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Review Article

Identification of Microsporidia

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Summary. Identification of microsporidia to genus level is described. Easily evaluated light microscopic, and to some extent electron microscopic characters, have been selected and typical character states are illustrated by light and electron micrographs. Two diagnostic tables are included. One is a survey of all genera where the description is detailed enough to allow them to be recognized. The second table exhibits where the various morphs of the polymorphic microsporidia of mosquitoes are found. The microsporidian genera are listed with sources to facilitate identification and with comments on diagnostic characters.

Key words: diagnostic characters, identification of genera, microsporidia.

INTRODUCTION

The phylum Microspora Sprague, 1977 comprises unicellular parasites reported from every major group of animals. Microsporidia are especially common in insects, crustaceans and fish, but have in later years also proven to be important parasites of man. Until Matsubayashi and colleagues reported the first human infection caused by microsporidia, this groups was considered of no interest for human medicine (Matsubayashi *et al.* 1959). Now most research on microsporidia deals with the medical aspects, and units for infectious diseases, all over the world, routinely screen for them. The number of parasitologists active with the identification of microsporidia has probably never been greater than it is today.

Comprising approximately 1,300 named species Microspora is a phylum of modest size. To identify and handle the classification of a group of that size should not cause particular problems. Nevertheless identification is obviously not easy. Tentative identification and temporary assignment into genus are not uncommon, and in addition, which has few counterparts in taxonomy, the classification of microsporidia recognizes one genus lacking diagnostic characteristics and a place in the classification: *Microsporidium* Balbiani, 1882. This genus was introduced by Sprague (1977), who actually is the *auctor*, when revising the microsporidian classification. *Microsporidium* functioned as a repository for species excluded from other genera, waiting for new information allowing proper classification. However, *Microsporidium* has also been used as a temporary genus when describing new species.

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There are many explanations to the problems with the identification of microsporidia. One is the minute size. When dealing with organisms only a few microns large, diagnostic characters are difficult to evaluate. New techniques have given access to new characters, and each introduction of new techniques has initiated a renaissance for studies of microsporidia. In the 1970-ies and 1980-ies investigations made great use of electron microscopy. In the 1990-ies molecular biology has been in focus. This does not mean that electron microscopy is out of use now. The ultrastructure is rich and varied, and today it is practically impossible to describe a new microsporidium without including ultrastructural data.

Another problem is the quality of descriptions. When describing new microsporidia authors use the most appropriate, or modern, techniques available. However, it is sometimes overlooked that a good description must be useful. Even if the rules of nomenclature are fulfilled when a new species or genus is defined in a strict way using brand new diagnostic criteria (International Code of Zoological Nomenclature 1985), the description of the new taxon will not always be good enough to also allow comparison. If the authors base the taxon on molecular differences, or on distinct ultrastructural characteristics, in order to make the description useful the "oldfashioned" light microscopic parameters, like how cells divide and the shape and size of spores, must also be described. The light microscopic characters are the only ones allowing comparison with all microsporidia, and good light micrographs, at least of living and stained spores, should always be included in the description of a new species. There are many recent descriptions of new microsporidia lacking this crucial information.

The third problem is that only a small fraction of species, and even genera, have been studied, described and named. In the last decade an average of five new genera have been established each year, and approximately 50% of the microsporidian genera are still monotypical, comprising only one species each. The rapid appearance of new genera, and the great proportion of monotypical genera, are clear indications of how little is known. When investigating microsporidia, the opportunity, or risk, that the specimen under study is undescribed is considerable.

While the classification of microsporidia has been the topic of a number of recent publications, there has been little interest in the problems with identification. A complete key to species has never been published, and as the number of species increases rapidly, after a short time it would be out of date. Treatises on identification have either been restricted to the microsporidia of selected host groups or focused on the identification of genera. Since the most recent identification key to genus level appeared (Larsson 1988b), approximately 50 new genera have been described, and this is of limited use today. This review tries to fill a gap. It summarizes the characters used for the discrimination of microsporidian genera, provides 2 diagnostic tables which in most cases will allow identification of the genus, and lists the genera recognized today, with comments on characteristics and problems with the identification.

HOW TO RECOGNIZE MICROSPORIDA?

Even if the microsporidia of certain invertebrates and fish induce pathogenic conditions recognizable with the naked eye, the small size of the organisms makes it necessary to apply great magnification to verify the microsporidian nature and find the characters necessary for identification. The spore, which is the stage in which the microsporidium is transmitted to the new host, is the only life cycle stage that easily can be recognized as a microsporidium (Figs. 1 A, B). Spores of most species are ovoid or pyriform and only a few microns long. The human parasite *Enterocytozoon bieneusi* belongs to the smallest species, with spores averaging 1 µm. The rod-shaped spores of *Bacillidium* species are the giants, exceeding 30 µm in length. Spores of fungi, especially yeasts (Figs. 1 C, D), and some types of sperms (Fig. 1 E) might be mistaken for microsporidia. While a sample of yeast cells show organisms of variable size, a sample of microsporidian spores is more uniform, containing cells of one or two distinct size classes.

The spore is one cell with one or two nuclei of normal eukaryotic type (Figs. 2 A, B, E). The spore wall is thick (with an external exospore layer, a median endospore, and an internal plasma membrane), making the living spore refractive. The spore is equipped with a unique infection apparatus, containing an extrusive organelle, the polar filament, an anterior system of membrane-lined cavities, the polaroplast, and a posterior vacuole. In most microsporidia the filament is much longer than the spore and arranged in a number of coils in the posterior half of the spore. The transversely sectioned coil exhibits concentric layers of different electron density and thickness (Fig. 2 C). By application of pressure, or by adding weak solutions of certain salts, acids or bases to a sample, the spores can be forced to eject the polar filament (Fig. 2 D). During this process the filament is reorganized into an

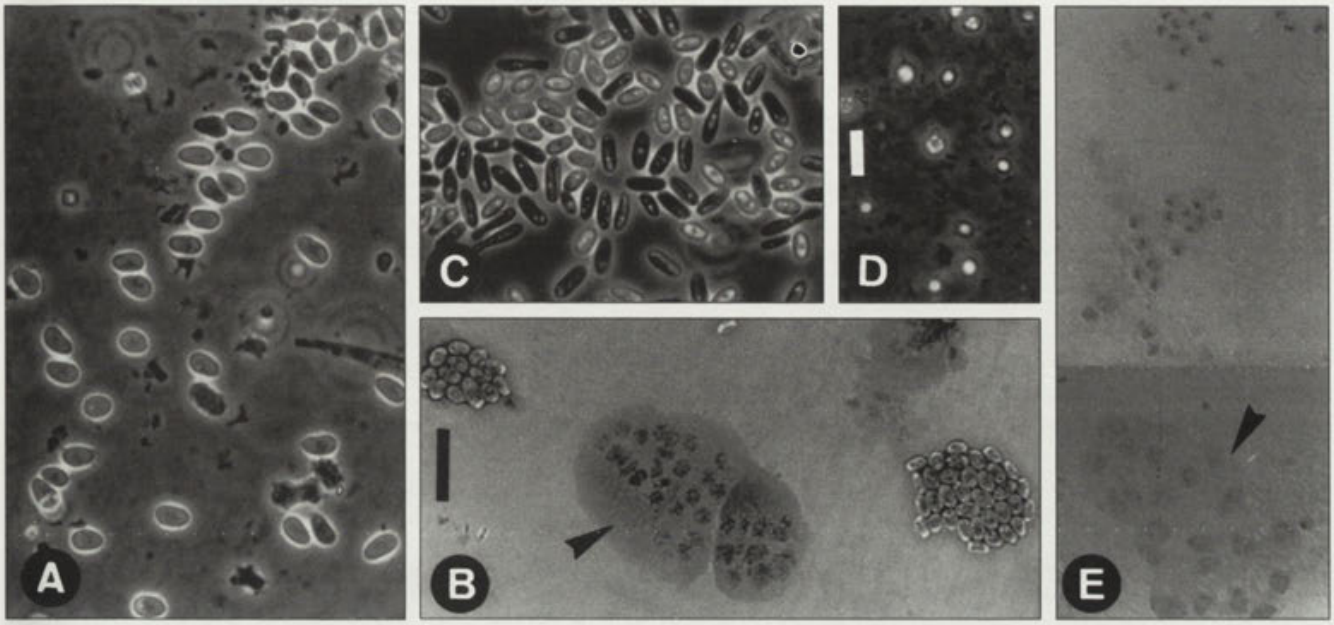


Fig. 1. Microsporidia and cells that can be mistaken for microsporidia. A - spores of the microsporidium *Flabelliforma magnivora*; B - the microsporidium *Glugea anomala*, spore groups and a sporogonial plasmodium (arrowhead) are visible; C - yeast cells (from *Endochironomus* sp.); D - yeast cells (from *Hylurgops palliatus*); E - free spermatocytes and a testicular sac (arrowhead) with developing spermatocytes (from a mite). A, C, D - phase contrast, B - Giemsa staining, E - Heidenhain´s hematoxylin. Scale bars - 10 µm (A, B, E with common bar on B; C, D with common bar on D)

invasion tube (Weidner 1976, 1982; Weidner and Byrd 1982; Weidner *et al.* 1995). When invading a new host the sporoplasm, the infectious stage of the microsporidium, is injected safely, through the tube, into a host cell (Lom and Vávra 1963, Dall 1983, Undeen 1990). When the nucleus and cytoplasm of the spore pass through the remainders of the polaroplast, the sporoplasm acquires a plasma membrane from the membranes of the polaroplast. The plasma membrane of the spore remains as the internal layer of the spore wall of the empty spore. When using light microscopy for the study of microsporidia, observed ejection of the polar filament is the definite proof of the microsporidian nature of the organism studied.

The cytology of microsporidia is aberrant. On one hand microsporidia possess a uniquely constructed infection apparatus, with polar filament, polaroplast and posterior vacuole, on the other hand they lack mitochondria, typical Golgi apparatus and microbody-like organelles (hydrogenosomes, peroxysomes). The ribosomes are not of the typical eukaryotic type but resemble ribosomes of prokaryotic organisms (Ishihara and Hayashi 1968, Curgy *et al.* 1980). Useful reviews of the biology of microsporidia include the first two volumes of the series "Comparative Pathobiology" (1976, 1977), Canning (1989), and "The Microsporidia and Microsporidiosis" (1999).

REPRODUCTION AND LIFE CYCLES

There are several ways for a microsporidium to reach a new host. Most species infect through the digestive tract. For many microsporidia of insects transovarial transmission, from mother to offspring, has been reported.

As soon as the new host has been invaded reproduction starts. In the literature on microsporidia the terminology for life cycle stages and events during the reproduction varies. For this review a conservative approach has been taken, and the terminology applied herein should be useful for most of the descriptions and reviews cited. Alternative terminology has been suggested by Sprague *et al.* (1992) and by Sprague and Becnel (1999b).

The initial reproductive phase is usually called merogony (Fig. 3 A). It yields daughter cells, merozoites. The mother cell for merogony is called a meront. One or more bouts of merogony follow each other. Some microsporidia, like *Metchnikovella* and *Chytridiopsis*, are believed to lack merogonic reproduction. Merozoites of the last generation mature to become sporonts, which enter another phase of reproduction, the sporogony, leading to the production of spores (Fig. 3 A). The final division products of the sporogony are called sporoblasts. These cells mature to become spores. Some microsporidia reproduce weakly in

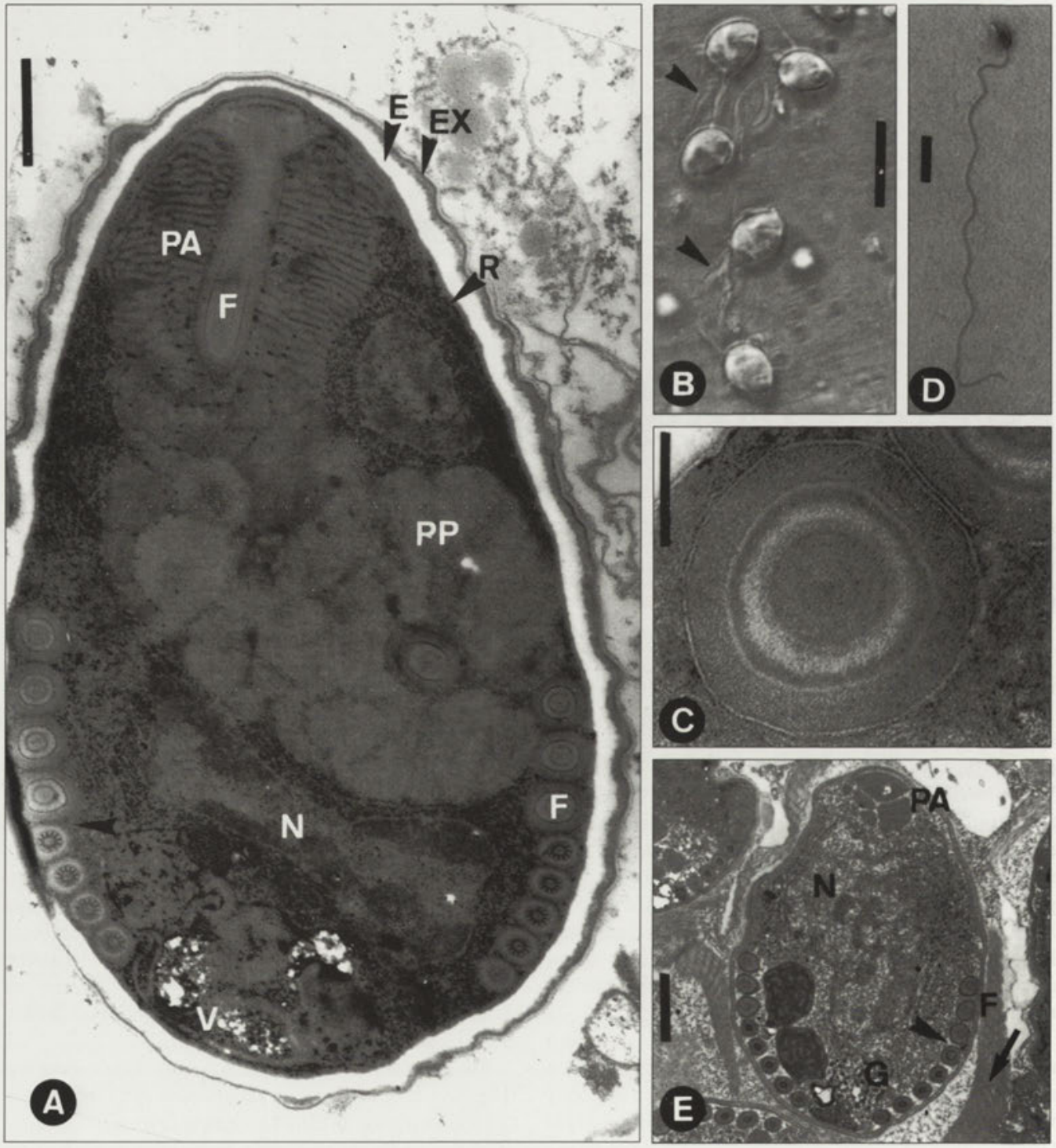


Fig. 2. Cytology of *Trichoctosporea pygopellita*. A - longitudinally sectioned spore showing nucleus, spore wall, and the extrusion apparatus; arrowhead indicates the position where the anisofilar filament tapers; B -living spores, arrowheads point at fibrous projections; C - a transversely sectioned polar filament coil, the layered material is distinct; D - partially ejected polar filament; E - immature spore; the polar filament has reached nearly full length, but the internal organization is still immature (arrowhead indicates where the narrow part begins); the first signs of the polaroplast are visible, the endospore layer is still lacking; arrow points at a fibrous projection. Abbreviations: E - endospore, EX - exospore, F - polar filament, G - Golgi apparatus, N - nucleus, PA - anterior part of the polaroplast, PP - posterior part of the polaroplast, R - ribosomes, V - posterior vacuole. A, C, E - electron microscopy, B - interference phase contrast, D - Giemsa staining. Scale bars - A - 0.5 μm; B, D - 10 μm; C - 100 nm; E - 1μm

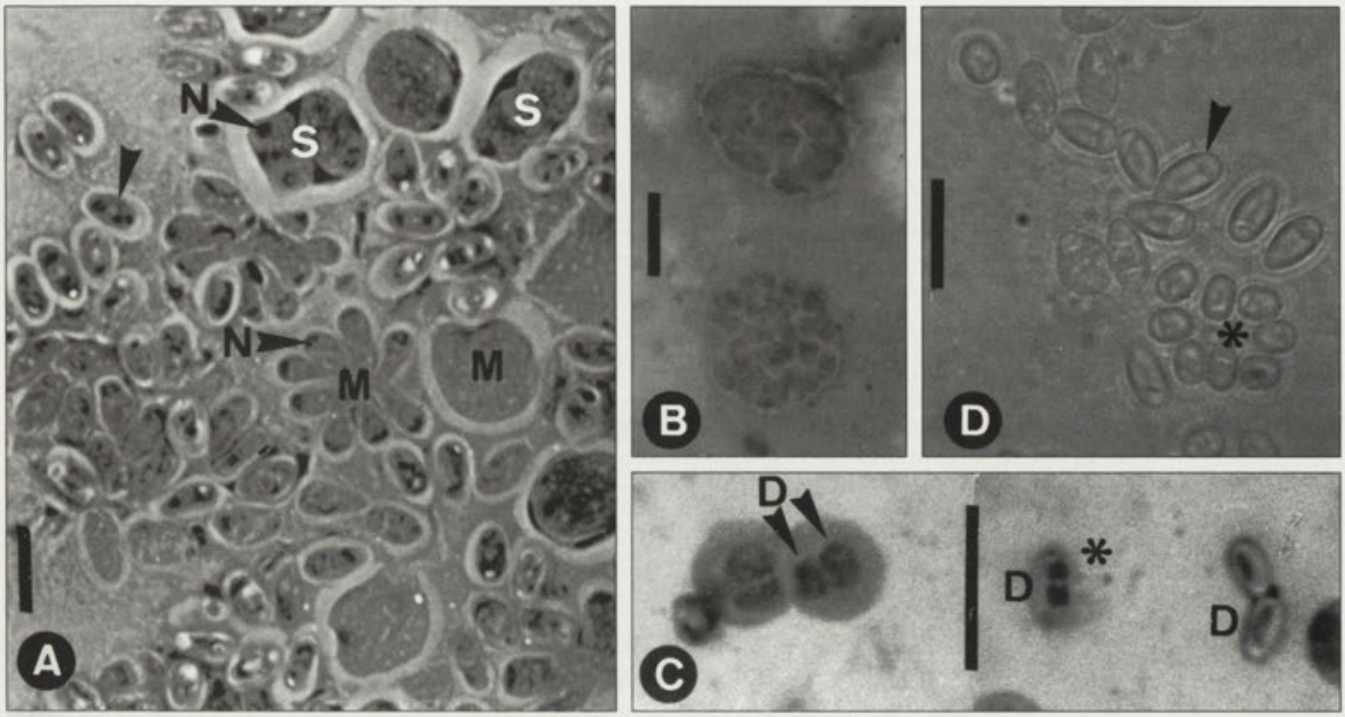


Fig. 3. Reproduction. A - rosette-like dividing merogonial plasmodia, sporogonial plasmodia in sporophorous vesicles, and mature spores (arrowhead) (*Systenostrema corethrae*). B - sporogonial plasmodia of *Vavraia holocentropi* dividing by plasmotomy. C - sporont dividing by binary fission, an immature spore (*) with distinct diplokaryon, and mature spores (not hydrolyzed, the two components of the diplokaryon are not distinct) (*Nosema tractabile*). D - free spores (arrowhead) and one sporophorous vesicle (*) with 8 spores of the dimorphic species *Amblyospora callosa*. Abbreviations: D - diplokaryon, M - merogonial plasmodium, N - nucleus, S - sporogonial plasmodium. A - Giemsa staining; B, C - Heidenhain´s hematoxylin, D - phase contrast. Scale bars - 10 μm

the merogonic phase. As a compensation great numbers of spores are produced. For other microsporidia the merogony is the principal reproductive phase, and each sporont yields only two spores. Sexual processes, and meiosis, have been revealed in the transition between merogony and sporogony (Loubès 1979, Hazard and Brookbank 1984, Hazard *et al.* 1979, Hazard *et al.* 1985). The spores are either produced free in the cytoplasm of the host cell or are enclosed in sac-like structures generated by the microsporidium (sporophorous vesicles of various types).

For rather few microsporidia complete life cycles have been elucidated. Most species are interpreted as being monomorphic, producing only one type of spores. Microsporidia of mosquitoes and some other hosts express complex life cycles, where two (dimorphic species, Fig. 3 D) or three (trimorphic species) different types of spores are produced. The different spore morphs occur together or are distributed into different host individuals. In the life cycles of some genera, like *Amblyospora*, two generations of the mosquito are involved (adult female and larva), together with a copepod as alternate host (Becnel and Andreadis 1999). The spore types of dimorphic and trimorphic microsporidia differ in several respects: the spores have different shape, different number of nuclei, different construction of the components of the extrusion apparatus, one type occurs free in the cytoplasm of the host cell another is enclosed in an enveloped produced by the microsporidium, and they are produced by different processes of reproduction.

Real dimorphism is sometimes mixed up with the production of an early generation of spores (Iwano and Ishihara 1989, 1991). These early spores are usually the result of a reproduction in the gut epithelium, normally the first tissue reached by the microsporidium. They hatch directly and their function is to transmit the parasite inside the host. Usually in a deeper tissue, like fat, gonads or glands, a second generation of spores is formed. These,

so called environmental spores, transmit the microsporidium to a new host. The two types are often described as being different, but in all cases known to me, the differences can be explained as different degree of maturation. The early spores differ from the environmental spores by having a thinner spore wall, with a weakly developed endospore layer, a less well organized polaroplast, and a polar filament with a smaller number of coils. The endospore layer and the polaroplast are the last structures of the spore to develop, and the polar filament increases in size until the spore is mature. The two spore types seem to be the microsporidian equivalent to the two kinds of oocysts produced by coccidia of the genus *Cryptosporidium* (Current 1989), or to the phenomenon of metazoa called neoteny. Where early spores have been looked for, they have usually been found, which indicates that the production of early spores might be a normal phase of the microsporidian life cycle. In this review, when microsporidia are characterized as dimorphic or trimorphic, the early spores are disregarded.

CLASSIFICATION

Absence of mitochondria has suggested a ranking of microsporidia in the kingdom Archeozoa Haeckel, 1894, which was resurrected by Cavalier-Smith (1983). This has never been generally accepted among microsporidiologists, and it has recently been proven that microsporidia have a protein (HSP70) suggesting that microsporidia once have possessed mitochondria (Germot *et al.* 1997, Hirt *et al.* 1997).

As it looks for the moment the phylum Microspora Sprague, 1977 is a monophyletic taxon with no evident relationship to other protists. Molecular biology has suggested fungal affinities (Müller 1997). At present time there is no fungal taxon in which the microsporidia could be placed.

The classification of microsporidia has been reviewed several times. Kudo (1924) summarized the earliest achievements. Sprague produced a major treatise, still indispensable for the microsporidiologist (Sprague 1977). This volume covers all species known at the time and it is a critical evaluation of the microsporidian classification. Weiser (1977) and Issi (1986) presented new classifications where several new genera were established. The most recent classification is by Sprague *et al.* (1992).

There are different opinions about the relationships of microsporidia, and more than one view on how to classify the phylum. A number of genera related with *Chytridiopsis* and *Metchnikovella* respectively exhibit an aberrant cytology, and obviously lack the merogonic reproduction. Three different groups of microsporidia can easily be revealed. One contains *Chytridiopsis* and a few related genera. The second consists of *Metchnikovella* and a few related genera. The third group houses the bulk of microsporidia, expressing "normal" cytology and reproduction. At present time approximately 145 genera are recognized.

IDENTIFICATION

Classification and identification are different processes. Characters of great weight for classification are not necessarily used for identification. The goal of identification is to recognize a certain taxonomic group, to find the correct name, by using as simple and reliable characters as possible. When dealing with organisms with complex life cycles, like many microsporidia, it is expected that it should be possible to identify the organism even if only a small fraction of life cycle stages are present.

Few publications have been devoted to the identification of microsporidia, and most of them were restricted to certain groups of hosts.

The microsporidia of insects and mites have been covered most extensively. General reviews include: Weiser (1961- keys to species); Weiser and Briggs (1971 - keys to genera); Hazard *et al.* (1981 - keys to genera); Weiser (1982, 1991 - microsporidia with insect control potential); Becnel and Andreadis (1999 - genera present in insects). Particular groups include: Collembola (Weiser and Purrini 1980, Maddox *et al.* 1982), Chironomidae (Coste-Mathiez and Tuzet 1977), Chrysomelidae (Toguebaye *et al.* 1988), Simuliidae (Maurand 1975, Issi *et al.* 1991), Tabanidae (Bykova and Issi 1991), and Oribatei (Purrini and Weiser 1981).

The microsporidia of Protozoa were reviewed by Vivier (1975), Platyhelminthes by Canning (1976), Crustacea by Voronin (1986), and the microsporidia of vertebrates by Canning and Lom (1986).

Publications on identification covering practically all genera are few. Larsson (1983a) published identification keys and a diagnostic table to all microsporidian genera with clear characteristics (in Swedish, translated into English in Canada). Weiser (1985) provided keys to families, but treated only one third of the genera. Issi (1986) treated all genera in a diagnostic table. Larsson

(1988b) provided keys and a table covering all genera with distinct characteristics (translated into Spanish in Costa Rica).

Characters used for identification

For the identification of microsporidia above the species level, characters from cytology, reproduction and hosts are useful. The spore is the most important life cycle stage. The spore is always present and provides abundant characters to evaluate.

Weiser´s important monograph on the microsporidia of insects included identification keys to all species known at the time (Weiser 1961). All characters used for identification were taken from associations with hosts and from the external cytology of the spore. Weiser (1982) evaluated in depth the same sources of characters, but included some internal cytological characters of the spore visible using light microscopy, and described 70 different character states visible to the keen eye. In the key to microsporidian genera found in insects or mites by Hazard *et al.* (1981) only spore characters, visible using light microscopy, were used. If Weiser, in 1961, had decided to extend his monograph to cover all microsporidia, the same set of characters would have been sufficient for identification. In 1981 that would not have been the case. In the time separating these publications a number of new genera were described, and many of them were distinguished using characters taken from the submicroscopic cytology. In the most recent identification keys to microsporidian genera it was necessary to use at least a minimum of ultrastructural characters (Larsson 1983a, 1988b).

In the identification table of this review (Table. 1) host groups and light microscopic characters have been used extensively, but it has also been necessary to include some ultrastructural characters. Electron microscopy is indispensable for the study of microsporidia.

Cytology

The characters used for identification are briefly explained below. More details about the cytology are found in reviews by Vávra (1976), Larsson (1986b) and Vávra and Larsson (1999).

Characters visible using light microscopy

Nuclei. The number of nuclei in different developmental stages is visible in living and stained preparations (Figs. 3 A, C). Nuclei of spores are distinct only in stained preparations, and it is necessary to hydrolyze the spores prior to staining. The nuclei of microsporidia are either isolated or coupled as diplokarya (Fig. 3 C). Spores are either uninucleate or binucleate (diplokaryotic).

The shape of the spore (Fig. 4) is characteristic for the genus, i.e. all species of a genus share a basic spore shape. While some genera have uniquely shaped spores (e.g. lageniform in *Cougourdella* (Figs. 4 L, M), horseshoe-shaped in *Toxoglugea* (Fig. 4 K) and *Toxospora,* and ovoid with a posterior collar in *Parathelohania*), the majority of genera produce spores of a shape shared with other genera. The basic shapes that can be recognized are: spherical (Fig. 4 C), ovoid (blunt anterior pole, Figs. 4 A, B), pyriform (usually with pointed anterior pole, Figs. 4 E-H), lanceolate (elongated pyriform and slightly curved), and rod-shaped (cylindrical, Figs. 4 I-J, N-Q). In addition spore projections of various types can be found, like long tails in *Caudospora* and *Jirovecia* (Figs. 4. N-O), or fibrous tufts and girdles in *Hirsutosporos*, *Cristulospora* and *Trichoctosporea* (Fig. 2 B). Sometimes microsporidian spores appear intermediate between distinct shapes, and it might be difficult to decide whether the spores are best characterized as ovoid or pyriform.

Polymorphism. In some genera, like *Pleistophora* and *Glugea* (Fig. 4 A), spores of two size classes occur together: microspores and macrospores. These spores are of almost identical shape. Microsporidia of mosquitoes, and a few other cases, exhibit more expressed polymorphism: two or three morphologically different spore types are formed in different parts of the life cycle (Fig. 3 D). The different spore morphs are either distributed between different hosts or occur together in the same host individual (Table. 2). In *Metchnikovella* (and related genera, Fig. 5 T) and *Chytridiopsis* (and related genera, Fig. 5 U) free spores occur together with spores enclosed in sporophorous vesicles. Free and enveloped spores are of identical construction, only differing in size.

Early spores and environmental spores, which obviously are immature and mature variants of the same spore type, normally occur in different tissues in the host. The early spores are found in the gut epithelium.

Polar filament. The construction of the polar filament can be revealed if living spores are forced to eject their filament. It is necessary to realize that the ejected filament seen not always has reached the completely extended state. It is usually necessary to stain the smear to reveal whether the filament has uniform thickness (isofilar filament, Figs. 6 B, H), is moderately wide in the anterior part (anisofilar filament, Figs. 2 A,E, 6 G) or has a stiff, straight and wide anterior portion (manubrium-like, Figs. 4 N-Q, 6 E). The genus *Mrazekia* has a unique manubrium with

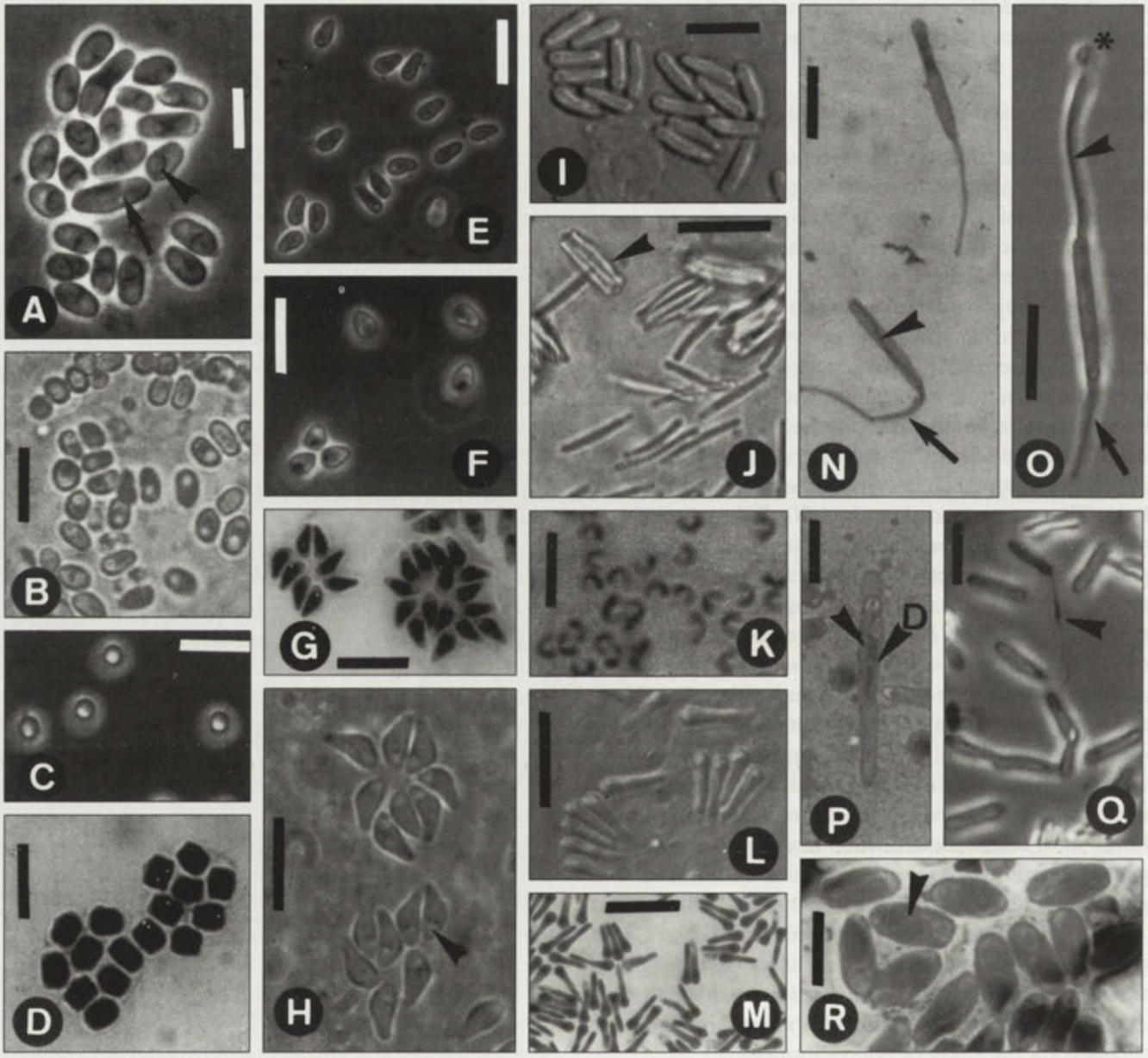


Fig. 4. Light microscopy views of mature spores. A - ovoid spores of *Glugea anomala*, arrow points at macrospore, arrowhead at posterior vacuole. B - ovoid spores of *Vavraia holocentropi*. C - spherical spores of *Coccospora micrococcus*. D - barrel-shaped spores of *Amblyospora undulata*. E - pyriform spores of *Flabelliforma magnivora*. F - pyriform spores of *Episeptum invadens*. G, H - stained and living pyriform angular spores of *Bohuslavia asterias*, 8-sporous groups occur together with 16-sporous groups, arrowhead points at posterior vacuole. I - rodshaped (cylindrical) spores in 8-sporous groups (*Resomeria odonatae*). J - narrow rod-shaped spores of *Cylindrospora fasciculata*, arrowhead points at an 8-sporous group. K - horseshoe-shaped spores of *Toxoglugea variabilis*. L, M - lageniform living and stained spores of *Cougourdella polycentropi*. N, O - rod-shaped stained and living spores of *Jirovecia caudata*, arrows point at tail-like posterior projection, arrowheads at the manubrium-like part of the polar filament; the filament in Fig. N is visible inside the spore, the filament of Fig. O is ejected and transformed into an invasion tube from which the sporoplasm (*) is leaving. P - rod-shaped spore of *Bacillidium criodrili*, the manubrium-like part of the polar filament (arrowhead) is visible (D - diplokaryon). Q - living rod-shaped spores of *Mrazekia cyclopis*, the ejected polar filament exhibits the posterior swelling of the manubrium-like part (arrowhead) and the narrow final portion. R - stained immature spores of *Janacekia adipophila*, the straight part and one coil of the polar filament is visible (arrowhead). A-C, E-F, H-J, L, O, Q - phase contrast; D, G, K, M, N, P, R - Heidenhain´s hematoxylin. Scale bars - A, K, P - 5 μm, others 10 μm

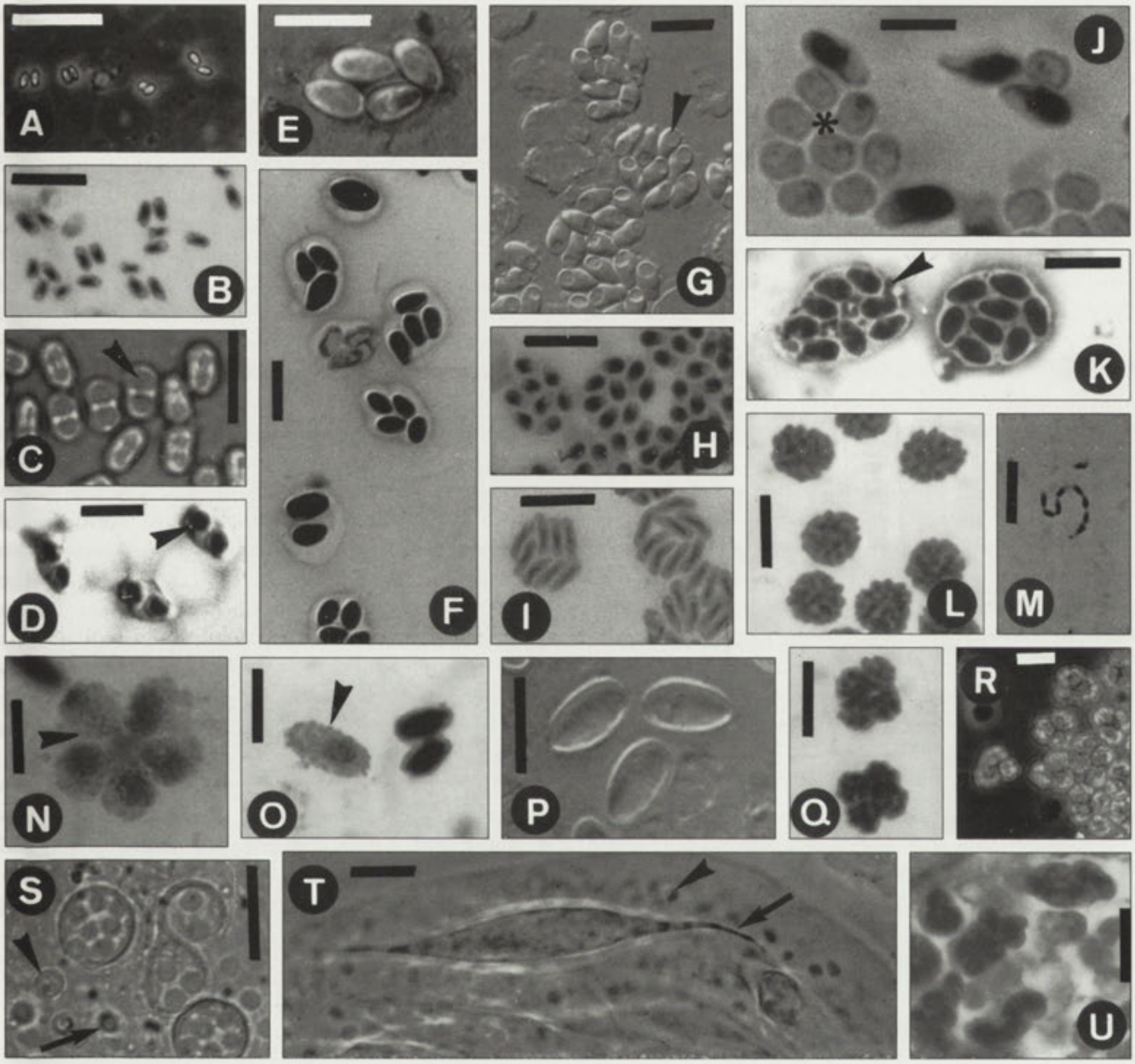


Fig. 5. Sporophorous vesicles. A, B - 2-sporous vesicles of *Berwaldia singularis*. C, D - 2-sporous vesicles of *Telomyxa glugeiformis*, arrowheads point at individual spores. E, F. - 4-sporous vesicles of *Gurleya dorisae*, macrospore-production yields and aberrant number of spores (Fig. F). G - living 8-sporous vesicles of *Napamichum dispersus*, no inclusions are visible (arrowhead indicates the posterior vacuole). H - 8-sporous vesicles of *Systenostrema candida*. I - 8-sporous vesicles of *Resiomeria odonatae*. J - 8-sporous vesicles (*) together with free spores of *Amblyospora callosa*. K - 8-sporous vesicles of *Napamichum dispersus*, arrow-head indicates granular inclusions. L - 16-sporous vesicles of *Agglomerata sidae*. M - spores of *Ordospora colligata* in chain-like configuration, no sporophorous vesicle. N-P - rosette-like sporogonial plasmodium (N), immature and mature stained spores (O), and mature living spores (P) of *Janeckia adipophila*, arrowheads point at darkly stained inclusions, revealing that spores are enclosed in individual sporophorous vesicles; inclusions disappear when the spores mature and are not visible on mature spores. Q, R - the 8-sporous vesicles of *Pegmatheca lamellata* are permanently associated. S - spherical vesicles of *Chytridiopsis trichopterae*; arrow points at a spore released from a vesicle, arrowhead indicates a spore of the not vesicle-bound sporogony. T - fusiform vesicles of *Amphiacantha longa*, arrow points at filiform terminal projection; spores of the not vesicle-bound sporogony indicated by arrowhead. U - the fusiform vesicles of *Napamichum dispersus* as seen in sectioned host tissue. A, C, R, S, T - phase contrast; E, G, P - interference phase contrast; B, F, I-K, N, O, Q, U - Heidenhain´s hematoxylin; D, H, L, M - Giemsa stain. Scale bars - D, J - 5 μm, others 10 μm

a posterior swelling (Fig. 6 E), visible using light microscopy (Fig. 4 Q). In large immature spores the developing polar filament might be visible (Fig. 4 R). In large cylindrical (rod-shaped) spores a manubrium-like filament might be visible also in mature spores (Figs. 4 N, P).

Spore groups are produced in many genera (Fig. 5). Sac-like structures, in modern literature usually called sporophorous vesicles, in older literature pansporoblasts, are produced by the microsporidium to collect the spores, most commonly the spore yield of one sporont. Sporophorous vesicles are more or less persistent, but can usually be revealed if preparations are made carefully. Spore groups with a constant number of spores are indicative of sporogony in sporophorous vesicles. The number of spores is often characteristic for a genus, and especially the configuration 8 spores per vesicle is found in several genera. Chain-like associated spores (Fig. 5 M) are usually not collected in a sporophorous vesicle.

If the microsporidium is studied in sections of host tissue other groups of spores might be mistaken for sporophorous vesicles. Parasitophorous vacuoles, produced by the host cell, collect spores in a similar way. In parasitophorous vacuoles spores are normally mixed with immature stages. Host cells can be completely filled with spores. From the gut epithelium cells filled with microsporidian spores are released into the gut lumen. Such cells look identical to multisporous sporophorous vesicles.

The shape of the sporophorous vesicle can be revealed by the shape of the spore groups. The envelope of the vesicle is rarely seen. Most sporophorous vesicles are spherical or ovoid (Figs. 5.F-L, S). Fusiform vesicles are characteristic for *Chapmanium, Napamichum* (Figs. 5 G, K, U) *Amphiacantha* and *Amphiamblys* (Fig. 5T) Sporophorous vesicles of some genera have thread-like projections, like *Trichoduboscqia*, *Mitoplistophora* and *Amphiacantha*. (Fig. 5 T). The 8-sporous sporophorous vesicles of *Pegmatheca* are permanently united to groups (Figs. 5 Q-R).

Inclusions. Sporogony in sporophorous vesicles is sometimes associated with production of inclusions visible in stained (Figs. 5 K, 6 B, F), and sometimes also in living preparations, most abundant in *Cryptosporina* and *Amblyospora*. When spores are enclosed in individual sporophorous vesicles, this can not usually be revealed using light microscopy. Spores of the genus *Janacekia* are an exception. Abundant production of inclusions, in stained preparations visible as dark spots on the surface of (sporogonial plasmodia) and the immature spores (Figs. 5 N-P), indicates the presence of an individual sporophorous vesicle.

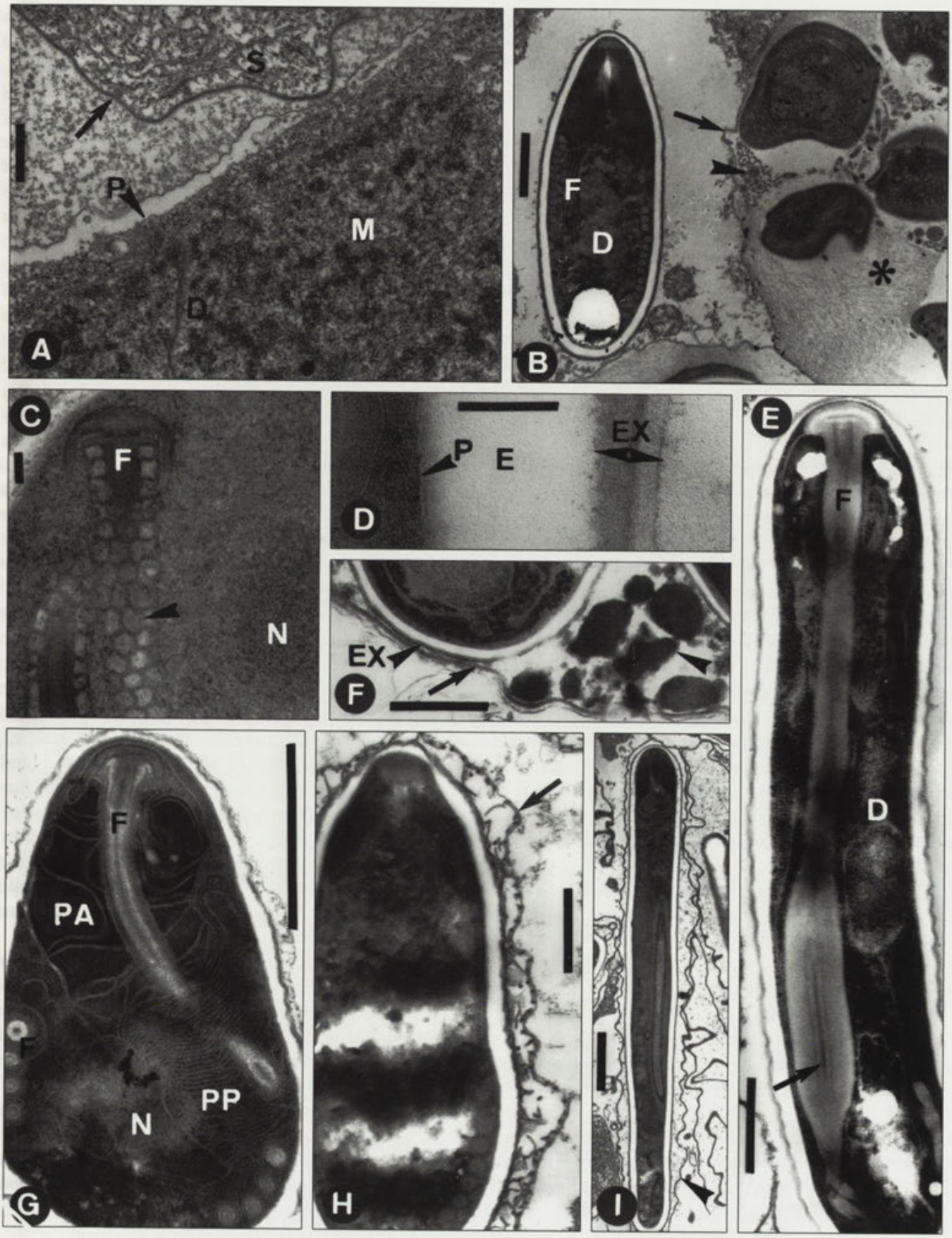
The mode of reproduction can also be revealed in carefully made squash preparations. Most microsporidia produce multinucleate stages, plasmodia, of various shapes (rounded, band-like, lobed etc.) prior to the release of daughter cells. If the reproduction is by binary fission associated cells are observed (Figs. 3 C, 5 A-D). The daughter cells can for some time remain connected as chains-like configurations. Plasmotomy is a reproduction where a plasmodium successively splits into daughter cells (Fig. 3 B). If the plasmodium divides in a finger-like fashion, to release all daughter cells simultaneously, the process is called rosette-like budding (Fig. 3 A).

In field-collected material the reproduction has often proceeded beyond merogony. If dividing merogonial stages are not present, groups of merozoites might be found, and the nuclear configuration of the merozoites can be evaluated. There are no clear cases described where the meront (mother cell) and the merozoites differ in nuclear configurations.

Characters which need electron microscopy

Merogony-sporogony. It is usually easier to distinguish between merogonial and sporogonial stages in ul-

Fig. 6. Ultrastructure of microsporidia. A - sporogonial stages differ from merogonial by a more complex cell wall, arrow points at the sporont wall (*Trichoctosporea pygopellita*). B - free spore with isofilar polar filament and immature spores in 8-sporous sporophorous vesicle (*), arrow points at the thin envelope of the vesicle, arrowhead at inclusions (*Amblyospora callosa*). C - spore of *Chytridiopsis trichopterae* with polar filament covered by a honeycomb-like layer (arrowhead). D - spore wall of *Trichoctosporea pygopellita* exhibiting a complex, layered exospore. E - longitudinally sectioned spore of *Mrazekia cyclopis*, arrow points at the posterior swollen part of the manubrium-like polar filament. F - sporophorous vesicle of *Cryptosporina brachyfila*, arrow points at the thick envelope of exospore construction, arrowhead at inclusions. G - longitudinally sectioned spore of *Episeptum circumscriptum* revealing the construction of the polaroplast. H -spore of *Tuzetia ecdyonuri* enclosed in an individual sporophorous vesicle (arrow). I - spore of *Jirovecia involuta* enclosed in an exospore-derived sac (arrowhead). Abbreviations: D - diplokaryon, E - endospore, EX - exospore, F - polar filament, M - meront, N - nucleus, P - plasma membrane, PA - anterior region of the polaroplast, PP - posterior region of the polaroplast, S - sporont. Scale bars - A, E-H - 0.5 μm; B, I - 1 μm; C, D - 100 nm



trathin sections than in light microscopic preparations (Fig. 3 A). In ultrathin sections merogonic stages have a plasma membrane without external reinforcements, while the plasma membrane of sporogonic stages is covered with uniform or layered electron-dense material (the primordium of the exospore layer of the spore wall, Fig. 6 A).

Nuclei. It is also easier to verify if nuclei in different developmental stages occur isolated or coupled as diplokarya when seen in ultrathin sections (Figs. 2 A; 6 B, D, G). It is necessary to be observant that sections through diplokarya not necessarily must show both nuclear components.

Spore wall. The spore wall is three-layered (if the internal plasma membrane is acknowledged as a layer belonging to the spore wall, Figs. 2 A, 6 D). The median layer, the chitinous endospore, has no particular structure and in ultrathin sections it looks like an empty space. The external layer, the exospore, has for each genus a characteristic construction, and some genera have exospore layers of unique construction. The simplest exospore is a uniform electron-dense layer. The greater part of the genera, for which the ultrastructure is known, has an exospore composed of layered material. The exospore is sometimes ornamented with projections visible only using electron microscopy, like *Trichotuzetia*. If the sporophorus vesicle contains fibrillar or tubular material, this material normally traverses the vesicle from exospore to envelope (like in *Tuzetia* (Fig. 6 H) and *Janacekia*).

Polar filament. The construction of the polar filament (isofilar, anisofilar, manubrium-like) is best evaluated in longitudinal ultrathin sections of spores (Figs. 2 A, 6 B, G, H). Even if the fixation and embedding have not been successful, the filament usually retains shape and arrangement.

Polaroplast. The construction of the polaroplast can only be evaluated using electron microscopy. This system of compartments can be organized in a more or less complex way. Microsporidia of *Chytridiopsis*- and *Metchnikovella*-types lack a polaroplast of traditional type. A honeycomb-like layer on the surface of the polar filament, like in *Chytridiopsis* (Fig. 6 C) and *Nolleria*, or projecting membrane-folds, like in *Buxtehudea*, might represent polaroplasts of unusual construction.

