observed in humid epiphytic moss samples from the "Khao Yai" National Park (St. 4).

The shell of *C. lithostoma* is composed of a mixture of small, siliceous shell plates and assorted flattened pieces of quartz arranged so that the shell is thin and



Figs. 1-2. *Cyclopyxis intermedia*. **1** - apertural view, **2** - lateral view showing the rough dorsal half of shell. Scale bars - **1, 2** - 50 μm

Table 2. Morphometrical characteristic of *Cyclopyxis intermedia* Kufferath*

Characters	$\bar{x}$	M	SD	$SE_{\bar{x}}$	CV	Min	Max	n
Diameter	211.2	212.3	6.8	1.6	14.6	190	232	21
Height	136.2	136.4	4.3	1.4	13.4	131	142	21
Aperture	65.4	66.1	2.8	1.3	8.1	57	70	21
Invagination of aperture	28.2	28.3	2.1	1.2	4.5	25	31	21

Abbreviations: $\bar{x}$ - arithmetical mean, M - median, SD - standard deviation, $SE_{\bar{x}}$ - standard error of the mean, CV - coefficient of variation, Min - minimum, Max - maximum, n - number of the measured specimens. *All measurements in μm

Table 3. Morphometrical characteristic of *Cyclopyxis lithostoma* Bonnet

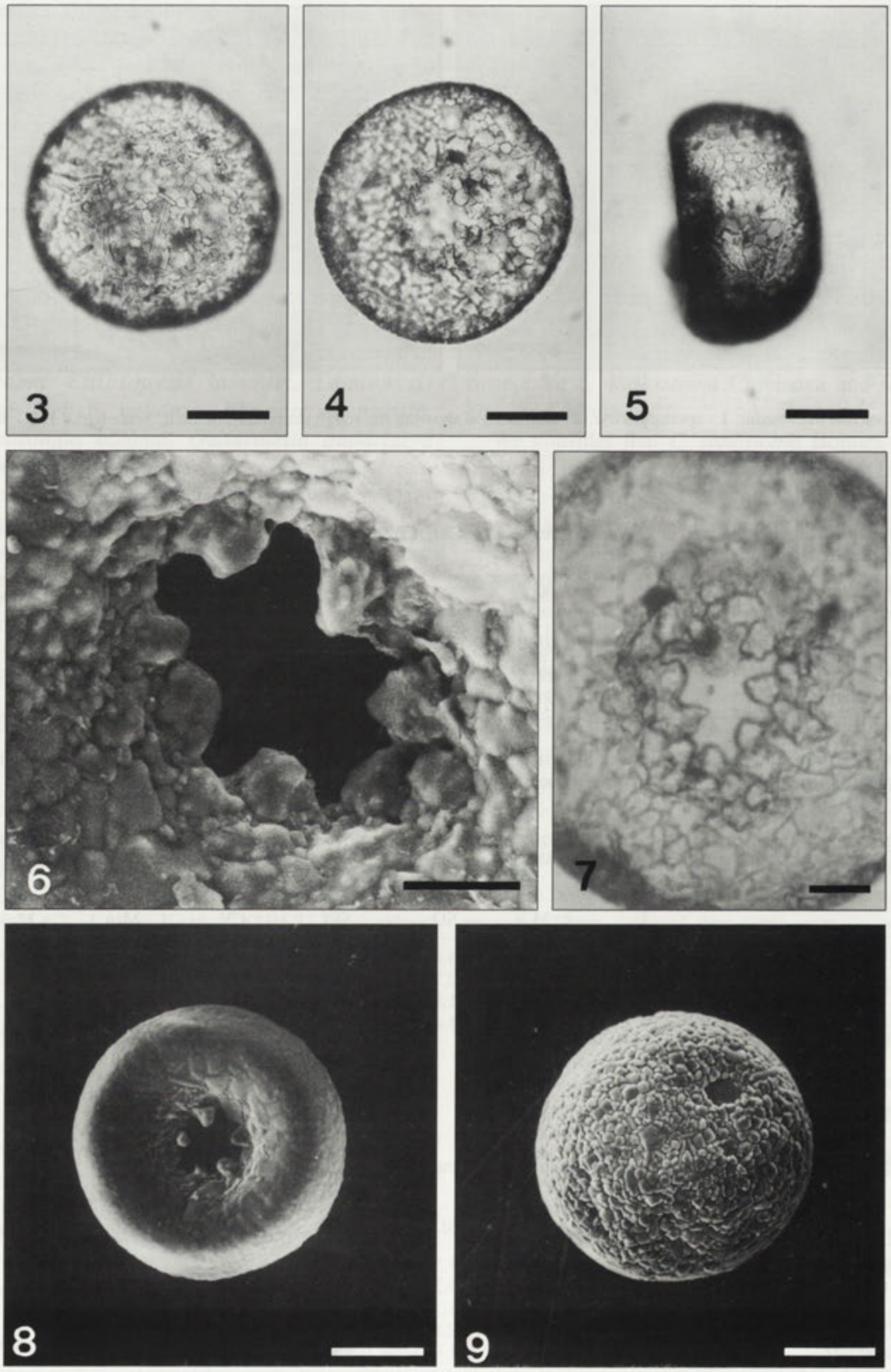
Characters	$\bar{x}$	M	SD	$SE_{\bar{x}}$	CV	Min	Max	n
Diameter	159.2	158.3	3.8	1.2	3.6	150	175	58
Height	88.4	89.1	2.3	1.1	2.4	84	95	34
Aperture	32.4	31.8	1.8	1.0	5.1	28	35	58
Invagination of aperture	28.2	28.5	1.4	0.9	1.5	26	32	34

