Monograph of the Spathidiida (Ciliophora, Haptoria)

Vol. I: Protospathidiidae, Arcuospathidiidae, Apertospathulidae

Wilhelm Foissner and Xudong Xu

The spathidiids belong to the ciliate subclass Haptoria (Protozoa, Ciliophora), that is, they are predators using toxicysts to overwhelm the prey. Spathidiid ciliates prefer terrestrial and semiterrestrial habitats, but many occur also in freshwater, and some are marine.

Over 200 nominal spathidiid species have been described, sometimes based on seemingly minute differences. Thus, many protozoologists considered them as indeterminable and claimed for a detailed revision. The present monograph carefully revises the taxonomy, nomenclature, and ecology of all nominal species and shows that spathidiid diversity has been greatly underestimated. Based on the reinvestigation of described species with modern methods (silver impregnation, scanning electron microscopy) and the first description of over 50 new species, the family Spathidiidae is split into four

MONOGRAPH OF THE SPATHIDIIDA (CILIOPHORA, HAPTORIA)

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Aims and Scope

The Monographiae Biologicae provide a forum for top-level, rounded-off monographs dealing with the biogeography of continents or major parts of continents, and the ecology of well individualized ecosystems such as islands, island groups, mountains or mountain chains. Aquatic ecosystems may include marine environments such as coastal ecosystems (mangroves, coral reefs) but also pelagic, abyssal and benthic ecosystems, and freshwater environments such as major river basins, lakes, and groups of lakes. In-depth, state-of-the-art taxonomic treatments of major groups of animals (including protists), plants and fungi are also elegible for publication, as well as studies on the comparative ecology of major biomes. Volumes in the series may include single-author monographs, but also multi-author, edited volumes.

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Monograph of the Spathidiida (Ciliophora, Haptoria)

Vol I: Protospathidiidae, Arcuospathidiidae, Apertospathulidae

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Contents

Preface

This book continues our series of ciliate monographs, several of which have been published or are in preparation: class Colpodea (FOISSNER 1993); suborder Hypotrichia (BERGER 1999, 2005; volume 3 in preparation); and order Oligotrichida (AGATHA, in preparation). Furthermore, there are several thematic monographs available, viz., on the ciliates used as bioindicators in water quality assessment (FOISSNER et al. 1991, 1992, 1994, 1995); on the ecology and taxonomy of limnetic plankton ciliates (FOISSNER et al. 1999); on soil ciliates from Namibia (FOISSNER et al. 2002); and on the generic names of the ciliates (AESCHT 2001). Altogether, we described and revised over 1000 ciliate species in these monographs, that is, circa one tenth of the species described, but only one thirtieth of the diversity proposed (FOISSNER 1999b, c, 2004a). Certainly, this is far from being complete, but our detailed revisions will be long-lived, and we are optimistic to do some further monographs, even in a time where molecular biology overwhelms most other biological disciplines.

The family Spathidiidae belongs to the subclass Haptoria, a group of rapacious, "lower" holotrichs. The family comprises about 200 described species, most belonging to the time-honoured genus *Spathidium*. Several colleagues doubted the validity of so many *Spathidium* species (BUITKAMP 1977b, FINLAY et al. 1996, WENZEL 1955). However, our monograph shows not only the validity of most described species, but adds 50 new species discovered in over 500 samples from terrestrial biotopes worldwide. Thus, the spathidiids are as diverse as proposed by KAHL (1930a, b) 75 years ago! Now, they consist of over 250 species distributed in four families and 20 (!) genera, several of which will be described in this monograph or were established rather recently. About half of the species have been described or redescribed with modern methods, and thus each needs an average of eight printed pages in the revision. Accordingly, the over 200 species will be not squeezed into a single, large volume with 1000 pages, but they are split into two parts which form a harmonic unit, but can be used also independently. Further, the split facilitates publication, which was considerably delayed because we had to perform basic investigations on ontogenesis, conjugation and resting cysts as well as to describe nearly 100 populations half of which represented new species.

Our monograph is also a first attempt to standardize ciliate species descriptions by fixing and/or quantifying as many features as possible, for instance, the shapes of body and extrusomes, distances, and other "abouts". Indeed, most ciliate descriptions are inprecise using, for instance, different names for the same shape. Botanists recognized the resulting problems very early and thus established descriptive baselines many years ago (STEARN 1992).