Most microsporidia have a polaroplast where the anterior region is composed of densely packed lamellae, the posterior region of wide and less regularly arranged lamellae (Fig. 2 A). Alternatively the compartment of the posterior part can be characterized as chambers or tubules. The genus *Episeptum* has a polaroplast where the posterior lamellae are the most densely packed (Fig. 6 G). In a few genera, like *Agglomerata*, three distinct regions of the polaroplast can be discriminated. In some genera, like *Ordospora*, the polaroplast has uniform lamellar construction, or, like in the lanceolate spore morph of polymorphic microsporidia of mosquitoes, the voluminous polaroplast consists of a system of wide, globular chambers.

When evaluating membraneous organelles, like the polaroplast, it is necessary to be observant on the plane of sectioning, and to evaluate critically how successful the fixation and embedding procedures have been. The polaroplast is easily damaged during the handling, and some of the aberrant polaroplasts described are clearly deformed by the techniques used. It is further necessary to have perfect longitudinal sections taken through the centre of the spore to see the construction of the polaroplast. The polaroplast is the last organelle of the spore to develop. If the spore wall lacks a distinct endospore layer it must be suspected that the spore is still immature (Fig. 2 E). In that case the polaroplast has not reached the mature construction.

Sporophoros vesicle. The presence of a sporophorous vesicle is obvious under the light microscope, but the nature of the vesicle is only seen in ultrathin sections. Normally, like in the *Thelohania*-like microsporidia, the sporophorous vesicle is formed by the sporont at the onset of sporogony (sporontogenetic sporophorous vesicle). In a few genera, like *Pleistophora*, *Vavraia* and *Trachipleistophora*, the vesicle is produced already during the merogony (merontogenetic sporophorous vesicle). A merontogenetic vesicle encloses the complete number of daughter cells originating from the meront, not only the daughter cells of the sporont (like in the sporontogenetic vesicle, Fig. 6. B). Individual sporophorous vesicles are only visible using electron microscopy (Fig. 6 H).

Chytridiopsis and related genera, and *Metchnikovella* and related genera, generate their sporophorous vesicles in an unusual way. The original cell wall of the sporont remains as the internal component of the envelope, and the daughter cells receive a new plasma membrane from vacuoles originating in the cytoplasm of the sporont.

Electron microscopy is necessary for the interpretation of the construction of the envelope. Most sporontogenetic sporophorous vesicles have thin uniform envelopes (Fig. 6 B). In the genera *Telomyxa* and *Cryptosporina*

the envelope is thick and layered. The envelope of *Cryptosporina* has a construction identical to the exospore (Fig. 6 F). Merontogenetic sporophorous vesicles (*Pleistophora*, *Vavraia*, *Trachipleistophora*) are thick-walled with a unique construction for each genus.

In the genera *Bacillidium*, *Jirovecia* (Fig. 6 I), *Alfvenia* and *Nelliemelba*, the surface layer of the exospore might be released to form a sac resembling an individual sporophorous vesicle. In *Alfvenia* and *Nelliemelba* this extra sheath is formed inside a normal sporontogenetic sporophorous vesicle.

Electron microscopy is also necessary for revealing the construction of the inclusions of the sporophorous vesicle (Figs. 6 B, F). Inclusions include crystals of various types, fibril bundles and various kinds of tubular structures.

Hosts

Microsporidia and hosts have co-evolved to such extent that many species are specific to a certain host species, and several genera appear restricted to a particular group of hosts. There are two distinct borders for microsporidia: between invertebrate-vertebrate hosts, and between poikilothermous-homoiothermous vertebrates. Each of these groups (invertebrates, poikilothermous vertebrates and homoiothermous vertebrates) hosts its own selection of microsporidian genera. A number of genera, like *Nosema, Glugea, Pleistophora,* and *Encephalitozoon*, have been reported from a great variety of hosts, both from invertebrates and vertebrates. All these genera are old, and when they were established only a small number of characters were used. They are further rich in species. As presently used these genera are polyphyletic, assemblages of not related species. Successively, with better knowledge, species are removed from them to become types species of new genera. In a number of cases, the identification of these and of some other genera has not been proven.

All microsporidia with complex polymorphic life cycles, using an alternate host, are exclusively parasites of invertebrates. Proven cycles alternate between mosquitoes and copepods (Becnel and Andreadis 1999). Life cycles with an invertebrate definite host and one or more vertebrate alternate hosts, like exhibited by many coccidia, have not been revealed from microsporidia.

The effect of the microsporidium on the host is useful for identification (particularly when dealing with species). Histological sections are normally necessary for evaluation of the pathogenic effects, and these characters are avoided herein. Especially the microsporidia of fish generate prominent tumor-like cytological anomalies, called xenomas, which are visible to the naked eye.

Techniques for identification

Techniques for making light- and electron-microscopic preparations, and biochemical and molecular biological methods, including specific staining reactions, have been described in several publications (Vávra and Maddox 1976, Hazard *et al.* 1981, Undeen 1997, Undeen and Vávra 1997, Weber *et al.* 1999, Vávra and Larsson 1999) and there is no need to recapitulate them herein. When making light microscopic preparations it is important not to destroy groups of spores or dividing stages. To make squash preparations is more rewarding than to make bacteriological smears (Larsson 1988b). Living spores are best studied on a thin layer of agar. Methanol or formaldehyde-based fixatives are suitable for light microscopic preparations. Fixation of spores causes up to 25% shrinkage. Routinely used histological stains, like Giemsa solution or hematoxylin, are usually sufficient for revealing nuclear configurations. Mallory´s trichrome and Feulgen techniques are less useful. To reveal the number of nuclei in spores it is usually necessary to hydrolyze using hydrochloric acid prior to staining. For electron microscopy routine fixations using glutaraldehyde and osmium tetroxide are suitable, but for spores it is normally necessary to prolong fixation times. During embedding it is necessary to allow the resin sufficient time to penetrate spores.

Electron microscopy can also be performed on formalin-fixed material and specimens embedded for paraffin sectioning (Larsson 1983b, 1989a). Membranes are partially destroyed but the general organization, including the construction of the polar filament, is visible.

IDENTIFICATION OF MICROSPORIDIAN GENERA

The characteristics of the microsporidian genera have been summarized in 2 diagnostic tables (Table 1 - covering all genera, Table 2 - restricted to polymorphic microsporidia of mosquitoes) and in an alphabetical survey of the genera.

The following genera, which have been established for organisms claimed to be microsporidia, have been omitted for the following reasons:

***Bertramia* Caullery and Mesnil, 1897 (b).** An investigation by Badalamente and Vernick (1973) claimed that *B. asperospora* was a microsporidium. Obviously a mixed

Table. 1. Discrimination of microsporidian genera

Table is an arrangement of genera according to characters useful for identification. If the different spore morphs of the polymorphic microsporidia appear in different host individuals, the genus appears in more than one numbered position in the table. The table is divided into 7 sections:
Spores of two or three morphologically different types simultaneously present - Section **A**
Spores of a single type (micro- and macrospores, and early spores disregarded) - Sections **B**-**G**
Spores cylindrical (rod-shaped), without projections visible using light microscopy (LM) - Section **B**
Spores spherical, without projections visible using LM - Section **C**
Spores pyriform, without projections visible using LM - Section **D**
Spores lanceolate, without projections visible using LM - Section **E**
Spores ovoid, without projections visible using LM - Section **F**
Spores of other shapes or with projections visible using LM - Section **G**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Section A: spores of two or three types simultaneously present														
VE	+	n(8)	OS	1	1	1	B	n	a	msv	n+w	o	**[1]**	*Trachipleistophora*
VE	+	2	O	1	1	1	B	2	i	ssv	l	os		*Trachipleistophora*
VE	abs.	abs.	abs.	1	1	1	?	n	i	abs.	n+w	o	**[2]**	*Spraguea*
VE	abs.	abs.	abs.	2	2	2	B	2	i	abs.	n+w	oc		*Spraguea*
IN	+	<8	S	1	2	2	R	<8	i?	ssv	l	b	**[3]**	*Edhazardia*
IN	+	1-2	O?	1	2	2	B	2	i	ssv	c	l		*Edhazardia*
IN	+	<8	S?	1	2	2	R	<8	a	ssv	n+w	b	**[4]**	*Culicosporella*
IN	+	1-8	S?	2	2	2	R	1-8	i	ssv	c	l		*Culicosporella*
IN	+	8	S	1	2	2	R	8	a	ssv	n+w	o	**[5]**	*Intrapredatorus*
IN	+	2	IR	1	2	2	B	2	i	ssv	c	l		*Intrapredatorus*
IN	+	8	F	1	2	2	P?	8	i	ssv	l?	op	**[6]**	*Heterovesicula*
IN	+	n	S	2	2	2	B	2	i	ssv	l?	oc		*Heterovesicula*
IN	+	8	S	1	2	2/1	R	8	i	ssv	l	p	**[7]**	*Krishtalia*
IN	+	n?	S?	2	2	2/1	R	n?	a	ssv	l	o		*Krishtalia*
IN	+	n	S	1	2	2	R	8-32	a	ssv	c	l	**[8]**	*Simuliospora*
IN	+	?	?	?	2	2	?	?	i	ssv	?	?		*Simuliospora*
IN	+	2-2	S	1	1	1	B	4	a	ssv	l	o	**[9]**	*Gurleyides*
IN	+	1	I	1	1	1	?	n	i	ssv	w+n	o		*Gurleyides*
IN	+	2	S	1	?	?	B	2?	?	ssv?	?	o	**[10]**	*Evlachovaia*
IN	abs.	abs.	abs.	2	2?	2?	?	?	?	abs.	?	o		*Evlachovaia*
IN	+	2,4	S	2	2	2	R?	2,4	i	ssv	l?	op	**[11]**	*Tabanispora*
IN	abs.	abs.	abs.	1	2	2	B	2	i	abs.	l?	op		*Tabanispora*
IN	+	4	O	1	?	?	R	4	a	ssv	n+w?	o	**[12]**	*Stempellia*
IN	abs.	abs.	abs.	1	?	?	R	8	a?	abs.	l?	p		*Stempellia*
IN	+	8	S	1	2	2	R	8	a	ssv	n+w	b	**[13]**	*Amblyospora*
IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	n+w	o		*Amblyospora*
IN	+	8	S	1	2	2	R	8	i	ssv	?	o	**[14]**	*Burenella*
IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	?	oc		*Burenella*
IN	+	8	S	1	2	2	R	8	i	ssv	n+w?	o	**[15]**	*Vairimorpha*
IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	n+w?	oc		*Vairimorpha*
IN	+	?	S	?	?	?	?	?	?	ssv?	?	p	**[16]**	*Auraspora*

Table 1 (contd)

IN	abs.	abs.	abs.	2	2?	2?	?	4?	i	abs.	l	p		*Auraspora*
IN	abs.	abs.	abs.	1	1	2	R	2-16	i	abs.	c	p	**[17]**	*Hazardia*
IN	abs.	abs.	abs.	2	2	2	B	2	a	abs.	l?	l		*Hazardia*
IN	abs.?	abs.?	abs.?	1	?	?	?	4-16	?	abs.?	n+w?	o	**[18]**	*Parastempellia*
IN	?	?	?	2	?	?	B?	2?	?	?	?	?		*Parastempellia*
Section B: spores cylindrical (rod-shaped) without projections														
IN	+	4	C?	2	2	2	R	4	i	ssv	l+s		**[19]**	*Scipionospora*
IN	+	8	C	1	2	2	PR	8	ir	ssv	n+w		**[20]**	*Cylindrospora*
IN	+	8	O	1	2	2	P?	8	a?	ssv	n+w		**[21]**	*Ormieresia*
IN	+	8	O	1	2	2	R	8	ir	ssv	n+w		**[22]**	*Helmichia*
IN	+	8	S	1	2	2	R	8	a	ssv	n+w		**[23]**	*Resiomeria*
IN	+	8	S?	1	2	2	?	8	ir	ssv	s		**[24]**	*Striatospora*
IN	+	8	S	2	2	2?	R	8	i	ssv	l		**[25]**	*Octosporea*
IN	+	n	S	1	1	1	R	16-32	i	ssv	n+w		**[26]**	*Tardivesicula*
IN	+	n	I	1	1	1	P	4-16	i	ssv	l		**[27]**	*Cystosporogenes*
IN	+	8n	O	1	2	2	RB	8	i	ssv	l+s		**[28]**	*Pernicivesicula*
IN	+	n	I	2	2	2	P	16-32	i	ssv	n+w+t		**[29]**	*Binucleospora*
IN	abs.	abs.	abs.	1	1	1	B	2/4	ir	abs.	l		**[30]**	*Baculea*
IN	abs.	abs.	abs.	1	1	1	B	2	i	abs.	n+w		**[31]**	*Canningia*
IN	abs.	abs.	abs.	1	1	1	B	2	i	abs.	l		**[32]**	*Nadelspora*
IN	abs.	abs.	abs.	2	2	2	B	2	m	abs.	l+t		**[33]**	*Rectispora*
IN	abs.	abs.	abs.	2	2?	2?	B	2	?	abs.	?		**[34]**	*Cristulospora*
IN	abs.	abs.	abs.	2	2	2	B	2	m	abs.	n+w		**[35]**	*Bacillidium*
IN	abs.	abs.	abs.	2	2	2	B	2	m	abs.	n+w		**[36]**	*Mrazekia*
PR	+	32	F	1	1	?	P	32	ml	sw?	?		**[37]**	*Desportesia*
PR	abs.	abs.	abs.	1	1	1?	B	2	i	abs.	l		**[38]**	*Ciliatosporidium*
Section C: spherical without projections														
IN	+	8	S	1	2	2	R	8	i	ssv	l+s		**[39]**	*Coccospora*
IN	+	8	S	1	2	2	P	8	i	ssv	l		**[40]**	*Pilosporella*
IN	+	n	S	1	1	?	PV?	n	i	sw?	abs.		**[41]**	*Burkea*
IN	±	?	?	1	1	?	PV	n	it	sw	abs.		**[42]**	*Buxtehudea*
IN	±	n	S	1	1	abs.?	P	n	it	sw	abs.		**[43]**	*Chytridiopsis*
IN	?	?	?	1?	1	1?	P	n	?	?	?		**[44]**	*Jiroveciana*
IN	+	n	S	1	1	abs.	V	n	it	sw?	abs.		**[45]**	*Nolleria*
IN	+	n	S	1	1	abs.?	V	n	at	sw	abs.		**[46]**	*Intexta*
IN	+	n	S	1	2	2	R?	8-32	i	ssv	?		**[47]**	*Spherospora*
IN	+	n	S	2?	2?	2?	P?	n	?	sw?	?		**[48]**	*Chytridioides*
IN	+	n?	S	1/2	2	2	P	n	i?	msv?	?		**[49]**	*Hessea*
PR	±	n	F	1	1	abs.?	P	n	m	sw?	?		**[50]**	*Amphiacantha*
PR	±	n	F	1	1	abs.?	PB	n	ml	sw?	?		**[51]**	*Amphiamblys*
PR	±	n	F	1	1	abs.?	P?	n	ml	sw?	?		**[52]**	*Metchnikovella*

Table 1 (contd)

Section D: spores pyriform without projections													
VE	+	8/16	S	1	1	2?	P?	8/≥16	i	msv	n+w?	**[53]**	*Heterosporis*
VE	abs.	abs.	abs.	1	1	1	R	4	m	abs.	l	op **[54]**	*Microfilum*
VE	abs.	abs.	abs.	1	1	1/2	RP	n	i	abs.	l	**[55]**	*Microgemma*
VE	abs.	abs.	abs.	1	1	2/1	P	n	a	abs.	n+w	**[56]**	*Neonosemoides*
IN	+	2	?	2	2?	2?	B	2	?	ssv	?	**[57]**	*Issia*
IN	+	2	O	1	?	?	B	2	?	ssv	l	op **[58]**	*Holobispora*
IN	+	2+2	O	1	1	1	B?	4	a	ssv	l	op **[59]**	*Norlevinea*
IN	+	4	S	1	1	1	R	4	a	ssv	w+n	**[60]**	*Episeptum*
IN	+	4	O	1	1	1	R	4	a	ssv	l+s	**[61]**	*Gurleya*
IN	+	8	F	1	2	2	R	8	a	ssv	l?	**[62]**	*Chapmanium*
IN	+	8	F	1	2	2	R	8	a	ssv	l+t	**[63]**	*Napamichum*
IN	+	8	S	1	2	2	R	8	a	ssv	n+w	**[64]**	*Hyalinocysta*
IN	+	8	SO	1	2	2	P	8	a	ssv	l	**[65]**	*Agmasoma*
IN	+	8	S	1	2	2	R	8	a	ssv	n+w	**[66]**	*Systenostrema*
IN	+	8	S	1	2	2	R	8	i	ssv	n+w	**[67]**	*Thelohania*
IN	+	8	O	2	2	2?	R?	8	i	ssv	l?	**[68]**	*Cryptosporina*
IN	+	8/16	S	1	2	2	R	8/16	i	ssv	c	ap **[69]**	*Bohuslavia*
IN	+	16/32	SP	1	2	?	R	16/32	i	ssv	n+w?	**[70]**	*Trichoduboscqia*
IN	+	n(16)	S	1	1	1	R	n(16)	a	ssv	w+n+t	**[71]**	*Agglomerata*
IN	+	n	L	1	1	1	R	n	i	ssv	n+w	**[72]**	*Flabelliforma*
IN	+	n	S	1	1	1/2	R	4-32	i	ssv	w+n	**[73]**	*Larssonia*
IN	+	n	S	1	1	1	P?	48,64	?	ssv?	?	**[74]**	*Parapleistophora*
IN	+	n	TP	?	?	?	?	n	?	ssv?	?	**[75]**	*Mitoplistophora*
IN	+	1	I	1	1	1	R	n	a	ssv	n+w?	**[76]**	*Nelliemelba*
IN	+	1	I	1	1	1	R	n	i	ssv	w+n+t	op **[77]**	*Lanatospora*
IN	+	1	I	1	1	1	R	n	i	ssv	n+w	**[78]**	*Tuzetia*
IN	+	1	I	1	1	1	R	n	i	ssv	n+w	**[79]**	*Trichotuzetia*
IN	abs.	abs.	abs.	1	1	1	P	8	i	abs.	l	**[80]**	*Ordospora*
IN	abs.	abs.	abs.	1	?	?	R	n	i?	abs.	l?	**[81]**	*Pleistosporidium*
IN	abs.	abs.	abs.	2?	2?	?	?	?	i	abs.	l?	**[82]**	*Semenovaia*
IN	abs.	abs.	abs.	2	2	2	R	n	i	abs.	l	**[83]**	*Parathelohania*
Section E: spores lanceolate without projections													
IN	+	4	O	1	1?	1?	R	4	i?	ssv?	c?	**[84]**	*Pyrotheca*
IN	+	8	O	1	1	1?	R	8	a	ssv	c	**[85]**	*Aedispora*
IN	+	n	O	1	2	2	R	2->8	i	ssv	c	**[86]**	*Culicospora*
IN	+	1	I	1	1	1	R	4	i	ssv	c	**[87]**	*Amblyospora*
IN	?	?	?	1	?	?	R	8	?	?	c?	**[88]**	*Parathelohania*
Section F: spores ovoid without projections													
VE	+	n	O	1	1	1	P	n?	i	ssv	n+w?	**[89]**	*Loma*
VE	+	n/8	S	1	1	1	PB	n/8	i	ssv	n+w	**[90]**	*Glugea*
VE	+	n	S	1	1	1	P	n	i	msv	n+w	op **[91]**	*Pleistophora*
VE	abs.	abs.	abs.	1	1	1	B	2	i	abs.	n+w?	**[92]**	*Encephalitozoon*

Table 1 (contd)

VE	abs.	abs.	abs.	1	1	1	R	n?	?	abs.	l+t?		**[93]**	*Alloglugea*
VE	abs.	abs.	abs.	1	1	1	R	4	i	abs.	n+w?	op	**[94]**	*Tetramicra*
VE	abs.	abs.	abs.	1	2	2?	R	n?	i?	abs.	l		**[95]**	*Enterocytozoon*
VE	abs.	abs.	abs.	1	2	2?	R	n?	i?	abs.	l		**[96]**	*Nucleospora*
VE	abs.	abs.	abs.	2	2	2	B	2	a	abs.	l+t		**[97]**	*Brachiola*
VE	abs.	abs.	abs.	2	2	2?	B	2	i	abs.	n+w?		**[98]**	*Ichthyosporidium*
VE	abs.	abs.	abs.	2	2	2	B	4-8	a	abs.	l?		**[99]**	*Vittaforma*
IN(VE)	abs.	abs.	abs.	1	1	1	R	n	i	abs.	n+w?	oc	**[100]**	*Nosemoides*
IN	+	2	OU	1	1	1	B	2	i	ssv	n+w		**[101]**	*Telomyxa*
IN	+	2	O	1	1	1	B	2	i	ssv	n+w		**[102]**	*Abelspora*
IN	+	2	IA	1	1	1	B	2	i	ssv	n+w		**[103]**	*Berwaldia*
IN	±	2	S	1	2	2	B	2	i	ssv	n+w		**[104]**	*Neoperezia*
IN	+	(4)8	S	1	2	2	R	(4)8	i	ssv	l+l		**[105]**	*Octotetraspora*
IN	+	8	SA	1	2	2	R	8	i	ssv	n+w		**[106]**	*Pegmatheca*
IN	+	8	S	1	2	2	R	8	a	ssv	l+s		**[107]**	*Trichoctosporea*
IN	+	8	S	1	2	2	RB	8	a	ssv	n+w	ob	**[108]**	*Amblyospora*
IN	+	16	O	1	1	1	B	16	?	ssv	?		**[109]**	*Duboscqia*
IN	+	32	O	1	2	2	P	32	i	ssv	l	os	**[110]**	*Ovavesicula*
IN	+	8n	S	1	2	2	P	8	i	ssv	n+w		**[111]**	*Polydispyrenia*
IN	+	n	I	1	1	1	P	n	i	ssv	n+w		**[112]**	*Glugoides*
IN	+	n	S	1	1	1	PR	n	a	msv	n+w?		**[113]**	*Vavraia*
IN	+	n	O	1	1?	1?	R	n	i?	ssv	l?	?	**[114]**	*Kinorhynchospora*
IN	+	n	S	1	1	1	R	8-32	i	ssv?	n+w?		**[115]**	*Johenrea*
IN	+?	n?	O?	1	2	2	P?	n	ir	ssv?	l?		**[116]**	*Merocinta*
IN	+	8n	S	1	2	2	?	8-32	a	msv	l?		**[117]**	*Pulicispora*
IN	+	n	S	2	2	?	P	n	i	sw?	n+w?		**[118]**	*Pseudopleistophora*
IN	+	1	I	1	2	2	R	n	i	ssv	n+w		**[119]**	*Alfvenia*
IN	+	1	I	1	2	2	R	n	i	ssv	n+w	op	**[120]**	*Janacekia*
IN	abs.	abs.	abs.	1	1	1	PR?	n	i	abs.	l	oc	**[121]**	*Endoreticulatus*
IN	abs.	abs.	abs.	1	1	1	B?	2?	i	abs.	n+w?	oc	**[122]**	*Larssoniella*
IN	abs.	abs.	abs.	1	1	1	B?	2?	i?	abs.	n+w?	op	**[123]**	*Oligosporidium*
IN	abs.	abs.	abs.	1	1	1	B	2	i	abs.	n+w?	op	**[124]**	*Unikaryon*
IN	abs.	abs.	abs.	1	1	1	P	2-8	?	abs.	n+w?		**[125]**	*Orthosomella*
IN	abs.	abs.	abs.	1	2	2	R	8	i	abs.	n+w		**[126]**	*Nudispora*
IN	abs.	abs.	abs.	1	2	2	P	n	i	abs.	?		**[127]**	*Perezia*
IN	abs.	abs.	abs.	1	2	2	B	n	i	abs.	n+w?		**[128]**	*Ameson*
IN	abs.	abs.	abs.	2	2	1?	P?	n	i	abs.	n+w		**[129]**	*Wittmannia*
IN	abs.	abs.	abs.	2	2	2	B	2	a	abs.	l+t		**[130]**	*Anncaliia*
IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	n+w		**[131]**	*Nosema*
IN	abs.	abs.	abs.	2	2	2	PB	≤4	i	abs.	l		**[132]**	*Amblyospora*
IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	l+v		**[133]**	*Edhazardia*
IN	abs.	abs.	abs.	2	2	2	B?	2?	i?	abs.	l+v?		**[134]**	*Culicosporella*
IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	l		**[135]**	*Culicospora*
IN	abs.	abs.	abs.	2	2	2	B?	2?	?	abs.	?	oc	**[136]**	*Merocinta*

Table 1 (contd)

IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	l		**[137]**	*Pilosporella*
IN	abs.	abs.	abs.	2	2	2	R	8	i	abs.	n+w?		**[138]**	*Ringueletium*
IN	abs.	abs.	abs.	2	2	2	R	n	i	abs.	l?	oc	**[139]**	*Parathelohania*
IN	abs.	abs.	abs.	2	2	2	P?	n	i	abs.	l		**[140]**	*Steinhausia*
PR	abs.?	abs.?	abs.?	1	2?	?	?	6/8	i?	abs.?	n+w?		**[141]**	*Geusia*
PR	abs.	abs.	abs.	1	2	2	P	n	i	abs.	?		**[142]**	*Perezia*
Section G: spores of other shapes or with projections														
IN	+	4	O	1	1	1	R	4	i	ssv	c	z	**[143]**	*Cougourdella*
IN	+	8	S	1	2	2	P	8	i	ssv	n+w	h	**[144]**	*Toxoglugea*
IN	+	8	S	1	2	2	P	8	a?	ssv	l+c	h	**[145]**	*Toxospora*
IN	+	8	S	1	2	2	R	8	a	ssv	n+w?	ok	**[146]**	*Parathelohania*
IN	+	8	S	1	2	2	R	8	a	ssv	l	ox	**[147]**	*Tricornia*
IN	+	8	S	1	2	2	R	8	i	ssv	n+w	oy	**[148]**	*Inodosporus*
IN	+	8	O	1	2	2	R?	8	i	ssv	l	oy	**[149]**	*Ortothelohania*
IN	+	8	S	1	2	2	R	8	?	ssv	?	of	**[150]**	*Cristulospora*
IN	+?	n	?	1	?	?	?	n	?	ssv?	?	or	**[151]**	*Weiseria*
IN	+?	?	O?	2	2	2?	B	2(4)	?	ssv?	?	g	**[152]**	*Campanulospora*
IN	abs.	abs.	abs.	2	2	1/2	?	?	a?	abs.	?	ot	**[153]**	*Hrabyeia*
IN	abs.	abs.	abs.	2	2	2	B	2	i	abs.	l?	of	**[154]**	*Hirsutosporos*
IN	abs.	abs.	abs.	2	2	2	B	2	m	abs.	n+w	ct	**[155]**	*Jirovecia*
IN	abs.	abs.	abs.	2	2	2	R	8	i	abs.	c?	ot	**[156]**	*Caudospora*
IN	abs.	abs.	abs.	2	2?	2?	R	4-16	?	abs.	?	ok	**[157]**	*Golbergia*

Characters and abbreviations used:
1 - Host: **IN** - invertebrate, **PR** - protist, **VE** - vertebrate.
2 - Spores in distinct groups: **+** - character present, **±** - grouped and ungrouped spores occur together, **abs.** - character absent.
3 - Number of spores per group.
4 - Shape of spore groups: **A** – associated, **C** – cylindrical, **F** – fusiform, **I** – individual,
L – lobed, **O** – ovoid, **P** – projections, **S** – spherical, **T** – triangular.
5 - Number of nuclei in the spore: 1, 2 (diplokaryon).
6 - Number of nuclei in the sporont: 1, 2 (diplokaryon).
7 - Number of nuclei in the meront: 1, 2 (diplokaryon).
8 - Mode of division in the sporogony: **B** - binary fission, **P** - plasmotomy, **R** - rosette-like multiple budding, **V** - vacuolation (endogeneously by vacuole formation).
9 - Number of sporoblasts, **n** - indicates an irregular number.
10 - Construction of the polar filament: **a** - anisofilar, **i** - isofilar, **l** - posterior lamella-like prolongation, **m** - manubrium-like (wide and uncoiled), **r** - reduced, **t** - external cover of tubules or a honeycomb-like layer.
11 - Envelope of microsporidian origin around the spores: **msv** - merontogenetic sporophorous vesicle, **ssv** - sporontogenetic sporophorous vesicle, **sw** - sporophorous vesicle incorporating the plasma membrane (the cell wall) of the sporont, **abs.** - character not present.
12 - Construction of the polaroplast (1-3 subdivisions): **c** – chambers; **l** - lamellae (either uniform lamellar, or the exact arrangement of lamellae unknown); **n** - narrow (densely packed) lamellae; **s** – sacs; **w** - wide lamellae.
13 - Spore shape when aberrant from the general shape of the group: **a** - angular, **b** - barrel-shape, **c** - cylindrical, **f** - fibrous tufts, **g** - bell-shaped (campanuloform), **h** - horseshoe-shape, **k** - collar-like prolongation, **l** - lanceolate, **o** - ovoid, **p** - pyriform, **r** - ridges, **s** - spherical, **t** - tail-like prolongation, **x** - horn-like projections, **y** - filamentous projections, **z** - lagenform.
14 - Number in [ ] referred to in the alphabetical list.
15 - Genus name.
? - The character is unknown or the construction is postulated

infection of a microsporidium and *B. asperospora* was studied.

***Caulleryetta* Dogiel, 1922.** Established for a probably microsporidian parasite of gregarines. Differences to *Metchnikovella* not clear.

***Marssoniella* Lemmermann, 1900.** Komárek and Vávra (1968) proved convincingly that the single species *M. elegans* was a microsporidium. The distinction between *Marssoniella* and *Gurleya* is not clear.

***Microsporidyopsis* Schereschewsky, 1925.** Established for a probably microsporidian parasite of gregarines. Differences to *Metchnikovella* not clear.

***Myxocystis* Mrázek, 1898.** The genus was established for the new species *M. ciliata*. In a later publication *M. ciliata* was associated with microsporidian spores of *Jirovecia* type (Mrázek 1910). Structures similar to *M. ciliata* have been observed in Swedish oligochaetes, both in combination with *Jirovecia* infection and in the absence of microsporidia (Larsson, unpublished). It has never been proven that *Myxocystis ciliata* has any microsporidian affinity.

***Pseudothelohania* Codreanu and Codreanu-Bălcescu, 1982.** This genus was clearly intended for a microsporidium, but no species belonging in the genus was mentioned in the description. The genus is not valid.

***Spiroglugea* Léger & Hesse, 1924.** *S. octospora* is the only microsporidium with s-shaped spores and clearly a microsporidium. The spores of *Toxoglugea* vary in shape and s-shaped spores occur together with the normal horseshoe-shaped spores (Larsson 1980a). Until a new investigation proves that *Spiroglugea* is a distinct genus, *S. octospora* is better placed in *Toxoglugea.*

Genera

The genera are listed in alphabetical order, with comments about the identification and with references to publications useful for identification.

The following information is included, using these abbreviations: ? - means that the information is lacking or has been extrapolated. References to the numbered position(s) in Table 1, and to figures used in this review, follow the *auctor(is)* of the genus in square brackets [ ]. **TS** - type species. Unless the new genus was established together with the description of the type species, the original genus, *auctor* and year of description are indicated. **S** - estimated number of named species. **TH** - type host. **G** - geographic location (continent and country) of the type species. Continents are abbreviated: **AF** - Africa, **AN** - Antarctis, **AS** - Asia, **AU** - Australia, **EU** - Europe, **NA** - North America, **SA** - South America. **T** - principal tissue or organ affected. **D** - type of characters used in the description, **EM** - electron microscopic, **LM** - light microscopic, **M** - molecular. **C** - comments pertinent to identification, including selected references which in addition to the description are useful for identification. **HG** - host groups from which the genus has been reported. When the group appears in brackets ( ) identification of the genus has been questioned.

***Abelspora* Azevedo, 1987 [102]. TS** - *A. portucalensis*. **S** - 1. **TH** - *Carcinus maenas* (Crustacea: Decapoda). **G** - EU, Portugal. **T** - Hepatopancreas. **D** - EM. **C** - Additional information by Rocha and Monteiro (1992). The polar filament was characterized as anisofilar but micrographs in both papers show an isofilar filament. The coupled spores of *Berwaldia* have a more pointed anterior pole (Figs. 5 A, B), *Neoperezia* has diplokaryotic presporal stages. The coupled spores of *Telomyxa* (Figs. 5 C, D) are cemented together and inseparable in a squash preparation. **HG** - Crustacea.

***Aedispora* Kilochitskij, 1997 [85]. TS** - *A. dorsalis*. **S** - 1. **TH** - *Aedes caspius*. **G** - EU, Ukraine. **T** - Fat. **D** - LM and EM. **C** - Characteristic lanceolate, uninucleate 8-spores in sporophorous vesicles. **HG** - Diptera: Culicidae.

***Agglomerata* Larsson & Yan, 1988 [71, Fig. 5 L]. TS** - Originally described as *Duboscqia sidae* Jírovec, 1942. **S** - 2. **TH** - *Sida crystallina* (Crustacea: Cladocera). **G** - EU, Czech Republic. **T** - Body cavity. **D** - Basically EM. **C** - The dense polysporous groups of pyriform spores and the construction of the polaroplast diagnostic (the polaroplast of *Binucleospora* has narrow anterior lamellae). Differs from *Larssonia* by the absence of diplokarya. **HG** - Crustacea.

***Agmasoma* Hazard & Oldacre, 1975 [65]. TS** - Originally described as *Thelohania penaei* Sprague, 1950. **S** - 1. **TH** - *Penaeus setiferus* (Crustacea: Decapoda). **G** – NA, U.S.A. **T** - Gonads. **D** - Basically EM. **C** - Differs from other 8-sporoblastic genera of crustaceans by lacking rosette-like budding in sporogony, and developing in gonads, not musculature. **HG** - Crustacea.

***Alfvenia* Larsson, 1984 [119]. TS** - *A. nuda*. **S** - 2. **TH** - *Acanthocyclops vernalis* (Crustacea: Copepoda). **G** - EU, Sweden. **T** - Fat. **D** - LM and EM. **C** - Spore shape, polysporoblastic sporogony, and ultrastructural cytology distinguish from the copepod morphs of the polymorphic microsporidia of mosquitoes. In stained preparations lacking the visible inclusions of the episporontal space typical of *Janacekia* (Figs. 5 N, O). **HG** - Crustacea.

***Alloglugea* Paperna & Lainson, 1995 [93]. TS** - *A. bufonis*. **S** - 1. **TH** - *Bufo marinus* (Amphibia: Anura). **G** - SA, Brazil. **T** - Intestine. **D** - LM and EM. **C** - The distinctive characters of this new genus are difficult to trace because the authors have mixed up the two poles of the spore. The three, probably immature,

spores visible in Fig. 33 (in: Paperna and Lainson 1995) are longitudinally sectioned and show fairly well the typical organization of microsporidian spores. The structure called "polaroblast" (in Fig. 40) is obviously the polar filament coils, and the "whorls of the polar filament" is the polaroplast. The "sub-apical depression" is a commonly observed shrinkage in the vacuole region at the posterior pole of the spore. It is remarkable that the new genus, which was established for "an apansporoblastic microsporidian", was included in the family Glugeidae, containing microsporidia sporulating in sporophorous vesicles. **HG** - Amphibia.

Amblyospora **Hazard & Oldacre, 1975 [13, 87, 108, 132; Figs. 3 D; 4 D; 5 J; 6 B]. TH** - Originally described as *Thelohania californica* Kellen & Lipa, 1960. **S** - 60. **TH** - *Culex tarsalis* (Insecta: Diptera), intermediate host *Mesocyclops leukarti* (Crustacea: Copepoda). **G** - NA, U.S.A. **T** – Fat. **D** - LM and EM. **C** - The genus, as defined for the present, contains mono-, di- and trimorphic species. 8-sporous sporogony with barrel-shaped spores (when stained) and prominent crystalline inclusions in the sporophorous vesicle shared by other mosquito parasites (Table. 2). However, in the other genera the complete number of 8 spores is rarely found (Table. 2). **HG** - 8-sporous sporogony reported from Diptera, Trichoptera and Crustacea: Amphipoda. Intermediate lanceolate spores in Crustacea: Cladocera.

Ameson **Sprague, 1977 [128]. TS** - Originally described as *Nosema michaelis* Sprague, 1970. **S** - 4. **TH** - *Callinectes sapidus* (Crustacea: Decapoda). **G** – NA, U.S.A. **T** - Musculature. **D** - LM and EM. **C** - Ultrastructure described by Sprague *et al.* (1968). Fibrous exospore projections distinctive at the EM level. **HG** - Crustacea, (Diptera).

Amphiacantha **Caullery & Mesnil, 1914 [50, Fig. 5 T]. TS** - *A. longa*. **S** - 3. **TH** - *Lecudina elongata* (Apicomplexa: Gregarinida). **G** - EU, France. **T** - Cytoplasm. **D** - LM. **C** - The fusiform spore sacs with thread-like poles are diagnostic. Free and sac-bound spores occur together and all life cycle stages are diplokaryotic (Larsson, unpublished). **HG** - Gregarinida.

Amphiamblys **Caullery & Mesnil, 1914 [51]. TS** - Originally described as *Metchnikovella capitellides* Caullery & Mesnil, 1897 (a). **S** - 6. **TH** - *Ancora* sp. (Apicomplexa: Gregarinida). **G** - EU, France. **T** - Cytoplasm. **D** - LM. **C** - Ormières, Loubès and Maurand (1983) described the ultrastructure of *A. bhatiellae*. The fusiform spore-sacs with blunt poles are diagnostic. Free and sac-bound spores occur together (visible in micrographs of *A. bhatiellae*). **HG** - Gregarinida.

Anncaliia **Issi, Krylova & Nicolaeva, 1993 [130]. TS** - Originally described as *Nosema meligethi* Issi and Radishcheva, 1979. **S** - 2. **TH** - *Meligethes aeneus* (Insecta: Coleoptera). **G** - EU, Russia. **T** - ?. **C** - EM. **C** - Ultrastructural differences to *Nosema*. **HG** - Coleoptera.

Auraspora **Weiser & Purrini, 1980 [16]. TS** - *A. canningae*. **S** - 1. **TH** - *Lepidocyrtus lignorum* (Insecta: Collembola). **G** - EU, Germany. **T** - ?. **D** - LM and EM. **C** - Two sporulation sequences occur together, observed in all specimens collected, according to the authors difficult to unite. The description gives the impression that the characteristic episporal secretions occur on the free *Nosema*-like spores. However, the electron micrographs show the enveloped sporogony, where the episporal secretions are visible. **HG** - Collembola.

Bacillidium **Janda, 1928 [35, Fig. 4 P]. TS** - *B. criodrili*. **S** - 4. **TH** - *Criodrilus lacuum* (Annelida: Oligochaeta). **G** - EU, Czech Republic. **T** - ?. **D** - LM. **C** - The present evaluation of distinctive characters has been based on surviving material collected by Janda and newly collected material (Larsson 1994a). No 8-sporoblastic species [*Bacillidium* sensu Issi (1986)] are acknowledged in the genus. The surface layer of the exospore is stripped off in some of the species, including the type species, which can be mistaken for an individual sporophorous vesicle. The large rod-shaped, ungrouped spores without projections are diagnostic. **HG** - Oligochaeta.

Baculea **Loubès & Akbarieh, 1978 [30]. TS** - *B. daphniae*. **S** - 1. **TH** - *Daphnia pulex* (Crustacea: Cladocera). **G** - EU, France. **T** - Intestine. **D** - EM. **C** - *Bacillidium* spores are similar, but diplokaryotic. **HG** - Crustacea.

Berwaldia **Larsson, 1981 (b) [103; Figs. 5 A, B]. TS** - *B. singularis*. **S** - 2. **TH** - *Daphnia pulex* (Crustacea: Cladocera). **G** - EU, Sweden. **T** - Fat, hypodermis. **D** - LM and EM. **C** - Spores of *Abelspora* have more blunt anterior pole, and the presporal stages of *Neoperezia* are diplokaryotic. Characteristic folded sporophorous vesicle with a distinct internal layer of tubular material. **HG** - Crustacea.

Binucleospora **Bronnvall & Larsson, 1995 [29]. TS** - *B. elongata*. **S** - 1. **TH** - *Candona* sp. (Crustacea: Ostracoda). **G** - EU, Sweden. **T** - Fat, connective tissues. **D** - LM and EM. **C** - Nuclei surrounded by multiple membranes breaking the diplokaryotic configuration.

Polaroplast with 3 regions, but different from *Agglomerata* (which has narrow lamellae in the central part). **HG** - Crustacea.

Bohuslavia **Larsson, 1985 (b) [69; Figs. 4 G, H].** **TS** - Originally described as *Thelohania asterias* Weiser, 1963. **S** - 3. **TH** - *Endochironomus nynchoides* (Insecta: Diptera). **G** - EU, Czech Republic. **T** - Fat. **D** - LM and EM. **C** - Ultrastructure described by Larsson (1985b). The spore groups are distinctive. **HG** - Diptera.

Brachiola **Cali, Takvorian & Weiss, 1998 [in Cali *et al.* (1998)] [97].** **TS** - *B. vesicularum*. **S** - 1. **TH** - *Homo sapiens* (Mammalia: Primates). **G** – NA, U.S.A. **T** - Musculature. **D** - EM. **C** - Similar to *Nosema* (never proven from vertebrates). **HG** - Mammalia.

Burenella **Jouvenaz & Hazard, 1978 [14].** **TS** - *B. dimorpha*. **S** - 1. **TH** - *Solenopsis geminata* (Insecta: Hymenoptera). **G** – NA, U.S.A. **T** - Hypodermis (free sporogony) and fat (8-sporous sporogony). **D** - LM and EM. **C** - The two sporogonies occur simultaneously but in different tissues. Similar to *Heterovesicula* and *Vairimorpha*. **HG** - Coleoptera, Hymenoptera.

Burkea **Sprague, 1977 [41].** **TS** - Originally described as *Coccospora gatesi* Puytorac & Tourret, 1963. **S** - 2. **TH** - *Pheretima hawayana* (Annelida: Oligochaeta) selected by Sprague and Becnel (1999a). **G** – NA, Hawaii. **T** - Body cavity, xenoma. **D** - LM and EM. **C** - Sporogony yields free and sac-bound spores, like in *Chytridiopsis*. **HG** - Oligochaeta.

Buxtehudea **Larsson, 1980 (b) [42].** **TS** - *B. scaniae*. **S** - 1. **TH** - *Petrobius brevistylis* (Insecta: Microcoryphia). **G** - EU, Sweden. **T** - Intestine. **D** - LM and EM. **C** - Enveloped and free spores occurs together (Larsson, unpublished). Characteristic polar filament projections. **HG** - Microcoryphia.

Campanulospora **Issi, Radischcheva & Dolzhenko, 1983 [152].** **TS** - *C. denticulata*. **S** - 1. **TH** - *Delia floralis* (Insecta: Diptera). **G** - AS, Kazachstan. **T** - Fat. **D** - LM and EM. **C** - Characteristic spore shape. **HG** - Diptera.

Canningia **Weiser, Wegensteiner & Žižka, 1995 [31].** **TS** - *C. spinidentis*. **S** - 2. **TH** - *Pityokteines spinidens* (Insecta: Coleoptera). **G** - EU, Austria. **T** - Fat. **D** - LM and EM. **C** - The slightly rod-shaped spores distinguish from *Unikaryon* (oval spores). **HG** - Coleoptera.

Caudospora **Weiser, 1946 [156].** **TS** - *Caudospora simulii*. **S** - 7. **TH** - *Simulium hirtipes* (Insecta: Diptera). **G** - EU, Czech Republic. H: Fat. **D** - LM. **C** - Ultrastructure described by Doby *et al.* (1965) and Vávra (1968). The spore shape unique. **HG** - Diptera: Simuliidae.

Chapmanium **Hazard & Oldacre, 1975 [62].** **TS** - *C. cirritus*. **S** - 3. **TH** - *Corethrella brakeleyi* (Insecta: Diptera). **G** – NA, U.S.A. **T** - Fat. **D** - LM and EM. **C** - The fusiform 8-sporous sporophorus vesicles with pyriform spores similar to *Napamichum* (exospore different). **HG** - Diptera, Heteroptera, Crustacea.

Chytridioides **Trégouboff, 1913 [48].** **TS** - *C. schizophylli*. **S** - 1. **TH** - *Schizophyllum mediterraneum* (Myriapoda: Diplopoda). **G** - EU, France. **T** - Intestine. **D** - LM. **C** - Not associated with the host nucleus in contrast to *Chytridiopsis*. Spores spherical with two associated components, possibly a diplokaryon ("noyaux formés de 2 grains en diplocoque"), and a rod-like structure which might be a manubroid polar filament (takes stain intensively). **HG** - Myriapoda.

Chytridiopsis **Schneider, 1884 [43; Figs. 5 S, 6 C].** **TS** - *C. socius*. **S** - 10. **TH** - *Blaps mortisaga* (Insecta: Coleoptera). **G** - EU, France. **T** - Intestine. **D** - LM. **C** - Ultrastructure of other *Chytridiopsis* species described by Purrini and Weiser (1984, 1985) and Larsson (1993). Spores of two size classes, free and sac-bound. Characteristic association with nucleus of the host cell. **HG** - Annelida, Arachnida, Myriapoda, Coleoptera, Trichoptera.

Ciliatosporidium **Foissner & Foissner, 1995 [38].** **TS** - *C. platyophryae*. **S** - 1. **TH** - *Platyophrya terricola* (Ciliophora). **G** - NA, U.S.A. **T** - Cytoplasm. **D** - EM. **C** - Ultrastructure characteristic. **HG** - Ciliophora.

Coccospora **Kudo, 1925 [39, Fig. 4 C].** New name for *Cocconema* Léger & Hesse, 1921. **TS** - *C. micrococcus* Léger and Hesse, 1921 [selected by Kudo (1925)]. **S** - 1. **TH** - *Tanypus setiger* (Insecta: Diptera). **G** - EU, France. **T** - Fat. **D** - LM. **C** - Ultrastructure described by Bylén and Larsson (1994a). The rosette-like sporogonial budding different from *Pilosporella* (linear plasmodium). **HG** - Diptera.

Cougourdella **Hesse, 1935 [143; Figs. 4 L, M].** **TS** - *C. magna*. **S** - 5. **TH** - *Megacyclops viridis* (Crustacea: Copepoda). **G** - EU, France. **T** - Fat, haemocoele. **D** - LM. **C** - Ultrastructure of *C. trichopterae* described by Larsson (1989b). Spore shape distinctive. **HG** - Crustacea, Trichoptera.

Cristulospora **Khodzhaeva & Issi, 1989 [34, 150].** **TS** - *C. sherbani*. **S** - 3. **TH** - *Culex modestus* (Insecta: Diptera). **G** - AS, Uzbekistan. **T** - Fat (larvae), ovary (adult

females). **D** - LM. **C** - Dimorphic in adult females and larvae. Characteristic 8-spores with plum-like appendages on both poles. See Table. 2. **HG** - Diptera: Culicidae.

Cryptosporina **Hazard & Oldacre, 1975 [68, Fig. 6 F]. TS** - *C. brachyfila*. **S** - 1. **TH** - *Piona* sp. (Arachnida: Acari). **G** – NA, U.S.A. **T** - Fat. **D** - LM and EM. **C** - Re-evaluation of type material suggests that the species is diplokaryotic and that the envelope of the sporophorous vesicle is a structure identical to the exospore (Larsson, unpublished). The shape of the sporophorous vesicle together with the abundant crystalline inclusions is distinctive. **HG** - Arachnida.

Culicospora **Weiser, 1977 [86, 135]. TS** - Originally described as *Thelohania magna* Kudo, 1920. **S** - 2. **TH** - *Culex pipiens* (Insecta: Diptera). **G** – NA, U.S.A. **D** - LM and EM. **C** - Becnel *et al.* (1987) evaluated life cycle and described the ultrastructure. See Table. 2. Differences to *Simuliospora* unclear. **HG** - Diptera: Culicidae.

Culicosporella **Weiser, 1977 [4, 134]. TS** - Originally described as *Stempellia lunata* Hazard & Savage, 1970. **S** - 1. **TH** - *Culex pilosus* (Insecta: Diptera). **G** - NA U.S.A. **D** - LM and EM. **C** - Life cycle and ultrastructure described by Hazard *et al.* (1984) and Becnel and Fukuda (1991). See Table. 2. **HG** - Diptera: Culicidae.

Cylindrospora **Issi, 1986 [20, Fig. 4 J]. TS** - *C. chironomi*. **S** - 2. **TH** - *Chironomus plumosus* (Insecta: Diptera). **G** - EU, Russia. **T** - Fat ?. **D** - LM and EM. **C** - Micrographs in Issi (1986) show two different organisms (light micrograph of *C. chironomi* in Fig. 7, electron micrographs of a different microsporidium in Fig. 9). Larsson (1987) described a second species. The information of Table.1 is taken from this investigation. The slender spores in tight 8-sporous bundles are distinctive. **HG** - Diptera.

Cystosporogenes **Canning, Barker, Nicholas & Page, 1985 [27]. TS** - Originally described as *Plistophora operophterae* Canning, 1960. **S** - 3. **TH** - *Operophtera brumata* (Insecta: Lepidoptera). **G** - EU, U.K. **T** - Silk glands. **D** - EM. **C** - Cytology also described by Canning *et al.* (1983 a). Ultrastructure and spore shape different from *Tardivesicula* (slightly bent spores). **HG** - Diptera, Hymenoptera, Lepidoptera.

Desportesia **Issi & Voronin, 1986 [in Issi (1986)] [37]. TS** - Originally described as *Amphiamblys laubieri* Desportes & Théodoridès, 1979. **S** - 1. **TH** - *Lecudina* sp. (Apicompexa: Gregarinida). **G** - EU, Ireland. **T** - Cytoplasm. **D** - EM. **C** - Elongated spore sacs with rod-shaped spores distinctive. **HG** - Gregarinida.

Duboscqia **Pérez, 1908 [109]. TS** - *D. legeri*. **S** - 5. **TH** - *Reticulitermes lucifugus* (Insecta: Isoptera). **G** - EU, France. **T** - Fat?. **D** - LM. **C** - Additional information by Kudo (1942). Ultrastructure unknown. Oval sporophorous vesicles with 16 oval spores distinctive. **HG** - Crustacea, Diptera, Isoptera.

Edhazardia **Becnel, Sprague & Fukuda 1989 [3, 133]** in Becnel, Sprague, Fukuda and Hazard (1989). **TS** - Originally described *Nosema aedis* Kudo, 1930. **S** - 1. **TH** - *Aedes aegypti* (Insecta: Diptera). **G** – NA, Puerto Rico. **D** - LM and EM. **C** - Johnson *et al.* (1997) added more information about life cycle and ultrastructure. See Table. 2. **HG** - Diptera: Culicidae.

Encephalitozoon **Levaditi, Nicolau & Schoen, 1923 [92]. TS** - *E. cuniculi*. **S** - 11. **TH** - *Oryctolagus cuniculi* (Mammalia: Lagomorpha). **G** - EU, France. **T** - Brain. **D** - LM. **C** - Ultrastructure described by Pakes *et al.* (1975). Only genus from homoiothermic vertebrates with unenveloped oval spores (formed by binary fission) and isolated nuclei in sporonts and spores. **HG** - Reptilia, Aves, Mammalia, (Collembola).