Abbreviations: see Table 2

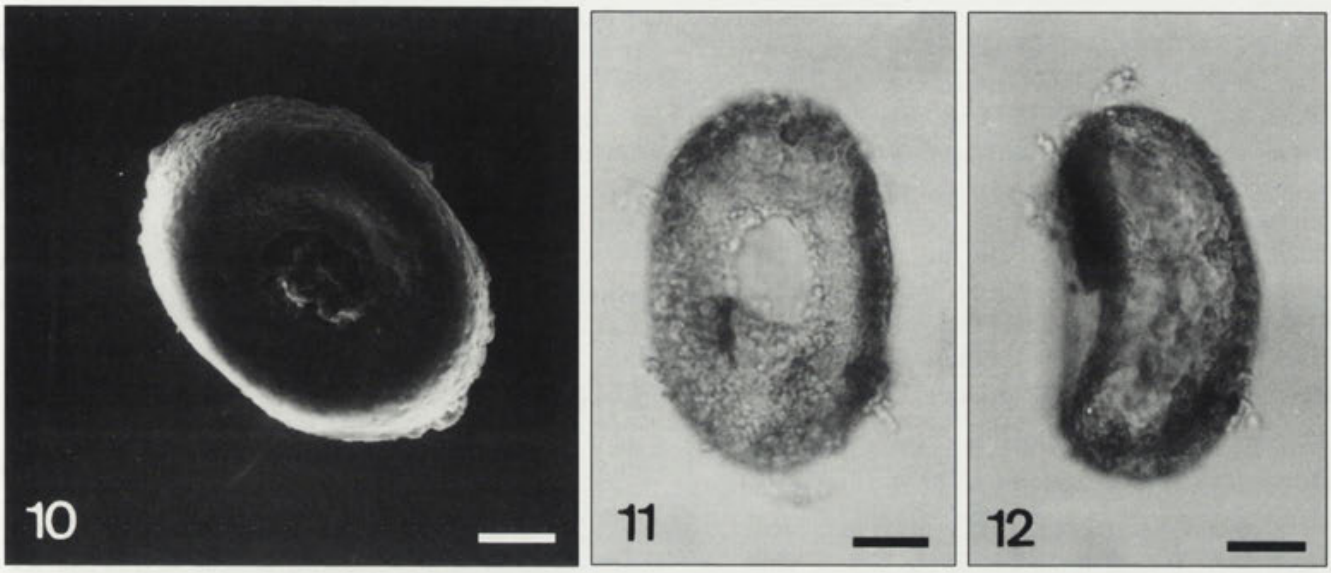
Table 4. Morphometrical characteristic of *Ellipsopyxis lamottei* Bonnet

Characters	$\bar{x}$	M	SD	$SE_{\bar{x}}$	CV	Min	Max	n
Large axis of the shell	105.2	107.1	5.3	1.4	7.6	98	114	9
Small axis of the shell	81.4	80.2	4.2	1.3	7.4	75	87	9
Height	54.1	54.3	4.3	1.2	6.7	52	57	9
Grand axis of aperture	33.0	32.8	2.1	1.1	2.3	30	35	9
Small axis of aperture	21.6	21.7	1.9	1.0	2.2	20	23	9