We earnestly hope that our revision will be of use for a long time, not only to taxonomists, but also to ecologists and molecular biologists who not yet recognized the bioindicative capacity of protozoa (FOISSNER 1987a, 1997b) and the many interesting species this group contains.

Salzburg, August 2005   Wilhelm Foissner
                                                                            Kuidong Xu

Acknowledgements

A   General Section

As explained in the preface, the monograph has been split into two volumes. This applies also to the general section. In volume I, the general morphology, the life cycle, and the principal investigation methods are described, while volume II will deal mainly with ecology and geographic distribution, phylogeny, evolution, and classification. Further, it will contain a user-friendly key to all species.

1 Morphology and Principal Terms

In this section, the principal morphology and terminology will be explained, using simple but well-defined figures. Indeed, this is a first step to the urgently needed general methodology of ciliate descriptions. Partly, figures and terms were taken from STEARN (1992), who reviewed the terms used in descriptive botany. There are still some uncertainties, also in botanical terminology, which should be clarified in a more comprehensive treatment of the matter.

For general ciliate terminology, we refer to the excellent compilations of CORLISS (1979) and MARGULIS et al. (1993), while the general ciliate morphology is exhaustively treated in CORLISS (1979) and PUYTORAC (1994).

1·1 Size and shape, morphometry (Fig. 1–3, 5)

The spathidiids range from about 60 × 10 μm to 400 × 50 μm in vivo. The volume of one of the largest species, that is, *Epispathidium securiforme* is 35 times larger than that of one of the smallest species, that is, *Edaphospathula minor*. This is a small range when compared to those found in colpodids (200 000, FOISSNER 1993) and oxytrichid stichotrichs (140, BERGER 1999). We have used seven categories of size (as reflected in body length; arbitrarily if unrealistically set up with non-overlapping ranges) as follows: very small, 10–30 μm; small, 30–60 μm; moderately small, 60–90 μm; medium sized, 90–150 μm; large, 150–300 μm; moderately large, 300–500 μm; very large, > 500 μm.

According to the name, spathidiids should be spatula-shaped. Unfortunately, this hardly applies (Fig. 1–3, 5)! Most are very narrowly to broadly bursiform with a more or less slanted anterior end and a rounded posterior. Others are cylindroidal, vermiform, clavate, axe-shaped etc. Thus, a huge variety of shapes exists, and the ratio of body length to body width ranges from about 1:1 to 30:1. Most species are slightly to distinctly flattened laterally, some are even leaf-like. Many of the terrestrial species are small and/or slender, as is typical for soil organisms in general (FOISSNER 1987a). However, those living in mosses and leaf litter may be large, for instance, the common *Epispathidium amphoriforme*. The shape is stabilized by bundles of cortical microtubules (WILLIAMS et al. 1981), but usually the cortex remains flexible and the shape may thus strongly deform in over- or under-nourished cells. Theronts and trophonts occur, but true polymorphism is lacking. Likewise, metaboly and pronounced contractility do not occur because myonemes are absent.

Invariably, the oral apparatus occupies the anterior body end, forming a more or less

broadly spatulate (l:w 3:2)

spatulate (l:w 2:1)

narrowly spatulate (l:w 3:1–6:1)

very narrowly spatulate (l:w 6:1–9:1)

cylindroidal (l:w 9:1–12:1)

rod-shaped and/ or vermiform (l:w > 12:1)

OB CK

clavate

cordiform

rostrate

Oral bulge (CK) slope:

slightly oblique < 30°

oblique 30°–60°

strongly oblique > 60°

amphoriform

knife-shaped (blade ≥ 1/3 of body length)