Endoreticulatus **Brooks, Becnel & Kennedy, 1988 [121]. TS** - Originally described as *Plistophora fidelis* Hostounský & Weiser, 1975. **S** - 2. **TH** - *Leptinotarsa undecimlineta* (Insecta: Coleoptera). **G** – NA, Cuba. **T** - Intestine. **D** - LM and EM. **C** - Clear identification needs knowledge of the ultrastructure. **HG** - Coleoptera, Lepidoptera.

Enterocytozoon **Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisse & Modigliani, 1985 [95]. TS** - *E. bieneusi*. **S** - 1. **TH** - *Homo sapiens* (Mammalia: Primates). **G** - EU, France. **T** - Intestine. **D** - EM. **C** - Ultrastructure of sporogonial plasmodium diagnostic. **HG** - Mammalia.

Episeptum **Larsson, 1986 (a) [60; Figs. 4 F, 6 G]. TS** - *E. inversum*. **S** - 3. **TH** - *Holocentropus picicornis* (Insecta: Trichoptera). **G** - EU, Sweden. **T** - Fat. **D** - LM and EM. **C** - Using LM indistinguishable from *Gurleya*. The characteristically inverted polaroplast and the surface layer of the exospore discriminative. **HG** - Trichoptera.

Evlachovaia **Voronin & Issi, 1986 [in Issi (1986)] [10]. TS** - *E. chironomi*. **S** - 2. **TH** - *Chironomus plumosus* (Insecta: Diptera). **G** - ?. **T** - ?. **D** - LM. **C** - The characters mentioned in the description are not sufficient for clear identification. **HG** - Diptera.

Flabelliforma **Canning, Killick-Kendrick & Killick-Kendrick, 1991 (b) [72; Figs. 1A, 4E]. TS** - *F. montana*. **S** - 3. **TH** - *Phlebotomus ariasi* (Insecta: Diptera). **G** - EU, France. **T** - Intestine. **D** - LM and EM. **C** - The fan-like dividing sporogonial plasmodium yielding pyriform spores, with one curved side, are distinctive. **HG** - Crustacea, Diptera.

Geusia **Rühl & Korn, 1979 [141]. TS** - *G. gamocysti*. **S** - 1. **TH** - *Gamocystis ephemerae* (Apicomplexa: Gregarinida). **G** - EU, Germany. **T** - Cytoplasm. **D** - LM and EM. **C** - The new genus was established because the new species could not be placed in *Nosema*. No discriminative characters are mentioned or visible, and no definition of the new genus was given. **HG** - Gregarinida.

Glugea **Thélohan, 1891 [90; Figs. 1 B, 4 A]. TS** - Originally described as *Nosema anomala* Moniez, 1887 (b). **S** - 35. **TH** - *Gasterosteus aculeatus* (Pisces: Teleostei). **G** - EU, France. **T** - Connective tissues, xenoma. **D** - LM. **C** - The ultrastructure was described by Canning, Lom and Nicholas (1982). The genus can often be recognized on the characteristic encapsulated parasitized cells. Similar genera: *Loma* (xenomas less densely encapsulated), *Ichtyosporidium* (lacking sporophorous vesicles) and *Spraguea* (binucleate spores, lacking sporophorous vesicles). No species from invertebrates have been proven to belong to the genus. **HG** - (Myriapoda), (Trichoptera), Pisces.

Glugoides **Larsson, Ebert, Vávra & Voronin, 1996 [112]. TS** - Originally described as *Pleistophora intestinalis* Chatton, 1907. **S** - 1. **TH** - *Daphnia magna*, selected by Sprague and Becnel (1999 a) (Crustacea: Cladocera). **G** - EU, France. **T** - Intestine. **D** - LM and EM. **C** - Clear identification needs knowledge of the ultrastructure. **HG** - Crustacea.

Golbergia **Weiser, 1977 [157]. TS** - Originally described as *Weiseria spinosa* Golberg, 1971. **S** - 1. **TH** - *Culex pipiens* (Insecta: Diptera). **G** - EU, Russia. **T** - Fat and salivary glands. **D** - LM. **C** - Additional LM characterization by Issi (1979). Spore shape possibly distinctive. **HG** - Diptera: Culicidae.

Gurleya **Doflein, 1898 [61; Figs. 5 E, F]. TS** - *G. tetraspora*. **S** - 21. **TH** - *Daphnia maxima* (Crustacea: Cladocera). **G** - EU, Germany. **T** - Hypodermis. **D** - LM. **C** - Friedrich *et al.* (1996) described the ultrastructure of a new *Gurleya* species, *G. daphniae*, from the hypodermis of *D. pulex*. These data have been accepted as diagnostic for *Gurleya*. The genus has been used to collect 4-sporoblastic species with pyriform spores. It should probably be restricted to parasites of Crustacea. The genus *Episeptum*, containing insect parasites, has different ultrastructure. **HG** - Ciliophora, Crustacea, Diptera, Ephemeroptera, Isoptera, Odonata.

Gurleyides **Voronin, 1986 [9]. TS** - *G. biformis*. **S** - 1. **TH** - *Ceriodaphnia reticulata* (Crustacea: Cladocera). **G** - EU, Russia. **T** - Fat. **D** - LM and EM. **C** - Enveloped spores similar to *Norlevinea*, free spores ultrastructurally different from *Tuzetia*. **HG** - Crustacea.

Hazardia **Weiser, 1977 [17]. TS** - Originally described as *Stempellia milleri* Hazard & Fukuda, 1974. **S** - 2. **TH** - *Culex pipiens quinquefasciatus* (Insecta: Diptera). **G** – NA, U.S.A. **T** - Hemocytes and fat. **D** - LM and EM. **C** - Dimorphic sporogony similar to *Krishtalia*. See Table. 2. **HG** - Diptera: Culicidae.

Helmichia **Larsson, 1982 [22]. TS** - *H. aggregata*. **S** - 4. **TH** - *Endochironomus* sp. (Insecta: Diptera). **G** - EU, Sweden. **T** - Fat. **D** - LM and EM. **C** - At LM level indistinguishable from *Striatospora* (characteristic exospore). *Resiomeria* differs by spherical sporophorous vesicles and anisofilar polar filament. **HG** - Diptera.

Hessea **Ormières & Sprague, 1973 [49]. TS** - *H. squamosa*. **S** - 1. **TH** - *Sciara* sp. (Insecta: Diptera). **G** - EU, France. **T** - Intestine. **D** - LM and EM. **C** - Together with *Chytridioides* and *Amphiacantha* (distinctive spore-sacs) the only genus with spherical binucleate spores. Ultrastructure of spore-sac characteristic. Uninucleate and binucleate spores occurring together. **HG** - Diptera.

Heterosporis **Schubert, 1969 (a) [53]. TS** - *H. fincki*. **S** - 4. **TH** - *Pterophyllum scalare* (Pisces: Teleostei). **G** – EU, Germany. **T** - Intestine, xenoma. **D** - EM (Schubert 1969a, b). **C** - Additional ultrastructural study by Michel *et al.* (1989). 8-sporous sporogony (rarely 16 microspores) in merontogenetic sporophorous vesicle discriminates from *Pleistophora*. **HG** - Pisces.

Heterovesicula **Lange, Macvean, Henry & Streett, 1995 [6]. TS** - *H. cowani*. **S** - 1. **TH** - *Anabrus simplex* (Insecta: Orthoptera). **G** – NA, U.S.A. **T** - Fat. **D** - LM and EM. **C** - Bisporous sporogony in the initial stage, 8-sporous sporogony predominately at later stages. 8-groups of spores obviously in sporontogenetic sporophorous vesicles, origin of envelopes around spores produced by binary fission unclear. Differences to *Burenella* and *Vairimorpha* not clear. **HG** - Orthoptera.

Hirsutosporos **Batson, 1983 [154]. TS** - *H. austrosimulii*. **S** - 1. **TH** - *Austrosimulium* sp. (Insecta: Diptera). **G** - AU. **T** - Fat. **D** - LM and EM. **C** - Spore with characteristic filamentous projections. **HG** - Diptera: Simuliidae.

Holobispora **Voronin, 1986 [58]. TS** - *H. thermocyclopis*. **S** - 1. **TH** - *Thermocyclops othonoides*

(Crustacea: Copepoda). **G** - EU, Russia. **T** - Connective tissue. **D** - LM (spores) and EM. **C** - Illustrations show wide, probably tubular, inclusions of the episporontal space, which seem to differ from other genera with coupled spores. **HG** - Crustacea.

Hrabyeia **Lom & Dyková, 1990 [153]. TS** - *H. xerkophora*. **S** - 1. **TH** - *Nais christinae* (Annelida: Oligochaeta). **G** - EU, Czech Republic. **T** - Coelomocytes. **D** - LM and EM. **C** - Oval spore with wide tail discriminates from *Jirovecia*. **HG** - Oligochaeta.

Hyalinocysta **Hazard & Oldacre, 1975 [64]. TS** - *H. chapmani*. **S** - 2. **TH** - *Culiseta melanura* (Insecta: Diptera). **G** – NA, U.S.A: **T** - Fat. **D** - LM and EM. **C** - Sporoblasts formed by rosette-like budding in combination with sporophorous vesicles devoid of inclusions discriminative. **HG** - Diptera.

Ichthyosporidium **Caullery & Mesnil, 1905 [98]. TS** - Originally described as *Glugea gigantea* Thélohan, 1895. **S** - 3. **TH** - *Crenilabrus melops* (Pisces: Teleostei). **G** - EU, France. **T** - Connective tissues, xenoma. **D** - LM. **C** - Ultrastructural data by Sprague and Vernick (1968) and Casal and Azevedo (1995). Diplokaryotic ungrouped spores distinguish from other genera in fish. **HG** - Pisces.

Inodosporus **Overstreet & Weidner, 1974 [148]. TS** - *I. spraguei*. **S** - 1. **TH** - *Palaemonetes pugio* (Crustacea: Decapoda). **G** – NA, U.S.A. **T** - Musculature. **D** - EM. Differences to *Orthothelohania* unclear. Characteristic spore projections shared by both genera. **HG** - Crustacea.

Intexta **Larsson, Steiner & Bjørnson, 1997 (b) [46]. TS** - *I. acarivora*. **S** - 1. **TH** - *Tyrophagus putrescentiae* (Arachnida: Acari). **G** - EU, Netherlands. **T** - Intestine. **D** - EM. **C** - Different from *Chytridopsis* and *Nolleria* by the lacking association with host nuclei and the anisofilar polar filament. **HG** - Arachnida.

Intrapredatorus **Chen, Kuo & Wu, 1998 [5]. TS** - Originally described as *Amblyospora trinus* Becnel & Sweeney, 1990. **S** - 2. **TH** - *Culex halifaxi* (Insecta: Diptera). **G** - AU. **T** - Fat. **D** - LM and EM. **C** - Lanceolate spores and 8-spores occurring together like in *Culicosporella*. The number of nuclei in lanceolate spores distinctive. See Table. 2. **HG** - Diptera: Culicidae.

Issia **Weiser, 1977 [57]. TS** - Originally described as *Perezia trichopterae* Weiser, 1946. **S** - 2. **TH** - *Plectrocnemia geniculata* (Insecta: Trichoptera). **G** - EU, Czech Republic. **D** - LM. **D** - Only genus with diplokaryotic coupled spores. **HG** - Diptera, Trichoptera.

Janacekia **Larsson, 1984 [120; Figs. 4 R, 5 N-P]. TS** - Originally described as *Plistophora debaisieuxi* Jírovec, 1943. **S** - 6. **TH** - *Simulium* sp. **G** - EU, Czech Republic. **T** - Fat. **D** - LM and EM. **C** - Ultrastructure described by Loubès and Maurand (1976). Stained sporogonial plasmodia and sporoblasts with characteristic spots revealing the presence of individual sporophorous vesicles. Characteristic wide tubules traverse the episporontal space. **HG** - Diptera.

Jirovecia **Weiser, 1977 [155; Figs. 4 N-O, 6 I]. TS** - Originally described as *Mrazekia caudata* Léger & Hesse, 1916. **S** - 7. **TH** - *Tubifex tubifex* (Annelida: Oligochaeta). **G** - EU, France. **T** - ?. **D** - LM. **C** - Ultrastructure described by Larsson (1990b). Rod-shaped, diplokaryotic spores with slender tails diagnostic. The surface layer of the exospore can be stripped off and mistaken for an individual sporophorous vesicle. **HG** - Oligochaeta, Diptera (verified by Larsson and Götz 1996)

Jiroveciana **Larsson, 1980 (a) [44]. TS** - Originally described as *Chytridiopsis limnodrili* Jírovec, 1940. **S** - 1. **TH** - *Limnodrilus misionicus* (Annelida: Oligochaeta). **G** - SA, Argentina. **T** - Intestine. **D** - LM. **C** - Genus insufficiently known with no information on the mature spore. **HG** - Oligochaeta.

Johenrea **Lange, Becnel, Razafindratiana, Przybyszewski & Razafindrafara, 1996 [115]. TS** - *J. locustae*. **S** - 1. **TH** - *Locusta migratoria capito* (Insecta: Orthoptera). **G** - AF, Madagascar. **T** - Fat. **D** - LM and EM. **C** - Resembling *Tardivesicula* (more elongate spores, sporoblasts formed by rosette-like budding) and *Polydispyrenia* (presporal stages diplokaryotic). **HG** - Orthoptera.

Kinorhynchospora **Adrianov & Rybakov, 1991 [114]. TS** - *K. japonica*. **S** - 1. **TH** - *Kinorhynchus yushini* (Kinorhyncha). **G** - AS, Sea of Japan. **T** - Intestine. **D** - EM. **C** - Characteristic dimorphic sporogony yielding bean-shaped (free?) spores and ovoid spores in sporophorous vesicles. **HG** - Kinorhyncha.

Krishtalia **Kilochitskij, 1997 [7]. TS** - *K. pipiens*. **S** - 1. **TH** - *Culex pipiens*. **G** - EU, Ukraine. **T** - Fat. **D** - LM and EM. **C** - Difference to *Hazardia* uncertain. See Table. 2. **HG** - Diptera: Culicidae.

Lanatospora **Voronin, 1986 [77]. TS** - Originally described as *Thelohania macrocylopis* Voronin, 1977. **S** - 3. **TH** - *Macrocyclops albidus* (Crustacea: Copepoda). **G** - EU, Russia. **T** - Fat. **D** - LM and EM. **C** - The ultrastructure was described in greater detail by Voronin (1989). Characteristic inclusions of the episporontal space. **HG** - Crustacea.

Larssonia **Vidtman & Sokolova, 1994 [73]. TS** - Originally described as *Plistophora obtusa* Moniez, 1887 (a). **S** - 1. **TH** - *Daphnia pulex* (Crustacea: Cladocera).

G - EU, France. **T** - ? **D** - LM and EM. **C** - Differs from *Agglomerata* by the presence of diplokarya at the end of merogony. **HG** - Crustacea.

Larssoniella **Weiser & David, 1997 [122].** **TS** - *L. resinellae*. **S** - 1. **TH** - *Petrova resinella* (Lepidoptera, Tortricidae). **G** - EU, Czech Republic. **T** - Silk glands, fat. **D** - LM and EM. **C** - Immature spores with tufts of tubules diagnostic. **HG** - Lepidoptera.

Loma **Morrison & Sprague, 1981 [89].** **TS** - *L. morhua*. **S** - 8. **TH** - *Gadus morhua* (Pisces: Teleostei). **G** – NA, Canada. **T** - Gills, xenoma. **D** - LM and EM. **C** - Differs from *Pleistophora* by the sporontogenetic sporophorous vesicles, and from *Glugea* by the less densely encapsulated xenomas. **HG** - Pisces.

Merocinta **Pell & Canning, 1993 (a) [116, 136].** **TS** - *M. davidii*. **S** - 1. **TH** - *Mansonia africana* (Insecta: Diptera). **G** - AF, Tanzania. **T** - Intestine (larvae), ? (adults). **D** - LM and EM. **C** - Possibly a third sporogony in copepods (Pell and Canning 1993b). Differs from other genera of mosquito parasites by ribbon-like merogonial plasmodia in larvae and polysporoblastic (not 8-sporoblastic) sporophorous vesicles in larvae. Two kinds of binucleate spores in adults differing in size and possibly in shape. **HG** - Diptera: Culicidae, Crustacea?

Metchnikovella **Caullery & Mesnil, 1897 (a) [52].** **TS** - *M. spionis*. **S** - 17. **TH** - *Polyrhabdina brasili* (Apicomplexa: Gregarinida). **G** - EU, France. **T** - Cytoplasm. **D** - LM. **C** - The ultrastructure of other *Metchnikovella* species was described by Hildebrand and Vivier (1971), Vivier and Schrével (1973) and Hildebrand (1974). Can be distinguished by the free and enclosed (in almost ovoid sporophorous vesicles) almost spherical uninucleate spores occurring together. **HG** - Gregarinida.

Microfilum **Faye, Toguebaye & Bouix, 1991 [54].** **TS** - *M. lutjani*. **S** - 1. **TH** - *Lutjanus fulgens* (Pisces: Teleostei). **G** - AF, Senegal. **T** - Hemocytes. **D** - EM. **C** - Spore shape and the manubrium-like polar filament (visible using LM) distinctive. **HG** - Pisces.

Microgemma **Ralphs & Matthews, 1986 [55].** **TS** - *M. hepaticus*. **S** - 3. **TH** - *Chelon labrosus* (Pisces: Teleostei). **G** - EU, U.K. **T** - Liver. **D** - EM. **C** - Differs from other fish microsporidia with pyriform spores in the following way: *Microfilum* has spores of unique shape, and *Heterosporis* sporulates in sporophorous vesicles. **HG** - Pisces.

Mitoplistophora **Codreanu, 1966 [75].** **TS** - *M. angularis*. **S** - 1. **TH** - *Ephemera danica* (Insecta: Ephemeroptera). **G** - EU, Roumania. **D** - LM. **C** - The angular sporophorous vesicles with filamentous projections characteristic (vesicles of *Trichoduboscqia* are spherical). **HG** - Ephemeroptera.

Mrazekia **Léger & Hesse, 1916 [36; Figs. 4 Q, 6 E].** **TS** - *M. argoisi*. **S** - 2. **TH** - *Asellus aquaticus* (Crustacea: Isopoda). **G** - EU, France. **T** - Fat. **D** - LM. **C** - The present diagnosis is mainly based on the species *M. cyclopis* which has been studied using LM and EM (Larsson *et al.* 1993). The lightly curved spores with swollen posterior section of the manubrium-like polar filament, visible using LM, are distinctive. **HG** - Crustacea.

Nadelspora **Olson, Tiekotter & Reno, 1994 [32].** **TS** - *N. canceri*. **S** - 1. **TH** - *Cancer magister* (Crustacea: Decapoda). **G** – NA, U.S.A. **T** - Musculature. **D** - EM. **C** - The genus can be recognized on the extremely narrow, needle-shaped spores. Polar filament surrounded by a unique cover of not membrane-lined spherical granules. **HG** - Crustacea.

Napamichum **Larsson, 1990 (a) [63; Figs. 5 G, K, U].** **TS** - Originally described as *Chapmanium dispersus* Larsson, 1985 (a). **S** - 2. **TH** - *Endochironomus sp.* (Insecta: Diptera). **G** - EU, Sweden. **T** - Fat. **D** - LM and EM. **C** - The second species, *N. aequifilum*, has uniform polar filament, interpreted as a reduction (Larsson 1990 a). *Napamichum* and *Chapmanium*, which have similar fusiform sporophorous vesicles with pyriform spores, can be distinguished by the construction on the exospore. **HG** - Arachnida, Diptera.

Nelliemelba **Larsson, 1984 [76].** **TS** - Originally described as *Tuzetia boeckella* Milner and Mayer, 1982. **S** - 1. **TH** - *Boeckella triarticulata* (Crustacea: Copepoda). **G** - AU. **T** - Musculature. **D** - LM and EM. **C** - Differs from copepod morphs of mosquito microsporidia by the spore shape, the construction of the polaroplast, and the extra coat of exospore material, and from *Lanatospora*, *Tuzetia* and *Trichotuzetia* by the anisofilar polar filament. **HG** - Crustacea.

Neonosemoides **Faye, Toguebaye & Bouix, 1996 [56].** **TS** - Originally described as *Nosemoides tilapiae* Sakiti & Bouix, 1987. **S** - 1. **TH** - *Tilapia guineensis* (Pisces: Teleostei). **G** - AF, Benin. **T** - Stomach. **D** - EM. **C** - Differs from *Microfilum* by the different spore shape. **HG** - Pisces.

Neoperezia **Issi & Voronin, 1979 [104].** **TS** - *N. chironomi*. **S** - 1. **TH** - *Chironomus plumosus* (Insecta: Diptera). **G** - EU, Russia. **T** - Fat. **D** - LM. **C** - The ultrastructure was published by Issi *et al.* (1985). The microsporidium occurs in a spring form where the spores are united by a gelatinous material (no sporophorous

vesicle) and an autumn-winter form where the two spores are enclosed in a sporophorous vesicle. A characteristic internal membrane fold is present in the sporophorous vesicle. The sporont differs from *Berwaldia* and *Abelspora* by being diplokaryotic. **HG** - Diptera.

Nolleria **Beard, Butler & Becnel, 1990 [45]. TS** - *N. pulicis*. **S** - 1. **TH** - *Ctenocephalides felis* (Insecta: Siphonaptera). **G** – NA, U.S.A. **T** - Intestine. **D** - EM. **C** - Absence of thick-walled sporophorous vesicles, no particular association with the host nucleus, and ultrastructure characteristic. **HG** - Siphonaptera.

Norlevinea **Vávra, 1984 [59]. TS** - Originally described as *Glugea daphniae* Weiser, 1947. **S** - 2. **TH** - *Daphnia longispina* (Crustacea: Cladocera). **G** - EU, Czech Republic. **T** - Ovaries. **D** - LM and EM. **C** - Spores are characteristically cemented together pairwise, two spore doublets present in each sporophorous vesicle. The genus can only be mixed up with the dimorphic genus *Gurleyides* (having similar spore doublets). **HG** - Crustacea.

Nosema **Naegeli, 1857 [131, Fig. 3 C]. TS** - *N. bombycis*. **S** - 215. **TH** - *Bombyx mori* (Insecta: Lepidoptera). **G** - ?. **T** - ?. **D** - LM. **C** - The description is practically void of diagnostic characters. The ultrastructure of the mature spore was described by Sato *et al.* (1982). The genus has been used to collect species with oval diplokaryotic spores. Probably restricted to invertebrates. **HG** - Myxozoa, Digenea, Cestoda, Nematoda, Annelida, Arachnida, Crustacea, Myriapoda, Coleoptera, Collembola, Diptera, Ephemeroptera, Heteroptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mecoptera, Neuroptera, Odonata, Orthoptera, Siphonaptera, Zygentoma, Mollusca, Bryozoa, (Pisces).

Nosemoides **Vinckier, 1975 [100]. TS** - Originally described as *Nosema vivieri* Vinckier, Devauchelle & Prensier, 1970. **S** - 4. **TH** - *Lecudina linei* (Apicomplexa: Gregarinida). **G** - EU, France. **T** - Cytoplasm. **D** - EM (also in Vinckier *et al.* 1971). **C** - Not enveloped uninucleate macro- and microspores distinctive. **HG** - Gregarinida, (Pisces).

Nucleospora **Docker, Kent, Hervio, Khattra, Weiss, Cali & Devlin, 1996 [96]. TS** - Originally described as *Enterocytozoon salmonis* Chilmonczyk, Cox & Hedrick, 1991. **S** - 1. **TH** - *Oncorhynchus tshawytscha* (Pisces: Teleostei). **G** – NA, U.S.A. **T** - Hemapoietic cells. **D** - M. **C** - The ultrastructure was described by Chilmonczyk *et al.* (1991) and Desportes-Livage *et al.* (1996). Molecular and ultrastructural differences to *Enterocytozoon*. **HG** - Pisces.

Nudispora **Larsson, 1990 (c) [126]. TS** - *N. biformis*. **S** - 1. **TH** - *Coenagrion hastulatum* (Insecta: Odonata). **G** - EU, Sweden. **D** - LM and EM. **C** - Characteristic sporogony yielding 8, not enveloped, oval, uninucleate spores. **HG** - Odonata.

Octosporea **Flu, 1911 [25]. TS** - *O. muscaedomesticae*. **S** - 19. **TH** - *Musca domestica* (Insecta: Diptera). **G** - AS, Surinam. **T** - Intestine. **D** - LM. **C** - The ultrastructure was described by Ormières *et al.* (1976). The rod-shaped, 8-sporoblastic, binucleate spores in a sporophorous vesicle are diagnostic. **HG** - Crustacea, Collembola, Diptera, Ephemeroptera, Heteroptera, Lepidoptera.

Octotetraspora **Issi, Kadyzova, Pushkar, Khodzhaeva & Krylova 1991 [105]. TS** - *O. paradoxa*. **S** - 3. **TH** - *Wilhelmia mediterranea* (Insecta: Diptera). **G** - AS, Uzbekistan. **T** - Fat. **D** - LM (spore) and EM. **C** - 4-sporoblastic sporogony occurs together with more common 8-sporoblastic. Differences to other *Thelohania*-like microsporidia uncertain. **HG** - Diptera.

Oligosporidium **Coderanu-Bălcescu, Codreanu & Traciuc, 1981 [123]. TS** - *O. arachnicolum*. **S** - 1. **TH** - *Xysticus cambridgei* (Arachnida: Araneae). **G** - EU, Roumania. **T** - Musculature. **D** - EM. **C** - The genus cannot be distinguished from *Unikaryon*. **HG** - Arachnida.

Ordospora **Larsson, Ebert & Vávra, 1997 [80, Fig. 5 M]. TS** - *O. colligata*. **S** - 2. **TH** - *Daphnia magna* (Crustacea: Cladocera). **G** - EU, U.K. **T** - Intestine. **D** - LM and EM. **C** - Ovoid, uninucleate spores in linear configurations are diagnostic. **HG** - Crustacea.

Ormieresia **Vivarès, Bouix & Manier, 1977 [21]. TS** - *O. carcini*. **S** - 1. **TH** - *Carcinus mediterraneus* (Crustacea: Decapoda). **G** - EU, France. **T** - Musculature. **D** - EM, spores also LM. **C** - Spores similar to *Bacillidium* but differ by being uninucleate and collected in 8-sporous sporophorous vesicles. **HG** - Crustacea.

Orthosomella **Canning, Wigley & Barker, 1991 (a) [125].** New name for *Orthosoma* Canning, Wigley & Barker, 1983(a). **TS** - Described as *Nosema operophterae* Canning, 1960. **S** - 2. **TH** - *Operophtera brumata* (Insecta: Lepidoptera). **G** - EU, U.K. **T** - Silk glands, intestine. **D** - LM and EM. **C** - The polysporoblastic sporogony by plasmotomy distinguishes from *Nosema*, *Oligosporidium*, *Unikaryon* and *Larssoniella*. **HG** - Coleoptera, Lepidoptera.

Ortothelohania **Codreanu & Bălcescu-Codreanu, 1974 [149]. TS** - Established for a microsporidium identified as *Thelohania octospora* Henneguy, 1892 (in Henneguy and Thélohan 1892). **S** - 1. **TH** - *Palaemon serratus* (Crustacea: Decapoda). **T** - Musculature. **D** - LM

and EM (illustrated by Codreanu *et al.* 1974). **C** - Indistinguishable from *Inodosporus*. Vivarès' (1975) investigation on *T. octospora* claimed in a more convincing way to deal with Thélohan´s species, and it shows a microsporidium different from the species for which *Orthotelohania* was created. **HG** - Crustacea.

Ovavesicula **Andreadis & Hanula, 1987 [110].** **TS** - *O. popilliae*. **S** - 1. **TH** - *Popillia japonica* (Insecta: Coleoptera). **G** – NA, U.S.A. **T** - Malpighian tubules. **D** - LM and EM. **C** - Ovoid groups with 32 ovoid, uninucleate spores distinctive. **HG** - Coleoptera.

Parapleistophora **Issi, Kadyzova, Pushkar, Khodzhaeva & Krylova, 1991 [74].** **TS** - *P. ectospora*. **S** - 1. **TH** - *Tetisimulium desertorum* (Insecta: Diptera). **G** - AS, Uzbekistan. **T** - Fat. **D** - LM (and EM?). **C** - Characteristic ovoid sporophorous vesicles with 1-3 projections. **HG** - Diptera.

Parastempellia **Issi, Kadyzova, Pushkar, Khodzhaeva & Krylova, 1991 [18].** **TS** - *P. odagmiae*. **S** - 1. **TH** - *Odagmia ferganica*. **G** - AS, Uzbekistan. **T** - Fat. **D** - LM and EM. **C** - Dimorphic sporogony yielding uni- and binucleate spores. **HG** - Diptera.

Parathelohania **Codreanu, 1966 [83, 88, 139, 146].** **TS** - Established for *Thelohania legeri* Hesse, 1904. **S** - 16. **TH** - *Anopheles maculipennis* (Insecta: Diptera). **T** - ?. **D** - LM. **C** - Hazard and Oldacre (1975) revised the genus and described the cytology. The life cycle and cytology of *P. anophelis* was described by Avery and Undeen (1990) and Garcia *et al.* (1993). Three spore types have been described. Octospores have characteristic shape. **HG** - Crustacea, Diptera.

Pegmatheca **Hazard & Oldacre, 1975 [106; Figs. 5 Q, R].** **TS** - *P. simulii*. **S** - 2. **TH** - *Simulium tuberosum* (Insecta: Diptera). **G** – NA, U.S.A. **T** - Fat. **D** - LM and EM. **C** - Characteristic associated 8-sporous sporophorous vesicles. **HG** - Diptera, Trichoptera.

Perezia **Léger & Duboscq, 1909 [127, 142].** **TS** - *P. lankesteriae*. **S** - 7. **TH** - *Lankesteria ascidiae* (Apicomplexa: Gregarinida). **G** – EU, France. **T** - Cytoplasm. **D** - LM. **C** - Ultrastructure described by Ormières *et al.* (1977). Linear sporonts like *Orthosomella* (all stages with isolated nuclei). **HG** - Gregarinida, Coleoptera, Orthoptera.

Pernicivesicula **Bylén & Larsson, 1994 (b) [28].** **TS** - *P. gracilis*. **S** - 1. **TH** - *Pentaneurella* sp. (Insecta: Diptera). **G** - EU, Sweden. **T** - Fat. **D** - LM and EM. **C** - Characteristic sporogonial divisions with lined sporoblasts protruding from a rounded plasmodium. The combination of distinctly cylindrical spores and an irregular number of spores per sporophorous vesicle are distinctive. **HG** - Diptera.

Pilosporella **Hazard & Oldacre, 1975 [40, 137].** **TS** - *P. fishi*. **S** - 3. **TH** - *Wyeomyia vanduzeei* (Insecta: Diptera). **G** – NA, U.S.A. **T** - Fat. **D** - LM and EM. **C** - Hazard *et al.* (1986) revealed the dimorphic life cycle of *P. chapmani*, with 8-sporous sporogony in larvae, bisporous sporogony in adults. The spherical spores are similar to *Coccospora*, but the shape of the sporogonial plasmodia is different (*Pilosporella* linear, *Coccospora* rosette-like). **HG** - Diptera.

Pleistophora **Gurley, 1893 [91].** **TS** - *P. typicalis*. **S** - 88. **TH** - *Myxocephalus scorpius* (Pisces, Teleostei). **G** - EU, France. **T** - Musculature. **D** - LM. **C** - Ultrastructure described by Canning and Nicholas (1980) and Canning and Hazard (1982). In poikilothermous hosts. Species reported from invertebrates do not belong in this genus. The merontogenetic polysporous sporophorous vesicles are diagnostic. **HG** - (Myxozoa), (Digenea), (Cestoda), (Nematoda), (Arachnida), (Crustacea), (Blattodea), (Coleoptera), (Dermaptera), (Diptera), (Ephemeroptera), (Hymenoptera), (Isoptera), (Lepidoptera), (Neuroptera), (Odonata), (Phasmida), (Plecoptera), (Mollusca), Pisces, Amphibia, Reptilia, (Mammalia).

Pleistosporidium **Codreanu-Bălcescu & Codreanu, 1982 [81].** **TS** - Originally described as *Pleistophora hyperparasitica* Codreanu, 1967. **S** - 1. **TH** - *Enterocystis rhitrogenae* (Apicomplexa: Gregarinida). **G** - EU, Roumania. **T** - Cytoplasm. **D** - LM and EM. **C** - Ultrastructure was described by Codreanu-Bălcescu (1997). No distinct characteristics. **HG** - Gregarinida.

Polydispyrenia **Canning & Hazard, 1982 [111].** **TS** - Originally described as *Nosema simulii* Lutz & Splendore, 1908. **S** - 2. **TH** - *Simulium venustum* (Insecta: Diptera). **G** - SA, Brazil. **T** - Fat. **D** - EM. **C** - Ultrastructure described by Maurand and Loubès (1978). Characteristic inclusions of the episporontal space. **HG** - Diptera.

Pseudopleistophora **Sprague, 1977 [118].** **TS** - *P. szollosi*. **S** - 1. **TH** - *Armandia brevis* (Annelida: Polychaeta). **G** – NA, U.S.A. **T** - Eggs. **D** - EM (Szollosi 1971). **C** - Binucleate spores of uniform size in polysporous sporophorous vesicles distinctive. **HG** - Annelida.

Pulicispora **Vedmed, Krylova & Issi, 1991 [117].** **S** - 1. **TS** - *P. xenopsyllae*. **S** - 1. **TH** - *Xenopsylla skrjabini* (Insecta: Siphonaptera). **G** - EU, Ukraine.

T - Fat. **D** - LM (spores) and EM. **C** - Merontogenetic sporophorous vesicles together with diplokaryotic presporal stages distinctive. **HG** - Siphonaptera.

Pyrotheca **Hesse, 1935 [84]. TS** - Originally described as *Gurleya cyclopis* Leblanc, 1930. **S** - 5. **TH** - *Cyclops albidus* (Crustacea: Copepoda). **G** - EU, Belgium. **T** - ?. **D** - LM. **C** - Ultrastructure unknown. At LM level similar to the copepod morphs of *Amblyospora* and other polymorphic microsporidia of mosquitoes. It cannot be excluded that the *Pyrotheca* species of copepods belong to polymorphic life cycles of *Amblyospora* and related genera, while the parasites of Trichoptera belong in *Cougourdella*. **HG** - Crustacea, Trichoptera.

Rectispora **Larsson, 1990 (d) [33]. TS** - *R. reticulata*. **S** - 1. **TH** - *Pomatothrix hammoniensis* (Annelida: Oligochaeta). **G** - EU, Sweden. **T** - Male gonads. **D** - LM and EM. **C** - The surface layer of the exospore is released and can be mistaken for an individual sporophorous vesicle. *Bacillidium* species similar at LM level, but their spores appear more cylindrical. **HG** - Oligochaeta.

Resiomeria **Larsson, 1987 [23; Figs. 4 I, 5 I]. TS** - *R. odonatae*. **S** - 1. **TH** - *Aeshna grandis* (Insecta: Odonata). **G** - EU, Sweden. **T** - Fat. **D** - LM and EM. **C** - Using LM *Cylindrospora* (more slender spores), *Octosporea* (diplokaryotic spores), *Ormieresia* (manubrium-like polar filament) can be distinguished from *Resiomeria*. EM characters discriminate from *Helmichia* and *Striatospora*. **HG** - Odonata.

Ringueletium **Garcia, 1990 [138]. TS** - *R. pillosa*. **S** - 1. **TH** - *Gigantodax rufidulum*, (Insecta: Diptera). **G** - SA, Argentina. **T** - Fat. **D** - LM and EM. **C** - Characteristic tubular exospore projections visible using LM. **HG** - Diptera.

Scipionospora **Bylén & Larsson, 1996 [19]. TS** - Originally described as *Mrazekia tetraspora* Léger & Hesse, 1922. **S** - 1. **TH** - *Tanytarsus* sp. (Insecta: Diptera). **G** - EU, France. **T** - Fat. **D** - LM and EM. **C** - Ultrastructural characters taken from an investigation of newly collected material (Bylén and Larsson 1996). This is the only genus with rod-shaped spores in 4-sporous sporophorous vesicles. **HG** - Diptera.

Semenovaia **Voronin & Issi, 1986 [in Issi (1986)] [82]. TS** - *S. chironomi*. **S** - 1. **TH** - *Chironomus plumosus* (Insecta: Diptera). **G** - EU, Russia ?. **T** - ?. **D** - LM and EM. **C** - 16-sporous sporogony in sporophorous vesicles together with free binucleate spores distinctive. The described polaroplast of free spores unique. **HG** - Diptera.

Simuliospora **Khodzhaeva, Krylova & Issi, 1991 [in Issi *et al.* (1991)] [8]. TS** - *S. uzbekistanica*. **S** - 2. **TH** - *Tetisimulium alajense*. **G** - AS, Uzbekistan. **T** - Fat. **D** - LM and EM. **C** - Lanceolate uninucleate spores similar to *Culicospora* and *Intrapredatorus* (simultaneous 8-sporous sequence distinctive). **HG** - Diptera.

Spherospora **Garcia, 1991 [47]. TS** - *S. andinae*. **S** - 1. **TH** - *Gigantodax chilense* (Insecta: Diptera). **G** - SA, Argentina. **T** - Fat. **D** - LM and EM. **C** - Only genus with spherical spores in thin-walled sporophorous vesicles containing a variable number of spores. **HG** - Diptera.

Spraguea **Weissenberg, 1976 [2]. TS** - Originally described as *Glugea lophii* Doflein, 1898. **S** - 1. **TH** - *Lophius piscatorius* (Pisces: Teleostei). **G** - EU, Italy. **T** - Nervous system. **D** - LM. **C** - Ultrastructure published by Loubès *et al.* (1979). Only dimorphic genus of fish. **HG** - Pisces.

Steinhausia **Sprague, Ormières & Manier, 1972 [140]. TS** - Originally described as *Haplosporidium mytilovum Field*, 1923. **S** - 2. **TH** - *Mytilus edulis* (Mollusca: Bivalvia). **G** – NA, U.S.A. **T** - Egg. **D** - Description based on LM, but in addition results of an ultrastructural study of *Coccospora brachynema* (see Richards and Sheffied 1971) were used. **C** - A recent investigation on *S. mytilovum* revealed that this species is not related with *S. brachynema* (see Sagristà *et al.* 1998). Genus not observed outside Bivalvia. **HG** - Mollusca.

Stempellia **Léger & Hesse, 1910 [12]. TS** - *S. mutabilis*. **S** - 6. **TH** - *Ephemera vulgata* (Insecta: Ephemeroptera). **G** - EU, France (EU). **T** - Fat. **D** - LM. **C** - The ultrastructure was described by Desportes (1976). The spore groups are characteristic. **HG** - Crustacea, Diptera, Ephemeroptera, Isoptera.

Striatospora **Issi & Voronin, 1986 [in Issi (1986)] [24]. TS** - *S. chironomi*. **S** - 1. **TH** - *Chironomus plumosus* (Insecta: Diptera). **G** - EU, Russia ?. **T** - Fat? **D** - LM and EM. **C** - Only ultrastructural differences to *Helmichia* and *Resiomeria*. **HG** - Diptera.

Systenostrema **Hazard & Oldacre, 1975 [66; Figs. 3 A, 5 H]. TS** - *S. tabani*. **S** - 5. **TH** - *Tabanus lineola* (Insecta: Diptera). **G** – NA, U.S.A. **T** - Fat. **D** - LM and EM. **C** - The genus was revised by Larsson (1988a). At LM level similar to *Thelohania* (polar filament different). *Systenostrema* is probably associated with insects, *Thelohania* with Crustacea. **HG** - Diptera, Odonata, Orthoptera.

Tabanispora **Bykova, Sokolova & Issi (1987?, 1991?) [in Bykova and Issi (1991)] [11]. TS** - *T. bacillifera*. **S** - 3. **TH** - *Hybomitra arpadi* (Diptera: Tabanidae). **G** - EU, Russia. **T** - Fat, hemolympha. **D** - LM and EM. **C** - It is uncertain whether the description

Table 2. Polymorphic microsporidia of Culicidae - distribution between the hosts and characteristics

Genus	Meiospores	"*Nosema*-morph"	"Copepod-morph"	Genus of mosquito host
Amblyospora	larva (ssv, ob, 8, 1n, a)	adult (abs., o-c, ≤4, 2n, i)	copepod (ssv, 4, l, 1n, i)	*Aedes, Aedomyia, Culex, Culiseta, Mansonia, Psorophora, Urotaenia*
Cristulospora	larva (ssv, of, 8,1n, ?)	adult (abs., 2, c, 2n, ?)		*Culex*
Culicospora		adult (abs., 2, o, 2n, i)	larva (ssv, 2-≤8, l, 1n, i)	*Aedes, Culex, Culiseta*
Culicosporella	larva (ssv, ≤8, ob, 1n, a)	adult (abs., 2?o, 2n, ?)	larva (ssv, 1-8, l, 2n, i)	*Culex*
Edhazardia	larva (ssv, ≤8, ob, 1n, a?)	adult (abs., 2, o, 2n, i)	larva (ssv, 2, l, 1n, i)	*Aedes*
Hazardia	larva (abs., 2-16, p, 1-n, i)		larva (abs., 2, l, 2n, a)	*Culex*
Intrapredatorus	larva (ssv, 8, o, 1n, i)		larva (ssv, 2, l, 1n, i)	*Culex*
Krishtalia	larva, adult? (ssv, 8, p, 1n, i)	larva, adult? (ssv, n? o, 2n, a)		*Culex*
Merocinta	larva (ssv, n, o, ?, i?)	adult (abs., 2?, o, 2n, ?)	copepod?	*Mansonia*
Parathelohania	larva (ssv, 8, ok, 1n, a)	adult (abs., n, o, 2n, i)	copepod (ssv?, 8, l, 1n, ?)	*Aedomyia, Anopheles*
Pilosporella	larva (ssv, 8, s, 1n, i)	adult (abs., 2, o, 2n, i)		*Aedes, Wyeomyia*

Information in brackets and abbreviations:
sporophorous vesicle (**ssv** - sporontogenetic sporophorous vesicle, **abs**. - absence);
number of spores;
spore shape (**c** - cylindrical, **l** - lanceolate, **o** - ovoid, **ob** - ovoid-barrel-shaped, **of** - ovoid with fibrous tufts, **ok** - ovoid with posterior collar-like part, **p** - pyriform, **s** - spherical);
number of nuclei;
polar filament (**a** - anisofilar, **i** - isofilar);
? indicates that the character-state is unknown or has been postulated.
Production of meiospores involves meiosis

dated 1987 was published in such way that it is available. This review has been based on the publication from 1991. The sporophorus vesicles with 1-4 spores and voluminous secretions are characteristic. **HG** - Diptera.

Tardivesicula **Larsson & Bylén, 1992 [26]. TS** - *T. duplicata*. **S** - 1. **TH** - *Limnephilus centralis* (Insecta: Trichoptera). **G** - EU, Sweden. **T** - Fat. **D** - LM and EM. **C** - Developing polar filament with wing-like extension. Differs by EM characters and rosette-like sporogony from *Cystosporogenes*. **HG** - Trichoptera.

Telomyxa **Léger & Hesse, 1910 [101; Figs. 5 C, D]. TS** - *T. glugeiformis*. **S** - 3. **TH** - *Ephemera vulgata* (Insecta: Ephemeroptera). **G** - EU, France. **T** - Fat. **D** - LM. **C** - Ultrastructure described by Codreanu and Vávra (1970) and Larsson (1981a). Characteristically coupled spores. **HG** - (Ciliophora), Ephemeroptera, Diptera, Trichoptera.

Tetramicra **Matthews & Matthews, 1980 [94]. TS** - *T. brevifilum*. **S** - 1. **TH** - *Scophthalmus maximus* (Pisces: Teleostei). **G** - EU, U.K. **T** - Connective tissues. **D** - LM (spores) and EM. **C** - Additional information by Figueras *et al.* (1992). 4-sporoblastic sporogony and more ovoid spores differ from *Microgemma*. **HG** - Pisces.

Thelohania **Henneguy, 1892 [67]. TS** - *T. giardi*. **S** - 75. **TH** - *Crangon vulgaris* (Crustacea: Decapoda). **G** - EU, France. **T** - Musculature. **D** - LM. **C** - Vivarès (1975, 1980) has treated the ultrastructure of other *Thelohania* species from Crustacea. *Thelohania* (restricted to Crustacea?) is at LM level similar to *Systenostrema* (restricted to Insecta?). **HG** - Cestoda,

Arachnida, Crustacea, Coleoptera, Collembola, Diptera, Ephemeroptera, Hymenoptera, Lepidoptera, Odonata, Trichoptera, (Pisces), (Mammalia).

Toxoglugea **Léger & Hesse, 1922 [144, Fig. 4 K].** New name for *Toxonema* Léger & Hesse, 1922. **TS** - *Toxonema vibrio* Léger and Hesse, 1924. **S** - 13. **TH** - *Ceratopogon* sp. (Insecta: Diptera). **G** - EU, France. **T** - Fat. **D** - LM. **C** - Ultrastructure of other *Toxoglugea* species published by Larsson (1980a) and Bylén and Larsson (1991). *Toxoglugea* and *Toxospora* can not be distinguished using LM. **HG** - Coleoptera, Diptera, Heteroptera, Homoptera, Odonata, Orthoptera, Plecoptera.

Toxospora **Voronin, 1993 [145]. TS** - *T. volgae*. **S** - 1. **TH** - *Corynoneura* sp. (Insecta: Diptera). **G** - EU, Russia. **T** - Fat. **D** - LM (spores) and EM. **C** - Only ultrastructural differences to *Toxoglugea*. **HG** - Diptera.

Trachipleistophora **Hollister, Canning, Weidner, Field, Kench & Marriott, 1996 [1]. TS** - *T. hominis*. **S** - 2. **TH** - *Homo sapiens* (Mammalia: Primates). **G** - AU. **T** - Musculature. **D** - LM and EM. **C** - Additional EM documentation by Weidner *et al.* (1997). Dimorphic sporogony described by Vávra *et al.* (1998). Only dimorphic genus from homoiothermous vertebrates. **HG** - Mammalia.

Trichoctosporea **Larsson, 1994 (b) [107, Fig. 2].** **TS** - *T. pygopellita*. **S** - 1. **TH** - *Aedes vexans* (Insecta: Diptera). **G** - EU, Sweden. **T** - Fat. **D** - LM an EM. **C** - The fibrous spore projections, visible in living but not in stained spores, are characteristic. **HG** - Diptera: Culicidae.

Trichoduboscqia **Léger, 1926 [70]. TS** - *Trichoduboscqia epeori*. **S** - 1. **TH** - *Epeorus torrentium* (Insecta: Ephemeroptera). **G** - EU, France. **T** - Fat. **D** - LM. **C** - Batson (1982) described the ultrastructural cytology. The spherical sporophorous vesicles (*Mitoplistophora* have angular vesicles) with a number of filamentous projections related to the number of spores is characteristic. **HG** - Ephemeroptera.

Trichotuzetia **Vávra, Larsson & Baker, 1997 [79].** **TS** - *T. guttata*. **S** - 1. **TH** - *Cyclops vicinus* (Crustacea: Copepoda). **G** - EU, Czech Republic. **T** - Gonads. **D** - LM and EM. **C** - The spore shape different from copepodmorphs of polymorphic microsporidia from mosquitoes. The fibrillar material of the sporophorus vesicles differs from *Tuzetia* and *Lanatospora*. **HG** - Crustacea.

Tricornia **Pell & Canning, 1992 [147]. TS** - *T. muhezae*. **S** - 1. **TH** - *Mansonia africana* (Insecta: Diptera). **G** - AF, Tanzania. **T** - Fat. **D** - LM and EM. **C** - The characteristic spore shape visible using EM. In LM preparations similar to *Amblyospora*. A microsporidian infection in copepods might constitute a third sporogony sequence (Pell and Canning 1993b). **HG** - Diptera: Culicidae.

Tuzetia **Maurand, Fize, Fenwick & Michel, 1971 [78, Fig. 6 H]. TS** - *Nosema infirmum* Kudo, 1921. **S** - 14. **TH** - *Cyclops albidus* (Crustacea: Copepoda). **G** – NA, U.S.A. **T** - Fat, gonads. **D** - LM and EM. **C** - Larsson (1984) provided additional information and revised the genus. The narrow tubules traversing the episporontal space are characteristic. **HG** - Crustacea, Coleoptera, Ephemeroptera, Odonata.

Unikaryon **Canning, Lai & Lie, 1974 [124].** **TS** - *U. piriformis*. **S** - 15. **TH** - *Echinostoma audyi* (Digenea). **G** - AS, Malaysia. **T** - Parenchyma. **D** - LM. **C** - Ultrastructural data on *Unikaryon* species from Digenea by Canning *et al.* (1983 b) and Azevedo and Canning (1987). *Unikaryon* is the invertebrate equivalent of the vertebrate parasitic *Encephalitozoon*. The present knowledge of the genera is not sufficient for discrimination between *Oligosporidium* and *Unikaryon*. **HG** - Digenea, Arachnida, Coleoptera, Lepidoptera.

Vairimorpha **Pilley, 1976 [15]. TS** - *Nosema necatrix* Kramer, 1965. **S** - 8. **TH** - *Pseudaletia unipunctata* (Insecta: Lepidoptera). **G** – NA, U.S.A. **T** - Hemolymph and fat. **D** - LM. **C** - Ultrastructural studies by Moore and Brooks (1992) and Mitchell and Cali (1993). Sporophorous vesicle-bound groups of 8 elongated oval spores together with diplokaryotic spores characteristic for *Vairimorpha*, *Burenella*, and *Heterovesicula*. **HG** - Hymenoptera, Lepidoptera.

Vavraia **Weiser, 1977 [113, Fig. 4 B]. TS** - *Plistophora culicis* Weiser, 1947. **S** - 5. **TH** - *Culex pipiens* (Insecta: Diptera). **G** - EU, Czech republic. **T** - Malpighian tubules. **D** - LM. **C** - Ultratructure described by Diarra and Toguebaye (1991). The invertebrate equivalent of the vertebrate parasitic *Pleistophora*. Ultrastructure (especially the merontogenetic sporophorous vesicle) characteristic. **HG** - Diptera, Trichoptera.

Vittaforma **Silveira & Canning, 1995 [99].** **TS** - *Nosema corneum* Shadduck, Meccoli, Davis & Font, 1990. **S** - 1. **TH** - *Homo sapiens* (Mammalia: Primates). **G** – NA, U.S.A. **T** - Cornea. **D** - LM and EM. **C** - Linearly arranged sporoblasts characteristic. **HG** - Mammalia.

Weiseria **Doby & Saguez, 1964 [151]. TS** - *W. laurenti*. **S** - 3. **TH** - *Prosimulium inflatum* (Insecta: Diptera). **G** - EU, France. **T** - Fat. **D** - LM. **C** - No ultrastructural studies known to me. Spore shape characteristic. **HG** - Diptera, Ephemeroptera.

***Wittmannia* Czaker, 1997 [129]. TS -** *W. antarctica*. **S -** 1. **TH -** *Kantharella antarctica* (Dicyemida). **G -** AN, Weddell Sea. **T -** Envelope around the axial cell. **D -** LM (spores) and EM. **C -** Resembling *Nosema* but polysporoblastic. **HG -** Dicyemida.