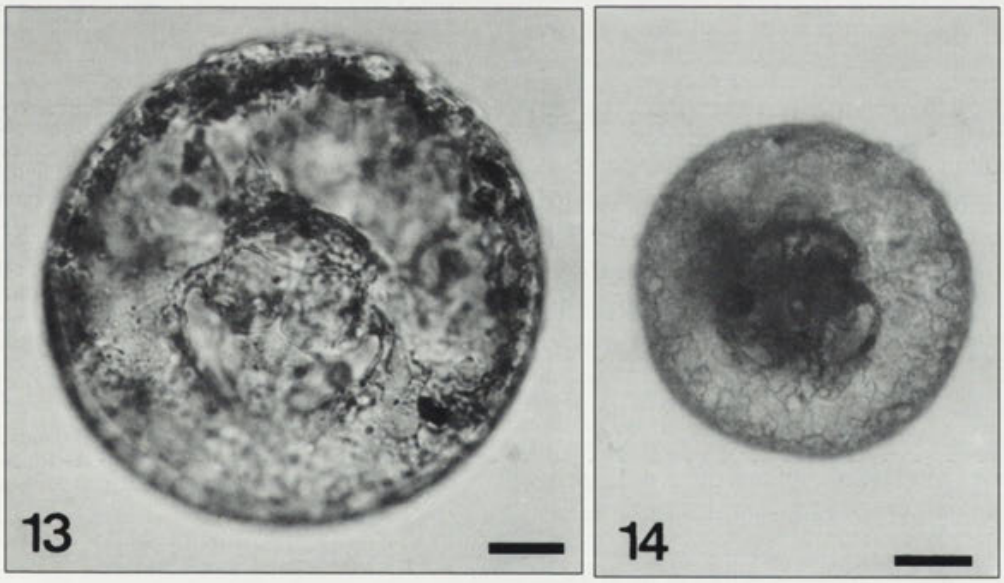
Abbreviations: see Table 2



Figs. 3-9. *Cyclopyxis lithostoma.* **3-5** - dorsal, ventral and lateral views showing the smooth surface of shell, **6** - apertural view showing the indented pseudostome, **7** - apertural view, **8, 9** ventral and dorsal views. Scale bars - **3-5** - 50 μm; **6, 7** - 15 μm; **8, 9** - 50 μm



Figs. 10-12. *Ellipsopyxis lamottei*. **10** - apertural view, **11, 12** - apertural and lateral views. Scale bars - **10-12** - 20 μm



Figs. 13, 14. *Lamtopyxis cassagnaui*. **13, 14** - apertural views showing the pseudostome area with three highly flattened teeth. Scale bars - **13** - 20 μm; **14** - 30 μm

Table 5. Morphometrical characteristic of *Lamtopyxis cassagnaui* Bonnet

Characters	$\bar{x}$	M	SD	$SE_{\bar{x}}$	CV	Min	Max	n
Diameter	135.4	136.1	3.7	1.5	5.4	130	141	15
Height	90.4	91.1	3.3	1.3	3.2	87	95	15
Aperture	36.7	37.4	2.8	1.2	2.2	35	42	15
Invagination of aperture	27.1	27.6	2.2	1.1	1.3	25	30	15

Abbreviations: see Table 2

smooth giving it a regular outline. The characteristic feature of *C. lithostoma* is the lack of large and rough xenosomes on the shell surface. Maybe that is the reason for the low coefficients of variation for the shell diameter and shell height (Table 3). Another feature of *C. lithostoma* is the presence of a rim of rough dent-like xenosomes around the shell aperture, which gives it an indented view (Figs. 6-8). In our investigations, we observed that these large and specialized apertural xenosomes on some specimens almost entirely obstructed the aperture (Fig. 7). This feature was emphasized also by Bonnet (1974) in the original description of the species.

***Ellipsopyxis lamottei* Bonnet, 1974 (Figs. 10-12)**

This rare species was found in two stations (St. 2, 3) with a relatively small number of specimens. The morphology of the observed specimens in Thailand is similar to the original description of the species based on the material from Africa (Bonnet 1974). The morphometrical characterizations of *E. lamottei* from Thailand are given in Table 4.

***Lamtopyxis cassagnaui* Bonnet, 1977 (Figs. 13, 14)**

Found in humid epiphytic and soil mosses (St. 3, 4). The specimens observed by us differ from the original description mainly in their larger shell dimensions (Table 5). Bonnet (1977) indicated an average shell diameter of 116.6 μm (specimens from Nepal) and 95.9 μm (specimens from Paraguay) and an average height of the shell about 2/3 of the diameter (about 62-76 μm). The specimens from Thailand have an average shell diameter of 135.4 μm and an average shell height of 90.4 μm (Table 5) and we accept that these differences are within the limits of the ecological variation of the species.