axe-shaped

Size (length) classification

very small: 10–30 µm

small: 30–60 µm

moderately small: 60–90 µm

medium sized: 90–150 µm

large: 150–300 µm

moderately large: 300–500 µm

very large: > 500 µm

Transverse views

circular
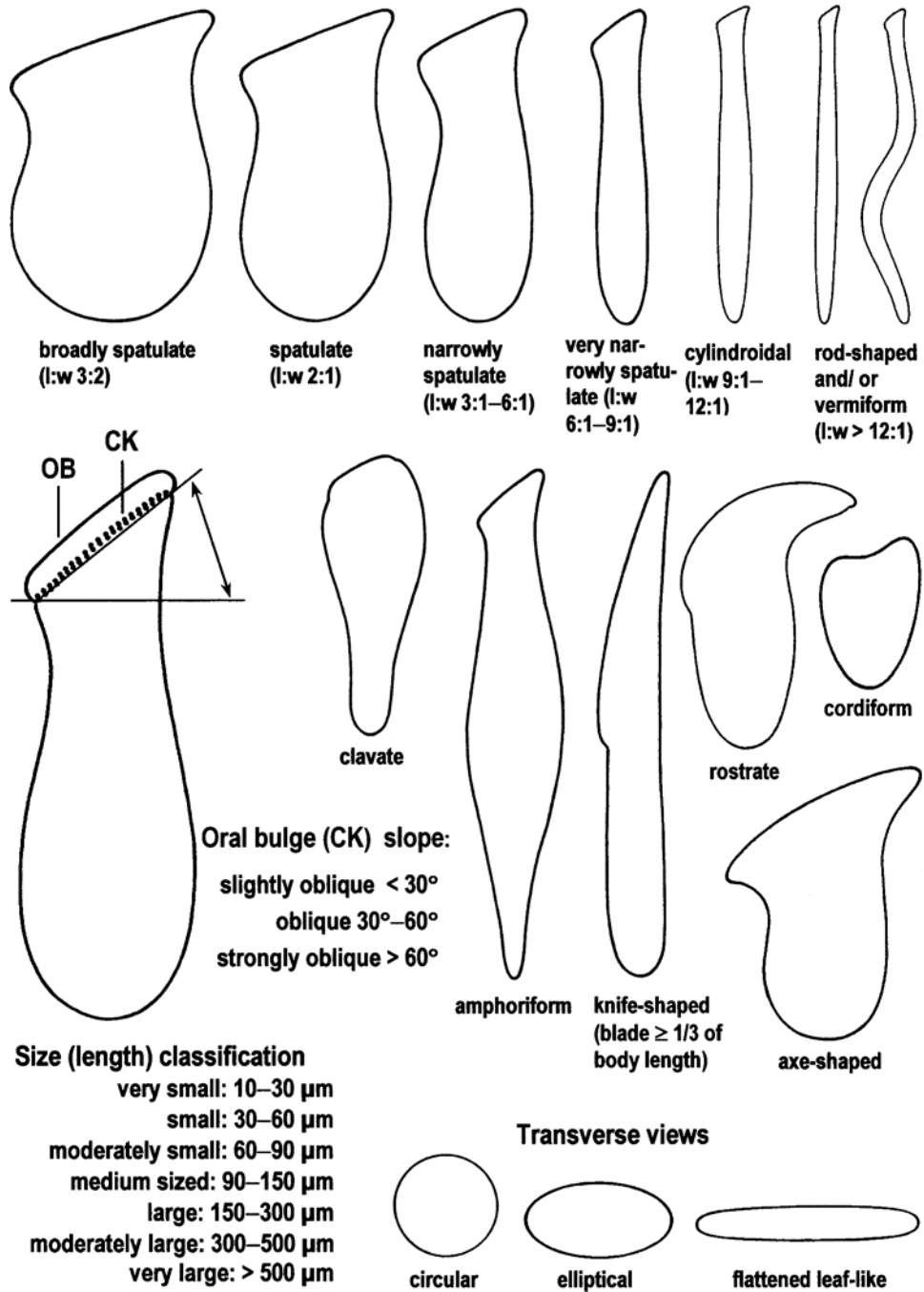
elliptical

flattened leaf-like

Fig. 1 Classification (terminology) of body shape in lateral and transverse view, size (length), and slope of oral bulge in spathidiid ciliates. CK – circumoral kinety, l:w – ratio of body length to width, OB – oral bulge.
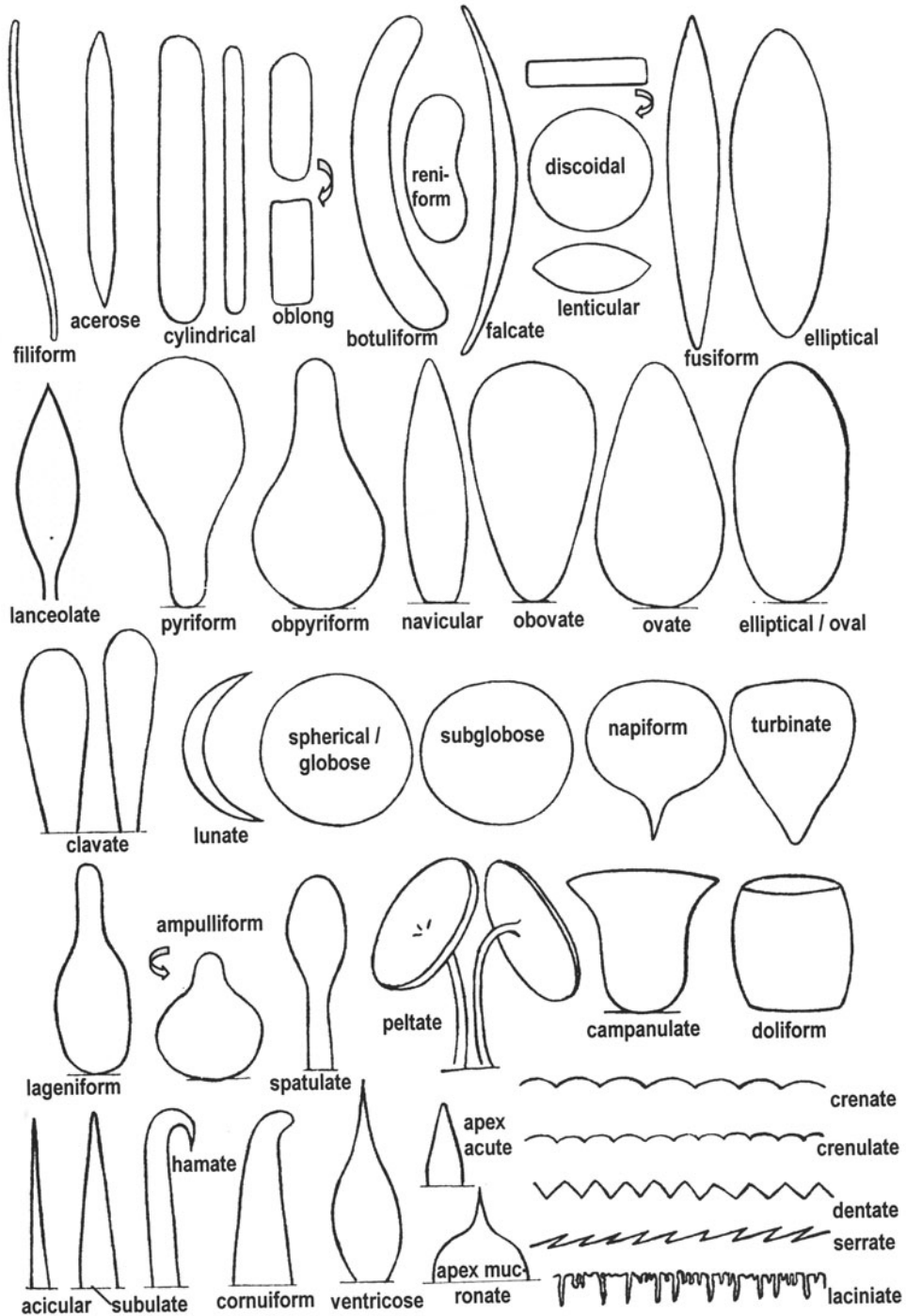