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ACTA
PROTOZOOLOGICA

A Program for Computer-Assisted Analysis for Creeping Behaviour of Ciliates and Similar Two Dimensional Movement Patterns

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Summary. Locomotion represents quite a complex phenomenon even in very simple forms like the ciliated protozoa. To improve the efficiency and the reliability of the already available semiautomatic program for the analysis of the tracks of creeping ciliates, the Interactive Computer Assisted Track Analysis (ICATA) program was written: it has a series of advantageous features: (A) the algorithms, specifically designed for the analysis of the tracks, ensure an extremely fast elaboration; (B) the "fitting error" of each step of the analysis is shown on the display: the operator can interact with ICATA in real time and choose to operate manually, if the biological meaning of the track is not appropriately interpreted by the automatic analysis; (C) it has been conceived as a modular program: new blocks can be added easily; (D) the output of the analysis (i.e. a series of files), processed by the utilities of ICATA itself, is also ready for analysis by commercial statistical software packages. Finally, ICATA, although conceived for originally analysing the locomotion of ciliates, has been shown to be a powerful tool also for the analysis of any bidimensional locomotion, from bacteria (*E. coli*) and metazoan cells (granulocytes) to simple metazoa (Gastrotricha).

Key words: behaviour, locomotion, track analysis.

INTRODUCTION

The important role played by behaviour in the biology of any protozoon was already acknowledged in 1906 by Jennings. The problem, which remained to be solved, was the way of accurately studying a phenomenon, which represents a peak of complexity also for protozoa, as discussed thoroughly by Bonner (1988). The conceptual solution was found in 1967 by Eibl-Eibesfeldt, who put forward the concept of the ethogram, namely "the catalogue as complete as possible of all of the behavioural patterns of a certain species". For protozoa, many ethograms have been drawn manually (Ricci 1981, 1982; Ricci *et al.* 1987) and, and more recently, several others have been drawn (Ricci and Tortorella 1990, Ricci and Verni 1988, Mugnaini *et al.* 1995) by means of a computer assisted program written by Russo *et al.* (1988). The first studies were quantitatively precise (but very slow, due to the problems of the manual measuring) and qualitatively somewhat subjective (due to the highly personalised interpretation of the tracks). The successive computer-assisted ethograms were more objective qualitatively and far more easily and rapidly drawn. However, this new generation of semi-automatic ethograms was not completely satisfying, because they were strongly determined and limited by the software itself, which carried out semiautomatically only an analysis of exactly the same kind as the manual one. These ethograms led to a renewed

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general perspective in the consideration of the behaviour of the ciliates (Ricci 1990), to a standard protocol of drawing an ethogram (Ricci 1992) and to a computer simulation of the tracks of creeping ciliates (Oliveira-Pinto *et al.* 1993). The further these studies went and the deeper our understanding of the behaviour became, the more inadequate the available software proved to be.

A new program became necessary which would allow for (i) a very rapid analysis, (ii) a very high degree of freedom of interpretation (as regards the choices made by the operator in successive steps of the analysis), (iii) a wide range of analytical potentialities and (iv) a series of new numerical and graphical elaborations, very important for a prompt understanding of various peculiarities of the behaviour. The essential improvement in the program's structure we wanted to achieve was a basic flexibility, namely a wide possibility of operator-program interactions to express directly the operator's experience in the analysis of the tracks, excluding the automatic routine (s) when necessary. It was assumed that the researcher is already perfectly acquainted with the behavioural patterns he wants to analyse. In conclusion, the structure of the program we were looking for was such a general and flexible one that the program had to be a suitable tool also for the analysis of the phenomena ranging from the cell motility of prokaryotes and mammal cells to the complex behaviour of entire, though simple, metazoa.

This paper deals with the experiments carried out to test the new program we prepared to solve the problems mentioned above. The results showed that it satisfies perfectly the need for a new type of behavioural analysis and data processing, and it also lends itself to the study of bidimensional tracks, regardless of the nature of the moving cell/organism studied.

MATERIALS AND METHODS

Biological preparations

To test the potential of ICATA (an acronym for "Interactive Computer Assisted Track Analysis") both marine and freshwater organisms were studied. Standard cultures of *Oxytricha bifaria*, a freshwater hypotrich ciliate grown according to Ricci *et al.* (1980), and of *Euplotes crassus*, a marine hypotrich ciliate grown in the artificial sea water prepared according to Allen's formula (Bidwell and Spotte 1985) with *Dunaliella tertiolecta* as food (Dini and Nyberg 1994), were used to study the tracks of creeping organisms under different experimental conditions. The creeping of *O. bifaria* was analysed at different temperatures (9, 14, 19 and 24 °C), the changing temperature representing a rather easy-to analyse experimental perturbation. The creeping of *E. crassus* was studied by treating it with different concentrations of Hg^{2+} (0.12 and 0.25 μM) over different periods of time (1 and 24 h), in order to test ICATA also in the field of etho-biomonitoring. In fact, as indicated by Olla *et al.* (1980 a, b) and by Kittredge (1980), the behaviour of ciliates had been shown to clearly reflect external changes (Mugnaini *et al.* 1995, Ricci *et al.* 1995). The ICATA program, once written by F. Erra, one of our group on the basis of his previous experience in the field of the study of protozoan behaviour, was tested experimentally for three years (1994-1996) by F. Barbanera and G. Mori, who were studying the behaviour of the two species used.

Quantification of ethograms

The ethograms were drawn according to the standard protocol and the indicated by Ricci (1992): this paper must be taken into account to understand the technique thoroughly and to become thoroughly acquainted with it. A scheme is given and explained briefly, to facilitate understanding of the present report (Fig. 1). Seven basic behavioural patterns were recognized and described, along the track of a creeping ciliate, like that shown in Fig. 1A and schematically analysed in Fig. 1B. Three patterns are referred to as Long Lasting Elements (LLE) and the other four as Short Lasting Elements (SLE) (Fig. 1C). The three LLE, namely the "rightward arcs" (A^+), the "leftward arcs" (A^-), and the "linear segments" (S), follow each other randomly forming a piecewise-linear track. The succession of many LLE to form a continuous track is obtained by the different SLE, which are the junctions of two contiguous LLE: they have been called the "Continuous Trajectory Change" (CTC), the "Smooth Trajectory Change" (STC), the "Rough Trajectory Change" (RTC), and the "Side Stepping Reaction" (SSR). The above reported order of the SLE reflects their capacity to change the creeping direction of wider and wider clockwise correction angles ($\alpha_{CTC} < \alpha_{STC} < \alpha_{RTC} < \alpha_{SSR}$). Finally (Fig. 1C), the LLE can be quantitatively characterized by measuring their parameters: the "relative frequency" (ν %), the "velocity of the creeping ciliate" (v), their "duration in time" (Δt), their "length" (l), and, only for the A^+ and the A^-, their "radius" (r) and their "central angle" (β°). Parameters such as "relative frequency" (ν %), and "correction angle " (α°) are considered in order to characterise the SLE. The length of the backward motion (BM) was also taken into consideration to quantify the SSR.

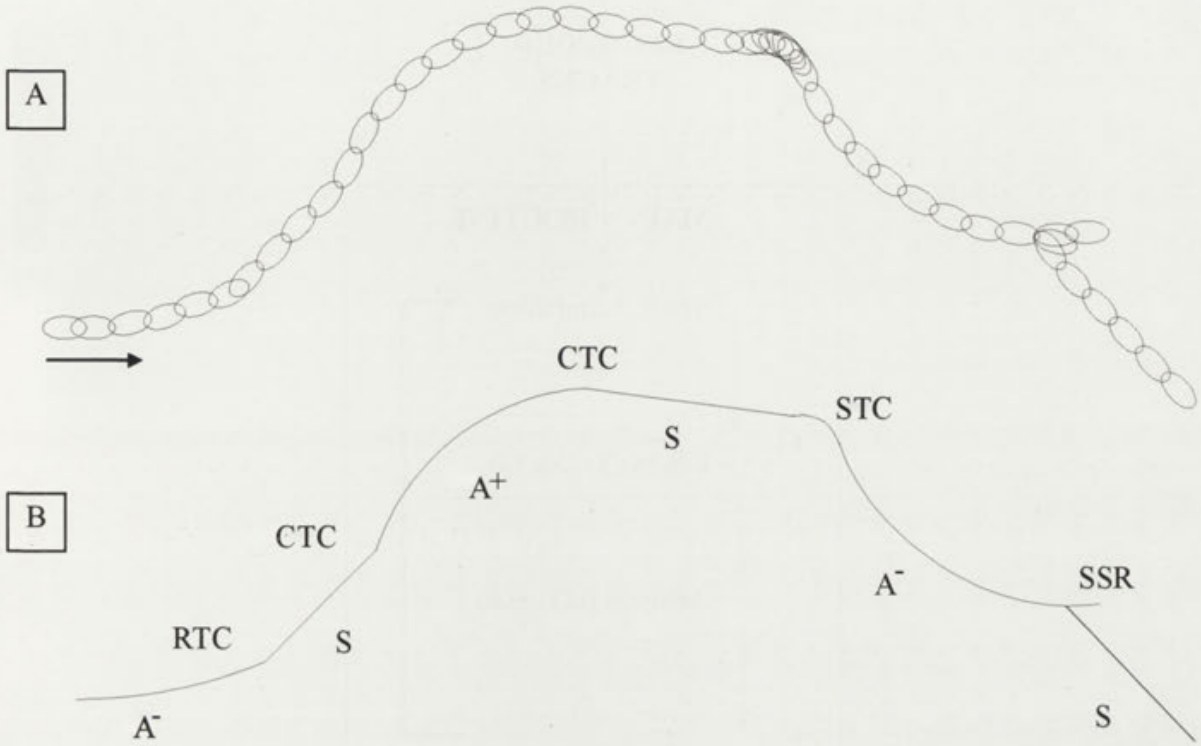
Computing equipment and software

Our basic philosophy was to prepare an easy to use software, for a basic hardware, available in whichever laboratory. The program was written in Professional Visual Basic for any PC utilising the DOS as the operating system and a digitising tablet as the device to acquire the successive positions of the organisms. The program may be forwarded to the students of the behaviour of ciliates: any economic matter will be defined with the Law Department of the University of Pisa.

RESULTS

1. General view of ICATA

ICATA was conceived from the start as a modular program, with the basic aim of preparing a new versatile and flexible, analytic tool. Beyond the long series of



C

LLE						
A^+	$\nu\%$	v	Δt	l	r	$\beta°$
A^-	$\nu\%$	v	Δt	l	r	$\beta°$
S	$\nu\%$	v	Δt	l	-	-

SLE			
CTC	$\nu\%$	$\alpha°$	-
STC	$\nu\%$	$\alpha°$	-
RTC	$\nu\%$	$\alpha°$	-
SSR	$\nu\%$	$\alpha°$	BM

Fig. 1. A - the track of a creeping ciliate, as recorded from the TV screen; B - its schematic interpretation in terms of Long Lasting Elements (LLE) and Short Lasting Elements (SLE); C - the three LLE (rightward arcs - A^+; leftward arcs - A^-; linear segments - S) and the four SLE (Continuous Trajectory Change - CTC; Smooth Trajectory Change - STC; Rough Trajectory Change - RTC; Side-Stepping Reaction - SSR) are shown together with the quantitative parameters characterising them. BM - the backward motion typical of the SSR

technical internal logic devices (often crucial to combine the needs of rapidity and precision) which obviously cannot be dealt within this paper, five clear-cut structural features of ICATA must be recalled.

A. Its modular structure, given in Fig. 2, consists of a series of independent blocks, which can work both in series and in parallel, according to the operator's needs and choices. Such a structure ensures not only a high degree of processing velocity, but also quite a precious saving, in terms of potential available for other operations.

B. The algorithms used to analyse the LLE and the SLE (based on new geometrical elaborations of these elements) were progressively improved and simplified with time. As an example, the automatic recognition of the type of LLE (i.e. A^+, A^-, S) at first was obtained by a number of steps roughly as large as 15 times the number of the final version.

C. For each quantitative measure the operator can choose different degrees of precision. The automatic procedure leads to the measurement of a certain param-

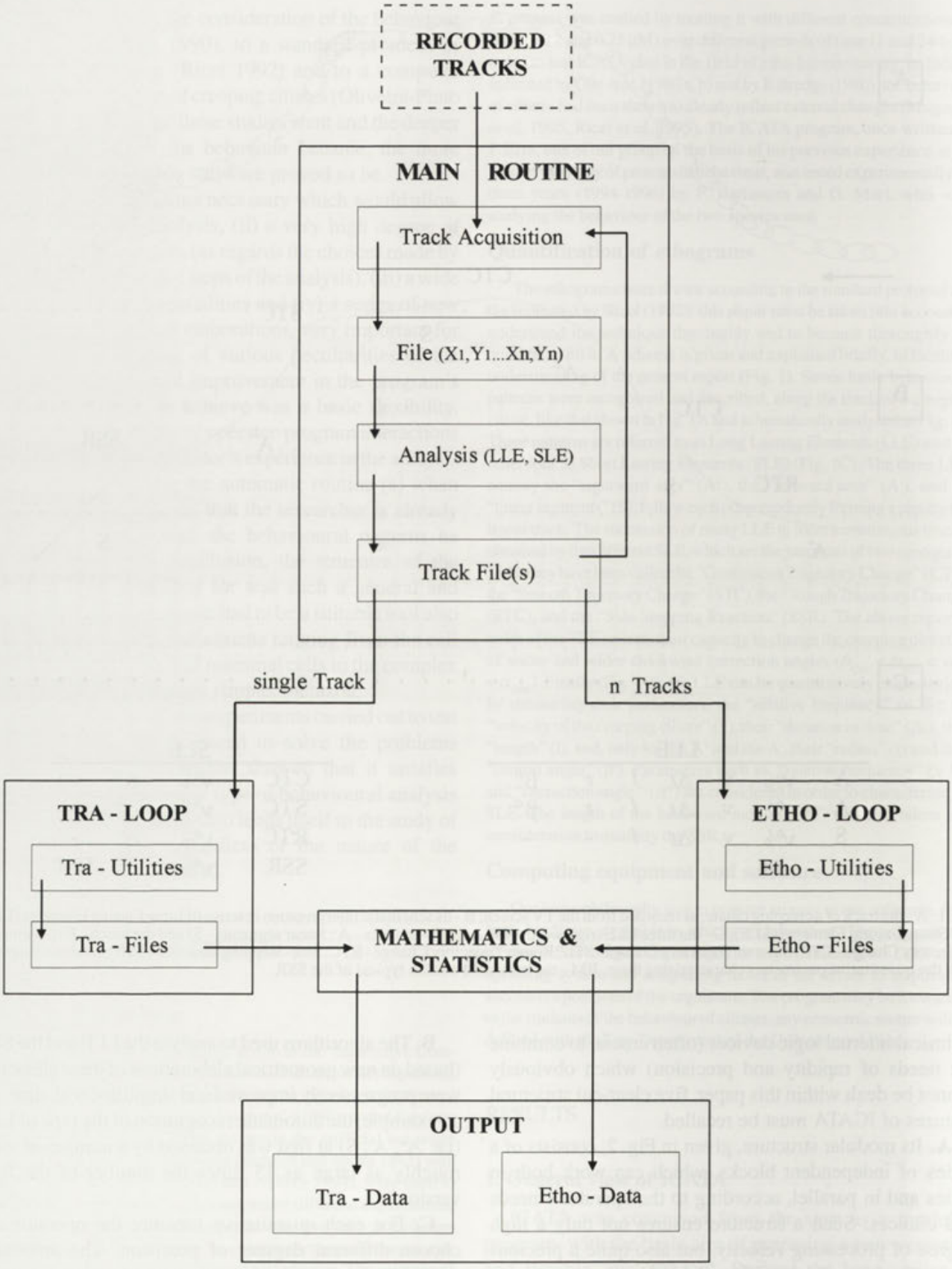


Fig. 2. The modular architecture of ICATA consists of the series of functional blocks indicated

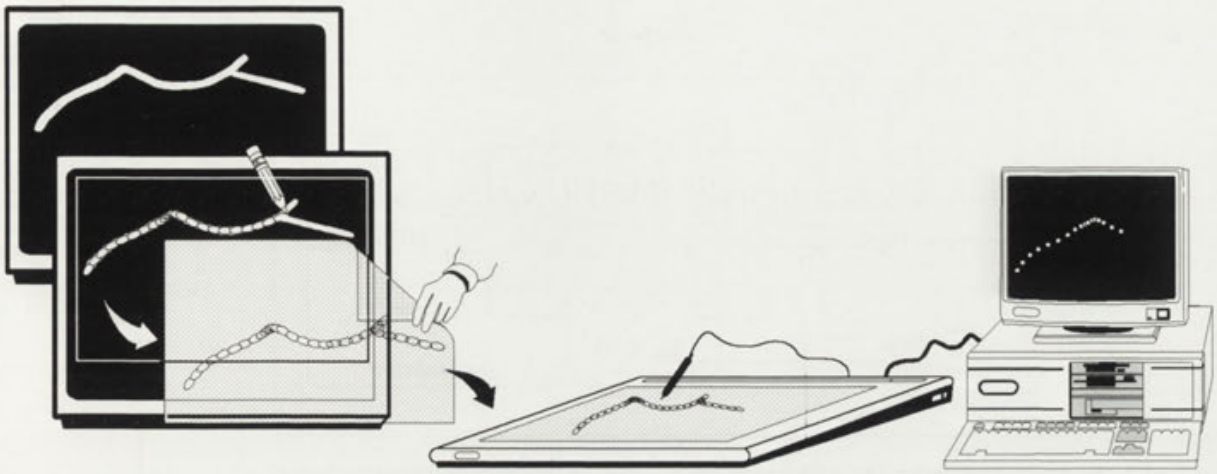


Fig. 3. The acquisition of a track. A - the TV recorded track (shown on the TV screen in the background) is recorded by hand on a triacetate sheet as a series of roundish forms; the transparent sheet is then transferred onto a digitising pad (B), where the barycentres of the successive positions of the ciliate are acquired by the magnetic pen and shown on the monitor as successive dots (C)

eter. The corresponding "fitting error", shown on the screen, is the lowest among those possible from a strictly mathematical point of view. The operator can exclude the automatic analysis whenever the results, (although mathematically the best) are not satisfying from a biological point of view. A detailed example of this kind of operator-computer interaction is discussed in the section dedicated to the "Analysis" of the tracks (see below, Fig. 5C).

D. The ICATA program is open: several utilities had already been added to its initial configuration in the first year of its use. As was expected, indeed, different experimental approaches to different biological topics brought into focus various analytical problems, very often easily solved by adding a specific new utility. The basic modular nature of ICATA allows a long series of added modules, without any appreciable loss in efficiency.

E. All of the data elaborated are arranged in files, in such a way that their immediate use by the specific utilities introduced for specific analyses is possible. Moreover these files can be processed by standard commercial statistical packages if required. The importance of this feature of ICATA is self-evident, because the operator can know immediately and in real time whether the sets of data possibly expressing different behaviours are actually different or not, thus having an immediate idea of their general trends and statistical significance.

2. The acquisition of the tracks

This first part of ICATA leads to the memorisation of all the successive positions of the organism along a certain track, each identified by its coordinates. Therefore the set of all the coordinates represents a file which describes the track point by point. As shown in Fig. 3A, the track must be first hand-recorded on a triacetate sheet directly from the TV screen, as a succession of elliptical forms, each representing one of the successive positions of the ciliate moving along that particular track, each recorded at well-defined time intervals. When the recording ends, the transparent sheet is transferred onto a digitising tablet, where the successive positions are acquired by means of the magnetic pen (Fig. 3B). The process is more difficult than expected, due to the precision required to limit error and bias. For this reason each position (or group of positions) can be deleted and re-acquired at any time, very easily and quickly. The track is also shown on the monitor (Fig. 3C), the successive positions appearing as soon as their barycentres are acquired.

3. Analysis of the acquired tracks

This block represents the basic part of ICATA. The structure is given in Fig. 4, where the operator (left column) guides the computer processes (central areas), in order to identify a series of ethological elements, together with the measurements of their parameters (right column). The complete set of these elements and measurements represents the final "track file".

The operator identifies the extremes of the first complete LLE, moving a cursor along the track previously acquired (Fig. 4, hexagon "Step 1": P1P2; Fig. 5A, left part: from P1 to P2). The program recognises the type of

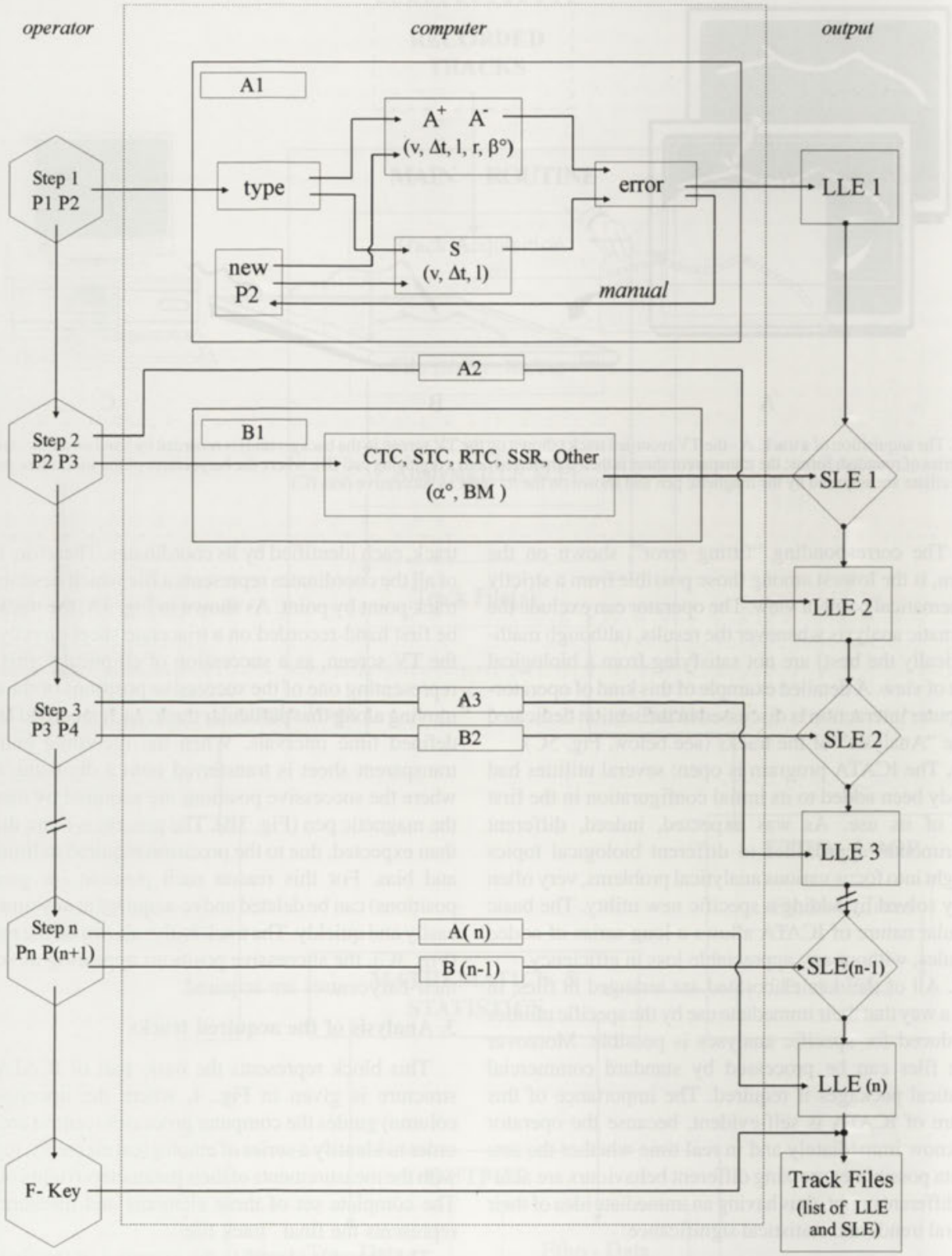


Fig. 4. The analysis of the tracks (corresponding to the unit "Analysis" of the block "Main routine" of Fig. 2). The hexagons on the left show the operator's steps, controlling the computer analysis to elaborate the output on the right. Each step consists of two different routines, A and B; the A-routine calculates the parameters of the LLE, while the B-routine analyses the SLE. The "track file" is generated when the operator, at the end of the analysis, (step n) presses an F-key

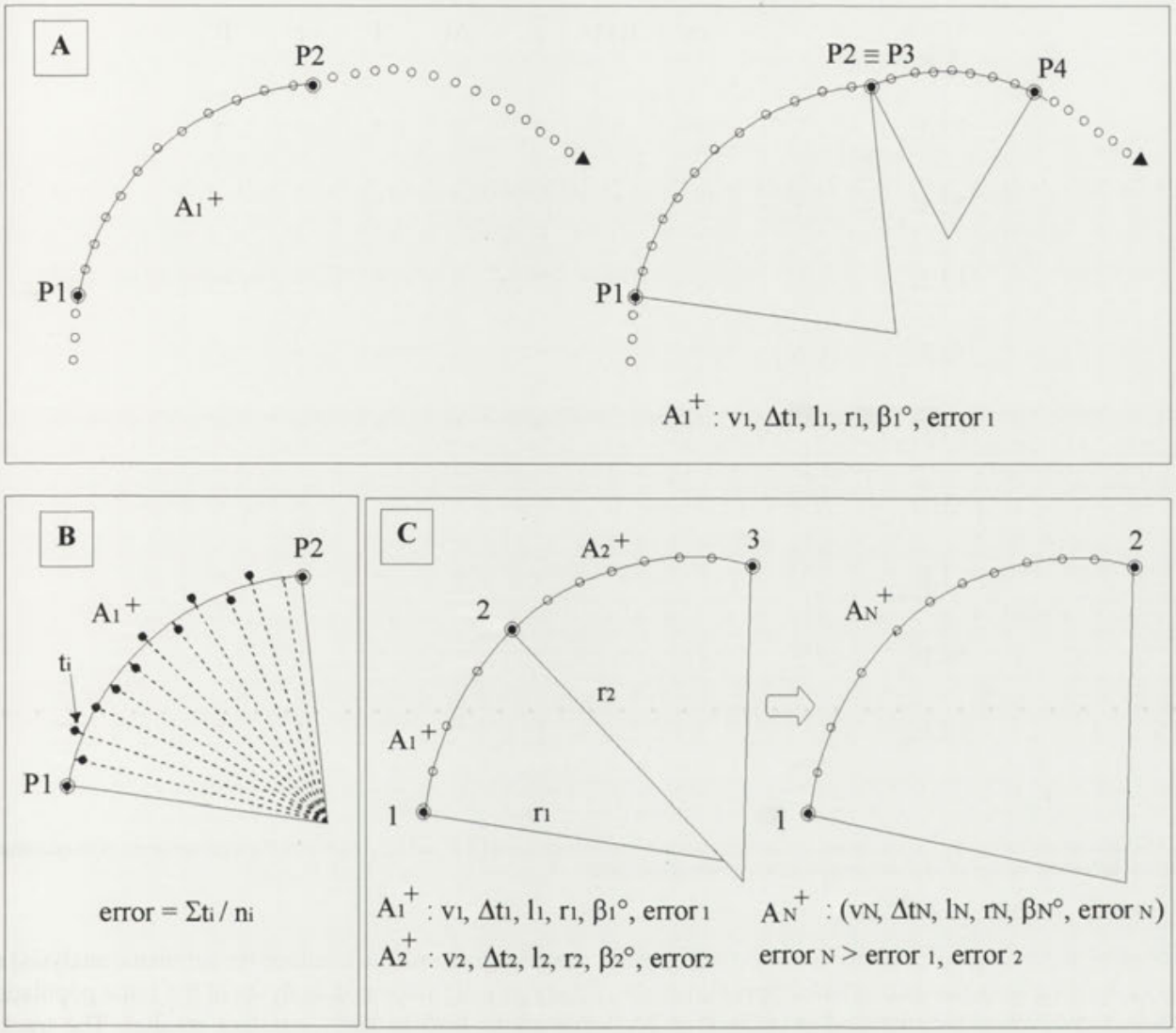


Fig. 5. The analysis of the LLE Panel A - the study of two successive A+: in the left part, the acquisition of the first A_1^+ from P1 to P2; in the right part, the parameters of A_1^+ and the initial phase of the analysis of the successive A+ are shown. Panel B - the calculation of the" fitting error of A_1^+"; t_i represents the distance between the position of the organism (black dot) and the theoretical arc calculated on the basis of all the positions from P1 to P2. Panel C - the operator, interacting with ICATA, chooses to combine A_1^+ and A_2^+ (on the left) into a unique, behaviourally more correct new A+, indicated as A_N^+ (on the right)

LLE, measures its parameters and shows the type of element itself (A+, in our example), together with its dimension and the degree of approximation of the fitting (Fig. 5A, right part: A_1^+; v_1, Δt_1, l_1, r_1, $\beta_1°$, $error_1$). At this point the operator can refuse the automatic analysis of the program, shifting to the loop "manual", indicated in Fig. 4, step 1, routine A1. In the latter case he can choose a different, more appropriate P2 (indicated as "new P2" in Fig. 4) and check whether the new, manual analysis describes the track better than the preceding automatic calculations. In this case he accepts the new elaboration or refuses and tries again. The analysis can be changed further in order to reach a final satisfactory description of the track. Figure 5B explains schematically the way the fitting error is calculated. As fitting error we mean the ratio between Σt_i and n_i, where t_i is the distance between each position along the track, indicated by the black dot, and the LLE calculated on the basis of all the positions, n_i, from P1 to P2.

Figure 5C schematically represents an example of a rather frequent case. Two LLE (in our example A_1^+ and A_2^+), slightly differing only in terms of their radii, follow each other joined by a CTC with $\alpha° \approx 0°$ (corresponding to position 2 in Fig. 5C). In this case the operator can choose to combine the two A+ to obtain better level of analysis, namely a unique, longer A+ (Fig. 5C, right part).

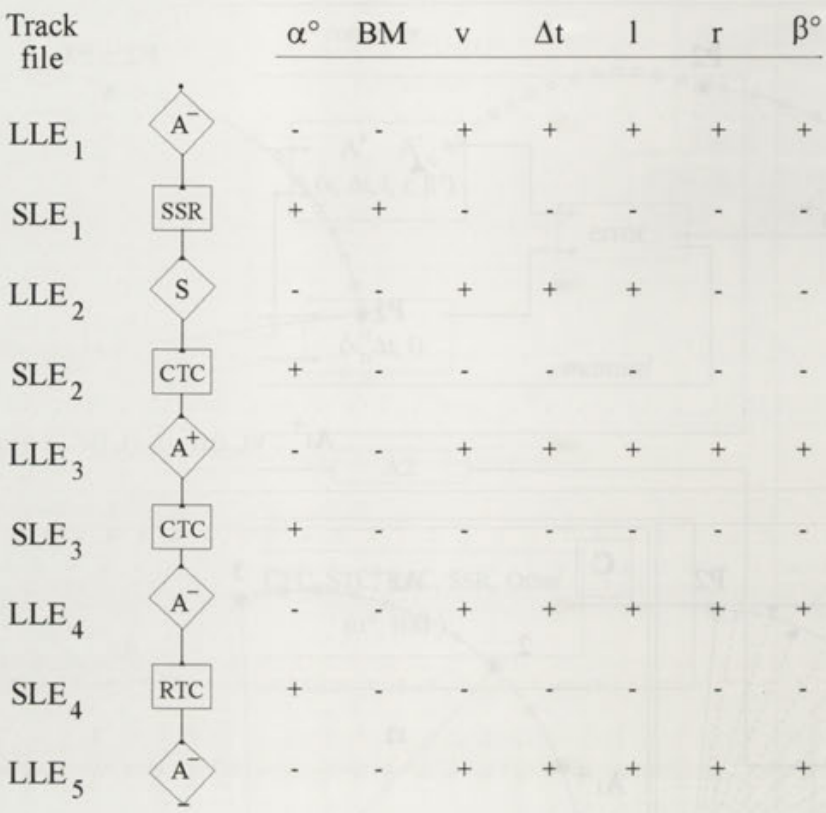


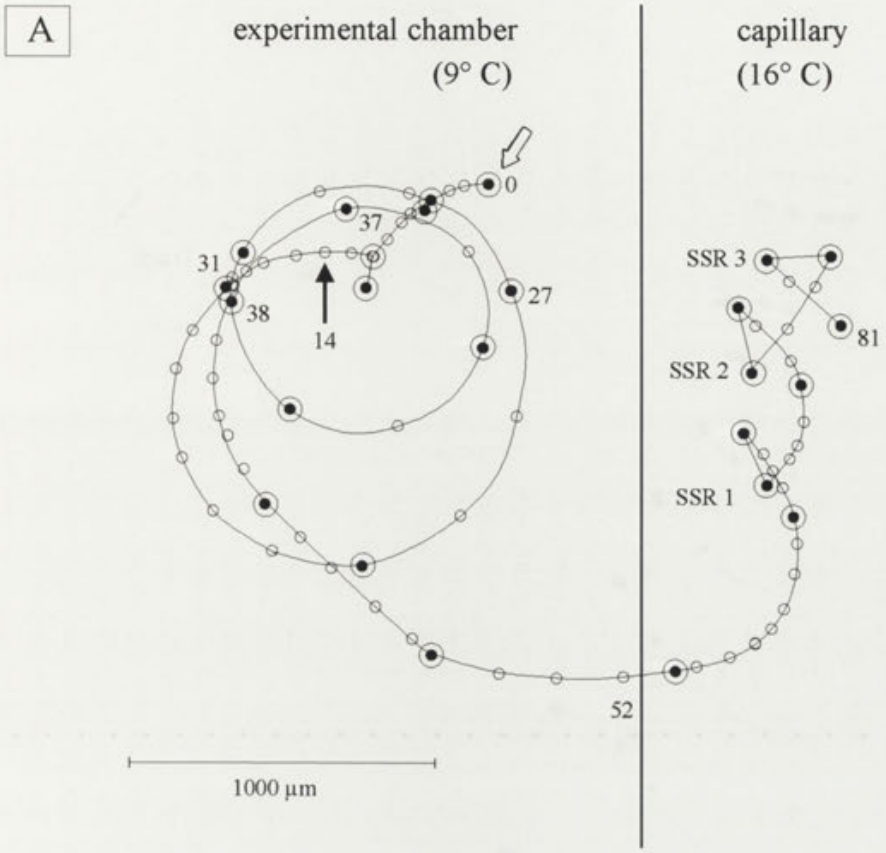
Fig. 6. An example of a "track-file" schematised by the succession of its elements from LLE_1 to LLE_5; each with the measurements of its quantitative parameters, shown at the top of each column. + - measurement available

Such possibilities of choice are important to avoid analytical errors deriving from the unavoidable approximations, occurring at the levels of both the hand recording (Fig. 3A) and the track acquisition (Figs. 2, 3B). After analysing the first LLE (Fig. 4, step 1, LLE1), the operator chooses P2 and P3 along the track and the program follows step 2 (Fig. 4, Step 2, P2P3), calculating the LLE2 by the routine A2. At this point, namely after analysing the first two LLE, the operator chooses manually (Fig. 4: step 2, routine B1) the proper kind of SLE (CTC, STC, RTC, SSR) and the SLE1 is shown on the screen together with the angle (α°) which the direction of the track has been changed. A regular succession of routines A and B (each forming one step) leads to the complete analysis of the track (Fig. 4, on the right), which is memorised as a "track file" when the operator, after step n, presses an F-key. In other words, ICATA enables us to conduct the complete analysis of a track in terms of objective elements very rapidly (less than a minute), very precisely (i.e. with fitting errors in an order of magnitude of 1% of the length of the LLE measured), very flexibly (the operator has to accept/refuse each stage in order to continue the automatic analysis) and very reliably (repeated analyses of the same populations of tracks lead to fairly constant results). The track is expressed by the succession of LLE1, SLE1, LLE2, SLE2,..... SLE(n-1), LLE(n), each with the measurements of its own parameters. The succession of elements and parameters, namely the "track-file" (Fig. 6), can be processed statistically. This phase concludes the part of ICATA corresponding to the classic analysis of ciliate locomotion leading to the ethogram.

4. The utilities of ICATA

This part of ICATA corresponds to the new analysis. It is articulated and it gives us instantaneously new elaborations not only of the data of each track file (Fig. 2, block TRA-LOOP), but also of entire populations of many track files (Fig. 2, block ETHO-LOOP).

A. The utility KI-TRA (KInematics of the TRAck) gives the values of the velocities between the successive positions of the ciliate along the track describing its trend. The data elaborated in this way are quite interesting for an



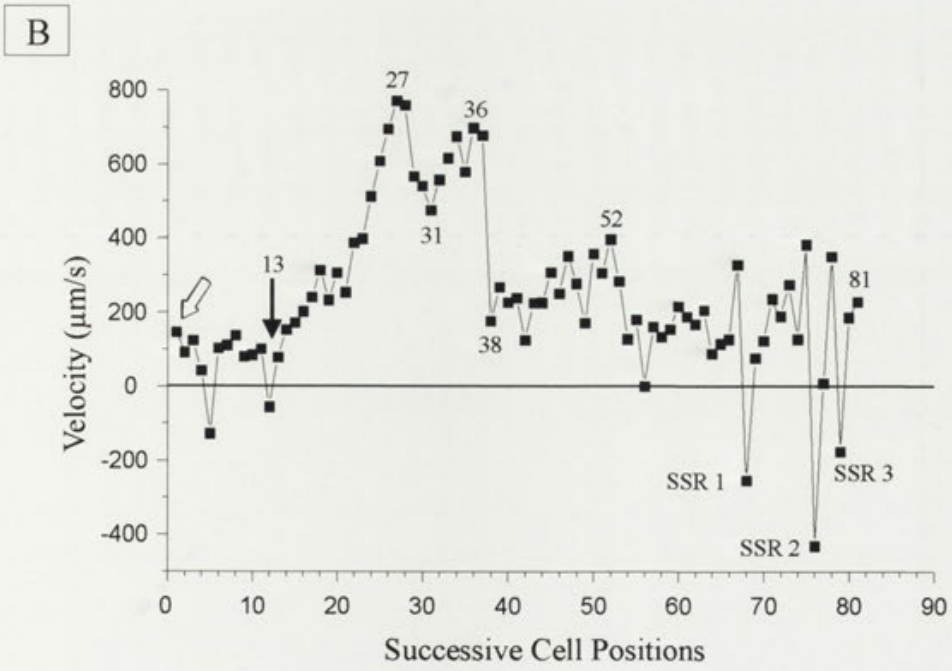


Fig. 7. A - the track of an *O. bifaria* kept at 9 °C creeping toward the region on the right, at 16 °C. B - the velocities along the same track. The white arrows indicate the starting point; the black arrows show the position of the organism when the 16 °C flow in the capillary was opened

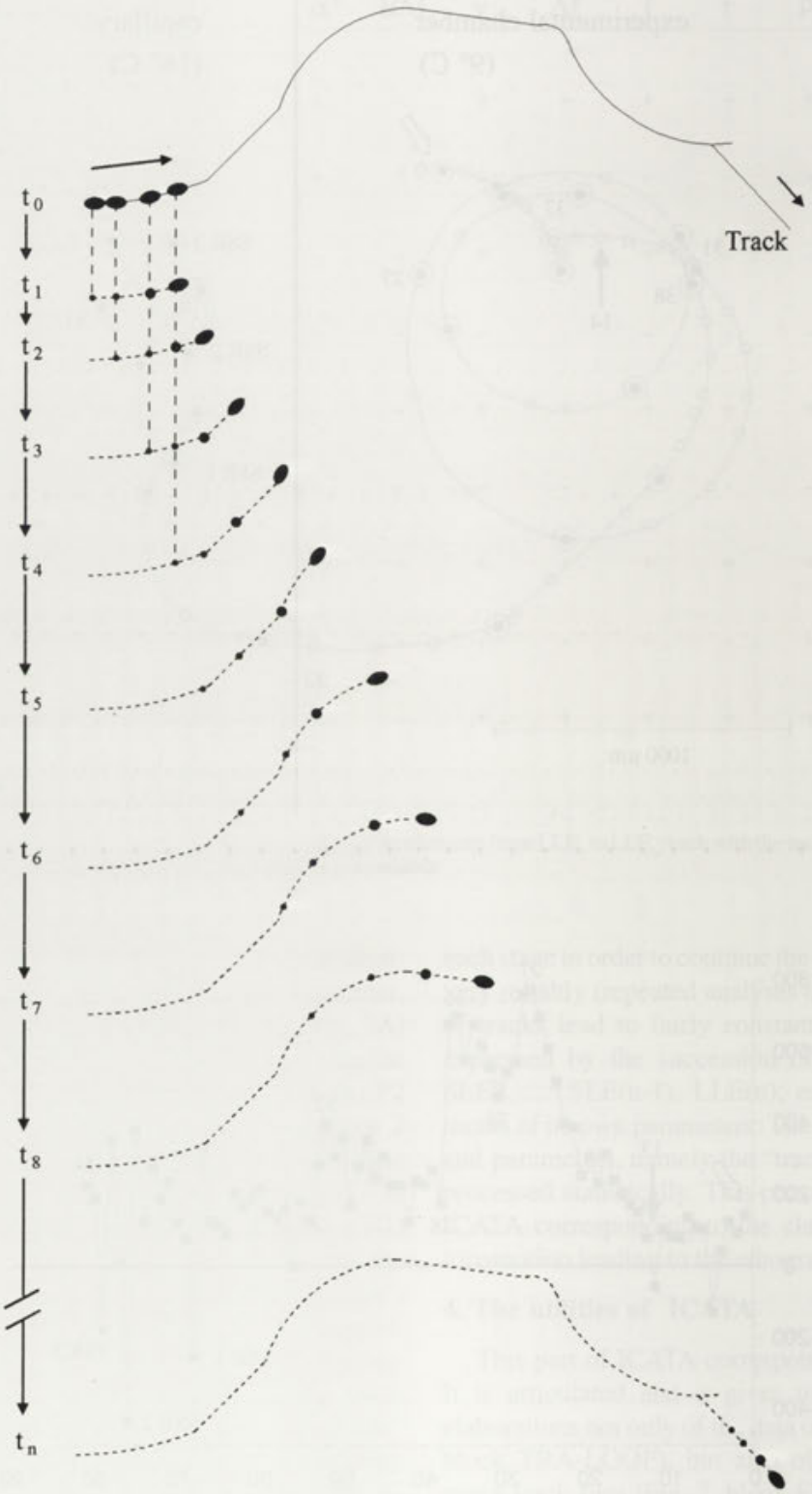


Fig. 8. The locomotion of an *E. crassus* along a track is reproduced in its temporal development. More details are given in the body of the paper

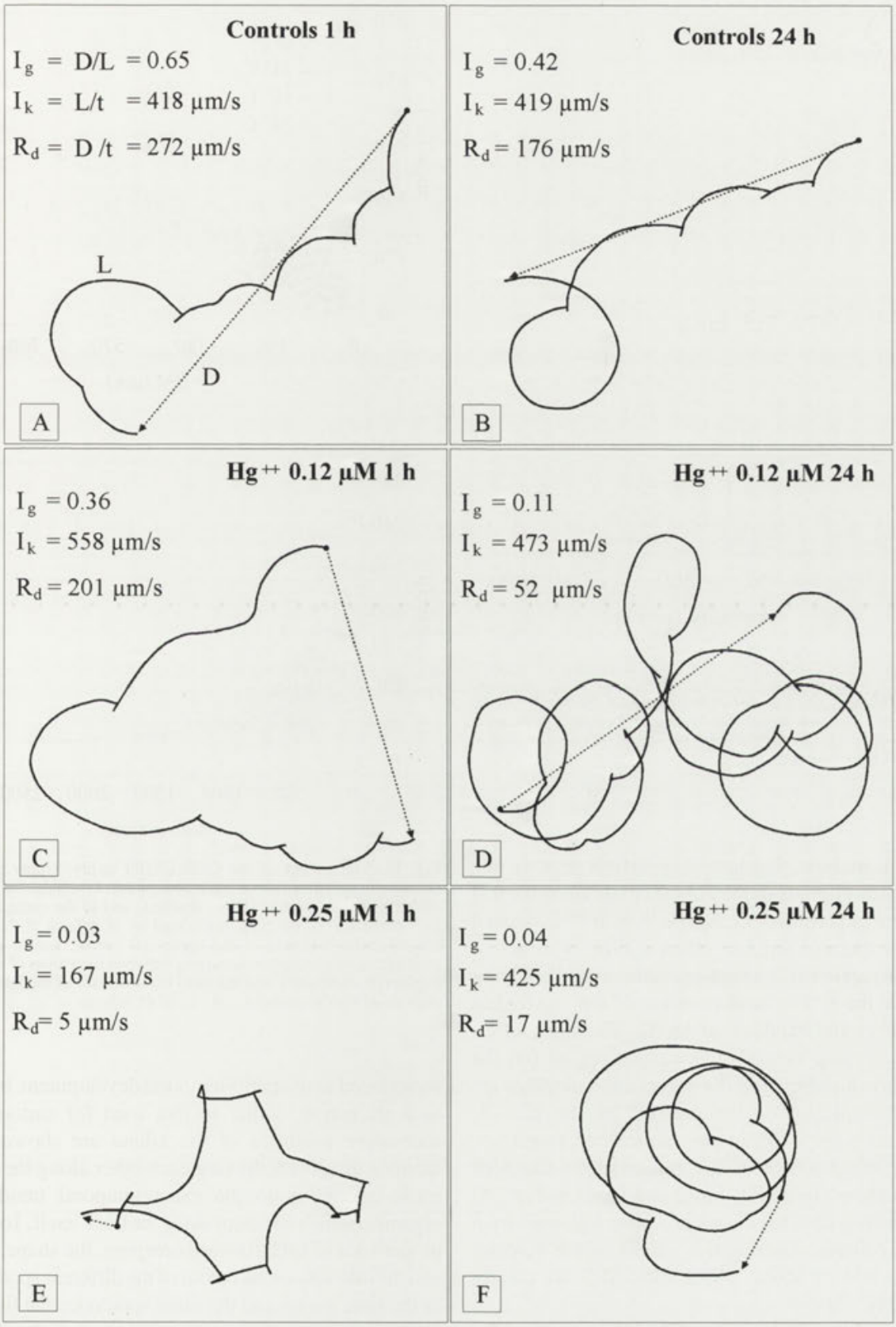


Fig. 9. The output of the IN-TRA utility. The indices shown in panel A are given for the tracks of the controls (1h - A, 24h - B) and the experimental populations, treated by 0,12 μM (1h - C, 24 h - D) and 0.25 μM Hg^{2+} (1h - E, 24 h - F)

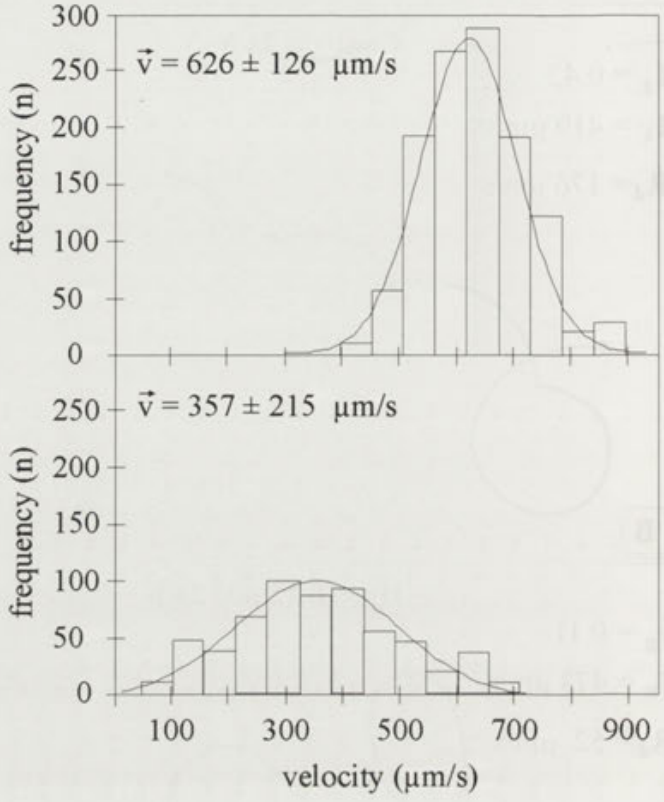


Fig. 10. The frequency histogram (on the ordinate: n, the number of the cases) of the velocities (on the abscissa - v, μms^{-1}) of a control population of *E. crassus* (upper graph) and of the experimental population treated with 0.25 µM Hg^{2+} (lower graph)

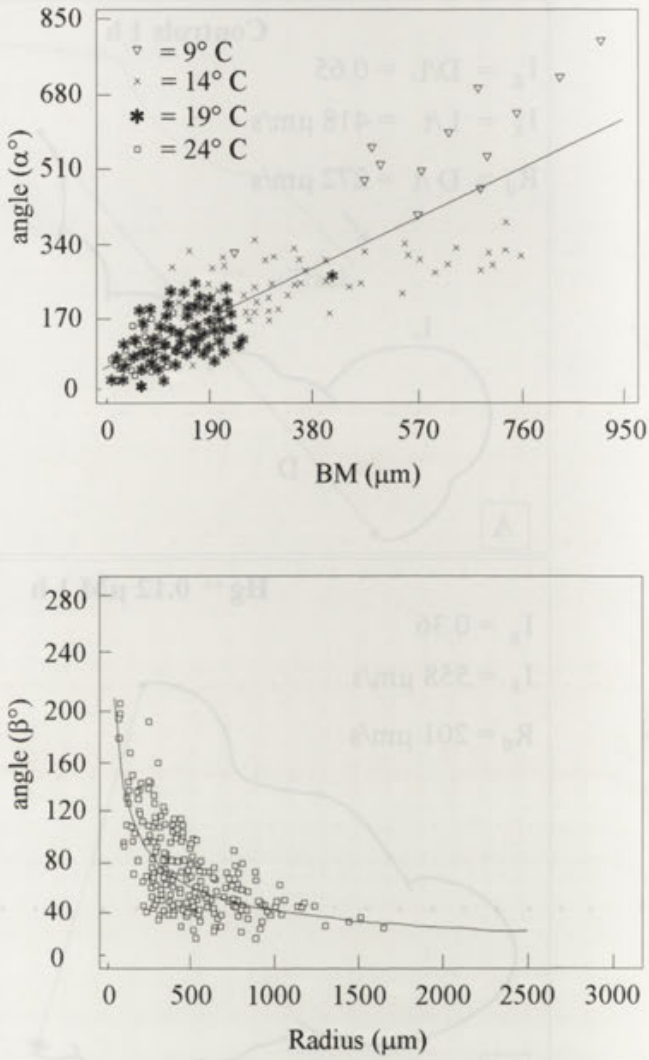


Fig. 11. The output of the COR-ETHO utility. Upper panel: the distribution and the positive linear correlation of the values of the length of the backward motion (BM - abscissa) and of the correction angle (α°- ordinates) of the SSR performed by *O. bifaria* at the different temperatures indicated in the upper left corner. Lower panel: the multiplicative correlation occurring between the values of the central angles (β° - ordinate), and the radii (r - abscissa) of the leftward arcs performed by the controls (24 °C) of *O. bifaria*

ethological analysis, because they reflect exactly the internal state of the physiology of the ciliate covering that track. In our experiments *O. bifaria* kept at 9 °C showed a clear-cut thermo-response when a flow of water at 16 °C (right part of Fig. 7A) was opened in a capillary lying underneath the 9 °C chamber: most of the oxytrichas crowded over the capillary at 16 °C. The track of an organism creeping towards the warmer region (on the right) is shown in Fig. 7A: the successive velocities are shown automatically on the monitor by the ICATA, KI-TRA utility (Fig. 7B). In the warmer area, moreover, a striking enhancement of the frequency of the SSR occurs (as shown by SSR1, SSR2 and SSR3 in Fig 7A) each consisting of a backward creeping followed by a clockwise rotation: the negative values of the velocity during the BM of SSR1, SSR2 and SSR3 are clearly shown in Fig. 7B.