A characteristic feature of the population from Thailand is that all specimens have three highly flattened teeth, which almost touch and fill up the pseudostome. This fact confirms the hypothesis of Bonnet (1983) that the species of the genus *Lamptopyxis* that occur in West-Gondwanan and Central-Gondwanan regions (South America and Africa) have three only lightly flattened teeth. Furthermore, the vestibule of their pseudostome is more open, whereas the species occurring in more southern oriental regions (Nepal, Thailand, Indonesia) have a complicated vestibule with three highly flattened teeth, some of which touch almost entirely.

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Received on 11th February, 2000; accepted on 27th July, 2000

Acta Protozool. (2000) 39: 345 - 347

ACTA
PROTOZOOLOGICA

Short Communication

Effect of Oxytocin and its Analogues on the Chemotaxis of *Tetrahymena*: Evolutionary Conclusions

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Summary. Oxytocin and its five analogues (arginin vasopressin included) were studied in chemotaxis assays using *Tetrahymena* as unicellular model cells. Exclusively the two real hormones (oxytocin and vasopressin) were consequently repellent, other analogues (with exchanged or missed amino acids) were neutral, attractant or unbalanced. *Tetrahymena* was not able to differentiate qualitatively between oxytocin and vasopressin, however it sharply discriminated the real hormones from the analogues. Considering that in earlier experiments oxytocin and vasopressin also equally influenced the function of the contractile vacuole, it is supposed that the uniformity of the effect of these hormones is not a chance event and they had to be diverged (in function) later, during the evolution.

Key words: chemotaxis, evolution, neurohypophyseal hormones, *Tetrahymena*.

INTRODUCTION

The unicellular organisms can react to the hormones of multicellular animals and can select between them (Josefsson and Johansson 1979; Csaba 1980, 1985, 1994; O'Neill *et al.* 1988; Renaud *et al.* 1991; Christopher and Sundermann 1995). Histamine, which has a phagocytosis stimulating effect in mammals, stimulates phagocytosis also in *Tetrahymena* (Csaba and Lantos 1973). Serotonin does the same and *Tetrahymena* can differentiate the animal hormone, serotonin (5HT) from the plant hormone indoleacetic acid, which are chemically related molecules. Thyroxin and its precursors (monoiodotyrosine, diiodotyrosine, triiodothyronin) enhance the growth of *Tetrahymena* with the advantage of the phylogenetically older molecules (Csaba and Németh 1980). Using oligopeptides and dipeptides as chemoattractant or repellent molecules, the *Tetrahymena* can differentiate between them thereby showing preference of some amino acids (Kőhidai *et al.* 1997, Kőhidai 1999).

Oxytocin and vasopressin are amino acid type (related) hormones, containing 9 amino acid residues (Frieden 1976). In *Tetrahymena* these exogeneously given hormones influence the function of the contractile vacuole, with the priority of the phylogenetically older oxytocin