B. The utility DEMO-TRA (DEMOnstration of the TRACK, Fig. 8): once acquired, each track can be reproduced in its spatio-temporal development, by means of a technique similar to that used for cartoons. The successive positions of the ciliate are shown on the monitor as spots following each other along the original track, according to the exact temporal trend of the organism which has crept along the track itself. To give the proper idea of such forward creeping, the shape, the size and the intensity of the colour of the different spots change as the time passes and the same spot, once the first in the series, progressively becomes the second, the third etc. (Fig. 8: $t_1 \rightarrow t_n$).

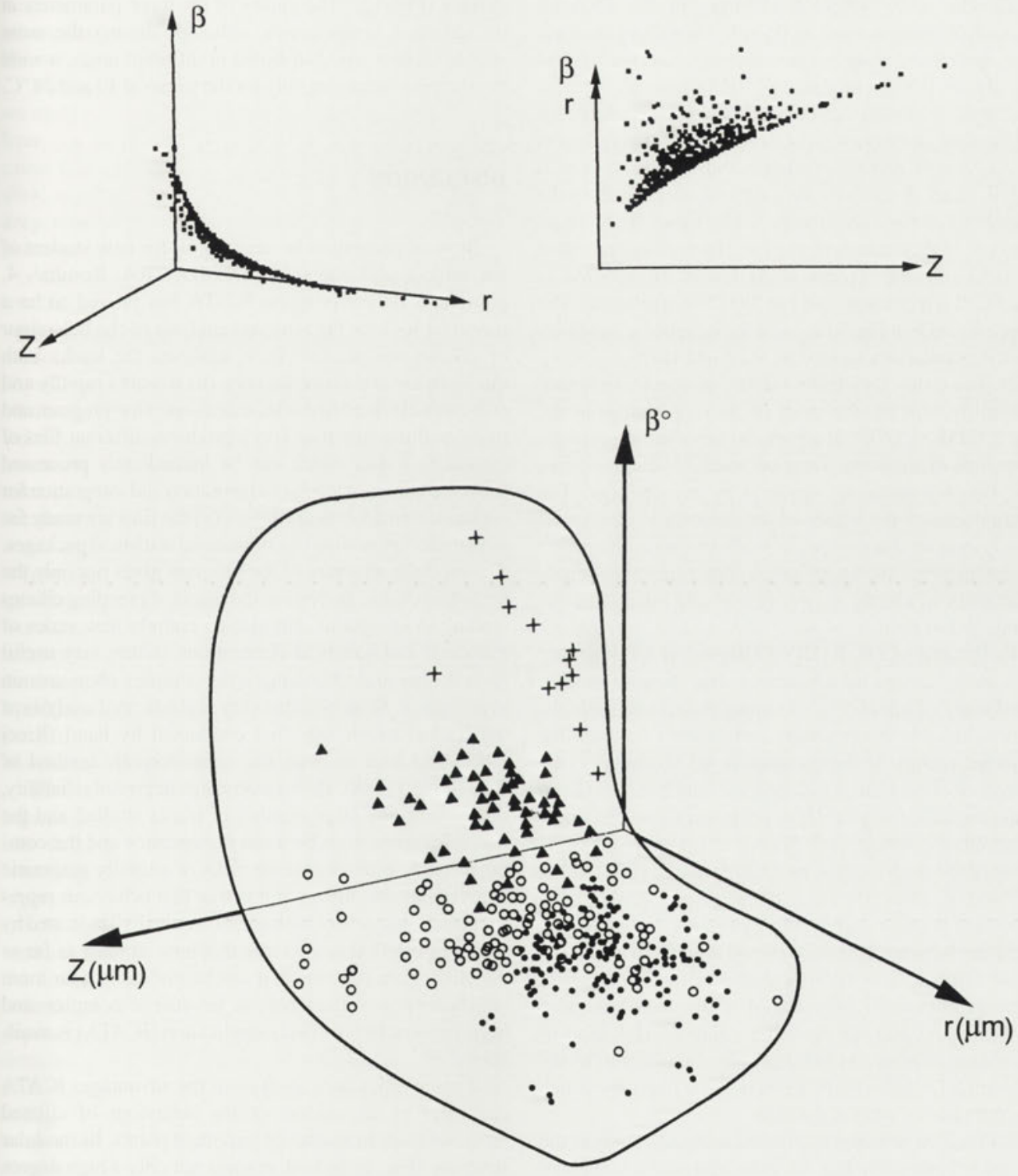


Fig. 12. The tridimensional distribution on a saddle surface of the three parameters (radius - r, μm; central angle - β, degrees; length - Z, μm) describing the leftward arcs performed by *O. bifaria* at the different temperatures studied; 9 °C - plus; 14 °C - triangle; 19 °C - open circle; 24 °C - closed circle. The same saddle surface is shown in the two upper graphs, in two different perspectives, for the sake of clarity

C. The utility IN-TRA (INdices of the TRAck). Recently 3 numerical indices (Fig. 9A) describing the track in its spatial and temporal development have been found (Ricci *et al.* 1998). The utility IN-TRA calculates them for each track (and with them creates new files, available for the operator, on request). As an example, 6 typical tracks of *E. crassus* and their relative indices are shown in Fig. 9. Panel A refers to the controls, after 1 h from the beginning of the experiments, while panel B shows an example of the controls after 24 h. The population treated by 0.12 μM Hg^{2+} creeps along tracks exemplified in Fig. 9C (1 h treatment) and Fig. 9D (24 h treatment). The panels E and F in Fig. 9 describe the equivalent examples for the populations treated by 0.25 μM Hg^{2+}.

D. The utility DIS-ETHO (DIStribution-ETHOgram: this utility, refers to the study of entire populations: cp. Fig. 2, ETHO-LOOP). It allows an immediate study of the frequency distribution of any parameter of the track files (cp. Fig. 6), whenever required by the operator. The distributions of the values of the velocity of *E. crassus* clearly indicate that the species is affected by 0.25μm Hg^{2+} treatment (Fig. 10: upper graph, control; lower graph, treatment), as clearly shown by both the means and the standard deviations.

E. The utility COR-ETHO (CORrelation-ETHOgram). This utility can be used whenever the operator needs a rapid study of linear (Fig. 11: upper panel) and multiplicative (Fig. 11: lower panel) correlation's between the different groups of the parameters which describe the behaviour of a certain population and which are contained in the track files. Figure 11, upper panel shows the linear correlation occurring in *O. bifaria* at 9, 14, 19 and 24 °C between the length of the backward motion (BM) of the SSR and its correction angle (α°). Figure 11, lower panel, shows the peculiar and extremely significant correlation occurring between the radius (r) and the central angle (β°) of the leftward arcs (A^-) measured in our experiments along the tracks of *O. bifaria* at 24° C (the same holds also for all the A^+ and for the other ciliates). This kind of correlation is defined as "multiplicative correlation" by the program STATGRAPHICS (version 3.0 Plus), by which it is possible to process the data.

F. Finally, a new approach to the analytical study of the A^+ and A^- is shown in Fig. 12. When the radii (r) are given on the abscissa, the corresponding central angles (β°) are on the ordinate and the relative lengths on the Z axis, the values at the different temperatures (9, 14, 19, 24 °C) are perfectly distributed on a tridimensional saddle surface (Fig. 12). The values of the three parameters at the different temperatures, although sharing the same saddle surface, are distributed in different areas, a wide overlapping occurring only for the values at 19 and 24 °C.

DISCUSSION

Beyond proving to be very useful for new student of the subject (didactic value, DEMO- TRA, Results - 4, paragraph B) the program ICATA has proved to be a powerful tool for the renewed analysis of the behaviour of ciliates we needed: (i) it analyses the tracks both qualitatively and quantitatively, (ii) it works rapidly and (iii) precisely due to the interactions operator-program and to its modular structure, (iv) it produces different files of quantitative data which can be immediately processed further at different levels of elaboration and integration for sophisticated analytical studies, (v) the files are ready for any further processing by commercial statistical packages. The modular structure of the program gives not only the complete classic analysis of the tracks of creeping ciliates leading to ethograms, but also an entirely new series of graphical and statistical elaborations, in turn very useful for a deeper understanding of the complex phenomenon investigated. Moreover this very sophisticated analysis of the tracks, which was first conducted by hand (Ricci 1981) and later on with the semiautomatic method of Russo *et al.* (1988), allows a very high degree of reliability, due to both the large number of tracks studied and the possible interactions between the operator and the computer itself, which avoid the risks of a totally automatic analysis. In conclusion, if it is true that behaviour represents that "pinnacle of biological complexity" indicated by Bonner (1988), it is also true that now, at least as far as the ciliates are concerned, it can be studied in a far more satisfactory way than before, because a complex and highly interactive software application (ICATA) is available.

A more articulated analysis of the advantages ICATA can offer to the student of the behaviour of ciliated protozoa leads to a series of important points. Its modular structure (Fig. 2), indeed, ensures not only a high degree of processing velocity, but also quite a precious saving in terms of potential available for other operation. Moreover the possibility of carrying out interactive behavioural studies, influenced in real time by the data analysed

themselves, makes ICATA a precious tool to study such a complex phenomenon like the behaviour is (Ricci 1981, Bonner 1988, Hinde 1996)

The fact that we still prefer to record by hand the tracks we study (instead of acquiring them automatically, directly from the videotape) must be discussed specifically because this phase of the analysis of the tracks, although slow, represents one of the most important phases for a deep, true knowledge and a complete understanding of the phenomenon by the operator.

Among the utilities, the KI-TRA utility deserves a specific consideration because it shows the trend of the velocity along the tracks, namely of the parameter directly expressing, point by point and instant by instant, the physiology of the creeping organism (Purcell 1977, Machemer and Dietmer 1987, Lueken *et al.* 1996). The velocity of the oxytricha creeping along the track (Fig. 7B) increases whenever it moves towards the favourable micropatch (positions: 13-27, 31-36, 38-52), while it decreases both when the organism moves away from the warm region (positions 36-38) and it has already reached it (position 36-38) and when it has already reached it (positions from 52 to the end of the track): Fig. 7B, in other words, reveals immediately the ethological mechanisms accounting for the adaptive crowding of the oxytricha on the warm micropatch: an oxytricha get closer by means of a positive orthokinesis and tends to remain in the favourable patch by a negative orthokinesis (coupled to a clear-cut klinokinetic behaviour, Fig. 7A, on the capillary).

The utility IN-TRA reveals immediately how the organism manoeuvres geometrically (i.e. along a more or less linear/coiled track: this reflects the state of the "steering wheel") and kinematically (namely moving at higher/lower velocities: this reflects the state of its "accelerator" in space and time). This easy way of analysing the tracks reveals immediately, for instance, that Hg^{2+} affects the locomotion of euplotes significantly. The effects, indeed, are both time (Fig. 9: D *vs.* C, F *vs.* E) and dose-dependent (Fig. 9: E *vs.* C and F *vs.* D) and they can be detected at the level of both the geometry and the kinetics of the tracks (cp the values of I_g and I_k in the six panels of Fig. 9: an ever clear analysis of the effects of Hg^{2+} can be made by comparing the values of the difference R_d (the displacement rate which combines I_g and I_k into a single figure; Fig. 9) and the distributions of the velocities (Fig. 10, lower panel: the treated populations creep at far lower and more widely distributed velocities than the controls).

The last utility to consider is COR-ETHO: the single linear correlation, occurring between the length of the BM of an oxytricha performing an SSR and the width of the correction angle of the same reaction at the four different temperatures tested suggests that a single sort of physiological response is at the basis of the diverse SSR performed differently under those experimental conditions. The multiplicative correlation occurring between the radius and the central angle of a certain arc has been found several years ago (Mugnaini *et al.* 1995): it is believed to reflect a basically physiological state of the experimental populations (Ricci 1996).

To draw a conclusion, it is possible to state that ICATA, although simpler from an algorithmic point of view than other more famous programs (Häder and Lebert 1985, Gualtieri *et al.* 1988, Baba *et al.* 1990, Häder and Vogel 1990) perfectly satisfies the needs of whomever may be interested into the analysis of the tracks, not only as far as the basic analysis producing the ethograms is concerned, but also from the point of view of the more sophisticated analyses possibly required by the operator. Such basically interactive and general purpose architecture, in turn, makes ICATA a tool perfectly suitable for the analysis of the tracks of any living object, provided that it moves on a bidimensional surface. In fact we analysed successfully by ICATA the tracks reported in the literature for the bacterium *E. coli* (Horward and Douglas 1972) and for granulocytes (Peterson and Noble 1972, Hall and Peterson 1979). Moreover during our comparative studies of the behaviour of *Heterolepidoderma* sp. (Gastrotricha, Chetonotoidea), the use of ICATA proved to be the critical link in the analytical chain which led us to describe its ethogram (Banchetti and Ricci 1998).

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ACTA
PROTOZOOLOGICA

Identification and Ontogenesis of the *nomen nudum* Hypotrichs (Protozoa: Ciliophora) *Oxytricha nova* (= *Sterkiella nova* sp. n.) and *O. trifallax* (= *S. histriomuscorum*)

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Summary. *Oxytricha nova* and *O. trifallax* were named and established as viable genetic systems (*via* frozen resting cysts) by molecular biologists, but never determined or described in a scientific way. Thus, their identity is unknown and both are *nomen nudum* species according to the International Code of Zoological Nomenclature. In the present paper, this bewildering situation is rectified by investigating offspring of the original populations. It is shown, by a detailed literature review and morphological and ontogenetical analysis, using live observation, silver impregnation and scanning electron microscopy, that both populations belong to a single morphotype, viz. *Sterkiella histriomuscorum* (Foissner, Blatterer, Berger and Kohmann, 1991), a cosmopolitan species very frequent in limnetic and terrestrial habitats. However, on the molecular level, *O. nova* and *O. trifallax* are very distinct, suggesting that they are different species. Thus, *S. histriomuscorum* is a complex of sibling species. For the sake of nomenclatural continuity and priority, we suggest identifying *O. trifallax* as *S. histriomuscorum* and establishing *O. nova* as a new species, *Sterkiella nova* sp. n. Both species are diagnosed by a combination of morphological, ontogenetical and gene sequence characters. Field populations of S. *histriomuscorum* should be designated as gene sequence "*Sterkiella histriomuscorum* complex" if no molecular data are available to decide whether they belong to *S. nova*, *S. histriomuscorum*, or to another not yet described species of the complex.

Key words: infraciliature, nomenclature, Oxytrichidae, *Oxytricha nova*, *Oxytricha trifallax*, sibling species, *Sterkiella histriomuscorum*, *Sterkiella nova* sp. n.

INTRODUCTION

Since self-splicing introns (ribozymes) were discovered in *Tetrahymena thermophila*, ciliates have become important models for molecular biologists and genome researchers (for review, see Cech 1990). Over the years, model systems have been established with several ciliate species to investigate important phenomena, such as gene scrambling and unscrambling, chromosome fragmentation, gene excision, and telomere function (Prescott 1994). Unfortunately, some of the models were based on organisms which had never been described in a scientific way, namely *Oxytricha nova* and *O. trifallax*. Although both organisms, which were obviously provisionally named,

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were used in many studies since 1980 (see list of synonyms in species descriptions), their identity is not known. Hence, they are *nomen nudum* species, according to articles 13 and 15 of the International Code of Zoological Nomenclature (1985). This situation is untenable not only because *nomen nudum* species do not exist in the official zoological literature but also because such species can hardly be re-sampled if the original strain should be lost.

In the present paper, the morphological identity of *Oxytricha nova* and *O. trifallax* is unscrambled, and both will be firmly established in accordance with the rules of the International Code of Zoological Nomenclature (1985). We shall show that they are sibling species of a *Sterkiella histriomuscorum* complex, which contains taxa that are very similar morphologically and ontogenetically but sufficiently different in several gene sequences to warrant recognizing at least two species, namely *Sterkiella nova* sp. n. and *S. histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991.

MATERIALS AND METHODS, NOMENCLATURE AND TYPE SLIDES

Origin of strains

Oxytricha nova (= *Sterkiella nova* sp. n.; see Nomenclature and Discussion): This species was recovered in 1995 from resting cysts frozen at -70°C by D. M. Prescott on 12.3.1986. It is not known whether the original culture was set up with one or several individuals, i.e. whether the cysts were from a clone or a population. For the morphological investigations, well-growing cultures were obtained by excysting several hundred cysts in Eau de Volvic (French table water) enriched with washed *Chlorogonium* cells and some crushed wheat grains to support growth of bacteria.

The original source of *S. nova* has been described by Klobutcher *et al.* (1981): "The organism used in work reported from this laboratory (University of Colorado at Boulder, Department of Molecular, Cellular, and Developmental Biology) before 1978 was isolated from a Boulder pond and was referred to as *Oxytricha* sp. This organism died out and was replaced in work reported in 1978 and since then by an organism isolated from water obtained from North Carolina and designated *Oxytricha nova*. The original *Oxytricha* sp. and the new *O. nova* are similar in most respects but sufficiently different to suggest that they are different species".

Oxytricha trifallax (= *Sterkiella histriomuscorum*; see Nomenclature and Discussion): a culture was obtained by S. M. Adl (University of British Columbia, Vancouver), who got the isolate from G. Herrick (Salt Lake City), one of the founders of *O. trifallax* (see below and Adl and Berger 1997). In our laboratory, the population was cultivated as described for *S. nova*.

Oxytricha trifallax was established as a viable (*via* frozen resting cysts) genetic system in the laboratory of G. A. Herrick by R. Hammersmith, who isolated it from the Jordan River in Indiana (USA) in the winter of 1985 (G. A. Herrick, Salt Lake City, Utah; pers. comm.). In the literature, *O. trifallax* was mentioned for the first time by Greslin *et al.* (1989) and Hunter *et al.* (1989). Later, Seegmiller *et al.* (1996) mentioned other sources and strains of *O. trifallax*: „Wild *O. trifallax* cells were collected from diverse limnetic sites in Indiana, cloned in the lab and placed into a single fertilely interbreeding group... A PCR screen for new IES-R alleles in 12 additional *O. trifallax* isolates...". These strains are, according to the molecular data, very similar to the Jordan River isolates of R. Hammersmith (see Fig. 3 in Seegmiller *et al.* 1996).

Morphological methods and terminology

Cells were studied *in vivo* using a high-power oil immersion objective (N.A. 1.32), differential interference contrast, and video microscopy. The infraciliature and other cytological details were revealed by protargol impregnation, methyl green-pyronin staining, and scanning electron microscopy. See Foissner (1991) for a detailed description of all methods mentioned.

Counts and measurements on silvered specimens were performed at a magnification of x 1000. *In vivo* measurements were conducted at a magnification of x 250 - 1000. Although these provide only rough estimates, it is convenient to give such data as specimens may shrink or become inflated in preparations (Table 1). Standard deviation and coefficient of variation were calculated according to statistics textbooks. Drawings of live specimens were based on free-hand sketches and videotape records, those of impregnated cells were made with a camera lucida.

Terminology is according to Berger and Foissner (1997). See this paper especially for numbering and designating of cirri and for diagnosis of genera presently assigned to the Oxytrichidae, to which *Sterkiella nova* (*Oxytricha nova*) and *S. histriomuscorum* (*O. trifallax*) belong.

Nomenclature

Nomenclature of the species and strains treated in this paper is extremely confused and difficult to follow for someone not familiar with the subject and the International Rules of Zoological Nomenclature. Thus, we provide an alphabetically sorted, two-sided index, which shows, in ***boldface***, the bonafide names and allows, for the sake of clarity, to dispense with quotation marks or complicated wordings in the following text. See Berger and Foissner (1997) for literature on original genus and species descriptions.

Histriculus Corliss, 1960: a valid oxytrichid genus characterized by a stiff body, confluent marginal cirral rows, and the lack of caudal cirri (see chapter "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*"). Type species (by original designation): *Paramaecium histrio* Müller, 1773.

Histriculus muscorum (Kahl, 1932) Corliss, 1960 is an outdated combination, that is, the species was assigned to the wrong genus; now it is ***Sterkiella histriomuscorum*** (see chapter "The *Sterkiella histriomuscorum* story").

Histrio Sterki, 1878: invalid because of homonymy (Corliss 1960). Type species (by original designation): *Histrio steinii* Sterki, 1878.

Histrio muscorum Kahl, 1932 is an invalid binomen because of homonymy; now it is ***Sterkiella histriomuscorum*** (see chapter "The *Sterkiella histriomuscorum* story").

Oxytricha Bory de Saint-Vincent, 1824: a valid genus characterized as described in Berger and Foissner (1997). See also chapter "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*"). Type species (by subsequent designation): *Oxytricha granulifera* Foissner and Adam, 1983.

Oxytricha nova, a *nomen nudum* species, is ***Sterkiella nova*** sp. n. in the present paper.

Oxytricha trifallax, a *nomen nudum* species, is ***Sterkiella histriomuscorum*** (Foissner *et al.*, 1991) Foissner *et al.*, 1991 in the present paper.

Sterkiella Foissner, Blatterer, Berger and Kohmann, 1991: Genus erected to contain some oxytrichid stylonychids erroneously assigned to *Histrio* and *Histriculus* (see chapters "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*" and "The *Sterkiella histriomuscorum* story"). Type species (by original designation): *Oxytricha cavicola* Kahl, 1935.

Sterkiella histriomuscorum (Foissner *et al.*, 1991) Foissner *et al.*, 1991 is the valid name for (i) *Histrio muscorum* Kahl, 1932, (ii) *Histriculus muscorum* (Kahl, 1932) Corliss, 1960, and (iii) the *nomen nudum* species *Oxytricha trifallax*.

Sterkiella histriomuscorum* complex** is presently composed of ***Sterkiella histriomuscorum and ***S. nova***.

Sterkiella nova sp. n. is the *nomen nudum* species *Oxytricha nova* in the previous literature.

Type slides

This chapter gives detailed information about the type material of the species and populations under discussion. All slides contain protargol-impregnated specimens and have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria (Natural History Museum of Upper Austria, Linz). Relevant specimens are marked by a black ink circle on the cover glass. The slides can be loan from the curator of the collections: Dr. Erna Aescht, Biologiezentrum des Oberösterreichischen Landesmuseums, Johann-Wilhelm-Klein-Strasse 73, A-4040 Linz, Austria.

Sterkiella nova Foissner and Berger, 1999 (this paper)

This is the *nomen nudum* species *Oxytricha nova* of the previous literature. The population deposited was obtained by D. M. Prescott as described in the Materials and Methods section of the present paper. Accession numbers: 1999/111 (Holotype; prepared with protocol B as described in Foissner 1991) and 1999/112 - 118 (Paratypes; prepared with protocols A and B as described in Foissner 1991). Remarks: The eight slides contain many excellently prepared morphostatic and dividing specimens from a pure culture.

Sterkiella histriomuscorum (Foissner *et al.*, 1991) Foissner *et al.*, 1991

(1) *Histriculus muscorum*, voucher slide from a population of a soil in the Austrian Central Alps. Accession number: 1981/10. Remarks: Detailed description of morphology in Foissner (1982). The slide contains several well-impregnated specimens of *H. muscorum* and many other ciliates because it has been made from material as obtained with the non-flooded Petri dish method.

(2) *Sterkiella histriomuscorum*, two voucher slides of the population from activated sludge in Austria. Accession numbers: 1993/75, 76. Remarks: Detailed description of morphology in Augustin and Foissner (1992). The slides contain many well-impregnated specimens (Foissner's method) of *S. histriomuscorum* and several other ciliates because they were made from a mixed sewage culture.

(3) *Histriculus muscorum*, four voucher slides of the populations investigated by Berger *et al.* (1985) from the Gastein area in Salzburg, Austria. Accession numbers: 1997/131 - 134. Remarks: The slides contain well-impregnated (Foissner's method) morphostatic and dividing specimens of *H. muscorum* and several other ciliates because they were made from non-flooded Petri dish cultures; only slide 1997/132 is from a more pure culture (population 4 in Berger *et al.* 1985), but contains only few dividers.

(4) *Sterkiella histriomuscorum*, one voucher slide from the Antarctic population studied by Petz and Foissner (1997). Accession number: 1997/130. Remarks: The slide contains many excellently prepared (Wilbert's method) morphostatic and dividing specimens from a wheat grain culture.

(5) *Sterkiella histriomuscorum* ("Oxytricha trifallax "), two **neotype** slides from the "Oxytricha trifallax" population described in the Materials and Methods section of the present paper. Accession numbers: 1999/109, 110. Remarks: The slides contain many excellently prepared (protocol A in Foissner 1991) morphostatic and dividing specimens from a pure culture.

Histriculus histrio (Müller, 1773) Corliss, 1960

This is the type species of the genus. Four **neotype** slides with protargol-impregnated (protocol A in Foissner 1991) specimens have been deposited. Accession numbers: 1999/61 - 64. Remarks: Detailed description in Berger and Foissner (1997) and Foissner and Gschwind (1998). The slides contain several well-impregnated morphostatic and

Table 1. Morphometric data from *Sterkiella nova*

Character[1]	Method[2]	x̄	M	SD	CV	Min	Max	n
Body, length	IV	122.0	119	9.7	7.9	106	137	29
Body, length	PF	98.1	97	7.2	7.4	87	113	29
Body, length	PW	128.5	132	17.2	13.4	85	150	29
Body, width	IV	57.2	58	4.4	7.7	50	62	29
Body, width	PF	46.5	48	7.0	15.0	25	60	29
Body, width	PW	59.8	60	8.9	14.9	36	75	29
Anterior somatic end to proximal end of adoral zone, distance	PW	52.7	55	7.0	13.2	40	62	29
Anterior macronuclear nodule, length	PW	28.6	29	5.1	17.8	17	39	29
Anterior macronuclear nodule, width	PW	14.0	14	2.6	18.3	9	18	29
Micronuclei, length	PW	3.9	4	0.6	14.5	3	5	29
Micronuclei, width	PW	3.8	4	0.6	14.8	3	5	29
Macronuclear nodules, number[3]	PW	2.0	2	0.0	0.0	2	2	29
Micronuclei, number	PW	1.4	1	0.7	47.4	1	4	29
Adoral membranelles, number	PW	34.2	34	2.0	5.7	30	39	29
Right marginal cirri, number	PW	21.5	21	1.7	7.7	18	25	29
Left marginal cirri, number	PW	19.9	20	1.4	6.9	17	23	29
Frontal cirri, number	PW	3.0	3	0.0	0.0	3	3	29
Frontoventral cirri, number	PW	4.0	4	0.0	0.0	4	4	29
Buccal cirri, number	PW	1.0	1	0.0	0.0	1	1	29
Postoral ventral cirri, number	PW	3.0	3	0.0	0.0	3	3	29
Pretransverse ventral cirri, number	PW	2.0	2	0.0	0.0	2	2	29
Transverse cirri, number[4]	PW	5.0	5	-	-	5	6	29
Caudal cirri, number	PW	3.0	3	0.0	0.0	3	3	29
Dorsal kineties, number	PW	6.0	6	0.0	0.0	6	6	29
Dikinetids in dorsal kinety 2, number	PW	19.7	20	1.4	7.0	16	22	29
Dikinetids in dorsal kinety 6, number	PW	5.8	6	1.2	20.3	4	8	29
4-day-old resting cysts, length	PW	45.6	47	4.5	9.8	38	57	34
4-day-old resting cysts, width	PW	45.4	47	4.6	10.2	38	57	34
4-weeks-old resting cysts, length	PW	44.6	44	4.3	9.6	36	53	42
4-weeks-old resting cysts, width	PW	43.5	44	4.4	10.0	36	53	42

[1] Data based on randomly selected, protargol-impregnated morphostatic specimens. Measurements in µm. CV, coefficient of variation in %; M, median; Max, maximum; Min, minimum; n, number of specimens investigated; SD, standard deviation; x̄ , arithmetic mean.
[2] IV, *in vivo* (from video tape records); PF, Foissner's (1991) protargol protocol; PW, Wilbert's (1975) protargol protocol.
[3] Of 286 specimens investigated, 265 had two macronuclear nodules, 13 had three, and 8 had only one.
[4] Of 30 specimens investigated, a single one had 6 transverse cirri.

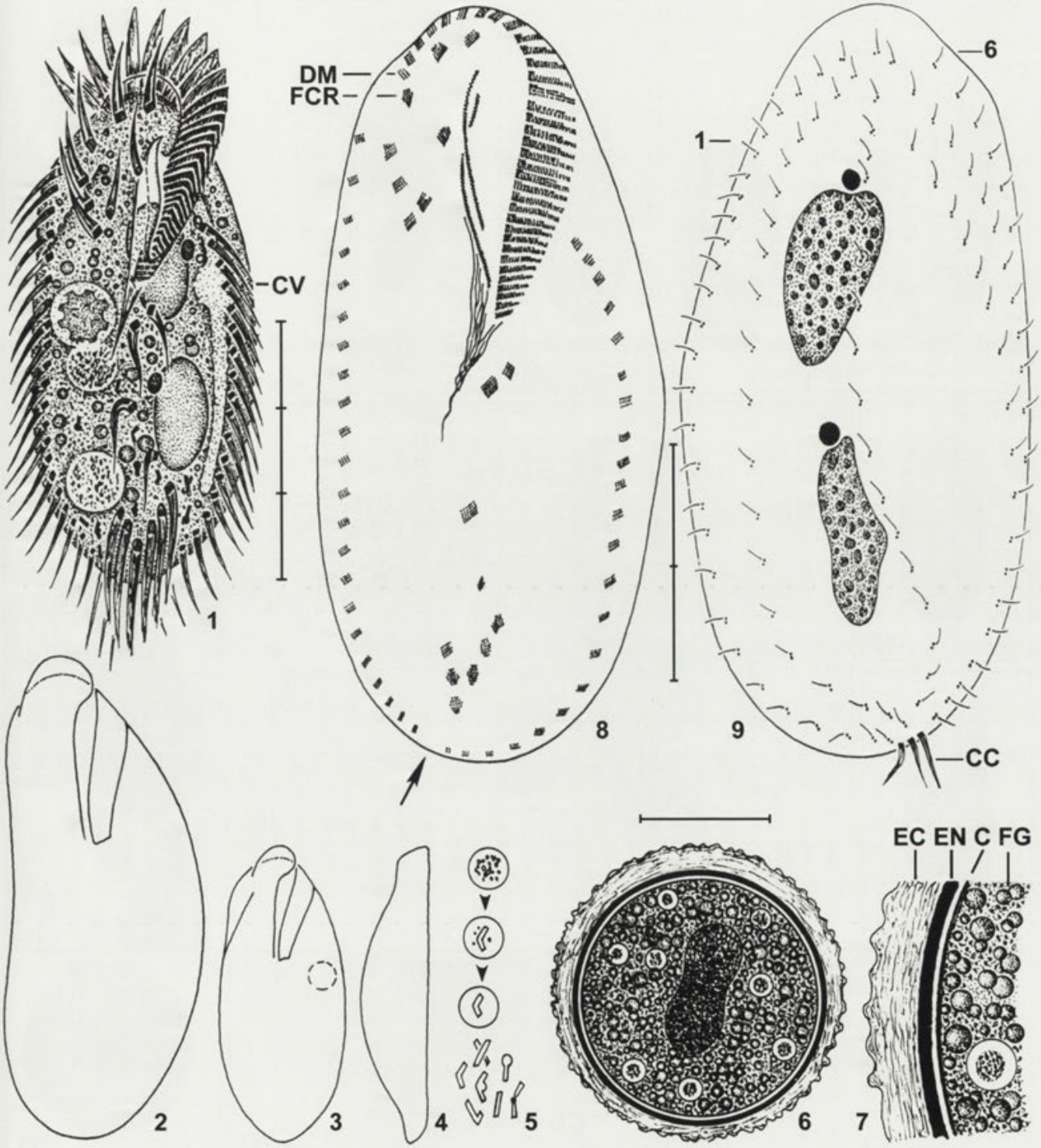
dividing specimens; they were obtained from field material and thus contain many other ciliate species.

RESULTS

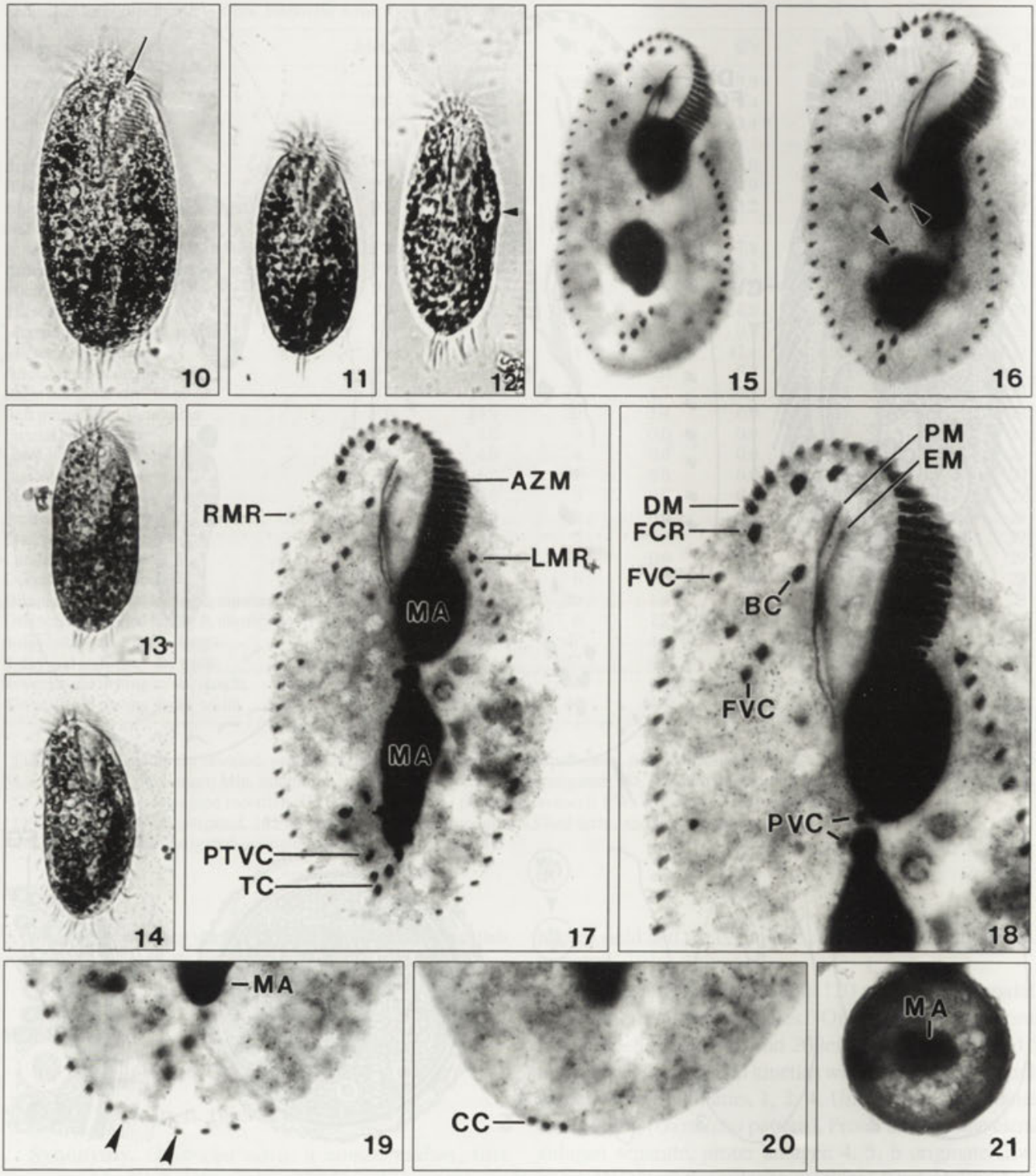
Sterkiella nova sp. n. (Figs. 1-30; Table 1)

Synonymy. *Oxytricha nova*, a *nomen nudum*, first mentioned in Klobutcher *et al.* (1981) and since then in many, mostly gene sequence and phylogenetic studies (see papers marked by asterisk in reference section). Very likely, Fig. 1b in Steinbrück (1986) does not show *O. nova* but a *Stylonychia* sp., as indicated by the large buccal field and the comparatively short, straight paroral membrane.

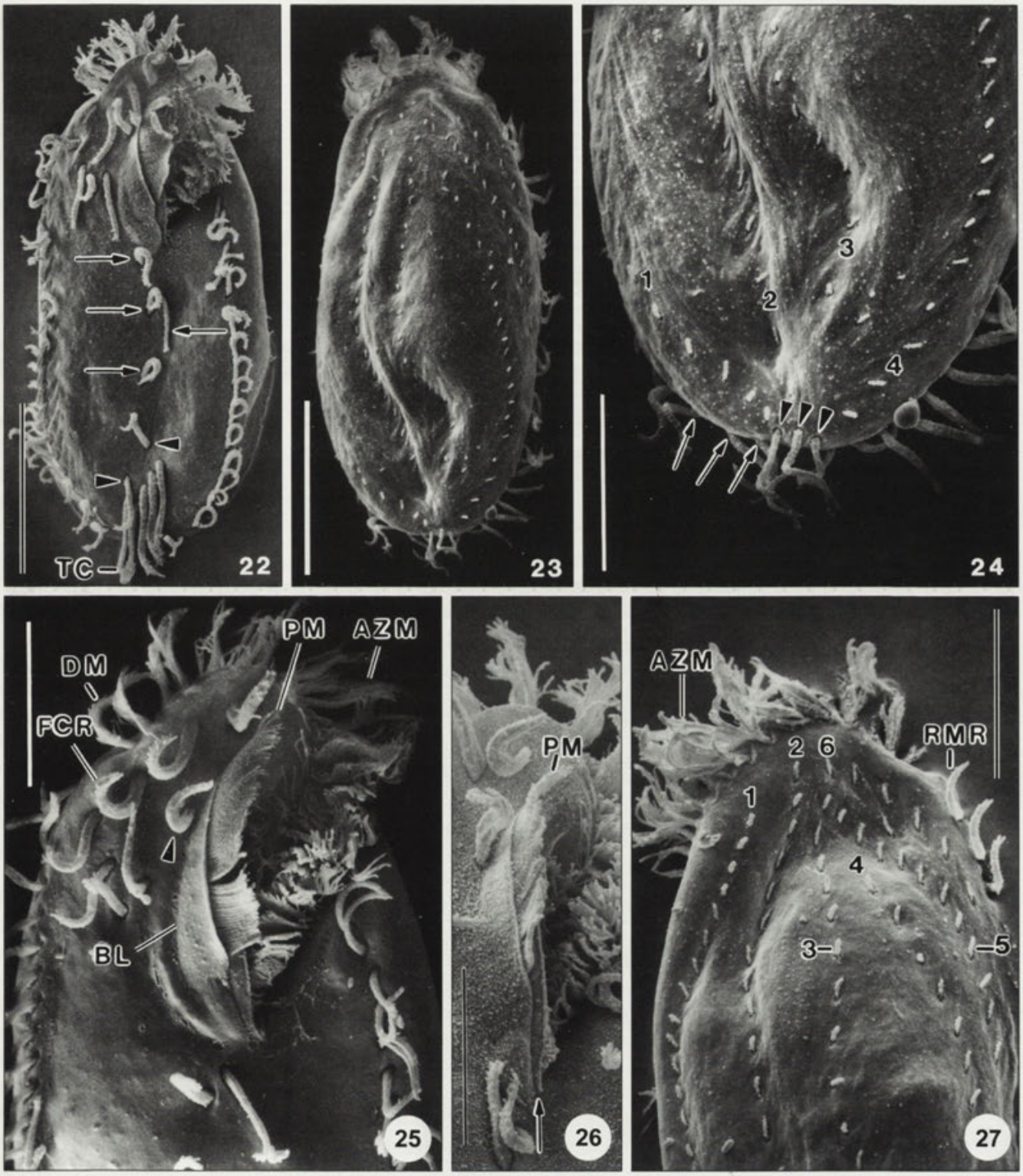
Diagnosis. Size *in vivo* about 120 x 60 µm, ellipsoidal. Two macronuclear nodules. On average 34 adoral membranelles, 21 right and 20 left marginal cirri, and 5 transverse cirri. 6 dorsal kineties with 1 caudal cirrus each associated with kineties 1, 2, 4. Undulating membranes intersecting (*Oxytricha* pattern). Proter and opisthe cirral anlagen separate, proter anlagen 4, 5, 6 originate from cirrus IV/3, opisthe anlagen originate from oral primordium (anlagen 1 - 3), cirrus IV/2 (anlage 4) and cirrus V/4 (anlagen 5, 6). Dorsal kineties generated in *Oxytricha* pattern. Complete nucleotide sequence of macronuclear DNA pol α gene described in Mansour *et al.* (1994) and



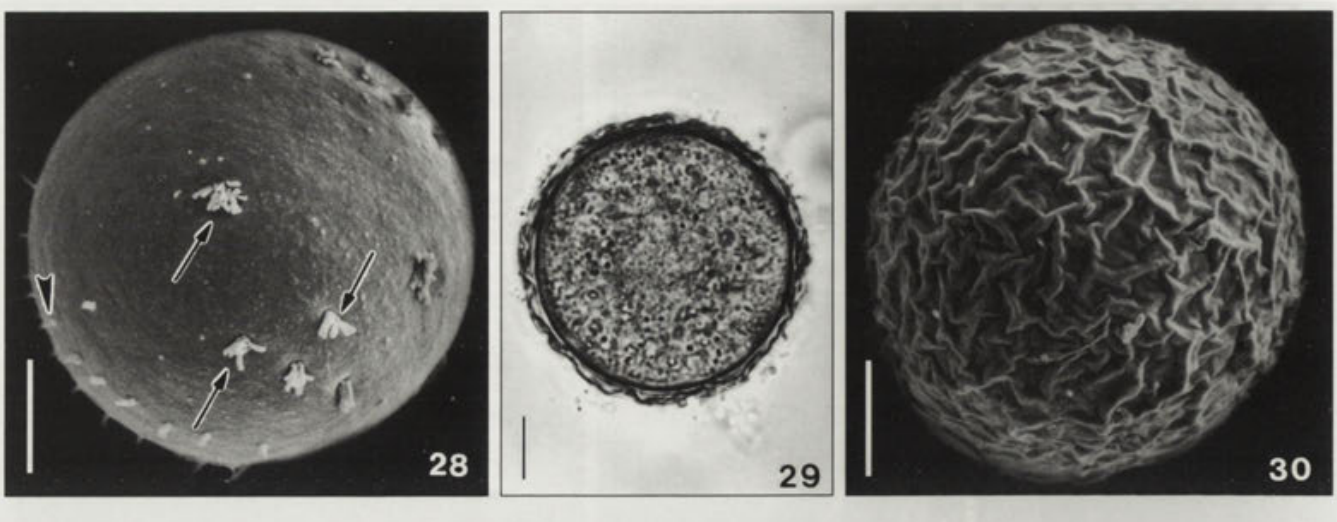
Figs. 1-9. *Sterkiella nova*, morphology of interphase cells and resting cysts from life (1-7) and after protargol impregnation (8, 9). 1 - ventral view of a representative specimen fed with a mixture of bacteria, *Chlorogonium* and wheat starch; 2, 3 - shape and size (135 μm, 110 μm) variants; 4 - narrow side view; *S. nova* is flattened dorsoventrally up to 2:1; 5 - the cytoplasmic crystals develop in small vacuoles; 6, 7 - one month old resting cyst with wrinkled surface and dumb-bell shaped macronucleus; 8, 9 - infraciliature of ventral and dorsal side. The posterior ends of the marginal rows are separated by a small gap (arrow), which is filled, on the dorsal side, by three inconspicuous caudal cirri. For detailed labeling of structures, see Figs. 17, 18, 25. C - cortex of cell, CC - caudal cirri, CV - contractile vacuole with two long collecting canals, DM - distalmost adoral membranelle, EC - ectocyst, EN - endocyst, FCR - right frontal cirrus III/3, FG - fat globules, 1, 6 - dorsal kineties. Scale bar division 20 μm



Figs. 10-21. *Sterkiella nova*, morphology of interphase cells and resting cysts from life (10-14), after protargol impregnation (15-20) and methyl green-pyronin staining (21). 10-14 - ventral views of freely motile specimens showing variability of shape and size (length 110-130 µm). Note narrow buccal field (arrow) and contractile vacuole (arrowhead); 15-18 - infraciliature of ventral side. Arrowheads denote postoral ventral cirri. Squashed, unmounted preparations, length of cells 110-140 µm; 19, 20 - posterior ventral and dorsal side to show location of caudal cirri (CC) on dorsal side between the ends of the ventral marginal rows (arrowheads); 21 - resting cyst showing fused macronuclear nodules. AZM - adoral zone of membranelles, BC - buccal cirrus, CC - caudal cirri, DM - distalmost adoral membranelle, EM - endoral membrane, FCR - right frontal cirrus III/3, FVC - frontoventral cirri, LMR - left row of marginal cirri, MA - macronucleus, PM - paroral membrane, PTVC - pretransverse ventral cirrus, PVC - postoral ventral cirri, RMR - right row of marginal cirri, TC - transverse cirri



Figs. 22-27. *Sterkiella nova*, morphology of interphase cells in the scanning electron microscope. 22, 23 - general ventral and dorsal view. Arrowheads mark pretransverse ventral cirri. The specimen shown in Fig. 22 has four postoral ventral cirri (arrows) instead of the usual three; 24 - posterior dorsal portion showing last cirri (arrows) of left marginal row, which are close to the caudal cirri (arrowheads) at the posterior dorsal margin of the cell (cp. Figs. 19, 20); 25, 26 - anterior ventral portion showing paroral membrane in cleft of buccal lip (arrow). Arrowhead marks buccal cirrus. 27 - anterior dorsal portion showing arrangement of dorsal kineties. AZM - adoral zone of membranelles, BL - buccal lip, DM - distalmost adoral membranelle, FCR - right frontal cirrus III/3, PM - paroral membrane, RMR - right row of marginal cirri, TC - transverse cirri. Numbers 1-6 denote dorsal kineties. Scale bars 40 μm (Figs. 22, 23) and 20 μm (Figs. 24 - 27)



Figs. 28-30. *Sterkiella nova*, resting cysts in the light (29) and scanning electron microscope (28, 30). 28 - encysting specimen with cirri (arrows) and dorsal bristles (arrowhead) still projecting from the forming, smooth cyst wall; 29, 30 - four weeks old resting cysts with wrinkled ectocyst. For labeling of structures, see Fig. 7. Scale bar division 10 μm

deposited in the Gene Bank sequence data base, accession number U 02001. Complete sequence of small subunit rRNA in Elwood *et al.* (1985).

Type location. Freshwater in North Carolina, USA.

Type specimens. One holotype slide and seven paratype slides (all protargol-impregnated) with morphostatic and dividing specimens of *S. nova* have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria, accession numbers 1999/111 - 118. Relevant specimens are marked by a black ink circle on the cover glass.

Etymology. "*nova*" (new) refers to a new isolate of an *Oxytricha* sp. (see Material section).

Interphase morphology (Figs. 1-30, Table 1). Morphometric data shown in Table 1 are repeated in this section only if needed for clarity. All observations are from cultivated material. Description will be very detailed, even containing generic characters, because it should serve not only ciliate taxonomists but also molecular biologists and biochemists usually not familiar with details of ciliate morphology and terminology.

Size in flourishing cultures *in vivo* about 100-140 x 45-65 μm, usually around 120 x 60 μm, very small specimens (< 100 μm) occur in declining cultures. Body ellipsoidal, right margin usually less convex than left, sometimes even concave (Figs. 2, 14), both ends broadly rounded, rarely bluntly pointed posteriorly (Figs. 3, 12, 14); dorsoventrally flattened up to 2:1, depending on nutritional state, ventral side flat, dorsal convex (Fig. 4). Body rather rigid, specimens with sharp-cornered injuries have been observed, very much like those known from *Stylonychia mytilus*. However, cells become rather flexible when overfed and, especially, when slightly squeezed by the cover glass. Hence, body rigidity must be observed in freely motile, untouched specimens and compared with that of common, flexible species, such as *Oxytricha* and *Urostyla* (for details on this character, see Berger and Foissner 1997). Macronuclear nodules in central portion of cell slightly left of midline, ellipsoidal (about 2:1), number slightly variable (Table 1), contain many 1-2.5 μm sized nucleoli. Micronuclei globular, near or attached to macronuclear nodules in variable positions, number highly variable (Table 1). Contractile vacuole slightly above midbody at left margin of cell, with one lacunar collecting canal each extending anteriorly and posteriorly (Fig. 1). No specific cortical granules. Cells colourless, however, well-fed specimens often appear dark in posterior half at low magnification (≤ 100 x) due to food inclusions and many fat globules 1-4 μm (usually 2-3 μm) across (Figs. 10, 11, 14); similarly, small cells from declining cultures usually contain black patches composed of hundreds of colourless to slightly yellowish, variably shaped crystals, which develop in small vacuoles from granular precursors and grow to a size of 2-5 μm (Figs. 5, 12). Feeds on green algae (*Chlorogonium*), bacteria, and wheat starch (Fig. 1). Movement moderately rapid, usually gliding to and fro on slide surface and bottom of culture dish, never rests.

Ventral and dorsal ciliary pattern (infraciliature) very constant, that is, 18 fronto-ventral-transverse cirri on

ventral side and 6 kineties (ciliary rows) on dorsal (Figs. 8, 9, 15-18, 22-24, 27; Table 1). Shape and size of cirral bases, as well as number of basal bodies (cilia) in individual cirri, in contrast, highly variable; specimens entirely identical in this respect were not observed, common structure shown in Figure 8.

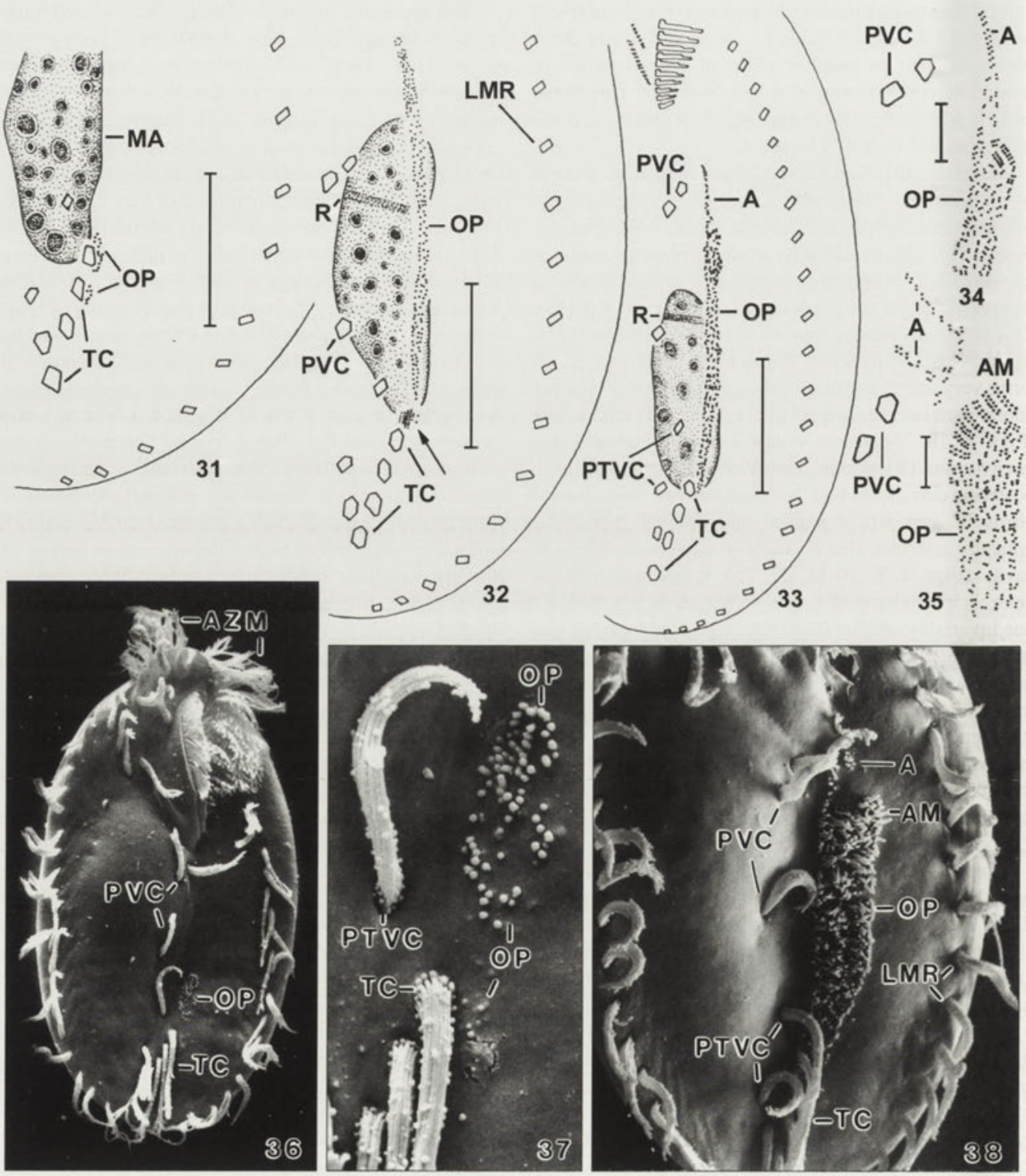
Marginal cirri *in vivo* about 18 µm long, size of cirral bases decreases posteriad, rather evenly spaced in one row each near right and left margin of cell, rows separated posteriorly right of midline by small, difficult to recognize gap seemingly occupied by caudal cirri, which, however, insert at posterior margin of dorsal side (Figs. 8, 9, 19, 20, 23, 24). Fronto-ventral-transverse cirri of similar size and length: frontal cirri about 20 µm long, rightmost (third) cirrus very near to and thus easily confused with distalmost adoral membranelle, especially in protargol preparations (Figs. 8, 18, 25); fronto-ventral cirri and buccal cirrus *in vivo* about 18 µm long, form V-shaped pattern because posterior cirri closer together than anterior ones; buccal cirrus in area where paroral and endoral membrane optically intersect, that is, slightly above mid of buccal cavity (Figs. 1, 8, 16-18, 22, 25); uppermost postoral ventral cirri underneath buccal vertex, close together, left one invariably smaller than right, separated by large gap from third (posterior) postoral ventral cirrus distinctly underneath mid-body; anterior pretransverse cirrus smaller than posterior one, which is very near to the rightmost transverse cirrus (Figs. 1, 8, 15-18, 22, 25); transverse cirri near posterior body end, *in vivo* 25-30 µm long and thus distinctly projecting beyond posterior body margin, distally frayed, form hook-like pattern (Figs. 1, 8, 10-17, 22).

Dorsal cilia *in vivo* 3-4 µm long, originate from anterior basal body of dikinetids comprising dorsal bristle rows (Figs. 9, 23, 24, 27). Rows 1-3 in left half of dorsal side, almost as long as body, follow curvature of body margin, except row 3, which curves right in posterior half producing rather large, barren area between kineties 2 and 3; row 4 commences subapically near midline of cell, curves to right margin in mid-body, and continues posteriad to right caudal cirrus; row 5 slightly shortened anteriorly, ends somewhat above or below mid-body; row 6 very short, on average comprising 6 dikinetids only (Table 1), terminates in anterior third of cell. Caudal cirri at posterior body margin right of midline, narrowly spaced, associated with dorsal kineties 1, 2 and 4 (see ontogenesis), inconspicuous because slender and only slightly longer (22 µm) than marginal cirri (Figs. 9, 19, 20, 23, 24, 60, 64).

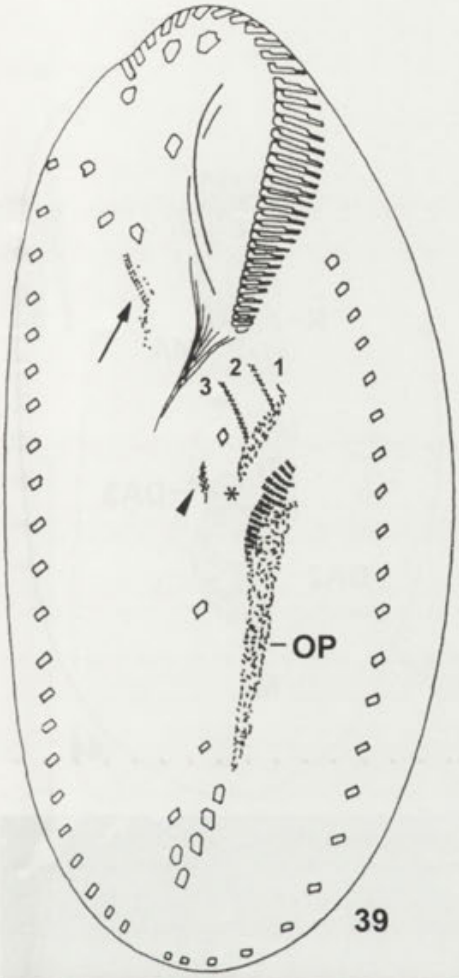
Oral apparatus in anterior left quadrant of cell, conspicuous because occupying about 41% of body length (Figs. 1, 8, 10-17, 22; Table 1). Adoral zone of membranelles commences subapically at right margin of body, curves along anterior body margin, and extends obliquely posteriad to midline of cell; adoral cilia *in vivo* about 20 µm long, bases of largest membranelles 11 µm wide, each membranelle composed of four ciliary rows with anterior rows successively shortened from left to right, frontal (distal) membranelles of different structure, as described by Augustin and Foissner (1992) in *S. histriomuscorum*: the distalmost membranelle, which is composed of three rows of equal length, is followed by four to five membranelles, which are also composed of three rows but have a fourth, shorter row attached to right mid-portion (Figs. 1, 8, 10-17, 22, 25, 67). Buccal cavity narrow and rather flat, slightly curved anteriorly, almost entirely covered by hyaline, lanceolate lip widening from anterior to posterior. Paroral and endoral membrane at right margin of buccal cavity, paroral near level of cell surface in deep cleft of buccal lip, endoral on bottom of buccal cavity, both slightly curved and possibly composed of tightly spaced dikinetids, intersect optically in anterior third of buccal cavity (Figs. 1, 10-14, 18, 22, 25, 26), as also evident from ontogenesis (Figs. 67, 69). Paroral cilia *in vivo* about 10 µm long, endoral cilia at least 15 µm long, form bundle beating into cytopharynx. Pharyngeal fibres inconspicuous, originate from posterior portion of endoral membrane and adoral zone of membranelles (Figs. 1, 8).

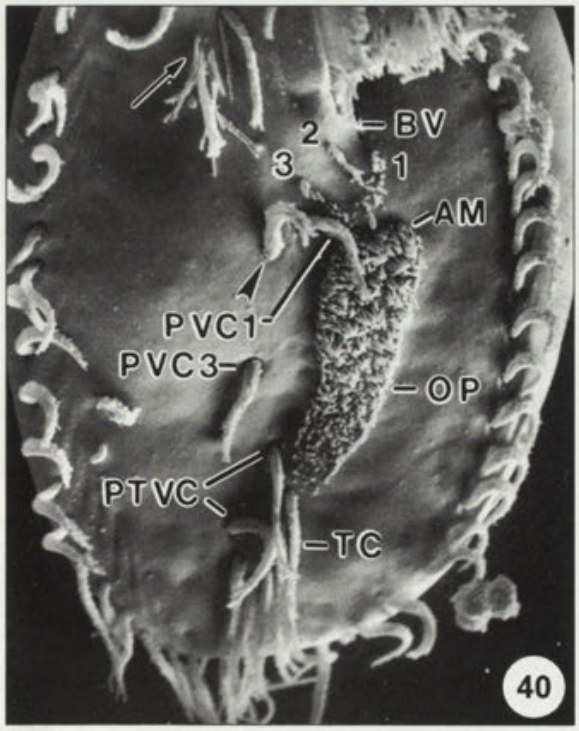
Resting cysts (Figs. 6, 7, 21, 28-30; Table 1). Permanent resting cysts spherical to slightly ellipsoidal, colourless, old cysts slightly smaller than young ones (Table 1). Ectocyst 1.5-3.5 µm, usually 2-3 µm thick, appears to be composed of many tightly spaced membranes, colourless and hyaline, surface smooth in very young cysts (Fig. 28), distinctly wrinkled when finished (Figs. 29, 30), stains lilac with methyl green-pyronin. Endocyst about 1 µm thick and with brownish shimmer, compact, separated from cortex of cell by narrow, hyaline zone. Cyst content comprises countless fat globules 1-2 µm across and some 3-4 µm sized vacuoles with granular, yellowish content, possibly food remnants. Macronuclear nodules fused to reniform or dumb-bell shaped mass (Figs. 6, 21).

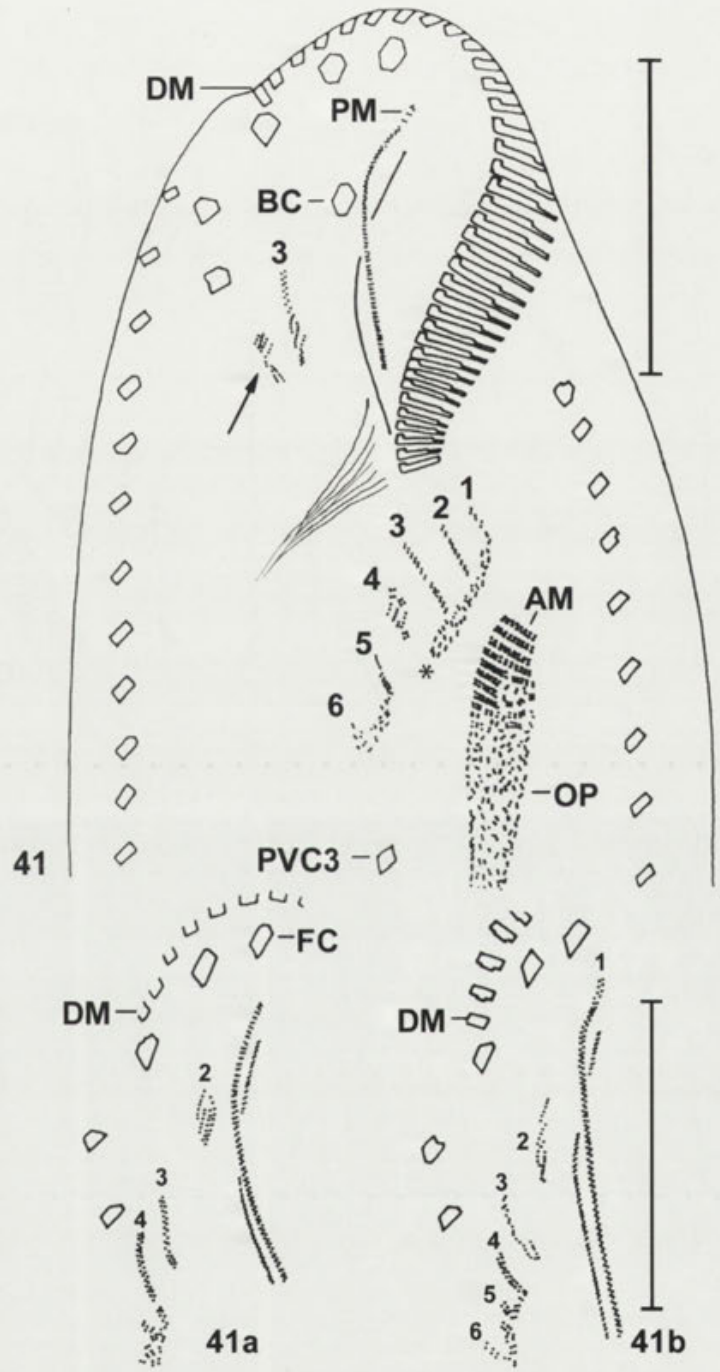
Divisional morphogenesis (Figs. 31-70). To make plain the changes during morphogenesis, old (parental) structures are depicted by contour, whereas newly formed structures are shaded black. For details, see also figure explanations.



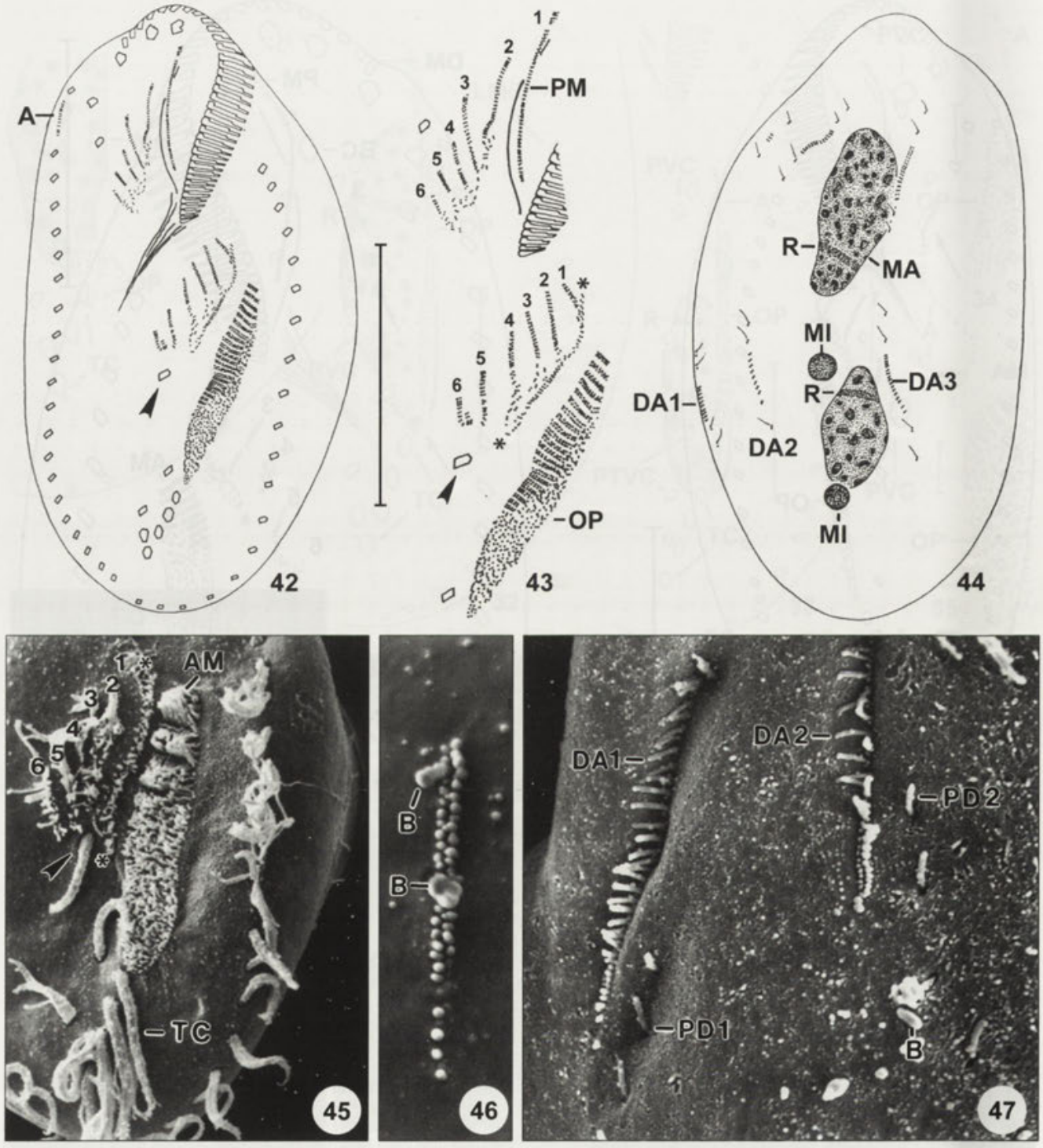
Figs. 31-38. *Sterkiella nova*, very early dividers after protargol impregnation (31-35) and in the SEM (36-38). 31, 36, 37 - basal bodies develop near the uppermost transverse cirri; 32 - an anarchic field of basal bodies develops between buccal vertex and transverse cirri. A supernumerary transverse cirrus (arrow) is incorporated into the oral primordium; 33 - 35, 38 - two cirral anlagen originate from the oral primordium. A - cirral anlagen, AM - adoral membranelles, AZM - adoral zone of membranelles, LMR - left marginal row, MA - posterior macronuclear bead, OP - oral primordium, PTVC - pretransverse ventral cirri, PVC - postoral ventral cirri, R - reorganization band, TC - transverse cirri. Scale bars 20 µm (Figs. 31-33) and 5 µm (Figs. 34, 35)



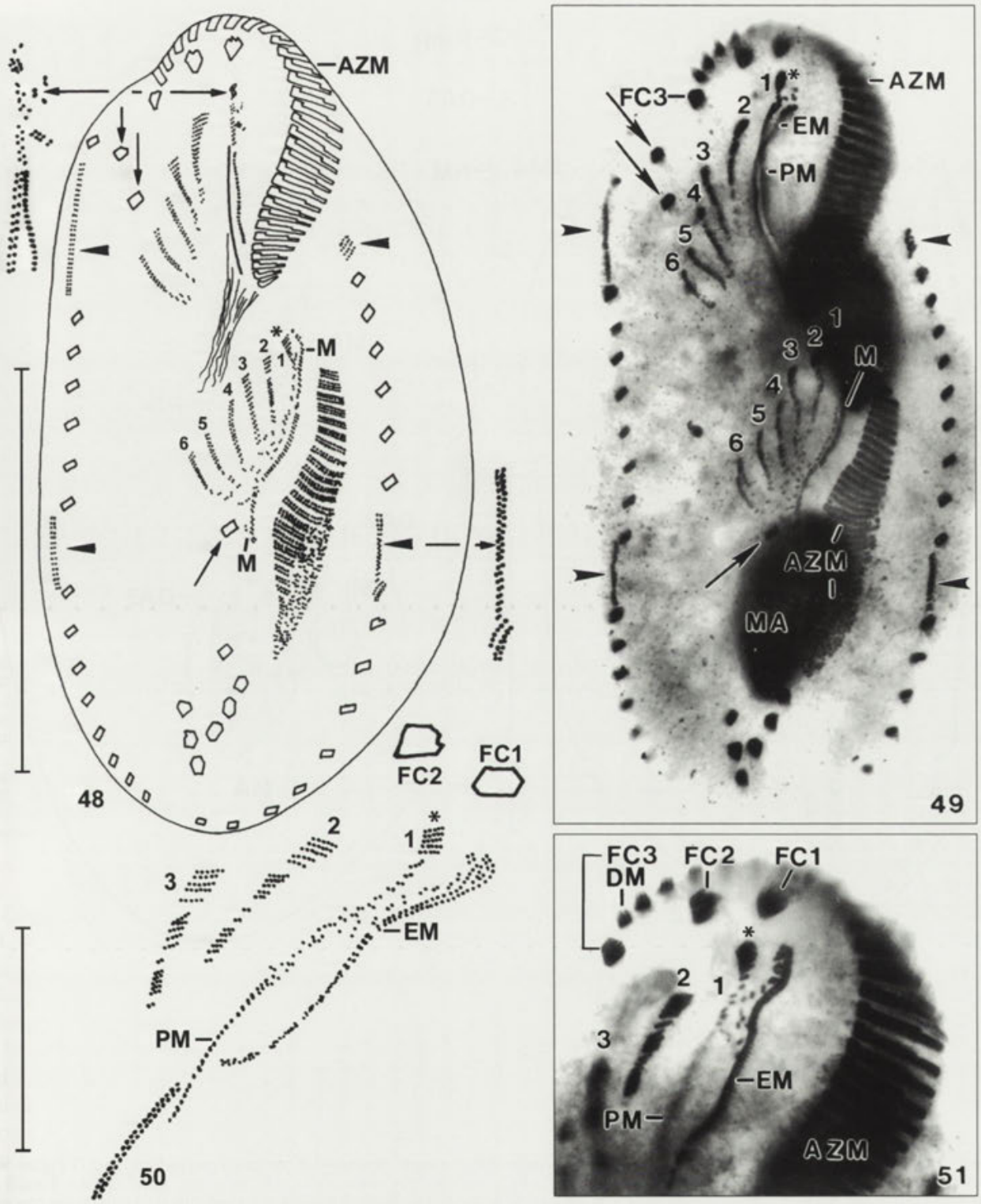




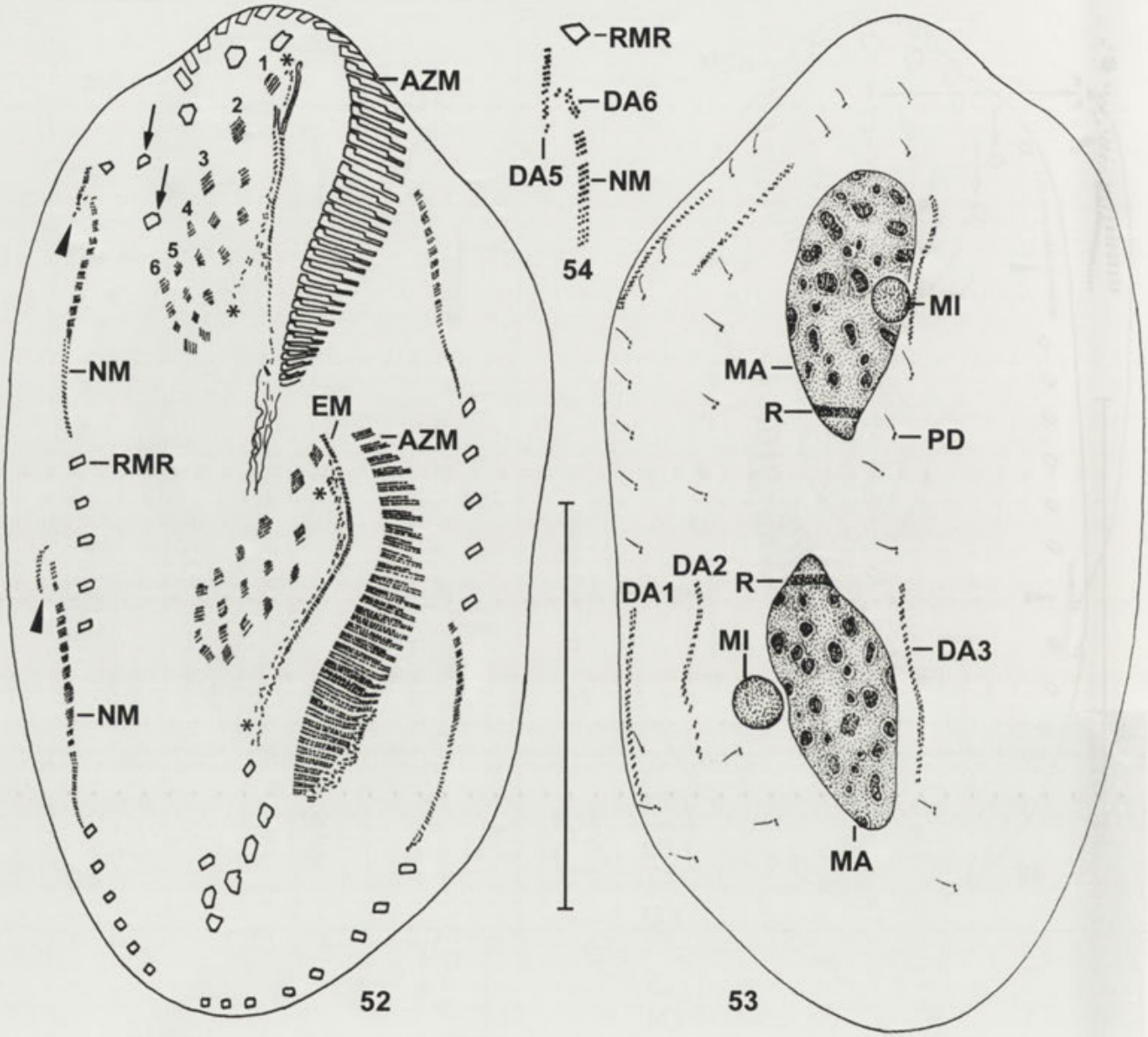
Figs. 39-41b. *Sterkiella nova*, ventral views of early dividers after protargol impregnation (39, 41, 41a, b) and in the SEM (40). Six (numbers 1-6) fronto-ventral-transverse cirral anlagen develop from parental cirri each in proter and opisthe. Arrows mark posteriormost frontoventral cirrus IV/3, which gives rise to proter cirral streaks 4, 5, 6 (Figs. 41a, b). Arrowheads denote postoral ventral cirrus V/4, from which opisthe's cirral streaks 5 and 6 originate. The parental paroral commences to reorganize at the anterior end (Figs. 41, 41b). In the opisthe, the new paroral and endoral are forming at the rear end of the cirral streaks (asterisks). AM - adoral membranelles, BC - buccal cirrus, BV - buccal vertex, DM - distalmost adoral membranelle, FC - frontal cirri, OP - oral primordium, PM - paroral membrane, PTVC - pretransverse ventral cirri, PVC 1, 3 - postoral ventral cirri, TC - transverse cirri. Scale bars 25 μm



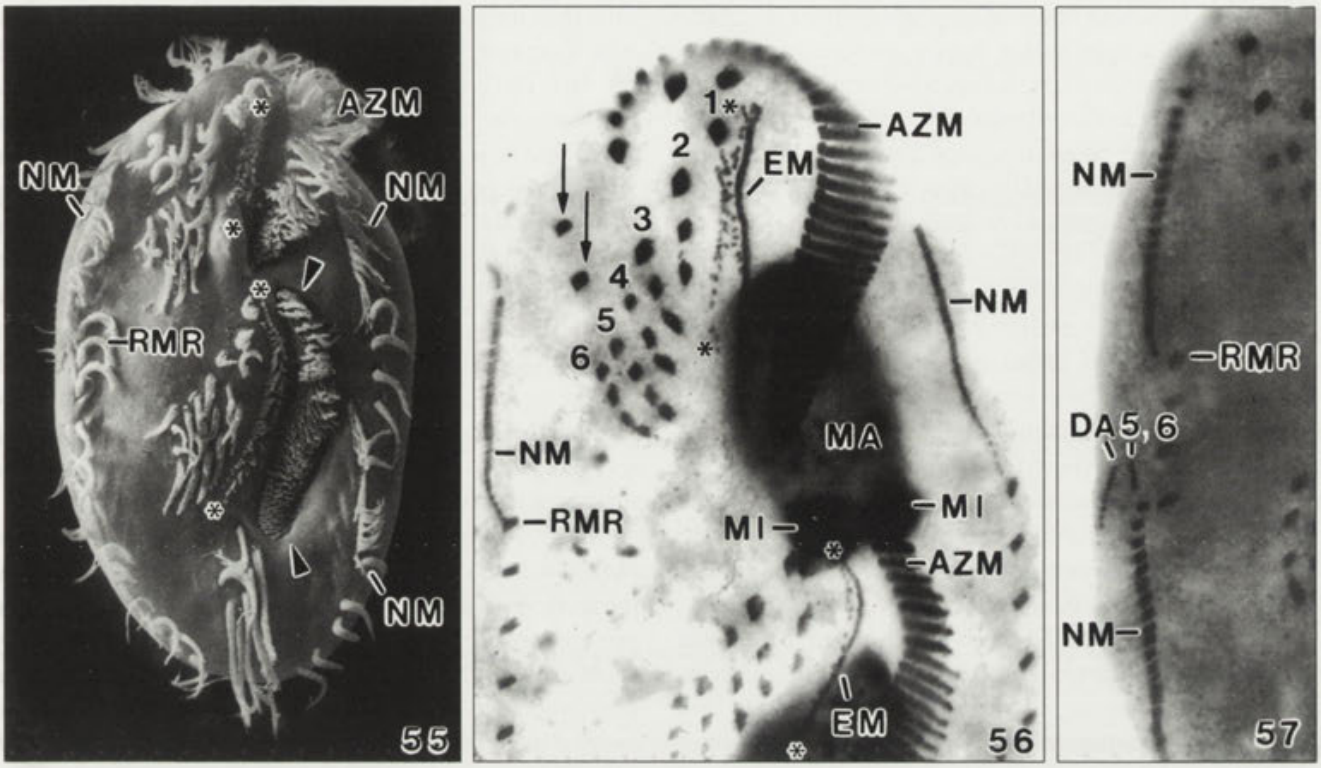
Figs. 42-47. *Sterkiella nova*, ventral (42, 43, 45) and dorsal (44, 46, 47) views of early-middle dividers after protargol impregnation (42 - 44) and in the scanning electron microscope (45-47). 42, 43, 45 - six fronto-ventral-transverse cirral anlagen each (numbers 1 - 6) are recognizable in the proter and opisthe, the second and third cirrus of the right marginal row form an anlage for a new marginal row, and a third row of basal bodies is added to the opisthe's anteriormost adoral membranelles, which commence to invaginate. Asterisks mark anlage for the opisthe's undulating membranes. Arrowheads mark third postoral ventral cirrus (V/3), which does not participate in anlagen formation; 44, 46, 47 - new dorsal kineties originate by anlagen formation within three parental rows. The parental bristles, which do not participate in anlagen formation (Fig. 47, not shown in Fig. 44), will be resorbed later. A - anlage, AM - adoral membranelles, B - bacterial rods from culture medium, DA 1 - 3 - anlagen for dorsal kineties, MA - macronuclear nodule, MI - micronuclei, OP - oral primordium, PD 1, 2 - parental dorsal bristle rows, PM - paroral membrane, R - reorganization band, TC - parental transverse cirri. Scale bar 50 μm (for Figs. 42, 44, which show the same specimen; Fig. 43 is an enlarged detail from Fig. 42)



Figs. 48-51. *Sterkiella nova*, ventral views of middle dividers after protargol impregnation. Figures 50 and 51 detail the reorganization of the proter's paroral and endoral membrane in a slightly later stage than shown in Figures 48 and 49. Six distinct fronto-ventral-transverse cirral anlagen each are now recognizable in the proter and opisthe. Arrows mark two frontoventral cirri (VI/3, VI/4) and a postoral ventral cirrus (V/3), which are morphogenetically inactive. The opisthe's undulating membranes are forming, while those of the proter are reorganizing. During these processes, the anterior end of the paroral splits Y-like; the right fork produces the new frontal cirrus I/1 (asterisks). Arrowheads denote anlagen within the marginal cirral rows; each of the anlagen will produce a new marginal cirral row. AZM - adoral zone of membranelles, DM - distalmost adoral membranelle, EM - proter endoral membrane, FC 1-3 - frontal cirri, M - opisthe undulating membranes, MA - macronuclear nodule, PM - proter paroral membrane, numbers 1-6 - anlagen for the fronto-ventral-transverse cirri. Scale bars 50 μm (Fig. 48) and 15 μm (Fig. 50)



Figs. 52-54. *Sterkiella nova*, ventral and dorsal view of a middle divider after protargol impregnation (cp. micrographs Figs. 55-57). Figure 54 is an enlarged part of Figure 52 and shows the anterior end of the new right marginal row of the proter, where the new dorsal kineties 5 and 6 are generated. The formation of adoral membranelles in the new adoral zone is almost finished and shaping of the individual membranelles proceeds from anterior to posterior. The endoral membrane forms slightly earlier than the paroral (asterisks), which shows a characteristic, oblique tail in the reorganizing proter. Cirri are forming in the fronto-ventral-transverse anlagen. Arrowheads denote anlagen for dorsal kineties 5 and 6 (for details, see Figs. 54, 57). Arrows mark morphogenetically inactive frontoventral cirri; note that cirrus V/3 has been resorbed (cp. Figs. 48, 49). The nuclear apparatus is still almost unchanged, except of the micronuclei, which are prophasic, and the reorganization band, which moved to the proximal end of the macronuclear nodules. AZM - adoral zone of membranelles, DA 1-6 - anlagen for dorsal kineties, EM - endoral membrane, MA - macronuclear nodules, MI - micronuclei, NM - anlagen for the new marginal rows, PD - parental dorsal kineties (only partially shown), R - reorganization band, RMR - parental right marginal row, numbers 1-6 - fronto-ventral-transverse cirral anlagen. Scale bar 50 µm



Figs. 55-57. *Sterkiella nova*, ventral views of middle dividers in the scanning electron microscope (55) and after protargol impregnation (56, 57). These micrographs supplement the camera lucida drawings shown in Figures 52-54. The new adoral zone of membranelles (arrowheads) is slightly but distinctly invaginated, while the reorganizing parental oral area has flattened. The forming (opisthe), respectively, reorganizing (proter) undulating membranes have shorter cilia than the six (numbers 1-6) fronto-ventral-transverse cirral anlagen, where cirri are forming. The endoral membrane forms slightly earlier than the paroral (asterisks), which has a characteristic, oblique tail in the reorganizing proter; the tail is unciliated and thus not recognizable in the scanning electron microscope (Fig. 55). Arrows mark morphogenetically inactive frontoventral cirri VI/3 and VI/4. Note large, prophasic micronuclei (Fig. 56) and anlagen for dorsal kineties 5 and 6, which are generated at the anterior end of the new right marginal rows (Fig. 57). AZM - adoral zone of membranelles, DA 5, 6 - anlagen for dorsal kineties 5 and 6, EM - endoral membrane, MA - macronuclear nodule, MI - micronuclei, NM - anlagen for the new marginal cirral rows, RMR - parental right marginal row, numbers 1-6 - fronto-ventral-transverse cirral anlagen

Stage 1 (Figs. 31, 36, 37). A few basal bodies develop left of the anteriormost transverse cirri, which appear intact both in the light and scanning electron microscope. Ciliary stubs are recognizable on these basal bodies, which belong to the oral primordium.

Stage 2 (Fig. 32). The basal bodies increase in number and form a long, narrow anarchic field (oral primordium), which extends between the buccal vertex and the transverse cirri. The macronuclear nodules show a reorganization band.

Stage 3 (Figs. 33-35, 38-40). A streak of basal bodies with ciliary stubs grows out from the right anterior end of the oral primordium, where dikinetidal adoral membranelles are formed from anterior to posterior (Figs. 33, 34, 38, 40). The streak then separates from the oral primordium, increases the number of basal bodies, and organizes to three oblique, dikinetidal cirral anlagen, which are connected posteriorly by scattered basal bodies, the prospective undulating membranes of the opisthe (Figs. 35, 39). While these cirral streaks are forming, the posteriormost frontal cirrus (IV/3) and postoral ventral cirrus V/4 disaggregate to cirral anlagen (Figs. 39, 40).

Stage 4 (Figs. 41-47). The oral primordium proceeds to differentiate adoral membranelles and a third row of basal bodies is added to the anteriormost membranelles, which slightly invaginate (Figs. 42, 43, 45). In the proter, the anterior portion of the paroral membrane and the buccal cirrus (II/2) generate cirral anlagen 1 and 2; cirral anlage 3 is formed by the penultimate frontoventral cirrus (III/2), and the anlagen 4, 5 and 6 are generated by the posteriormost frontal cirrus (IV/3), which disintegrates to a rather long, oblique streak (Figs. 39, 41a) assuming the shape of an extended letter W, when the anlagen grow out (Figs. 41b, 42, 43). The origin of proter anlagen 4-6 is

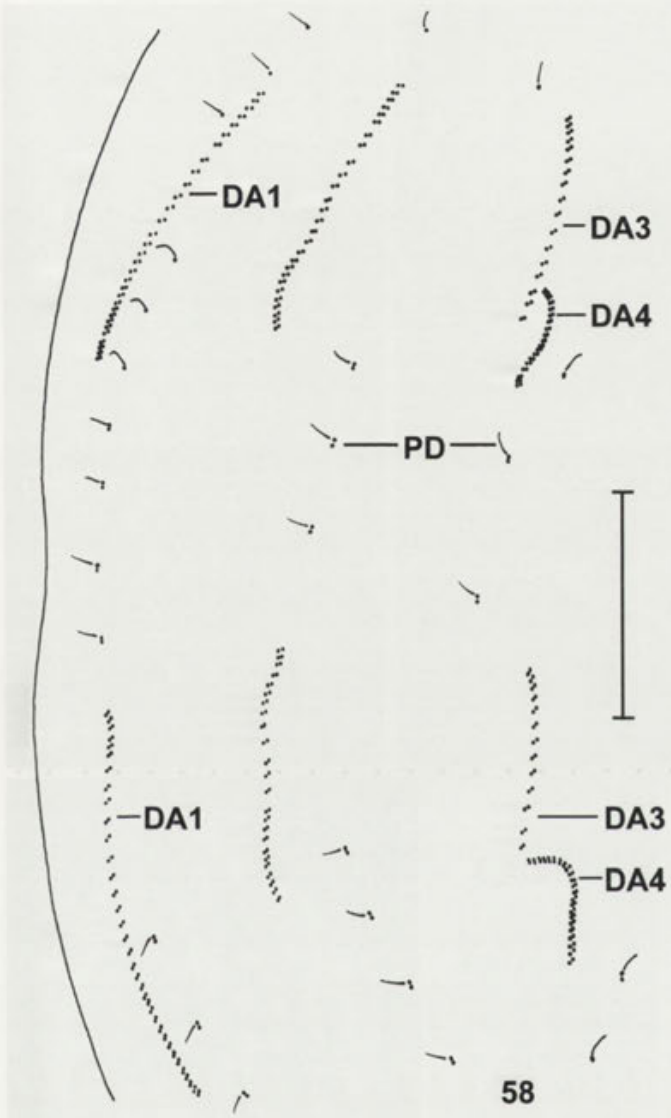
difficult to ascertain because they are formed comparatively fast, and appropriate stages are thus rare in the slides. In the opisthe, cirral anlagen 1-3 originate from the oral primordium as described above, anlage 4 is generated by the uppermost postoral ventral cirrus (IV/2), and the anlagen 5 and 6 are formed by cirrus V/4 (Figs. 41-43, 45). Thus, six cirral anlagen are recognizable in each the proter and opisthe (Figs. 42, 43). All anlagen lengthen by continued production of basal bodies, and ciliary growth proceeds posteriad in each anlage (Fig. 45). In all stages, the cirral streaks of proter and opisthe are distinctly separate. The second and third cirrus of the right marginal row reorganize to a dikinetidal anlage, which will become a new marginal row (Fig. 42). Dikinetids are proliferated intrakinetally in dorsal kineties 1-3 above and below the prospective division furrow; both basal bodies of the newly produced dikinetids generate cilia from anterior to posterior (Figs. 44, 46, 47).

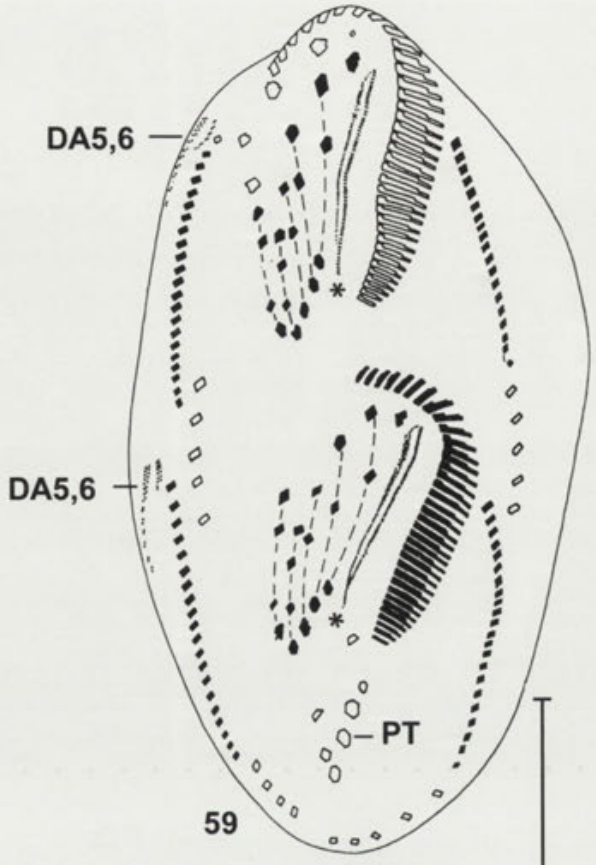
Stage 5 (Figs. 48-51). The formation of adoral membranelles in the oral primordium is still in progress. The cirral anlagen are now very distinct, cuneate, and commence to organize the individual cirri. Frontoventral cirri VI/3 and VI/4 and the posteriormost postoral ventral cirrus (V/3) are ontogenetically inactive and will be resorbed (Figs. 48, 49). The scattered dikinetids at the posterior end of the opisthe cirral anlagen arrange to a long streak, the prospective undulating membranes, right of the forming adoral zone. The parental undulating membranes reorganize completely from anterior to posterior, and the anterior portion of the paroral primordium generates the left frontal cirrus (I/1) in both proter and opisthe (Figs. 48, 50, 51). Four anlagen are now recognizable in the marginal rows (Figs. 48, 49): the proter anlagen develop at the anterior end of the rows, while the opisthe anlagen originate slightly underneath the prospective division furrow. These anlagen, which develop from parental marginal cirri and from anterior to posterior, will generate the new marginal rows.

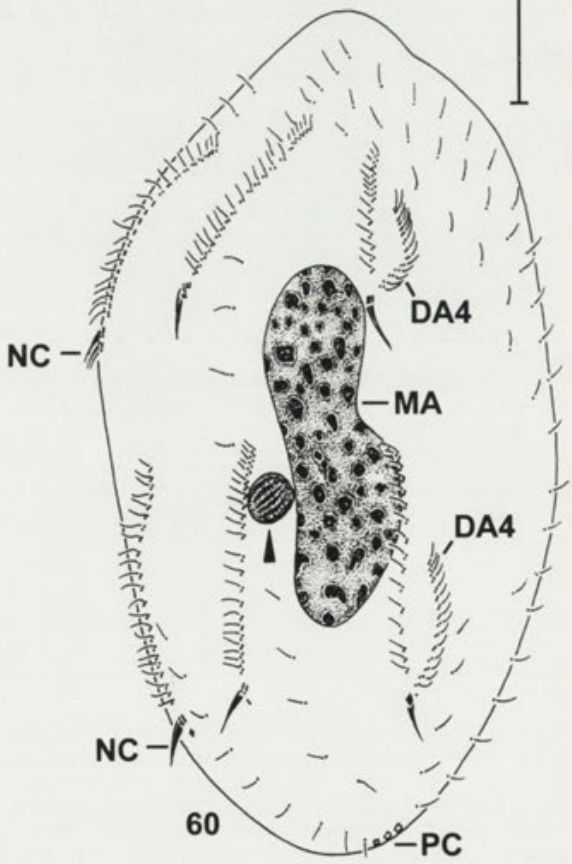
Stage 6 (Figs. 52-57). The new adoral zone of membranelles commences to invaginate (Fig. 55). The formation of adoral membranelles is complete, except for the posterior 5-10 membranelles. Shaping of the individual membranelles proceeds from anterior to posterior, that is, a third, slightly shorter row of cilia is added to each membranelle. The endoral membrane forms slightly earlier than the paroral, which shows a characteristic, oblique tail in the reorganizing proter, whose oral area has flattened (Figs. 52, 55, 56). The forming opisthe, respectively, reorganizing proter undulating membranes have shorter cilia than the six fronto-ventral-transverse cirral anlagen, which organize to cirri from anterior to posterior (Figs. 52, 55, 56). The anlagen for the marginal rows are complete and form cirri from anterior to posterior. At the anterior end of the new right marginal rows two short, dikinetidal streaks, the prospective dorsal kineties 5 and 6 develop (Figs. 52, 54, 57). These streaks do not evolve from parental marginal cirri, which are far away, but either *de novo*, or from the anterior end of the new marginal rows. The other dorsal anlagen are as described in stage 4, but slightly lengthened. The nuclear apparatus is still almost unchanged, except for the micronuclei, which are prophasic and thus rather large (Figs. 53, 56). The reorganization band has moved to the proximal ends of the macronuclear nodules.

Stage 7 (Figs. 58 - 65). The new adoral zone has obtained the final number of membranelles and its anterior third curves right and behind a minute, upright cortical process, the frontal scutum (Figs. 59, 61); a fourth, very short row of basal bodies is added to the mid-zone membranelles. In both proter and opisthe, the paroral and endoral membrane separate and lie side by side (Figs. 59, 61). The newly formed fronto-ventral-transverse cirri have been completed, that is, possess cilia as long as in interphase specimens, and are migrating to their mature positions (Figs. 59, 61). Likewise, the dorsal ciliature is completed by fragmentation of kinety 3, that is, the posterior third proliferates additional dikinetids, separates, and migrates to the left and then anteriad, forming dorsal kinety 4. Thus, the dorsal kineties of *S. nova* originate as follows (Figs. 58-60, 62-65): kineties 1-3 are generated by intrakinetal proliferation of dikinetids, kinety 4 originates by fragmentation of kinety 3, and kineties 5 and 6 are generated at the anterior end of the new marginal row. Caudal cirri are formed by condensation of dikinetids at the posterior end of kineties 1, 2, 4 (Figs. 60, 63-65). The cilium of the posterior basal body of the newly formed dorsal dikinetids has been reduced. The macronuclear nodules have fused and the micronuclei show distinct spindle microtubules (Fig. 60).