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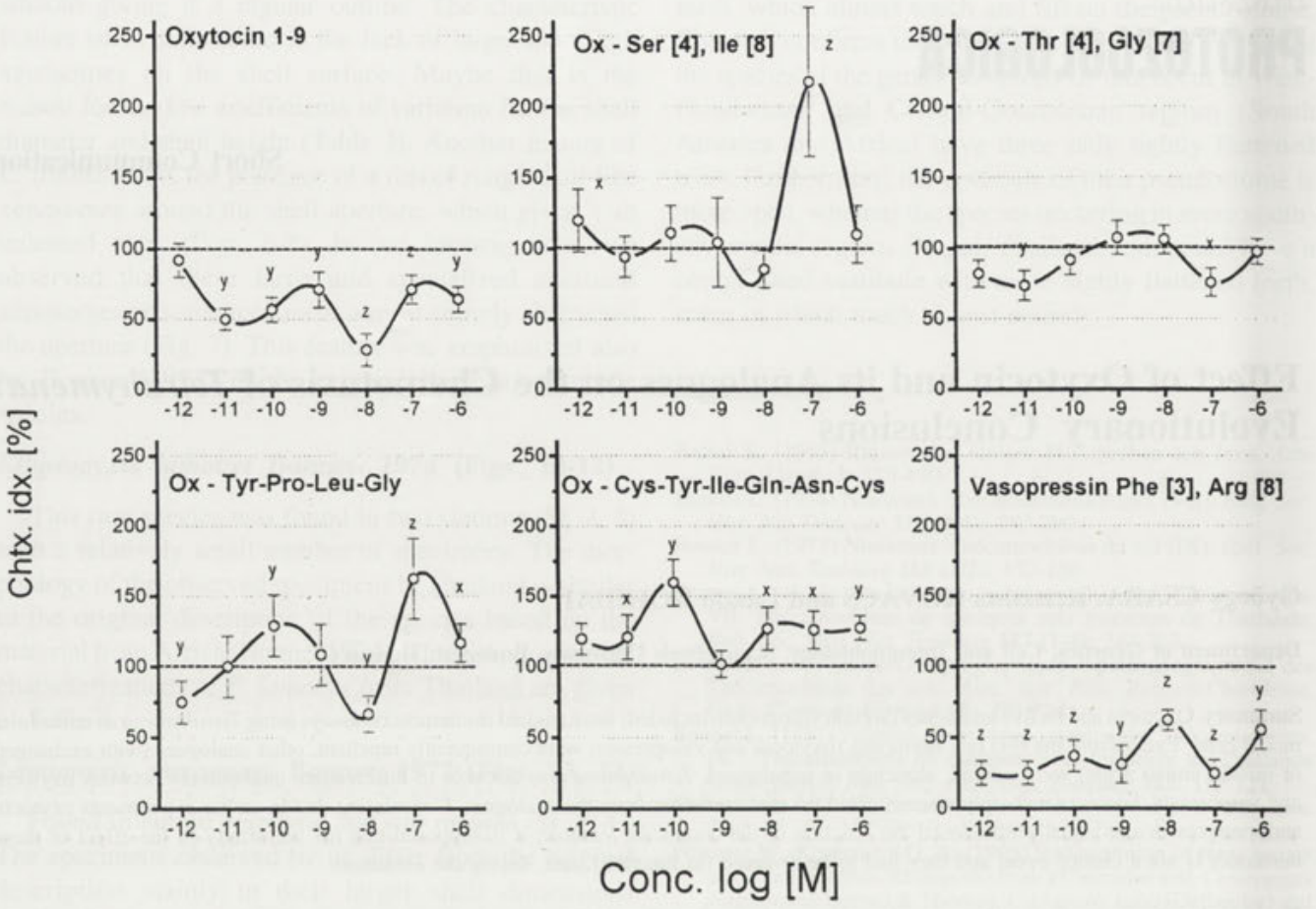

Fig.1. Chemotactic index (Chtx. idx) in percents (ordinate) related to the control as 100%. In the abscissa the concentrations of the materials are shown

(Csaba and Kovács 1992). In our present experiments these two hormones and their analogues were chosen for studying the effect of the alteration of molecules' amino acid composition in a chemotactic model system. Chemotaxis is an ancient and vital reaction of cells and single-celled animals, as a result of interaction between receptors and ligands (the chemotaxis provoking molecules - Kőhidai 1999). So the chemotactic index can give information on the interaction mentioned above neglecting the molecules' functional specificity at higher evolutionary level.

MATERIALS AND METHODS

Cells and culturing: *Tetrahymena pyriformis* GL cells were cultured in axenic cultures containing 1% Tryptone and 0.1% yeast extract (Difco, Michigan, USA). Cells of logarithmic growth phase (48 h) were assayed. Cell density was 10^4 cell/ml.

Hormones and buffers: The hormones and analogues used were: Oxytocin (OX) 1-9; OX Ser [4], Ile [8] (isotocin); OX Thr [4], Gly [7]; OX 6-9; OX 1-6 (tocinoic acid); vasopressin: OX Phe [3], Arg [8]. NaCl-phosphate buffer (PBS) 0.05 M phosphate buffer containing 0.9% NaCl at pH 7.2 was also used.

Chemotaxis assay: The chemotactic activity of *Tetrahymena* cells was evaluated with a two-chamber, capillary chemotaxis assay (Leick and Hell 1983, Levandowsky *et al.* 1984) modified by one of us (Kőhidai 1999). In this set-up, an 8-channel-micropipette served as the inner chamber of the system filled with the test substances (buffers containing different concentrations of the actual oxytocin), while the outer chamber, a microtitration plate was filled with the cells. The incubation time was 20 min., this relatively short time was necessary to measure the pure chemotactic responses and prevented the contamination of our samples by chemokinetic responder cells. In the concentration course study the chemotactic responses were tested in the range 10^{-12} - 10^{-6} M. Fresh culture medium served as control substance in the simultaneous runs. After incubation the samples of the inner chamber were fixed in 4% formaldehyde diluted in PBS. The number of cells was determined using Neubauer haemocytometer.