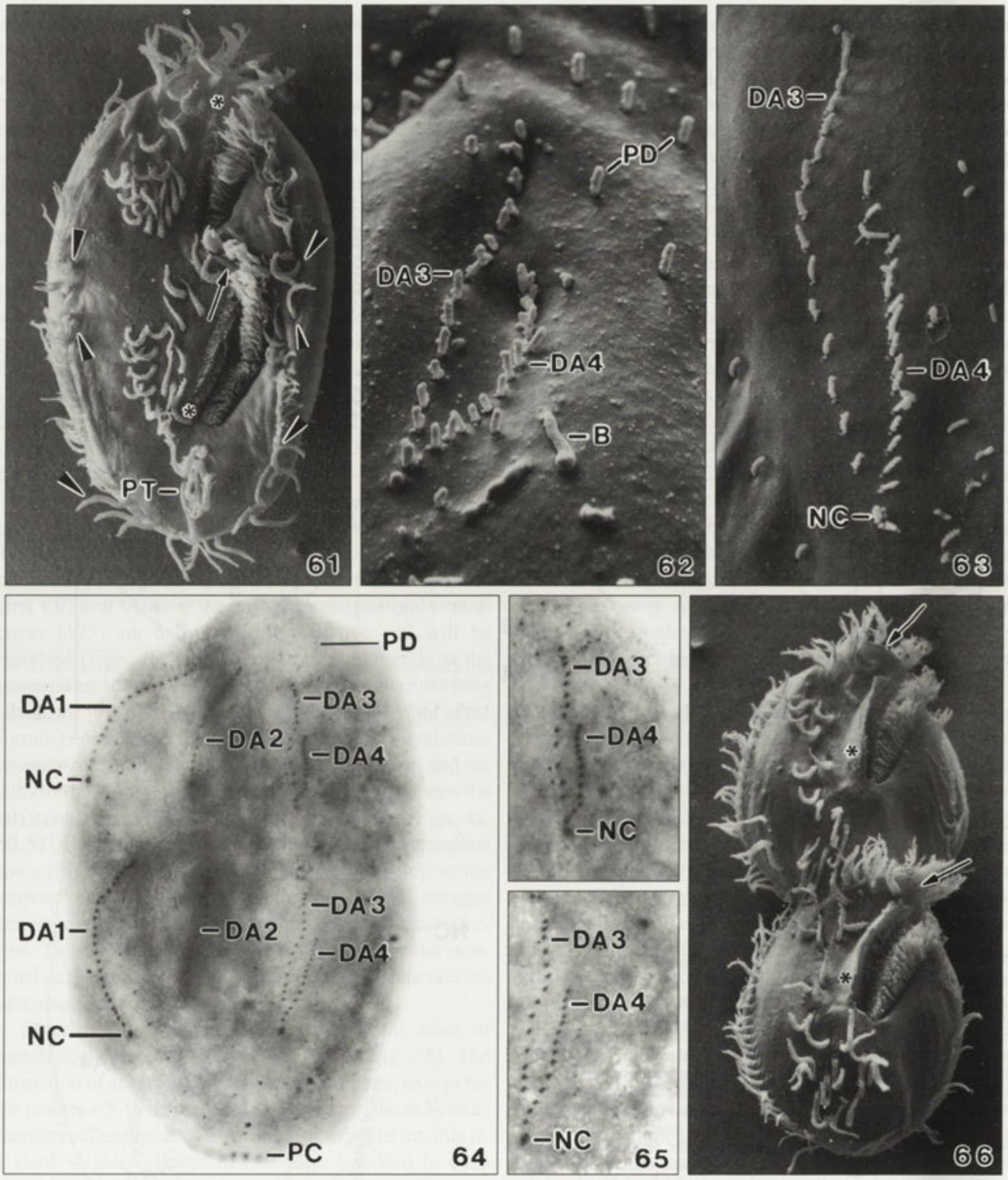
Stage 8 (Figs. 66-70). When cytokinesis commences, shaping of the new adoral membranelles and of the buccal cavity is completed in both proter and opisthe. The shaping of the buccal cavity causes the paroral and endoral membrane to become superimposed and to slightly but distinctly intersect optically in the anterior third when the cell is viewed ventrally (Figs. 66, 67, 69). The fronto-ventral-transverse cirri migrate apart (Fig. 67), obtaining the species-specific pattern and shape only after separa-



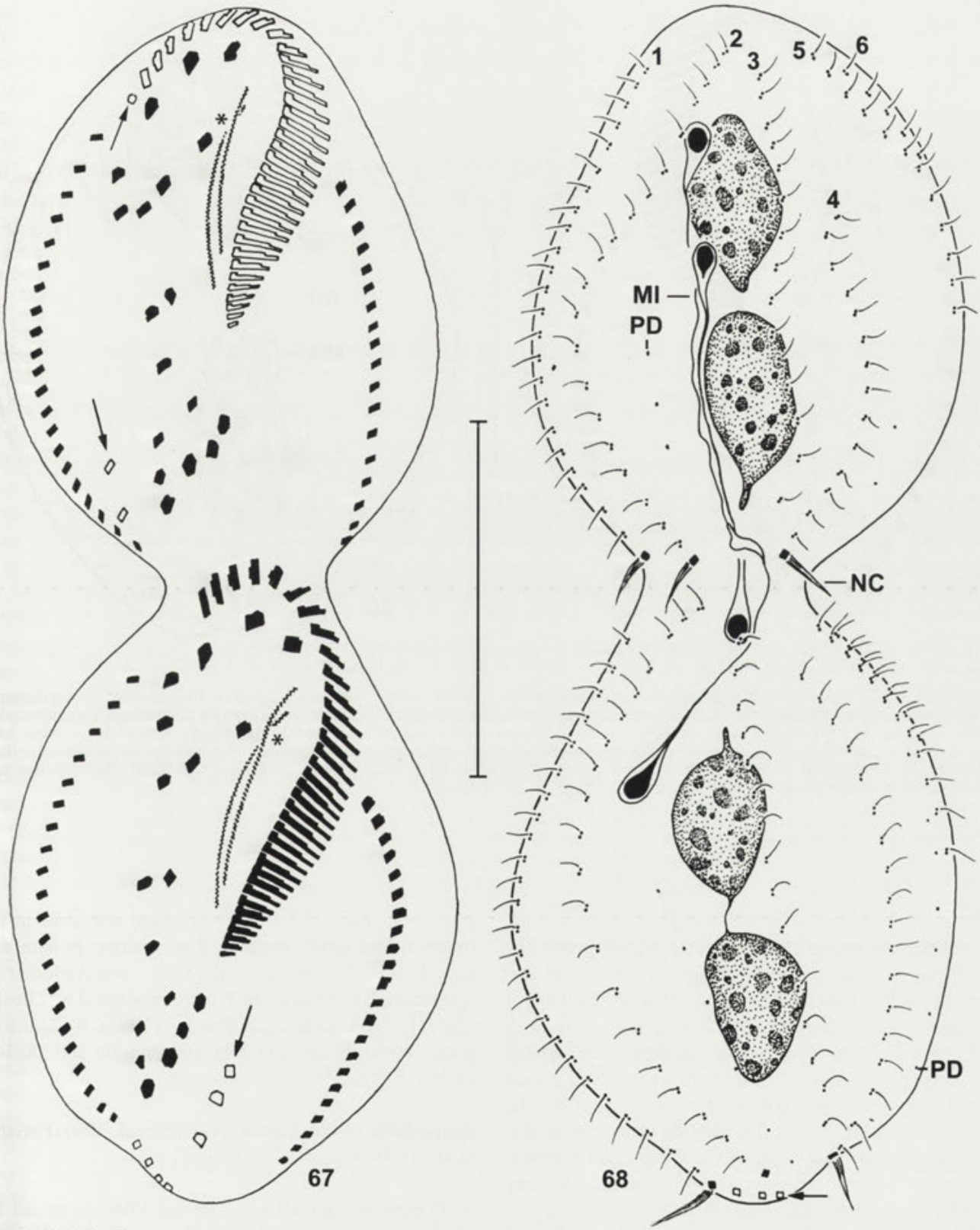




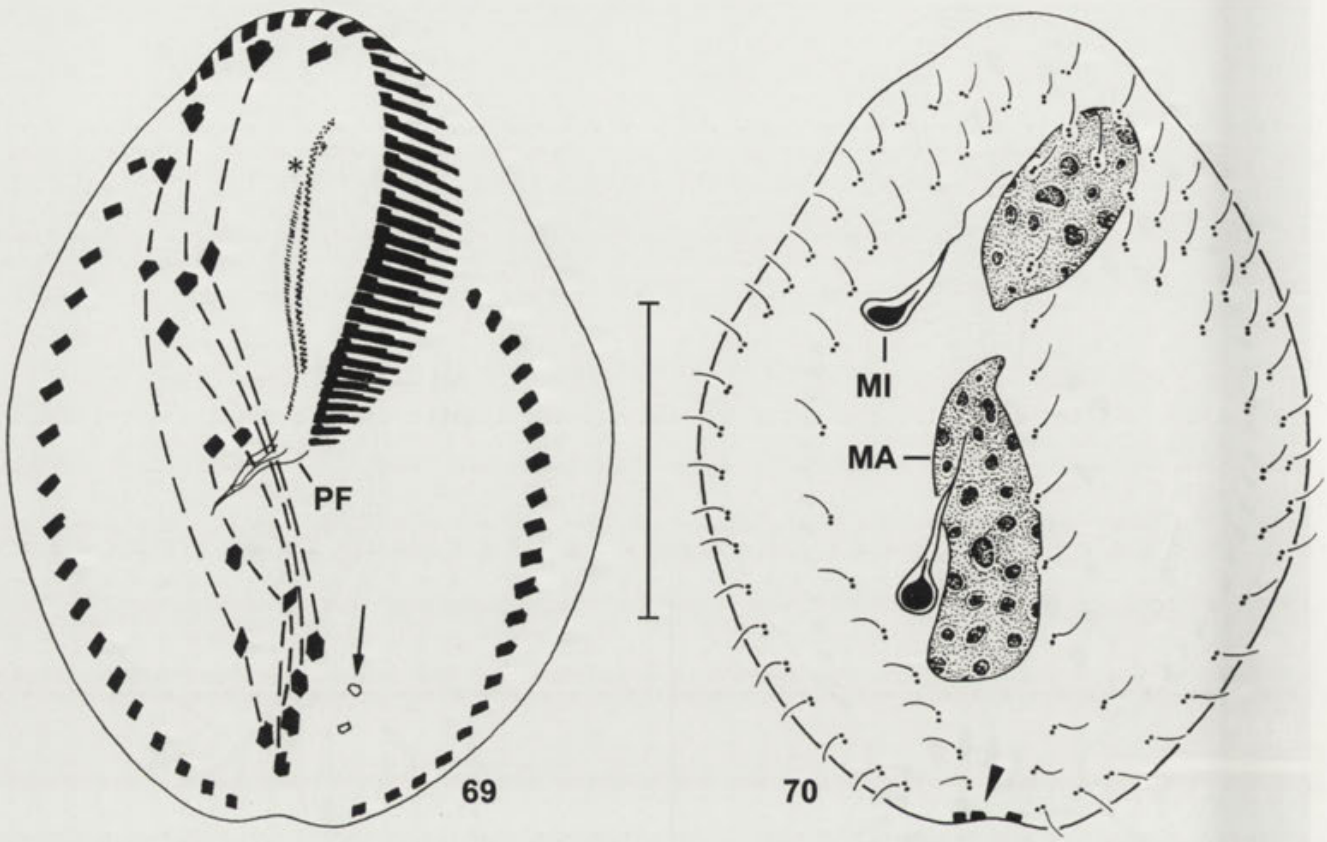
Figs. 58-60. *Sterkiella nova*, dorsal (58, 60) and ventral (59) views of middle dividers after protargol impregnation (cp. micrographs, Figs. 61-65). In both proter and opisthe, dorsal kineties 1 - 3 originate by intrakinetal proliferation of dikinetids (58, 60), dorsal kinety 4 is generated by fragmentation of kinety 3 (58), and dorsal kineties 5 and 6 originate at the anterior end of the new marginal rows (59). Caudal cirri are formed by condensation of dikinetids at the posterior end of dorsal kineties 1, 2, 4. Note that the posterior cilium of the new dorsal dikinetids has been resorbed (cp. Figs. 46, 47). Three main events occur on the ventral side (Fig. 59): the newly formed cirri migrate to their mature positions (hatched), the anterior portion of the new adoral zone of membranelles curves right, and the paroral and endoral membrane separate (asterisks). The macronuclear nodules have fused and the micronuclei (the specimen depicted in Fig. 60 has only one, arrowhead) show distinct spindle microtubules (60). DA 1-6 - anlagen for dorsal kineties, MA - macronucleus, NC - newly formed caudal cirri, PC - parental caudal cirri, PD - parental dorsal kineties, PT - parental transverse cirri. Scale bars 20 μm (Fig. 58) and 40 μm (Figs. 59, 60, which show the same specimen). Parental structures in contour, new shaded black



Figs. 61-66. *Sterkiella nova*, dividers in the SEM (61-63, 66) and after protargol impregnation (64, 65). 61 - ventral view of a middle divider (cp. Fig. 59): the newly formed cirri migrate to their mature positions, the frontal scutum develops (arrow), and the undulating membranes separate (asterisks). Arrowheads mark remnants of parental marginal rows; 62 - 65 - dorsal views of middle dividers (cp. Figs. 58, 60): dorsal kinety 4 originates by fragmentation of kinety 3. Figure 65 is an enlaged detail from Figure 64; 66 - ventral view of a late divider showing the frontal scutum (arrows) and shaping of the oral apparatus (asterisks). B - bacterial rod, DA 1-4 - anlagen for dorsal kineties, NC - newly formed caudal cirri, PC - parental caudal cirri, PD - parental dorsal kineties, PT - parental transverse cirri



Figs. 67, 68. *Sterkiella nova*, ventral and dorsal view of a late divider after protargol impregnation (cp. Figure 66). The shaping of the buccal cavity (Fig. 66) causes the paroral and endoral membrane to intersect optically (asterisks). Some parental cirri (arrows) which did not participate in anlagen formation are still present. The fused macronuclear nodules (Fig. 60) and the micronuclei divided. MI - micronuclei, NC - newly formed caudal cirri, PD - parental dorsal bristles, 1-6 - newly formed dorsal kineties. Scale bar 50 μm. Parental structures in contour, new shaded black



Figs. 69, 70. *Sterkiella nova*, ventral and dorsal view of an early opisthe postdivider after protargol impregnation. Early postdividers are distinctly smaller and broader than mature interphase specimens. Fronto-ventral-transverse cirri, which originated from the same anlage, are connected by hatched lines (cp. Fig. 59); most of them have not yet obtained the final location and shape as shown by the irregular outline. Arrow marks some not yet resorbed parental transverse cirri; arrowhead denotes newly formed caudal cirri. Note that the anterior portions of the paroral and endoral membrane intersect optically (asterisk), which is an important difference to *Stylonychia*. MA - macronuclear nodule, MI - micronucleus with adhering spindle microtubules, PF - growing pharyngeal fibres. Scale bar 30 μm

tion of the daughters (Fig. 69); the dorsal infraciliature, however, is complete already in late dividers (Fig. 68). The six fronto-ventral-transverse anlagen in the proter and opisthe each produce 18 cirri, the typical number for oxytrichids s. str. (Berger 1999): 1(1), 2(3), 3(3), 4(3), 5(4), 6(4). The parental cirri and dorsal bristles, which did not participate in anlagen formation, are resorbed in very late dividers and early postdividers (Figs. 67, 69). The fused macronuclear mass (Fig. 60) divides twice so that each offspring obtains two nodules. The divided micronuclei are still connected by a long, fine strand, possibly spindle microtubules (Figs. 68, 70).

Molecular data. The complete nucleotide sequence of the macronuclear DNA pol α gene, genes encoding actin I, histone H-4, β telomere protein and the telomere binding protein, number of internal eliminated segments in the micronuclear gene encoding β telomerase protein and actin I, as well as intron length of the α and β telomerase protein have been described and/or reviewed by Greslin *et al.* (1989), Hoffman and Prescott (1997a, b), Mansour *et al.* (1994), Prescott (1994), and Prescott and DuBois (1996). For details, see Discussion.

Sterkiella histriomuscorum (Foissner et al., 1991) Foissner et al., 1991 (Figs. 71-78, Table 2)

Synonymy (according to Berger 1999; as an aid for non-taxonomists, some explanations are included). 1932 *Histrio muscorum* Kahl, Tierwelt Dtl., 25: 617 (original description, Fig. 71); 1938 *Stylonychia curvata* - Giese

and Alden, J. exp. Zool., 78: 117 (misidentification); 1953 *Histrio similis* (Quennerstedt 1867) - Wenzel, Arch. Protistenk., 99: 113 (misidentification); 1954 *Oxytricha minor* - Mote, Proc. Iowa Acad. Sci., 61: 578, 588 (misidentification); 1956 *Opistotricha terrestris* Horváth, Arch. Protistenk., 101: 275 (synonym); 1957 *Oxytricha histrioides* Gellért, Annls Inst. biol. Tihany, 24: 20 (synonym); 1958 *Histrio macrostoma* Gellért and Tamás, Annls Inst. biol. Tihany, 25: 229, 240 (synonym); 1960 *Histriculus muscorum* (Kahl, 1932) - Corliss, J. Protozool., 7: 275 (replacement name for genus because *Histrio* was preoccupied); 1970 *Opistotricha terrestris* Horváth - Delhez and Chardez, Annls Spéléol., 25: 135 (redescription); 1970 *Histriculus muscorum* Kahl, 1932 - Dragesco, Annls Fac. Sci. Univ. féd. Cameroun (numéro hors-série): 116 (redescription); 1972 *Oxytricha bimembranata* Shibuya, 1929 - Matis and Danišková, Acta Fac. Rerum nat. Univ. comen. Bratisl., 17: 49 (misidentification); 1982 *Histriculus muscorum* Kahl, 1932 - Foissner, Arch. Protistenk., 126: 80 (Figs. 72, 73; authoritative redescription; 1985 *Histriculus muscorum* (Kahl, 1932) - Berger *et al.*, Protistologica, 21: 303 (morphometric comparison of four populations and description of morphogenesis); 1986 *Histriculus muscorum* Kahl, 1932 - Dragesco and Dragesco-Kernéis, Faune tropicale, 26: 483 (brief review); 1986 *Oxytricha terrestris* (Horváth, 1958) - Dragesco and Dragesco-Kernéis, Faune tropicale, 26: 471; 1989 *Oxytricha trifallax*, a *nomen nudum* species, first mentioned in the paper by Hunter *et al.* (1989), and since then used in the following studies: DuBois and Prescott (1995), Greslin *et al.* (1989), Klobutcher and Herrick (1997), Lingner *et al.* (1994), Prescott and DuBois (1996), Seegmiller *et al.* (1996, 1997), and Williams *et al.* (1993); 1991 *Oxytricha histriomuscorum* nom. nov. - Foissner *et al.*, Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, 1/91: 311 (replacement name for species because of objective homonymy); 1991 *Sterkiella histriomuscorum* (Foissner, Blatterer, Berger and Kohmann, 1991) nov. comb. - Foissner *et al.*, Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, 1/91: 311 (brief review and transfer to genus *Sterkiella* established in the same paper with *Oxytricha cavicola* Kahl, 1935 as type); 1992 *Histriculus muscorum* Kahl - Zou and Zhang, Acta zool. sin., 38: 345 (morphology and morphogenesis); 1992 *Sterkiella histriomuscorum* (Foissner, Blatterer, Berger and Kohmann, 1991) Foissner, Blatterer, Berger and Kohmann, 1991 - Augustin and Foissner, Arch. Protistenk., 141: 279 (redescription of a population from activated sludge); 1994 *Histriculus muscorum* Kahl, 1932 - Shin and Kim, Korean J. Zool., 37: 115 (redescription); 1997 *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) - Adl and Berger, Europ. J. Protistol., 33: 99 (life cycle); 1997 *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991 - Petz and Foissner, Polar Record, 33: 323 (morphology and morphogenesis of an Antarctic population).

Improved diagnosis. Morphology and morphogenesis very similar to *Sterkiella nova* (= sibling species). Differences in nucleotide sequences of the complete pol α gene and the small subunit rDNA significant, however (for details, see Discussion). Complete nucleotide sequence of macronuclear DNA pol α gene described in Hoffman and Prescott (1997b) and deposited in the Gene Bank sequence data base, accession number U59426.

Type material. No type material is available from Kahl's population of *Histrio muscorum* (now *Sterkiella histriomuscorum*, see Discussion). Clearly, Kahl's species needs an unambiguous identity to put an end to the existing confusion. Thus, we suggest fixing the *nomen nudum* species *Oxytricha trifallax* as neotype of *Histrio muscorum* Kahl, 1932. Two neotype slides with protargol-impregnated morphostatic and dividing specimens of *Oxytricha trifallax* (now *Sterkiella histriomuscorum*, see Discussion) have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria, accession numbers: 1999/109, 110.

Morphological and molecular biological characterization. The morphology and ontogenesis of *S. histriomuscorum*, as described by Berger *et al.* (1985) and Petz and Foissner (1997), and of its *nomen nudum* synonym, *Oxytricha trifallax*, are very similar to those of *S. nova* described above. Hence, there is no need for a detailed (re)description; main morphometric and morphologic characters of nine populations are compiled in Table 2 and Figures 72 - 78. The life cycle and standardized growth conditions are described in Adl and Berger (1997). Note that the *Histriculus muscorum* of Matsusaka's group is another species, namely *Sterkiella cavicola* (Foissner *et al.* 1991, Nakamura and Matsusaka 1991).

As concerns *Oxytricha trifallax* (now *Sterkiella histriomuscorum*, see nomenclature), the following small differences to *Sterkiella nova* should be mentioned: (1) the caudal cirri of *O. trifallax* are slightly larger than those of *S. nova*; (2) the buccal cirrus is often nearer to the anterior end of the paroral in *O. trifallax* than in *S. nova*; (3) the paroral and endoral intersect near mid of buccal cavity in *O. trifallax* and in the anterior third in *S. nova*;

(4) *Oxytricha trifallax* is, on average, slightly smaller than *S. nova* in most morphometric characters (Table 2), including the resting cysts (diameter 35-45 µm, x̄ 38.9, SD 2.8, CV 7.1%, n 28). However, data must not be over-interpreted because they were obtained with different methods. For instance, specimens prepared with Foissner's protargol protocol are distinctly smaller (length 98 µm) than those prepared with Wilbert's protocol (length 129 µm, Table 1). The first value (98 µm) matches most other data well (Table 2).

Ontogenesis is also very similar in *Oxytricha trifallax* and *S. nova*, although there are small differences in the temporal relationships of the events. A representative example is shown in Figures 41 and 76: both populations (species) agree in the opisthe development but differ in the development of the proter anlagen 4, 5, 6, which are more advanced in *O. trifallax* than in *S. nova*.

The molecular composition of *O. trifallax* has been described and/or reviewed by Doak *et al.* (1997), Hoffman and Prescott (1997a, b), Klobutcher and Herrick (1997), Prescott (1994), Prescott and DuBois (1996), Seegmiller *et al.* (1996), and Witherspoon *et al.* (1997). For details, see Discussion.

Sterkiella histriomuscorum is very frequent in limnetic and, especially, terrestrial habitats. It has been recorded from all main biogeographical regions (Foissner 1998). Very likely, it has a broad ecological range; however, some of the range might be caused by different, morphologically inseparable species.

Conjugation

In both, *Sterkiella nova* (*Oxytricha nova*) and *S. histriomuscorum* (*O. trifallax*), conjugation was never observed under the culture conditions used. Likewise, no sexual processes occurred when cultures of *S. nova* and *S. histriomuscorum* were mixed, indicating that they cannot mate. Prescott (1994) could not find mating types, but observed selfing from time to time in laboratory cultures. Usually, all of the cells that result from selfing die without resuming vegetative growth.

DISCUSSION

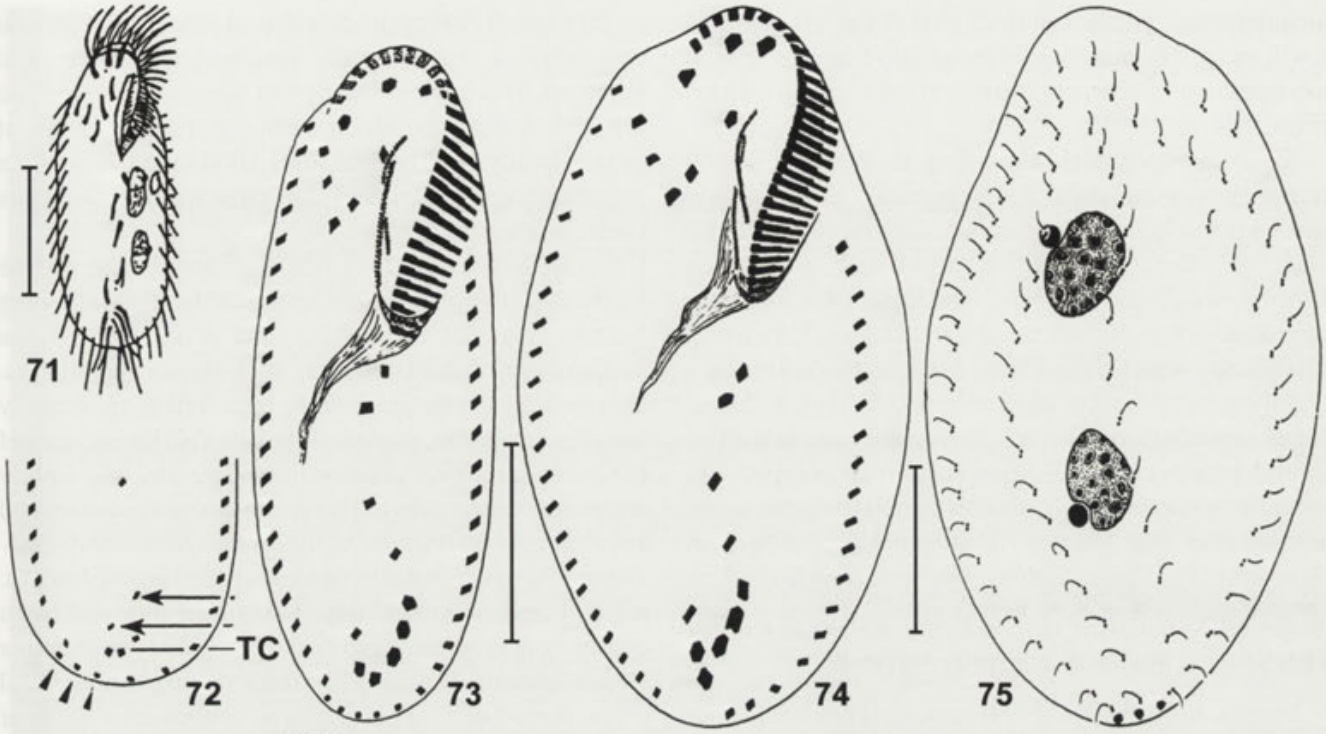
Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*

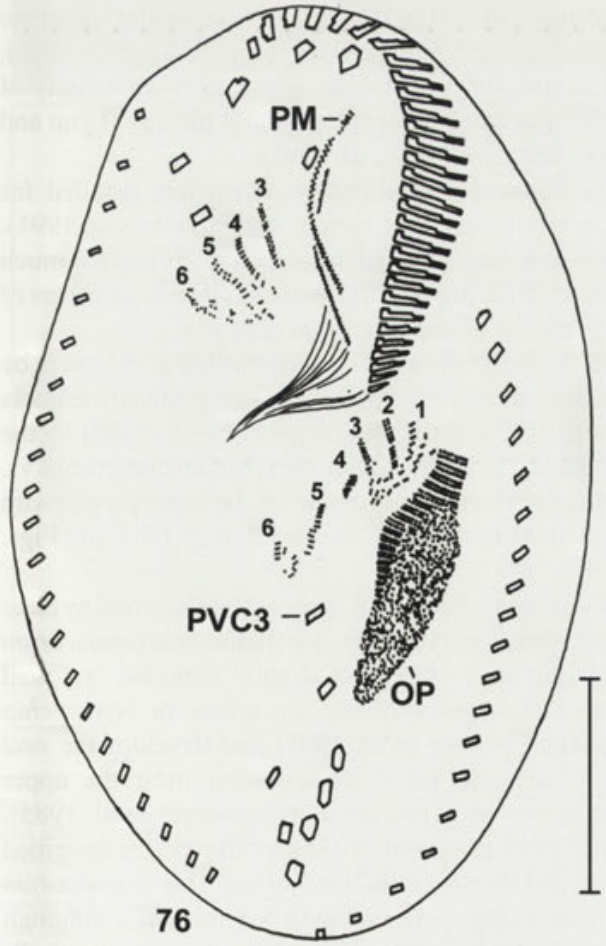
Species of these genera frequently look alike to untrained workers. Thus, they have often been confused (for review, see Berger 1999). Recently, however, Berger and Foissner (1997) solved the puzzle by using morphological and ontogenetic traits, which clearly distinguish these and other oxytrichid genera from each other. Briefly, *Oxytricha* is distinctly different from the other genera by its morphogenetically active postoral cirrus V/3 (Berger and Foissner 1997) and the small subunit ribosomal RNA gene sequences (Schlegel *et al.* 1991). Thus, it belongs to the subfamily Oxytrichinae, whereas the other genera, in which cirrus V/3 does not participate in anlagen formation, belong to the Stylonychinae. Within this group, only *Histriculus* lacks caudal cirri and has confluent marginal cirral rows, clearly separating it from *Sterkiella* and *Stylonychia*. The latter genera differ morphologically, at the present state of knowledge, mainly in the arrangement of the undulating membranes (intersecting in *Sterkiella*, parallel in *Stylonychia* Figs. 85, 86) and the buccal field (narrow in *Sterkiella*, rather broad-triangular in *Stylonychia*; Figs. 79, 80, 83, 84). The ontogenetic difference noted by Berger and Foissner (1997), namely, that cirral streaks V and VI of the opisthe originate de novo, holds only for *Sterkiella cavicola*, type of the genus. In *S. histriomuscorum* (*Oxytricha trifallax*) and *S. nova*, these anlagen are generated by cirrus V/4, as in *Stylonychia* spp. (Wirnsberger *et al.* 1985, 1986). However, *Sterkiella nova* and *Stylonychia mytilus* (type of the genus) differ distinctly in certain gene sequences (Hoffman and Prescott 1997b) and the allozyme pattern (Schlegel 1985, Schlegel and Steinbrück 1986), that is, are distinct genera, in spite of the rather inconspicuous morphological and ontogenetic differences.

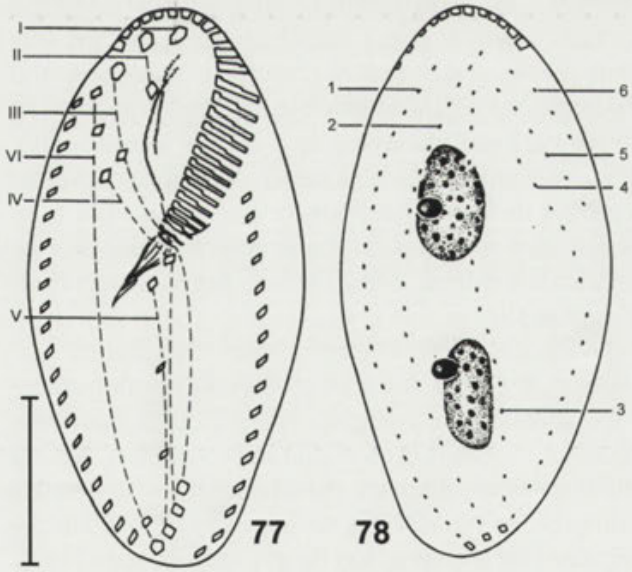
Identification of *Sterkiella histriomuscorum*

The original description of *S. histriomuscorum* (*Histrio muscorum* in Kahl 1932, p. 617) is brief and rather general (translated from German; includes characters mentioned in the subgenus description and the key to species): "Length *in vivo* 100-150 µm, length:width ratio slightly variable. Body rather rigid and distinctly flattened, parallel-sided with posterior end broadly rounded. Rightmost transverse cirri 1/3-1/2 projecting beyond posterior body margin. Last three cirri of left marginal row form rather distinct bristles, which, however, are soft and only slightly elongated. Frequently found in mosses from the German Alps and California ".

Fortunately, Kahl (1932) provided an excellent figure (Fig. 71), which not only perfectly matches later redescriptions (Foissner 1982, Shin and Kim 1994) but also *Oxytricha nova* (Figs. 1, 13) and *Oxytricha trifallax* (Fig. 74). Kahl's description of the caudal cirri, which he







Figs. 71-78. *Sterkiella histriomuscorum* (71-73, 77, 78) and its *nomen nudum* synonym *Oxytricha trifallax* (74-76) from life (71) and after protargol impregnation (72-78). 71- ventral view of type population (from Kahl 1932); 72, 73 - ventral views of soil populations from the Austrian Alps (from Foissner 1982). Note that there are three to five transverse cirri. Arrows mark pretransverse ventral cirri, arrowheads denote caudal cirri; 74, 75 - infraciliature of ventral and dorsal side of *O. trifallax*; 76 - early divider of *O. trifallax* showing that cirral anlagen (numbers 1-6) originate as in *S. nova*; 77, 78 - infraciliature of ventral and dorsal side of the soil population investigated by Berger *et al.* (1985); most specimens have only four transverse cirri. Roman numerals denote cirri originating from same anlage. Arabic numerals denote dorsal kineties. OP - oral primordium, PM - paroral membrane, PVC 3 - postoral ventral cirrus V/3, which does not participate in anlagen formation, TC - transverse cirri. Scale bars 40 µm (Fig. 71) and 20 µm (other figures)

misinterpreted as „the last three cirri of the left marginal row", exactly matches our observations; they are indeed inconspicuous and easily misidentified as marginal cirri (Figs. 1, 8, 9, 19, 20, 23, 24).

Sterkiella comprises, according to Berger's (1999) recent revision of oxytrichid hypotrichs, seven reliable species. Most have more than two macronuclear nodules and are thus easily distinguished from the *S. histriomuscorum* complex, which has two. The only other species with two macronuclear nodules is *S. tricirrata* (Buitkamp), which differs from *S. histriomuscorum* by a slightly reduced number of transverse cirri (3 vs. 3 - 5) and dorsal kineties (5 vs. 6). Thus, this species might well be another member of the *S. histriomuscorum* complex. As concerns separation from species of related genera, see next chapter and chapter "Distinguishing the genera *Oxytricha*, *Sterkiella*, *Stylonychia*, and *Histriculus*".

Proposed synonymy of *Sterkiella histriomuscorum* with *Stylonychia pustulata/vorax* rejected

Very recently, Eigner (1999) vaguely speculated about synonymy of *S. histriomuscorum* with *Stylonychia pustulata* and/or *S. vorax*, for which he erected the new genus *Tetmemena*. Indeed, such speculations and misidentifications are common in this kind of hypotrichs (Borror 1972 and synonymy list by *S. histriomuscorum*), whose separation needs a rather sophisticated set of characters difficult to experience by workers not fully familiar with the group. It is thus necessary to discuss the subject in some detail, using Eigner's paper as a representative example.

(1) Eigner (1999) correctly states that Berger and Foissner (1997) put much emphasis on the arrangement of the undulating membranes (parallel in *Stylonychia*, optically intersecting in *Sterkiella* and *Oxytricha*). We still hold this view, although the character is sometimes inconspicuous, because it is supported by a lot of ontogenetic data (for a review, see Berger and Foissner 1997). Eigner (1999) argues that, depending on the preparation conditions and the orientation of the specimens, the undulating membranes may appear parallel or intersecting in *Bakuella pampinaria*. We agree and thus do not use this character in large and soft hypotrichs, but only in the medium-sized oxytrichids *s. str.* Furthermore, it is quite common that the same character has different weight in different groups and exceptions exist within the group. Thus, Eigner (1999) mixes two different subjects.

(2) Eigner (1999) overlooked that *Stylonychia vorax* in Wirnsberger *et al.* (1985) is a misidentified *S. bifaria* (Foissner *et al.* 1991, p. 334).

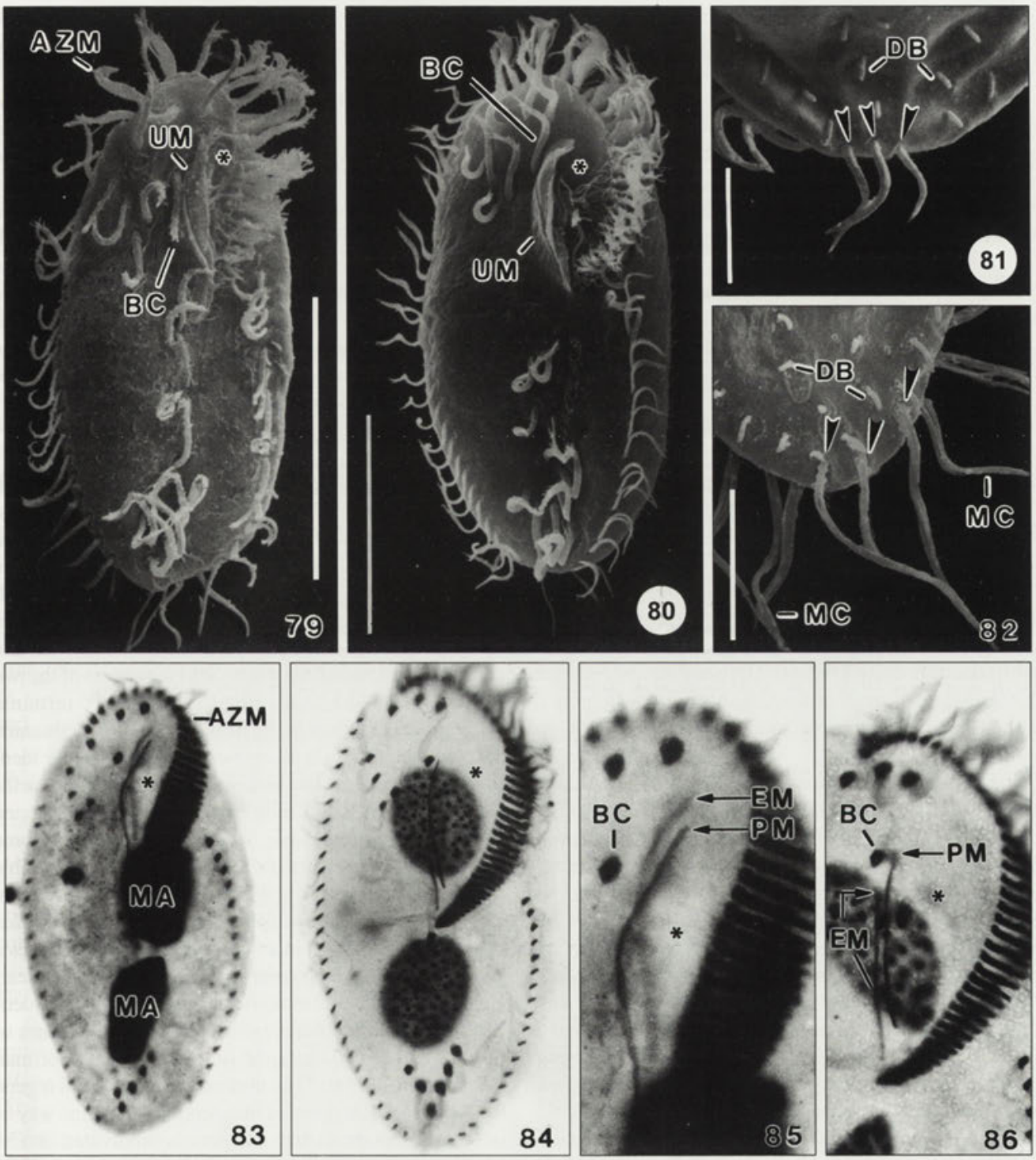
(3) Eigner (1999) considers almost entirely ontogenetic characters in separating the species under discussion. However, species are usually not separated by specific ontogenetic features, which are of significance mainly at genus, family, and ordinal level (Foissner 1996). As a consequence, Eigner (1999) lost most species characters (see following paragraph).

(4) *Stylonychia pustulata* differs from members of the *Sterkiella histriomuscorum* complex by the following features (Figs. 79 - 86 and Foissner *et al.* 1991): (i) the arrangement of the undulating membranes (parallel vs. intersecting), (ii) the shape of the buccal field (moderately broad-triangular vs. narrow elliptical), (iii) the location of the buccal cirrus (at anterior end of paroral vs. slightly above mid-buccal cavity), (iv) dorsal kinety 4 (unshortened anteriorly vs. shortened), (v) the arrangement and distinctiveness of the caudal cirri (widely spaced and distinct vs. narrowly spaced and indistinct), (vi) the resting cyst (with conspicuous tubercles or spines vs. irregularly wrinkled or almost smooth; Figs. 29, 30), (vii) the origin of the oral primordium (near upper postoral ventral cirri vs. near transverse cirri; Figs. 31, 32, 37), (viii) the allozyme pattern (Schlegel 1985, Schlegel and Steinbrück 1986), and (ix) the 16S-like rRNAs, which show an identity of 99,3%, indicating relatedness but not identity (Lynn and Sogin 1988, Schlegel *et al.* 1991).

(5) Data are, unfortunately, much less detailed for *Stylonychia vorax* (for a review, see Foissner *et al.* 1991). However, the studies available show clearly that it is much more similar to *Stylonychia pustulata* than to members of the *Sterkiella histriomuscorum* complex.

(6) *Stylonychia bifaria* (misidentified as *S. vorax* by Wirnsberger *et al.* 1985, see above) differs from members of the *Sterkiella histriomuscorum* complex mainly by the arrangement of the transverse cirri (two distinct groups vs. single group) and the structure of the resting cyst (with conspicuous tubercles vs. wrinkled; Kay 1945 and Figs. 29, 30).

(7) Although Petz and Foissner (1997) showed by clear figures that the Antarctic *Sterkiella histriomuscorum* population has a smooth or slightly wrinkled cyst wall (with conspicuous tubercles or spines in *Stylonychia pustulata*; Foissner *et al.* 1991) and develops the oral primordium near the transverse cirri (near the upper postoral cirri in *S. pustulata*; Wirnsberger *et al.* 1985), Eigner (1999, p. 45) states: "At least the species described by Petz and Foissner (1997) as *Sterkiella histriomuscorum* is probably *Stylonychia vorax* or *S. pustulata*". Although one can be of different opinion about the generic significance of these characters (Berger and Foissner 1997,



Figs. 79 - 86. Comparison of the *Sterkiella histriomuscorum* complex with *Stylonychia pustulata* in the scanning electron microscope (79-82) and after protargol impregnation (83-86). These species, which look similar at first glance (79, 80, 83, 84), are frequently confused or even synonymized, although they differ in many features, some of which are shown in the micrographs: (i) arrangement of undulating membranes (UM; endoral [EM] and paroral [PM] optically intersecting in mid-buccal cavity vs. parallel), (ii) buccal field (asterisk; narrow-elliptical vs. moderately broad-triangular), (iii) location of buccal cirrus (BC; slightly above mid-buccal cavity vs. anterior end of paroral), and (iv) arrangement and distinctiveness of caudal cirri (arrowheads; narrowly spaced and short vs. widely spaced and long). AZM - adoral zone of membranelles, BC - buccal cirrus, DB - dorsal bristles, EM - endoral membrane, MA - macronuclear nodules, MC - marginal cirri, PM - paroral membrane, UM - undulating membranes (paroral + endoral). Scale bars 40 µm (79, 80) and 10 µm (81, 82)

Eigner 1999), they unequivocally distinguish species. Thus, Eigner's speculation is groundless.

All data mentioned above were available to Eigner (1999). He did not discuss, why he discarded most of them. Furthermore, he neglected a basic "rule" in species taxonomy, namely, to reinvestigate the available type material. Slides with protargol-impregnated specimens of most populations under discussion are deposited in the Museum of Natural History in Linz (LI), Austria, and available to any worker. In sum, Eigner's proposed synonymies must be rejected because they are based on highly selected characters and insufficient literature and slide (species) knowledge.

Ontogenetic comparison

Ontogenetic data are available from four populations of *S. histriomuscorum*, which occurred in soils from Austria (Berger *et al.* 1985) and continental Antarctica (Petz and Foissner 1997) and in freshwaters from Spain (Nieto *et al.* 1984) and China (Zou and Zhang 1992). However, detailed illustrations were provided only by Berger *et al.* (1985) and Petz and Foissner (1997). In spite of this, it is obvious that ontogenesis is similar in all populations and to that described here for *S. nova*. Our data largely agree with those of Zou and Zhang (1992) and Petz and Foissner (1997), although small differences occur in the temporal relationships of the processes, similar as between *Oxytricha nova* and *O. trifallax*.

Berger *et al.* (1985), who studied a population usually having only four transverse cirri, could not unambiguously clarify the origin of proter's anlagen 4, 5 and 6. However, the figures provided show that they very likely originate as in *S. nova*, that is, from cirrus IV/3. This stage is difficult to observe, as explained in the Result section. On the other hand, the population studied by Berger *et al.* (1985) is clearly different from *S. nova* in that some fronto-ventral-transverse cirral anlagen of the proter and opisthe are confluent during the early morphogenetic stages, while all anlagen are distinctly separate in *S. nova* and *O. trifallax*. This might indicate that *S. histriomuscorum* populations with four transverse cirri are not conspecific with those having five transverse cirri (Petz and Foissner 1997).

The micrographs and description by Nieto *et al.* (1984) are more difficult to interpret: „The next step is the formation of the fronto-ventral-transverse primordium and paroral primordium of the future opisthe. As two ciliary streaks extend from the right anterior margin of the oral primordium, two ventral cirri disaggregate and the subsequent stringing out of their kinetosomes forms two primordial streaks each. From these six streaks, the closest cirral streak to the oral primordium will form the paroral primordium and the remainder one will give rise to the fronto-ventral-transverse system". However, micrographs 7 and 8 in Nieto *et al.* (1984) indicate that the oral primordium develops three anlagen and cirrus IV/2 only one, as in *S. nova* and *S. histriomuscorum* (Petz and Foissner 1997). Furthermore, Nieto *et al.* (1982, 1984) very likely misidentified the species. The figures show an organism which highly resembles *Stylonychia pustulata* (for review, see Foissner *et al.* 1991) in the location of the buccal cirrus (at anterior end of paroral), in the shape and size of the buccal field (broadly triangular), and the arrangement of the undulating membranes (parallel).

Molecular comparison

This has been performed by Hoffman and Prescott (1997b) who state: "Molecular data from both actin I and DNA pol α show that *Oxytricha* (now *Sterkiella*) *nova* and *O. trifallax* (now *Sterkiella histriomuscorum*) are different species". Specifically, Hoffman and Prescott (1997b) emphasize the following differences in the macronuclear DNA pol α polypeptides of *O. nova* and *O. trifallax*: "Overall the amino acid sequences of the two proteins are ~68% identical; the amino terminal ~350 amino acids extending from the initiator methionine to conserved region E diverge considerably (~46% identity) compared to the remaining ~1150 amino acids (~72% identity). The 12 conserved domains are separated by "spacers" of variable sequence and length; some "spacers" are more variable than others are. It is possible to define a core catalytic domain extending from region E through region V, in which the *O. nova* and *O. trifallax* polypeptides are 80% identical. This domain separates a highly variable amino-terminal domain (46% identical) and a less variable carboxy-terminal domain (63% identical)". Likewise, the micronuclear DNA pol α genes of *O. nova* and *O. trifallax* are distinctly different (Hoffman and Prescott 1997a): "The micronuclear DNA pol α gene in *O. trifallax* is scrambled in essentially the same way as in *O. nova*, but the *O. trifallax* gene is subdivided into 51 MDSs by 50 IESs, compared to 45 MDSs and 44 IESs in *O. nova*. PCR experiments failed to detect any non-scrambled or alternatively scrambled copies of the gene in the micronuclear genome. The first 1234 bp in the *O. trifallax* gene are subdivided into four non-scrambled MDSs, and the first 1233 bp in *O. nova* are subdivided into three non-scrambled MDSs. In *O. nova* the 3'-end of the

gene is divided into MDSs 44 and 45 by a single long IES of 223 bp, but in *O. trifallax* this region is divided into MDSs 49-51 by two short IESs of 69 and 10 bp. The other four additional MDSs in *O. trifallax* compared to *O. nova* are scrambled. The eight MDSs that are missing from the main body of the micronuclear gene of *O. trifallax* correspond closely in position in the ORF to the eight MDSs that are missing from the cloned micronuclear gene of *O. nova*. One scrambled MDS (MDS 8) is not present in the cloned micronuclear PCR product from *O. trifallax*. There is no corresponding MDS in *O. nova*. The micronuclear DNA pol α gene in *O. trifallax* contains an inversion in the same position as in *O. nova*. This strongly suggests that the DNA pol α gene became scrambled before *O. trifallax* and *O. nova* diverged from their common ancestor". There are also distinct differences in the internal eliminated sequences of these species (Prescott and DuBois 1996).

Neighbour-joining trees from the amino acid and DNA pol α sequences place *O. nova* and *O. trifallax* in the same clade, which is distinctly distant from the *Stylonychia mytilus/lemnae* clade; in contrast, actin I sequences separate *O. nova* from *O. trifallax*, which forms a clade with *S. mytilus/lemnae* (Hoffman and Prescott 1997b). However, 16S-like ribosomal RNA trees unambiguously show *O. nova* as sister group of *Stylonychia pustulata* (Lynn and Sogin 1988, Schlegel *et al.* 1991), which is in accordance with morphological and ontogenetical findings (Wirnsberger *et al.* 1985, 1986).

The *Sterkiella histriomuscorum* story

Before establishing *Sterkiella histriomuscorum* and *S. nova* as sibling species of a *Sterkiella histriomuscorum* complex, it seems appropriate to present in detail the complex history of the genus *Sterkiella*. The chapter is based on recent reviews (Berger 1999, Berger and Foissner 1997).

In 1878 Sterki established the genus *Histrio* to separate stylonychid hypotrichs with (*Stylonychia*) or without (*Histrio*) caudal cirri and with posteriorly open (*Stylonychia*) or confluent (*Histrio*) marginal cirral rows. Unfortunately, the generic name was preoccupied by *Histrio* Fischer, 1813, a fish. Thus, Corliss (1960) replaced the homonym by naming the ciliate genus *Histriculus* and combining, among others, *Oxytricha (Histrio) muscorum* Kahl, 1932 with the new name to *Histriculus muscorum* (Kahl, 1932) Corliss, 1960. Kahl (1932) and Borror (1972) added to *Histrio* and *Histriculus* species with inconspicuous caudal cirri and indistinctly separated marginal rows, obviously assuming that Sterki (1878) misinterpreted these characters in the type species *Histrio steinii* Sterki, 1878, a junior (objective?) synonym of *Histriculus histrio* (Müller, 1773). However, ontogenetic studies proved that species, as added by Kahl (1932) and Borror (1972) to *Histriculus*, indeed have caudal cirri and open marginal cirral rows, especially *Histriculus muscorum* (now *Sterkiella histriomuscorum*, see below), one of the most widespread oxytrichids (Berger *et al.* 1985). Thus, separation of *Histriculus* from *Stylonychia* became indistinct, suggesting synonymy (Wirnsberger *et al.* 1986). It was only recently that Foissner *et al.* (1991) and Berger and Foissner (1997) showed the existence of stylonychid hypotrichs without caudal cirri, as defined by Sterki (1878), requiring that species assigned to *Histrio* and *Histriculus* mainly by Kahl (1932) and Borror (1972) be referred to a new genus, *Sterkiella* Foissner *et al.*, 1991. Thus, a single species has accumulated three generic combinations over time: *Oxytricha (Histrio) muscorum* Kahl, 1932; *Histriculus muscorum* (Kahl, 1932) Corliss, 1960; and *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991. In the latter binomen, the species name has also changed because Kahl (1932) named three other species *Oxytricha muscorum*, namely *Oxytricha (Opisthotricha) muscorum*, *O. (Steinia) muscorum*, and *O. (Stylonychia) muscorum*. All these are primary homonyms because a subgeneric name does not affect homonymy [article 57(d) of the ICZN (1985)]. Thus, three species had to be renamed, among them also *Histrio muscorum* Kahl, 1932, now called *Sterkiella histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991. The doubling of "Foissner *et al.*, 1991" behind the species name is caused by a formal requirement, viz. that we had to combine the species with *Oxytricha* (to *Oxytricha histriomuscorum* nom. nov.) before transferring it to *Sterkiella*, which is indicated by parentheses [article 51(c) of the ICZN (1985)]. In other words, *Histrio muscorum* Kahl, 1932 (p. 617) was renamed because of homonymy of the genus name and of primary homonymy of the species name [thus, Kahl loses authorship of the species; article 60 of the ICZN (1985)], and then transferred to the genus *Sterkiella* because of new taxonomic findings.

When we discovered and rectified homonymy in 1991, we did not consider *Opisthotricha terrestris* Horváth, 1956, *Oxytricha histrioides* Gellért, 1957 and *Histrio macrostoma* (Gellért and Tamás, 1958) as junior synonyms of *Histrio muscorum* Kahl, 1932. Recently, however, Berger (1999) suggested this synonymy (see Results

section). Thus, the oldest name (*O. terrestris*) could be used as a replacement name for *Histrio muscorum*, respectively, *Sterkiella histriomuscorum* [articles 52 (b) and 60 of the ICZN (1985)]. However, changing the name again would not only cause further instability but could require changing back the name in future, if other authors reach a different conclusion, viz. that the above mentioned synonyms are distinct, valid species. Thus, we maintain our replacement name from 1991, which is unique and ensures stability.

Oxytricha nova and *O. trifallax*, sibling species of a *Sterkiella histriomuscorum* complex

Fortunately, detailed morphological and morphometric data are available from many populations of *S. histriomuscorum* collected worldwide in mainly terrestrial habitats. Except the population from activated sludge in Austria, all are very similar to each other and to *O. nova* and *O. trifallax* (Table 2). The mean values for the number of adoral membranelles, one of the best species

Table 2. Morphometric comparison of main characters of 9 populations of *Sterkiella histriomuscorum* (formerly *Histriculus muscorum*) with *Sterkiella nova* sp. n. and *Oxytricha trifallax*

Character[1]	Reference[2]	Species[3]	Method[4]	$\bar{x}$	SD	CV	Min	Max	n
Body, length	1	SH	PF	67.4	3.8	5.7	60	73	10
	1	SH	PF	71.6	6.0	8.3	60	80	8
	2	SH	PF	81.7	4.5	5.5	72	88	15
	2	SH	PF	73.6	8.4	11.5	52	82	15
	2	SH	PF	71.5	6.2	8.7	61	82	15
	2	SH	PF	64.9	5.4	8.3	57	75	15
	3	SH	PF	99.0	13.2	13.3	85	129	20
	4	SH	PW	105.8	4.8	4.6	100	114	10
	5	SH	PW	83.7	8.4	10.1	66	102	31
	6	SN	PW	128.5	17.2	13.4	85	150	29
	6	OT	PF	83.7	5.4	6.5	73	96	29
Adoral zone of membranelles, length	1	SH	PF	26.3	1.2	4.5	24	28	10
	1	SH	PF	30.5	1.9	6.3	27	33	8
	2	SH	PF	31.1	2.6	8.2	27	37	15
	2	SH	PF	29.4	2.8	9.4	22	33	15
	2	SH	PF	28.1	2.0	7.1	24	31	15
	2	SH	PF	27.5	1.9	6.9	25	32	15
	3	SH	PF	45.2	3.8	8.5	39	53	20
	4	SH	PW	43.4	2.0	4.6	41	47	10
	5	SH	PW	37.4	3.5	9.4	31	45	31
	6	SN	PW	52.7	7.0	13.2	40	62	29
	6	OT	PF	35.7	2.1	5.9	32	40	29
Adoral membranelles, number	1	SH	PF	31.2	2.1	6.7	28	35	10
	1	SH	PF	30.1	1.2	3.9	28	32	8
	2	SH	PF	31.0	2.3	7.4	26	34	9
	2	SH	PF	31.2	1.4	4.5	29	33	9
	2	SH	PF	30.1	3.2	10.6	24	34	9
	2	SH	PF	29.2	1.7	5.9	27	32	9
	3	SH	PF	39.3	2.9	7.3	34	44	20
	4	SH	PW	28.4	1.3	4.5	27	31	10
	5	SH	PW	27.9	1.1	4.0	26	31	31
	6	SN	PW	34.2	2.0	5.7	30	39	29
	6	OT	PF	29.7	1.3	4.5	26	32	29
Right marginal cirri, number	1	SH	PF	19.9	1.4	7.3	17	22	10
	1	SH	PF	22.6	1.3	5.8	20	25	8
	2	SH	PF	20.7	2.1	10.2	17	24	12
	2	SH	PF	18.8	1.1	5.6	17	21	12
	2	SH	PF	18.4	1.5	8.2	16	22	12
	2	SH	PF	19.0	1.0	5.5	17	21	12
	3	SH	PF	27.2	2.0	7.4	24	32	20
	4	SH	PW	21.6	1.2	5.4	20	23	10
	5	SH	PW	20.5	1.1	5.3	18	22	31
	6	SN	PW	21.5	1.7	7.7	18	25	29
	6	OT	PF	21.8	1.1	5.2	20	24	29

Table 2 (contd)

Character[1]	Reference[2]	Species[3]	Method[4]	$\bar{x}$	SD	CV	Min	Max	n
Transverse cirri, number	1	SH	PF	3.8	-	-	3	4	10
	1	SH	PF	5.0	0.0	0.0	5	5	8
	2	SH	PF	4.0	0.4	10.2	3	5	13
	2	SH	PF	3.9	-	-	3	4	13
	2	SH	PF	3.9	-	-	3	4	13
	2	SH	PF	4.2	-	-	4	5	13
	3	SH	PF	4.7	0.6	12.6	4	6	20
	4	SH	PW	4.0	0.0	0.0	4	4	10
	5	SH	PW	5.0	0.0	0.0	5	5	31
	6	SN	PW	5.0	-	-	5	6	29
	6	OT	PF	5.0	0.0	0.0	5	5	29
Dorsal kineties, number	1	SH	PF	6.0	0.0	0.0	6	6	10
	1	SH	PF	6.0	0.0	0.0	6	6	8
	2	SH	PF	6.0	0.0	0.0	6	6	12
	2	SH	PF	6.0	0.0	0.0	6	6	12
	2	SH	PF	6.0	0.0	0.0	6	6	12
	2	SH	PF	6.0	0.0	0.0	6	6	12
	3	SH	PF	6.2	-	-	6	7	20
	4	SH	PW	6.0	0.0	0.0	6	6	8
	5	SH	PW	5.8	-	-	5	6	31
	6	SN	PW	6.0	0.0	0.0	6	6	29
	6	OT	PF	6.0	0.0	0.0	6	6	29

[1] Data based on randomly selected, protargol-impregnated morphostatic specimens. Measurements in µm. CV - coefficient of variation in %, Max - maximum, Min - minimum, n - number of specimens investigated, SD - standard deviation, $\bar{x}$ - arithmetic mean.
[2] 1- Foissner (1982): two populations from soils of the Austrian Central Alps, field material; 2 - Berger *et al.* (1985): four populations from soils of the Austrian Central Alps, field material (populations 1 - 3) and a cultivated strain (population 4) were investigated; 3 - Augustin and Foissner (1992): from activated sludge in Austria, cultivated on sludge; 4 - Shin and Kim (1994): from soil of cultivated field in Korea, cultivated on bacteria; 5 - Petz and Foissner (1997): from Antarctic soil, cultivated on bacteria; 6 - this paper, see "Materials and Methods" and "Type location".
[3] SH - *Sterkiella histriomuscorum*; SN - *Sterkiella nova*; OT - *Oxytricha trifallax*.
[4] PF -Foissner's (1991) protargol protocol; PW - Wilbert's (1975) protargol protocol.

characters in hypotrichs, vary between 28 and 34 only, if the population from activated sludge (39.3) is excluded. Similar low variation is obtained for the number of marginal and transverse cirri. Furthermore, all populations have two macronuclear nodules, six dorsal kineties, and three inconspicuous caudal cirri. Metric parameters, like length of body and adoral zone of membranelles, vary to a greater extent. However, this is at least partially caused by the different preparation and culture methods used (Table 1), and should thus not be over-interpreted (see Results section).