The experiments were repeated five times in two parallels. Statistica and Origin 4.0 were used to statistically analyze data.

Values of t-probe are shown on the figures: x = $p<0.05$; y = $p<0.01$; z = $p<0.001$.

RESULTS AND DISCUSSION

Of the six molecules studied, only the two real hormones (oxytocin and arginine vasopressin) exhibited repellent activity in each concentration. The effect of vasopressin (OX Phe [3], Arg [8]) was more expressed. The change of glycine and proline to threonin and glycine, respectively (OX Thr [4], Gly [7]) neutralized the repellent effect in each concentration. Similar, however less balanced effect was produced by OX 6-9 and OX Ser [4], Ile [8]. These two molecules had a strong chemoattractant effect at 10^{-7} M concentration. OX 1-6 produced a mild, nevertheless constant attractant activity.

Repellence means that the cells recognize the surrounding molecules and escape. The two hormones-oxytocin and vasopressin- were uniformly recognized and a very low concentration (10^{-11}, 10^{-12} M) was enough for provoking running away. The effect of these hormones in higher organism is different, mainly influencing smooth muscle contraction and water resorption in mammals, respectively (Sawyer and Pang 1979). However, in earlier experiments (Csaba and Kovács 1992), in *Tetrahymena* both hormones influenced the function of contractile vacuole similarly, as was done also in the present case. This means that the *Tetrahymena* does not differentiate qualitatively between the two hormones, however it sharply discriminate the non-hormone analogues from the hormones. In addition it can distinguish between the non-hormone analogues, however this distinction is not so sharp.

The ring of oxytocin composed by six amino acids [1-6] alone has an attractant effect. The influence of the „tail" [7-9] combined with Tyr [6] is unbalanced and mostly attractant (in this case the aromatic amino acid, Tyr also could have a role). However, it seems to be likely that the ring and tail together (OX 1-9 or vasopressin) is needed for the repellent effect expressed by the real hormones.

It can be concluded that uniform and characteristic response was provoked oniy by the two molecules, which are hormones at higher level of evolution (Sawyer and Pang 1979). This means that selection of molecules for being hormones is not a chance event and this selection is started at a very low level of phylogeny. In the higher steps of evolution a functional refinement will happen according to the actual requirements (divergence of oxytocin and vasopressin).

Acknowledgement. This work was supported by the National Research Fund (OTKA T-024064 and T-017773), Hungary

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Received on 3rd July, 2000

Acknowledgement

The editors wish to acknowledge the help of the following colleagues who have served as reviewers for one or more manuscripts submitted to publication in our journal in 2000.

Erna Aescht
Javier Alba-Tercedor
O. Roger Anderson
Sihem Bahri
Christian F. Bardele
Greg Barrit
Louis Beyens
Tamara Beyer
F. Bringaud
Søren Tvorup Christensen
John C.Clamp
John Corliss
Charles F. Delviche
Livage Isabelle Desportes
Jean Dragesco
Donald W. Duszynski
Colette Febvre-Chevalier
Wilhelm Foissner
Joseph Frankel
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ACTA PROTOZOOLOGICA

NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY
WARSAW, POLAND

2000

VOLUME 39
ISSN 0065-1583

Polish Academy of Sciences
Nencki Institute of Experimental Biology
and
Polish Society of Cell Biology

ACTA PROTOZOOLOGICA
International Journal on Protistology

ACTA PROTOZOOLOGICA appears quarterly.

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This publication is supported by the State Committee for Scientific Research

Desktop processing: Justyna Osmulska, Data Processing Laboratory of the Nencki Institute
Printed at the MARBIS, ul. Kombatantów 60, 05-070 Sulejówek, Poland

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Journals supported by the Polish Society of Cell Biology

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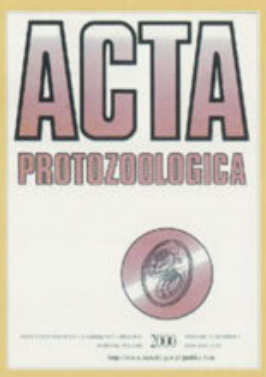

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ACTA
PROTOZOOLOGICA

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2000 NOVEMBER
VOLUME 39 NUMBER 4