Certainly, the populations listed in Table 2 cannot be reliably separated by morphological and morphometric criteria because the extreme values highly overlap in most cases. Furthermore, the ontogenesis pattern is very similar in several *S. histriomuscorum* populations (see above) and in *O. nova* and *O. trifallax*. On the other hand, *O. nova* and *O. trifallax* are clearly different in their genetic material (see Discussion above), suggesting that *S. histriomuscorum* is a complex of sibling species, like *Stylonychia mytilus* (Ammermann and Schlegel 1983, Steinbrück and Schlegel 1983), *Tetrahymena pyriformis* (Nanney and McCoy 1976), and *Paramecium aurelia* (Sonneborn 1975). Looking at the data compiled in Table 2 in more detail, it seems reasonable to assume that the complex contains more than two species, that is, *Sterkiella nova* (formerly *Oxytricha nova*) and *Sterkiella histriomuscorum* (formerly *Oxytricha trifallax*). For instance, the population from activated sludge, which has a distinctly higher number of adoral membranelles, and some alpine populations, which usually have only four transverse cirri, might be sufficiently different at the molecular level to give them species status. *Sterkiella tricirrata*, mentioned above, might belong to the complex, too. And Seegmiller *et al.* (1996) and Witherspoon *et al.* (1997) mention "two sibling *Oxytricha* species, *O. fallax*

and *O. trifallax*". However, the molecular differences between these populations are less distinct than those between *O. nova* and *O. trifallax*.[1]

Taxonomic - nomenclatural consequences

There is no possibility of knowing whether the populations of *S. histriomuscorum* studied by Kahl (1932) and others were *O. nova*, *O. trifallax*, or other (sibling) species. Thus, one could argue to classify both, *O. nova* and *O. trifallax*, as species nova, considering their molecular distinctiveness. However, this certainly would break the spirit of the International Code of Zoological Nomenclature (1985) to maintain nomenclatural continuity and priority. There can be no doubt that *O. nova* and *O. trifallax* are, from a morphological point of view, populations of Kahl's *Histrio muscorum*. Thus, we arbitrarily identify *O. trifallax* as *Histrio muscorum* Kahl, 1932 (now *Sterkiella histriomuscorum*) and establish *O. nova* as a new species, *Sterkiella nova*, simply because *O. nova* was used in more studies than *O. trifallax*. Furthermore, we maintain the specific epithet "*nova*" (although it is rather trivial) to ensure continuity with the previous literature. Synonymization of *O. trifallax* with *S. histriomuscorum* is not necessary, simply because the former is a *nomen nudum* and thus non-existent in the official zoological literature.

Our proposal follows that used by Nanney and McCoy (1976) for the *Tetrahymena pyriformis* complex. Furthermore, we suggest to follow Corliss and Daggett (1983) in designating field populations of *S. histriomuscorum* as "*Sterkiella histriomuscorum* complex", if molecular data are lacking. Alternatively, one can follow the concept of Génermont and Lamotte (1980) and designate it as "*Sterkiella* supraspecies *histriomuscorum* (Foissner *et al.*, 1991) Foissner *et al.*, 1991".

As mentioned above, the complex very likely contains more than the two species diagnosed here. If such species should be discovered, probably the synonyms of *S. histriomuscorum* (see synonymy list in Results section) should be used for naming. This would be an important contribution to reduce the vast number of species names. However, the name "*trifallax*" should be abandoned forever to avoid further confusion.

CONCLUDING REMARKS

The long-practiced cavalier assignment of a taxon name to random ciliate isolates by molecular biologists has generated tremendous confusion which will contaminate the literature for all time. Morphologists also contributed to the confusion by unjustified synonymies and nomenclatural mistakes. To order this mass and find moderate solutions for the problems, was a difficult job. Thus, we hope that our suggestions will be followed by both molecular biologists and classical taxonomists, otherwise the confusion will increase to an unresolvable mass. Furthermore, a more close and fair co-operation between morphologists and molecular biologists is needed.

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[1]It is beyond the scope of the present paper to discuss the *Oxytricha fallax* problem in detail. However, some background information is necessary to fully understand the rationale of the suggestions in the following chapter. Unfortunately, the morphological identity of the *O. fallax* population now used by molecular biologists is also not known because it is a re-isolate, that is, not that studied by Grimes (1972) and then used by Hammersmith during the 1970s (Hammersmith, pers. comm.). The population studied by Grimes died out, although cysts are still maintained in Hammersmith's laboratory (pers. comm.). The organism studied by Grimes and determined by A. C. Borror as "unnamed subspecies of *Oxytricha fallax* Stein" (Grimes 1972, p. 428) is about 80 µm long and possibly not very flexible, whereas Stein's (1859) *O. fallax* is 150 - 180 µm long and highly flexible and contractile (Stein 1859: "*Oxytricha fallax* is a real *Oxytricha* because it is highly flexible and contractile and thus cannot be assigned to *Stylonychia*. If specimens get between two obstacles, they contract or extend trying to force themselves through the obstacles by winding left and right. Such movements cannot be performed by any *Stylonychia*".). Thus, the organism studied by Grimes (1972) and Grimes and Adler (1976) cannot be identical with Stein's species. The data provided suggest that it was a member of the *Sterkiella histriomuscorum* complex.

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ACTA
PROTOZOOLOGICA

Licnophora limpetae sp. n. (Ciliophora: Heterotrichea) Ectosymbiont of South African Limpets

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Summary. During a survey of the intertidal symbionts of marine invertebrates, along the South African coast, a heterotrichous ciliate was found attached to the gills of limpet species. This ciliate conforms to the morphology of the genus *Licnophora* Claparède, 1867, but differs from all the known species, based on body morphology, details of the macronuclear apparatus and is described as a new species, *L. limpetae* sp. n. This description is based on light and scanning electron microscopy.

Key words: heterotrichous ciliate, *Licnophora limpetae* sp. n., marine mollusc.

INTRODUCTION

The monotypic family Licnophoridae Bütschli, 1881, with the genus *Licnophora* Claparède, 1867, is represented by nine species occurring on a variety of hosts (Cohn 1866; Claparède 1867; Stevens 1901, 1904; Dustin 1915; Fauré-Fremiet 1937; Villeneuve-Brachon 1939, 1940; Meng and Yu 1985). The type species was originally described as belonging to the genus *Trichodina*, i.e. *T. auerbachii* Cohn, 1866. The following year Claparède erected the genus *Licnophora* with the type *L. auerbachii* (Cohn, 1866). Stevens (1901) recognised that *L. auerbachii* was probably not the first licnophorid described. Claus (1862) and later Meyer and Moebuis (1865) recorded *Trichodina*-like organisms, without providing a specific identification. According to Stevens (1901) these were probably also licnophorids, based on the figures.

In a comprehensive survey of the micro-symbionts associated with the South African limpets, a new species of an ectosymbiotic peritrich, *Mantoscyphidia branchi* Van As, Basson and Van As, 1998 was described (Van As *et al.* 1998). During the same survey, a second symbiont was also found on the gills of these limpets. This heterotrichous ciliate conforms to the morphological features of the family Licnophoridae and the genus *Licnophora*. It differs from all the known *Licnophora* species based on characteristics of the nuclear apparatus, general body morphology and host preference. It 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.

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MATERIALS AND METHODS

Limpets were collected at low tide on the rocky shores of South Africa. On the west coast species of *Patella* Linnaeus, 1758 were collected at Mc Dougall's Bay (29° 45'S, 16° 45'E). *Patella* species occurring on the south coast were collected at De Hoop (34° 28'S, 20° 30'E) and Goukamma (34° 20'S, 22° 55'E) Nature Reserves.

Specimens were taken to a field laboratory where wet smears were prepared and examined. Positive smears were fixed in Bouin's and transferred to 70% ethanol. Some were stained with Mayer's hematoxylin for studying the nuclear apparatus and for obtaining body measurements. Other smears were impregnated with protargol, using a combined method as described by Lee *et al.* (1985) and Lom and Dyková (1992).

For scanning electron microscopy sections of fresh gills were fixed using different fixatives, i.e. 10% buffered neutral formalin, 2.5% glutaraldehyde or Parducz. Gills fixed in formalin were washed with tap water and those fixed in glutaraldehyde or Parducz were washed in a phosphate buffer, the gills were dehydrated through a series of ethanol concentrations and critical point dried. The gills were then mounted on a stub, sputter coated with gold and studied at 5 kV, using a JEOL WINSEM JSM 6400 scanning electron microscope (SEM).

Body measurements and number of macronuclear segments were obtained from microscope projection drawings. 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 macronuclei), standard deviation (only in n>9) and number of specimens measured. The type material is in the collection of the National Museum, Bloemfontein, South Africa.

RESULTS AND DISCUSSION

Licnophora limpetae sp. n. (Figs. 1-5)

Hosts: *Patella barbara* Linnaeus, 1785; *P. cochlear* Born, 1778; *P. compressa* Linnaeus, 1758; *P. longicosta* Lamarck, 1819; *P. miniata miniata* Born, 1778.

Position on host: gills.

Localities: Mc Dougall's Bay and the Olifants River Mouth on the west coast, De Hoop and Goukamma Nature Reserves on the south coast.

Type-specimens: holotype slide, S95/9/5-32 (NMBP 119), Paratype slides, S95/9/9-20 (NMBP 120); 95/9/10-11 (NMBP 121) in the collection of the National Museum, Bloemfontein, South Africa.

Type host and locality: *P. barbara*, Mc Dougall's Bay (29° 45'S, 16° 45'E).

Etymology: named after the common name for the host, limpet.

Description

Body elongated, total length 50-95 μm (72.5±11.1, 58), consists of three regions; oral region connected by neck to basal region (Figs. 1-3). Oral region egg-shaped, length 25-57 μm (37.0±8.3, 58), diameter at broadest part 25-60 μm (36.3±7.6, 58), tapering towards neck (Figs. 1-3). Adoral side of oral region fringed by broad band of adoral zone of membranelles (AZM), originating at neck region, describing spiral of 270°, before plunging into infundibulum (Figs. 1-3). AZM comprising 70-100 rows of membranelles, each four kinetids wide. Centre of aboral surface smooth, fringed by AZM. Neck short, 6-22 μm (12.3±3.6, 58), diameter 13-43 μm (27.7±7.0,

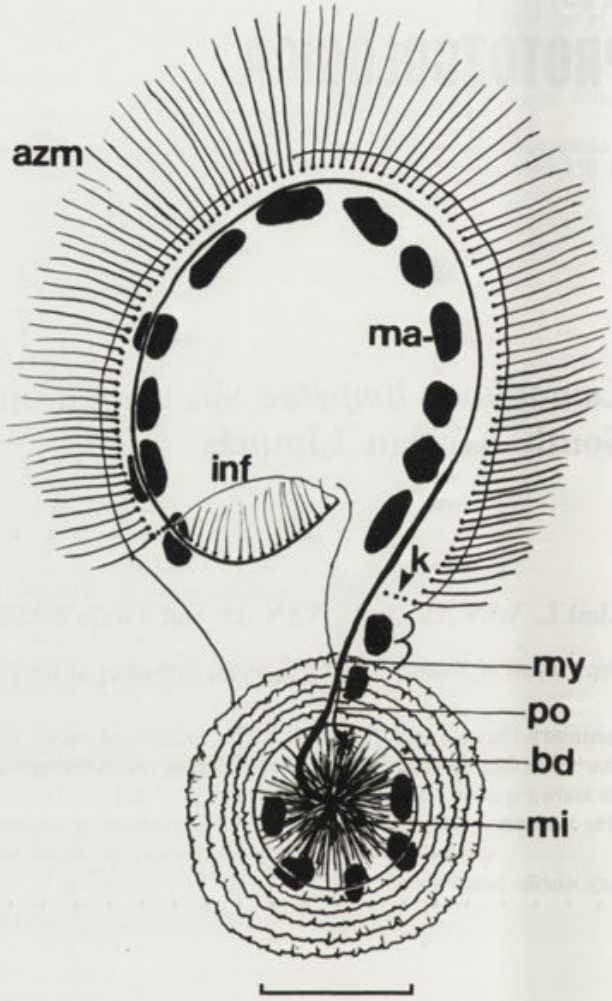
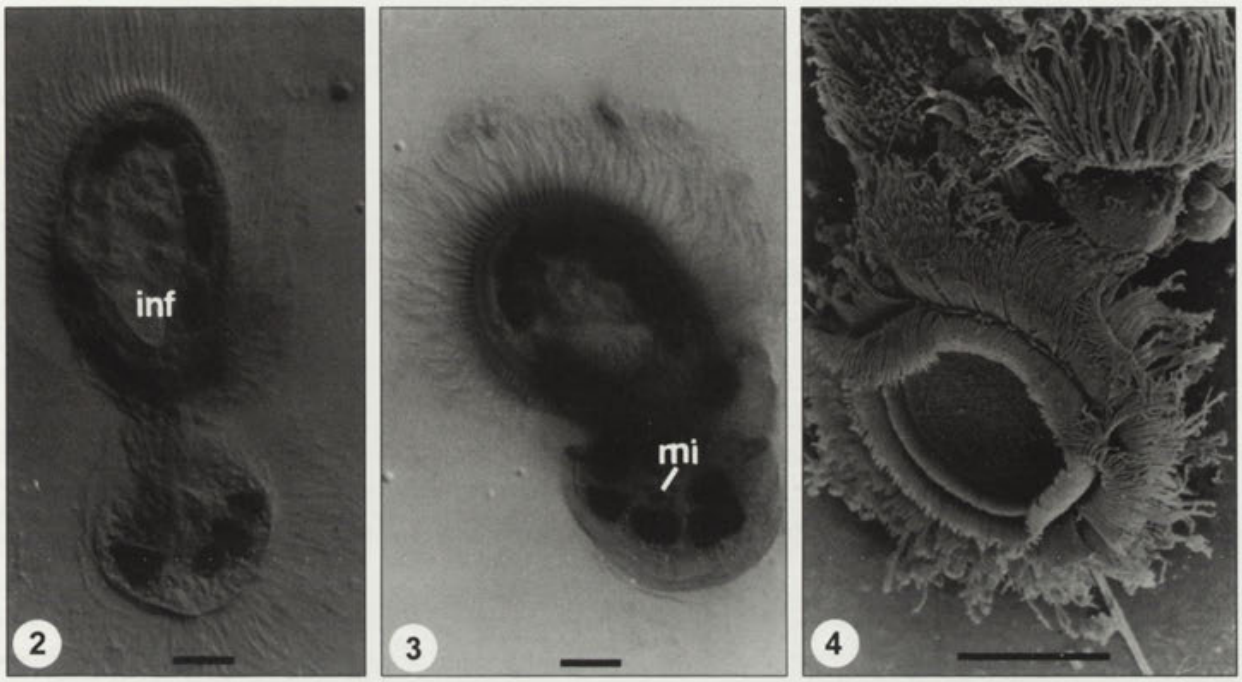


Fig. 1. Microscope projection drawings (based on a combination of observations made from material which were stained with hematoxylin, specimens that were impregnated with protargol, as well as photographs from SEM material) of *Licnophora limpetae* sp. n. occurring on the gill filaments of *Patella barbara* Linnaeus, 1785 collected at Mc Dougall's Bay, South Africa. azm - adoral zone of membranelles, bd - basal disc, inf - infundibulum, k - kinetids, ma - macronuclear segments, mi - micronucleus, my - myoneme, po - paroral organelle. Scale bar -30 μm



Figs. 2-4. Photomicrographs of hematoxylin stained specimens (2,3) and scanning electron micrograph (4) of *Licnophora limpetae* sp. n. collected from limpets from Mc Dougall's Bay. 2- macronuclear segments and infundibulum (inf) [host *Patella cochlear*], 3- macronuclear segments and micronucleus (mi) in basal disc [host *P. cochlear*], 4- aboral view of basal region and part of oral region [host *P. compressa*]. Scale bars -10 μm

58), without cilia. Basal region, slightly concave, irregular. Basal disc diameter 19-42 μm (30.7±5.2, 58), disc surrounded by five circular rings of cilia, proximal row shortest, distal row longest (Figs. 1, 4). Myoneme extends from centre of basal disc following curve of the AZM, before plunging into infundibulum. Paroral organelle extends from inner periphery of basal disc towards infundibulum, not always visible.

Macronucleus chain consists of round, sometimes slightly elongated, separate nuclei, varying in number between 13 and 25 (18, 58), distributed as an eight-shape through out oral region, neck and basal region (Figs. 1-3). Number of macronuclear segments in oral region 8-19 (12, 58), in neck 1-3 (1, 16) and in basal region 2-9 (5, 58). Micronucleus small, round, situated more or less in centre of basal disc (Figs. 1, 3) not always visible. No food vacuoles observed, endoplasm with granular appearance. No contractile vacuole found.

Intraspecific variation

Body measurements and the number of macronuclear segments in the different populations of *L. limpetae* sp.n., occurring on the gill filaments of limpets from different localities, are summarised in Table 1. In each population some variation was observed. *L. limpetae* sp.n. from Mc Dougall's Bay were found associated with different limpet species. Those from *P. barbara* and *P. cochlear* included the largest specimens found during this study. Those occurring on *P. compressa* and *P. miniata miniata* were considerably smaller. *L. limpetae* sp.n. collected from *P. barbara* and *P. longicosta* on the south coast were intermediate in size, between these two extremes.

In comparing the number of macronuclear segments in the different body regions (Fig. 5), a remarkable consistency was observed between populations from different hosts. This suggests that it is probably an important taxonomic characteristic.

Remarks

Licnophora auerbachii was described from a nudibranch, from Helgoland. Since then this species was recorded from a variety of host and localities (Wallengren 1894, Stevens 1904, Villeneuve-Brachon 1940, Williams 1954, Owen 1980, Silva Neto 1994). This species (80-120 μm) has a nuclear chain consisting of 10-25

Table 1. Body measurements (μm) and number of macronuclear segments of specimens of *Licnophora limpetae* sp. n. from the West coast (Mc Dougall's Bay) and South coast (De Hoop and Goukamma Nature Reserves) of South Africa

	Mc Dougall's *Pattella barbara*	Mc Dougall's *P. cochlear*	Mc Dougall's *P. compressa*	Mc Dougall's *P. miniata miniata*	De Hoop *P. barbara*	Goukamma *P. longicosta*
TBL	50-95 (72.5±11.1, 58)	54-95 (75.2, 8)	42-66 (52.5±6.0, 57)	42-61 (51.4±5.3, 23)	54-70 (61.9±4.5, 31)	61-82 (71.2±6.5, 21)
ORL	25-57 (37.0±8.3, 58)	24-55 (39.8, 8)	17-32 (24.8±4.1, 57)	19-32 (24.6±4.1, 23)	29-45 (36.6±4.1, 31)	33-57 (42.6±5.1, 21)
ORD	25-60 (36.3±7.6, 58)	25-40 (31.6, 8)	19-39 (25.5±4.0, 57)	19-38 (24.3±4.2, 23)	21-33 (28.1±2.7, 31)	29-40 (33.0±3.6, 21)
BDD	19-42 (30.7±5.2, 58)	25-37 (29.3, 8)	15-30 (23.9±3.3, 57)	21-30 (25.2±3.2, 23)	16-33 (24.7±3.6, 31)	23-40 (30.5±4.0, 21)
NL	6-22 (12.3±3.6, 58)	15-36 (25.5, 8)	5-15 (8.2±2.1, 51)	3-12 (6.9±2.2, 23)	2-12 (5.6±2.1, 30)	3-11 (6.5±1.6, 19)
ND	13-43 (27.7±7.0, 58)	9-18 (12.3, 8)	7-30 (18.8±4.4, 51)	17-30 (20.0±2.6, 23)	10-28 (15.2±3.7, 31)	15-28 (20.2±3.4, 20)
MaOR	8-19 (12, 58)	7-15 (11, 8)	7-17 (9, 57)	7-16 (12, 23)	8-17 (12, 31)	9-18 (10, 21)
MaN	1-3 (1, 16)	1-2 (2, 4)	1-2 (2, 4)	2-2 (2, 4)	1-2 (2, 11)	1-2 (1, 8)
MaBR	2-9 (5, 58)	1-7 (4, 8)	3-8 (4, 57)	4-8 (6, 23)	5-10 (7, 31)	4-8 (7, 21)
TMa	13-25 (18, 58)	10-21 (16, 8)	12-23 (16, 57)	14-23 (18, 23)	16-27 (20, 31)	14-24 (20, 21)

TBL - total body length, ORL - oral region length, ORD - oral region diameter, BDD - basal disc diameter, NL - neck length, ND – neck diameter, MaOR - number of macronuclei in oral region, MaN - number of macronuclei in neck, MaBR - number of macronuclei in basal region, TMa - total number of macronuclei

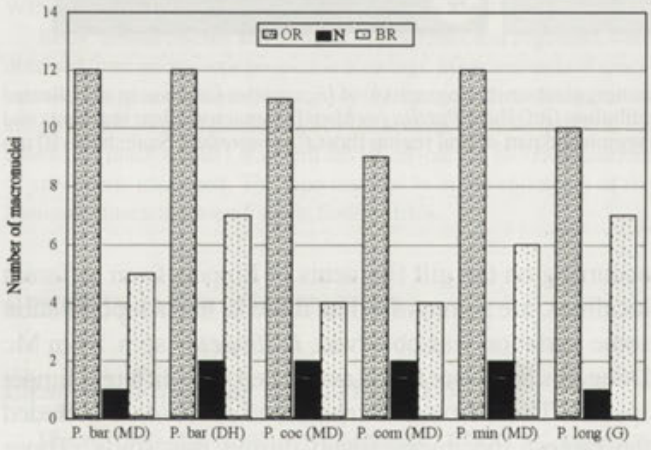


Fig. 5. Frequency histograms of number of macronuclear segments (mode) in the oral region [OR], neck [N] and basal region [BR] of specimens of *Licnophora limpetae* sp. n. occurring on the gill filaments of *Patella barbara* (P. bar), *P. cochlear* (P. coc), *P. compressa* (P. com), *P. miniata miniata* (P. min) and *P. longicosta* (P. long) collected at Mc Dougall's Bay (MD), De Hoop (DH) and Goukamma (G) Nature Reserves

segments. *L. cohnii* Claparède, 1867 (55-60 μm) was described from the gills of polychaetes from Italy, with the characteristic of the nuclear apparatus unstated.

The next two species, i.e. *L. setifera* Maskell, 1886 and *L. europaea* Garbini, 1889 were described from freshwater habitats. Their descriptions included very little morphological information. Bütschli (1889), Stevens (1901, 1904) and much later Balamuth (1941) questioned the validity of these two species. In the absence of any valid record of this genus from freshwater habitats, we believe that the genus *Licnophora* is most likely restricted to marine habitats.

Stevens (1901) described *L. macfarlandi* Stevens, 1901 as an endosymbiont of Holothuroidea, with an elongated body (67-96 μm) from the Californian waters. The nuclear apparatus consists of a broken chain of 25-30 spherical to oval or elliptical segments. Villeneuve-Brachon (1940) found licnophorids, what he identified as *L. macfarlandi*, on sea cucumbers from France. Balamuth (1941) recorded *L. macfarlandi* from yet another holothuroidea species, from southern California. Stevens (1904) described *L. conklini* Stevens, 1904 (100-135 μm) from a slipper snail, *Crepidula plana*, from Woods Hole, as a species with four nuclear segments in the body. Villeneuve-Brachon (1940) also found *L. conklini*, but from keyhole limpets in France. *L. bullae* Dustin, 1915, the second species described from a gastropod host, i.e. *Bulla hydatis* from France, has 15-20 macronuclear segments with a body length varying between 123-130 μm. The only species so far known from a non-zoological host is *L. lyngbycola* Fauré-Fremiet, 1937, which is described from filaments of the colourless Cynanophycea from Woods Hole. This species (100 μm) is characterised by a beaded macronuclear chain, with three large segments in the basal disc and 8-10 smaller segments in the rest of the body. Villeneuve-Brachon (1939, 1940) described two species from France, i.e. *L. chattoni* Villeneuve-Brachon, 1939 and *L. biecheleri* Villeneuve-Brachon, 1940. *L. chattoni* (70-90 μm) was found on the branchial surface of an ascidian host and has 10-20 macronuclear segments. *L. biecheleri*

(90-100 μm) described from a cnidarid host is characterised by 40-50 macronuclear segments. This species was found on the anterior half in the gastric cavity of the host. The only species known from a vertebrate host, i.e. *L. hippocampi* Meng and Yu, 1985, was described from the gills and skin of a seahorse. This species (50-87 μm) is also characterised by 7-10 macronuclei segments.

Licnophora limpetae sp.n. is the tenth licnophorid species and the third found on a gastropod host. Other species with a comparable number of macronuclear segments are *L. auerbachii, L. bullae* and *L. chattoni*. Both *L. auerbachii* and *L. bullae* are considerably larger than *L. limpetae* sp.n., with *L. chattoni* a little smaller. *L. auerbachii* was recorded from a broad spectrum of hosts and localities throughout Europe as well as South America. *L. bullae,* was described from *Bulla hydatis* from France and *L. chattoni* was described from ascidians. It is highly unlikely that licnophorids found on nudibranchs, echinoderms, gastropods or ascidians in central Europe and in the America's can be the same as those recorded from endemic limpets from southern Africa.

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ACTA
PROTOZOOLOGICA

Planhoogenraadia bonneti sp. n. and *Centropyxis thailandica* sp. n. (Rhizopoda: Testacea), Two New Testaceans from Thailand

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Summary. Two new testaceans: *Planhoogenraadia bonneti* sp. n. and *Centropyxis thailandica* sp. n., were found during a faunistic study on testate amoebae in epiphytic mosses on forest trees in Thailand. The morphology and biometry of these testate amoebae were described by LM and SEM investigations.

Key words: biometry, *Centropyxis thailandica* sp.n., morphology, *Planhoogenraadia bonneti* sp.n., taxonomy, testate amoebae.

INTRODUCTION

Thailand is one of those Asiatic countries whose testacean fauna has been poorly studied. There are only two publications on the testaceans of Thailand, where a very rich and original soil testacean fauna has been shown to occur (Bonnet 1981, 1987). Many cosmopolitan and eurybiotic testaceans have been observed there, among them several genera of Gondwana origin, such as *Distomatopyxis*, *Lamtopyxis*, *Deharvengia*, *etc.*

During our investigations on the testate amoebae in Thailand we also found a very rich testacean fauna, including 2 unknown species, namely *Planhoogenraadia bonneti* sp. n. and *Centropyxis thailandica* sp. n. The morphometric descriptions of the 2 new species are the subject of the present paper.

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MATERIALS AND METHODS

The material for the present investigation was collected in July 1995 from humid epiphytic mosses on tree trunks in the "Khao Yai" National Park, Thailand. This National Park is located 180 km Northeast from Bangkok in the Dongrak Mountains. Its altitude ranges from 250 to 1351 m a.s.l. and more than 85 % of its surface is covered with typical tropical forests. The annual rainfall is about 2270 mm and this assures very high humidity and abundant growth of soil and epiphytic mosses.

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

DESCRIPTION AND DISCUSSION

Planhoogenraadia bonneti sp. n. (Figs. 1-10, Table 1)

Description: the shell is brown, a type of simple cryptostomy, with a flattened ventral side. In dorsal view it is ovoid or ovoid-elongate, and very regular (Figs. 3, 8). In lateral view the shell is feebly bulging, with a maximum

height near the middle of the length. The invagination of the aperture is shallow (1/6 to 1/5 of the height). The inner lip of the aperture is depressed and the outer lip is incurvate, forming a well differentiated visor (Figs. 2, 7, 10). In ventral view the aperture is invaginated, oval, sub-terminal and easily visible (Figs. 1, 6, 9). The shell has a comparatively thick wall and it is mainly composed of small, flattish siliceous particles. The arrangement of these particles makes its surface smooth, with a well defined outline.

Biometry: see Table 1.

Ecology: humid epiphytic mosses on tree trunks, height 1-2 m.

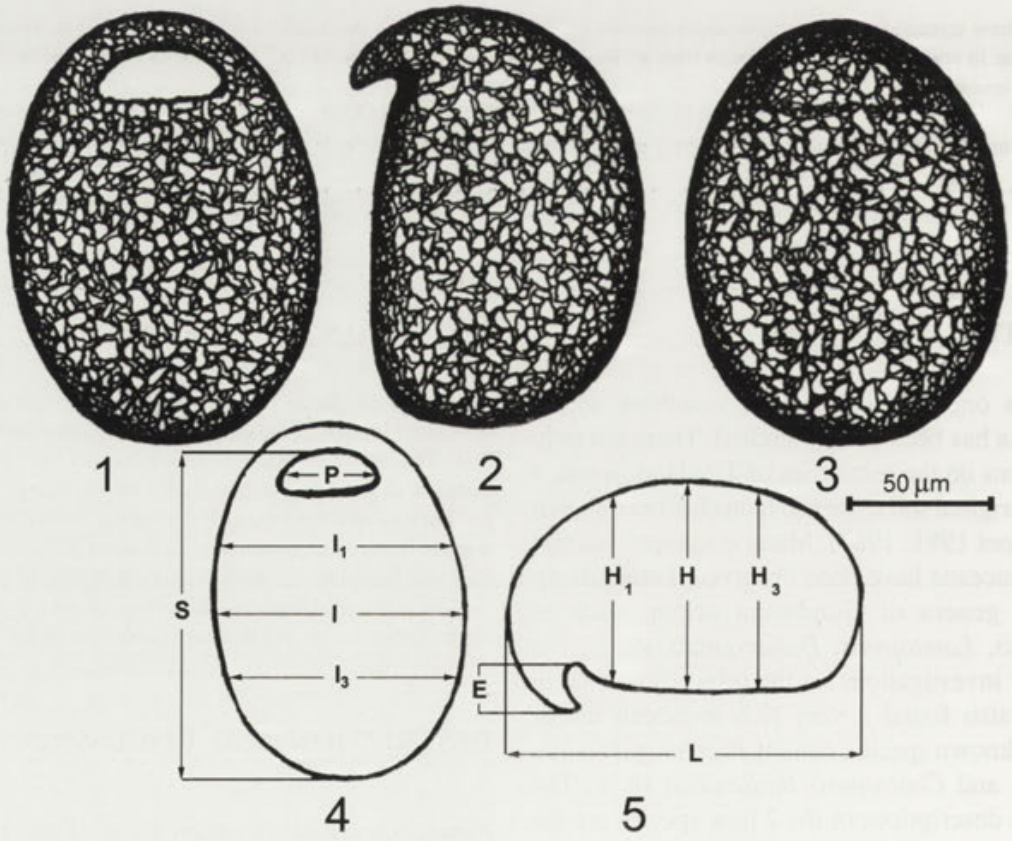
Locality: Thailand, "Khao Yai" National Park, 06.07.1995.

Type: holotype, preparation T-15/1995, in the collection of Dr. M. Todorov, Institute of Zoology, Sofia.

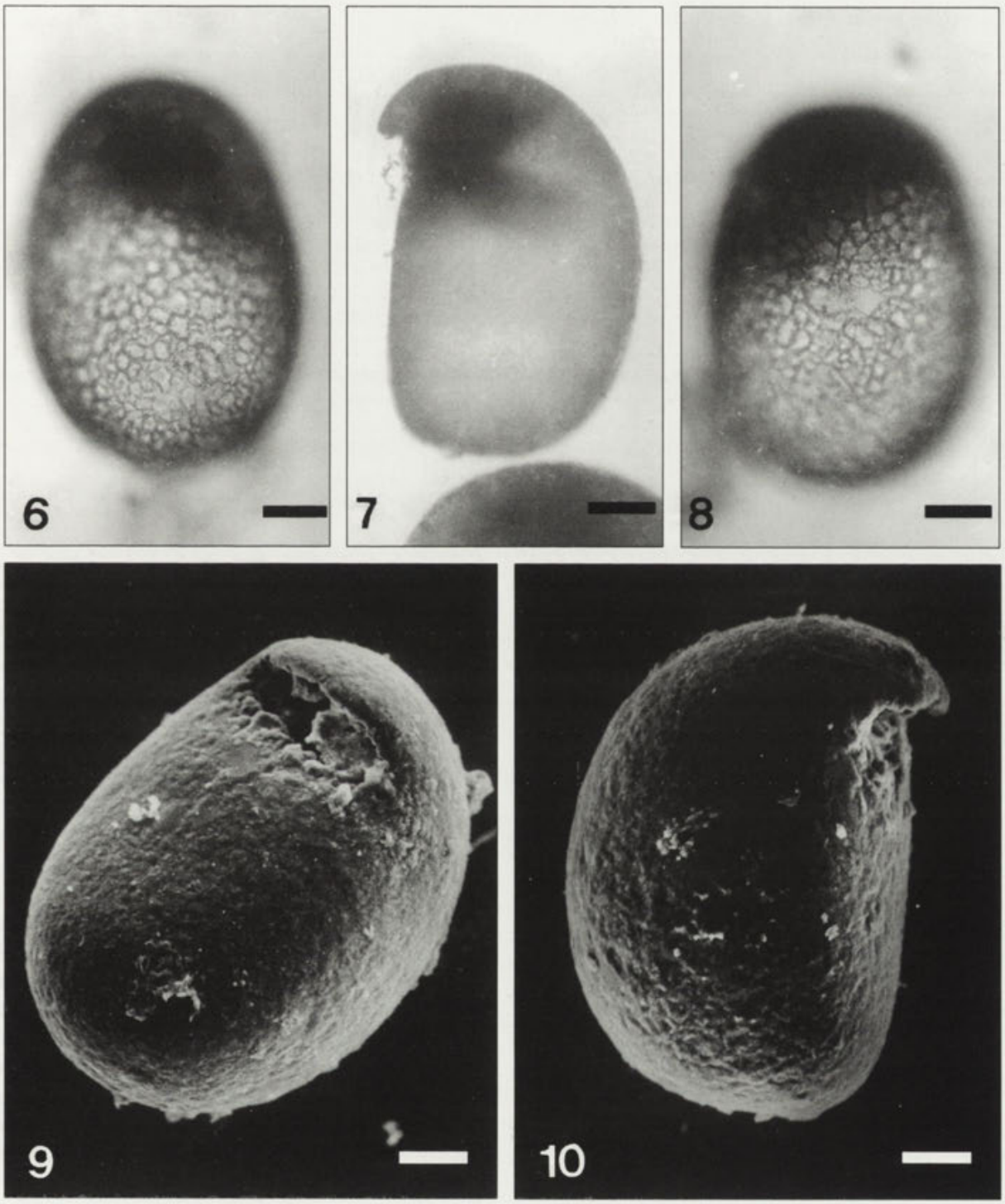
Etymology: this species is dedicated to Prof. Dr. L. Bonnet from Toulouse University, in recognition of his contributions to the study of moss and soil testaceans.

Discussion: the form and dimensions are relatively constant, as a very low coefficient of variation of all taxonomic features characterises the measured shells of *P. bonneti* sp. n. (Table 1). *P. bonneti* sp. n. differs distinctly from the other species of the genera *Planhoogenraadia* and *Hoogenraadia* by its biometric characterisation. *Planhoogenraadia alta* Bonnet, 1984, and *Hoogenraadia humicola* Bonnet, 1974, are the most closely related species to *P. bonneti* sp. n.

Planhoogenraadia bonneti sp. n. differs from *P. alta* by its wider and lower shell. Bonnet (1984) gave the average proportions for *P. alta*, as follows: L/l - 1,6 and H/l - 1,05. These proportions for *P. bonneti* sp. n. are L/l - 1,3 and H/l - 0,87, respectively. Furthermore



Figs. 1-5. *Planhoogenraadia bonneti* sp. n. 1 - apertural view; 2 - lateral view; 3 - aboral view; 4, 5 - measurements. E - depth of the aperture invagination; H - height; H_1, H_3 - height at the first and the third quarters of the shell; l - breadth; l_1, l_3 - breadth at the first and the third quarters of the shell; L - length; P - breadth of aperture; S - length of the flattened ventral side



Figs. 6-10. *Planhoogenraadia bonneti* sp. n. 6-8 - LM photographs (scale bars - 25 µm), 6 - apertural view, 7 - lateral view, 8 - aboral view; 9, 10 - SEM photographs (scale bars - 20 µm), 9 - apertural view, 10 - lateral view

Table 1. Biometric characterisation of *Planhoogenraadia bonneti* sp. n.*

Characters	$\bar{x}$	M	s	$s\bar{x}$	v	Min	Max	n
Length (L)	162.4	162.5	4.3	1.3	2.7	157	168	16
Breadth (l)	125.4	125.6	29	1.3	2.3	122	130	16
Height (H)	109.2	109.8	1.3	1.4	1.2	107	112	11
Breadth at the first quarter of the shell (l_1)	116.7	117.0	2.6	1.4	2.2	113	120	16
Breadth at the third quarter of the shell (l_3)	116.5	116.3	1.6	1.4	1.4	114	118	16
Height at the first quarter of the shell (H_1)	100.2	100.4	1.2	1.1	1.2	99	102	11
Height at the third quarter of the shell (H_3)	99.0	98.8	1.0	0.8	1.0	98	100	11
Breadth of aperture (P)	71.6	71.4	1.6	1.0	2.2	70	75	11
Length of flattened ventral side (S)	138.7	138.2	3.4	1.3	2.4	134	144	11
Depth of aperture's invagination (E)	11.0	11.2	1.1	0.9	6.1	10	13	11

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

Table 2. Biometric characterisation of *Centropyxis thailandica* sp. n.*

Characters	$\bar{x}$	M	s	$s\bar{x}$	v	Min	Max	n
Length	243.4	248.0	7.4	1.4	3.0	233	260	22
Breadth	198.0	202.5	5.4	2.1	2.6	190	212	22
Diameter of body	198.0	202.5	5.4	2.1	2.6	190	212	22
Length of visor	54.0	56.0	4.3	1.4	8.0	48	65	22
Large axis of aperture	113.7	113.3	2.3	1.6	2.0	110	117	14
Small axis of aperture	62.0	62.4	1.8	1.1	2.9	60	65	14
Dorso-ventral distance	135.1	135.4	2.8	1.5	2.0	130	140	22
Invagination of aperture	46.1	46.6	2.3	1.4	4.9	43	50	14

Abbreviations: see Table 1

P. bonneti sp. n. differs from *P. alta* by a comparatively thick wall of the shell, a brown colour, higher shell dimensions and an absence of the small teeth on the dorsal lip of the visor. The two species are distinguished from each other also by their ecology. Bonnet (1984) observed *P. alta* in forest soils and fertile soils which were rich in organic matter, whereas *P. bonneti* sp. n. inhabited humid epiphytic mosses.

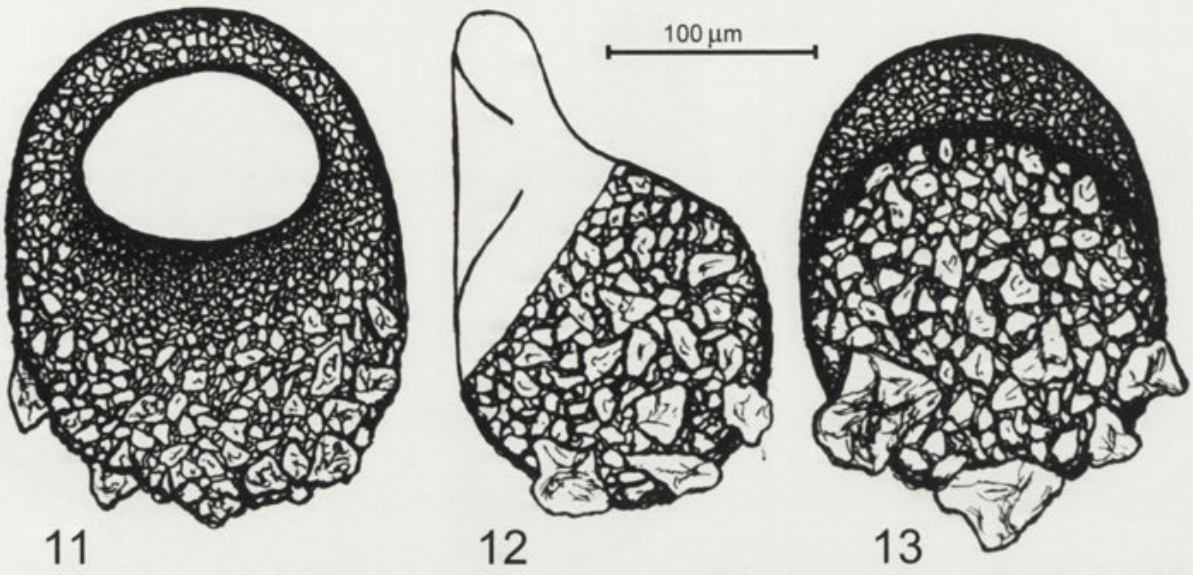
Planhoogenraadia bonneti sp. n. differs from *H. humicola* by its flattened ventral side and by the larger shell dimensions. Furthermore, the ecology of these species is different, too. Bonnet (1974) indicated that *H. humicola* inhabited soils rich in organic matter in dense forests of river outfall from tropical regions, whereas *P. bonneti* sp. n. was a moss-inhabiting species.

Eight species of *Planhoogenraadia* have been described till now and all of them inhabit different types of humid soils in Venezuela, Mexico, Guadeloupe, Nepal, the Philippines, New Guinea (Papua), Indonesia, Thailand, Spain and France (Bonnet 1984, 1987). Bonnet (1987) observed two unnamed species of *Planhoogenraadia*, also living in humid tropical soils (especially in Thailand), but he had not described their morphology.

So far *Planhoogenraadia bonneti* sp. n. is the only known moss-inhabiting species. From morphological point of view it is also interesting that this species has larger shell dimensions (L: 157-168 μm) in comparison with the rest of soil-inhabiting species.

Centropyxis thailandica sp. n. (Figs. 11-18, Table 2)

Diagnose: the shell is large, brown, a type of plagiostomy with a visor. In ventral and dorsal views it is ovoid. The aperture is large, ovoid and sub-terminal (Figs. 11, 14, 17). In lateral view there is one constriction which clearly separates the spherical body from a strongly flattened visor. The flattened ventral side is well differentiated. The



Figs. 11-13. *Centropyxis thailandica* sp. n. 11 - apertural view, 12 - lateral view, the anterior part of the shell is given without xenosomes in order to make visible the structure of the aperture, 13 - aboral view

inner lip of the aperture is feebly curved inside. The outer lip is incurvate and forms a strongly flattened visor (Figs. 12, 15). The shell surface on the ventral side is usually smooth, composed mainly of small siliceous particles, pasted together with yellow-brown organic cement (Figs. 11, 14, 17). The shell surface on the dorsal side is rough, usually composed of bigger quartz particles of various sizes. A few very large quartz particles are visible on the posterior extremity on the dorsal side (Figs. 13, 16, 18).

Biometry: see Table 2.

Ecology: humid epiphytic mosses on tree trunks, height 1-2 m.

Locality: Thailand, "Khao Yai" National Park 06.07.1995.

Type: holotype, preparation T-11/1995, in the collection of Dr. M. Todorov, Institute of Zoology, Sofia.

Etymology: the name of this species indicates that it is dedicated to the country where it was found for the first time.

Discussion: *C. thailandica* sp. n. is easily distinguished from the remaining known species of the genus *Centropyxis* by its biometric characteristics (Table 2). By its general form this big species resembles only *Centropyxis ventricosa* Bartoš, 1963. *C. thailandica* sp. n. differs from the other known species by the larger shell dimensions. Bartoš (1963) indicated an average length of 100 µm and an average breadth about 63 µm for *C. ventricosa*, whereas the same dimensions of *C. thailandica* sp. n. were 243 µm and 190 µm, respectively, i.e. almost three times larger. Furthermore, these species are also distinguished from each other by the structure of the visor. The visor of *C. ventricosa* is composed of two ranges of angular quartz particles and its outer lip is indented, whereas the visor of *C. thailandica* sp. n. is composed by many small siliceous particles and its outer lip is not indented.

So far more than 140 species of the genus *Centropyxis* have been described from different parts of the globe. Most of them are cosmopolitan. As a rule, the species with a shell larger than 200 µm are aquatic or inhabit the equatorial and tropical regions with relatively high degree of soil and air humidity.

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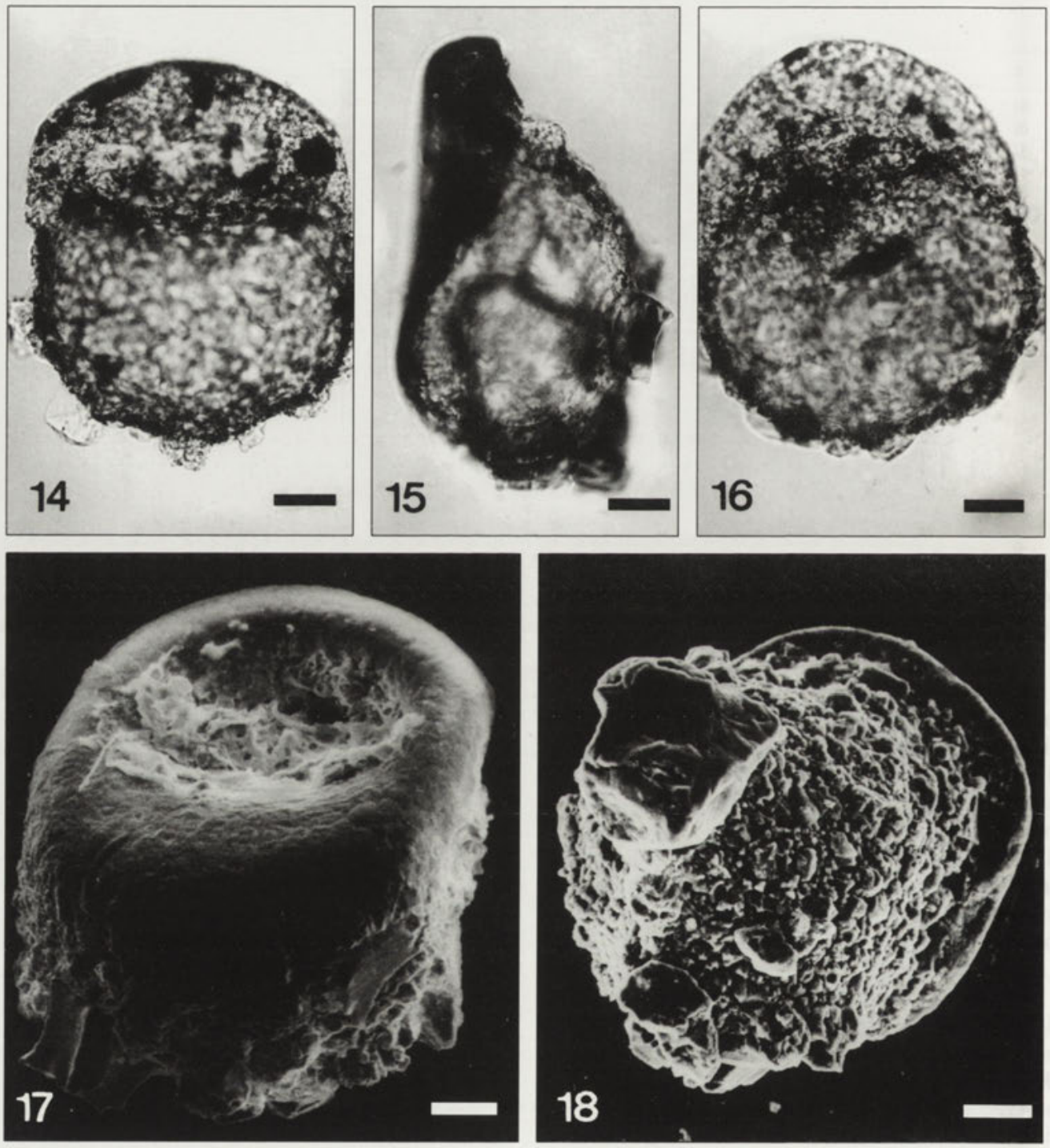

Figs. 14-18. *Centropyxis thailandica* sp. n. 14-16 - LM photographs (scale bars - 40 μm), 14 - apertural view, 15 - lateral view, 16 - aboral view, 17, 18 - SEM photographs (scale bars - 25 μm), 17 - apertural view, 18 - aboral view

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