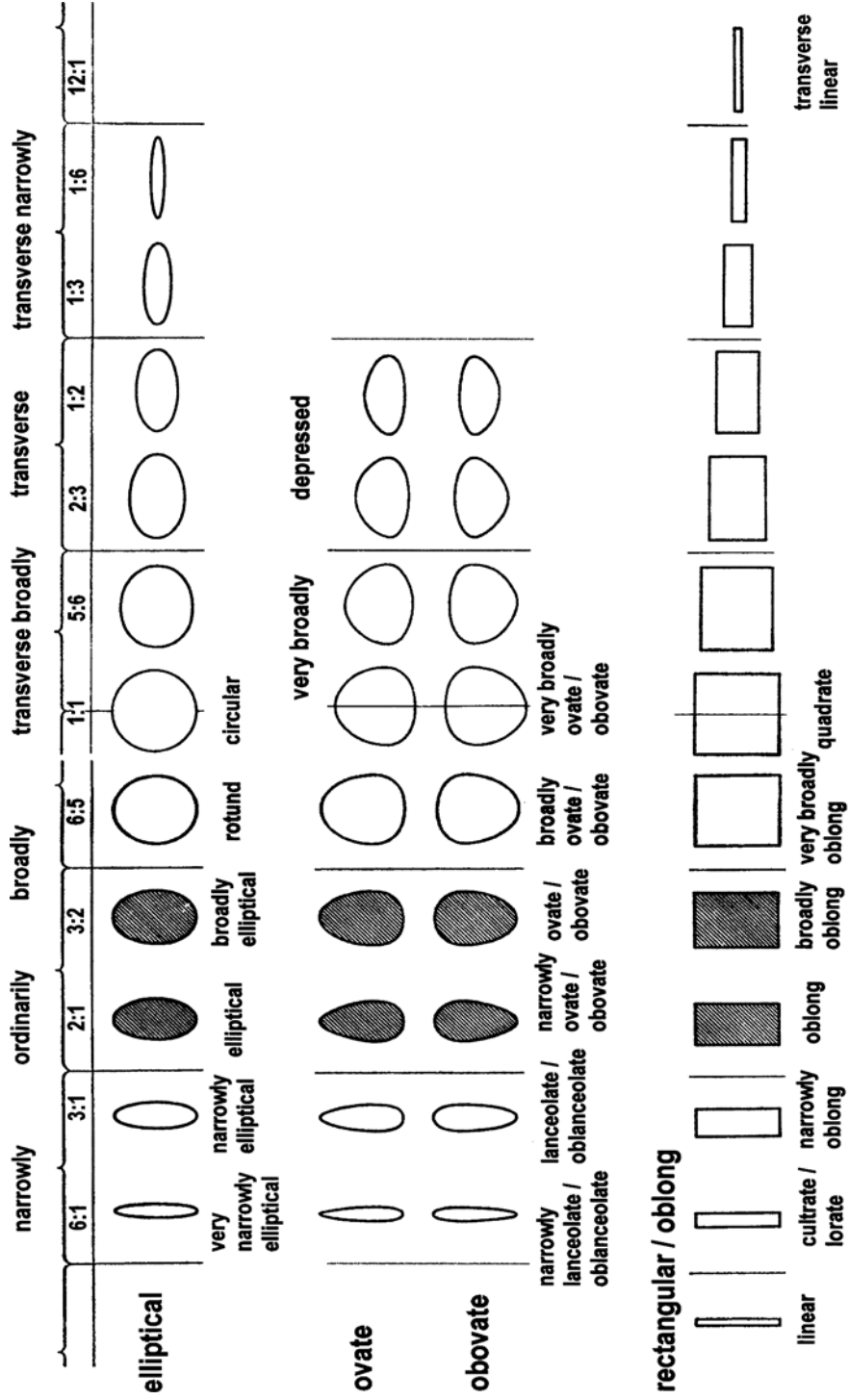**Fig. 2** Terminology of shapes/outlines. From STEARN (1992), modified.

Fig. 3 Shape outlines. From STEARN (1992), modified.

distinct bulge rarely extending to posterior body end (genus *Rhinothrix*). The bulge, respectively, the anterior body end is more or less slanted. Thus, bulge length is a composite of body width and bulge slope. We have used three categories of bulge slope as follows: slightly oblique, < 30°; (ordinarily) oblique, 30–60°; strongly oblique, > 60° (Fig. 1).

Detailed morphometrics are available from about 150 populations belonging to circa 100 species, mainly due to the investigations of FOISSNER (1984), FOISSNER et al. (2002), and the present monograph. These data show that the mean coefficients of variation are very similar to those found in colpodids, stichotrichine hypotrichs, and other kinetofragminophoran ciliates (BERGER 1999, FOISSNER 1984, 1993, FOISSNER et al. 2002). Accordingly and in the contrast to the widespread assumption (e.g., WENZEL 1955), spathidiids are as variable or stable as other ciliate groups. The impression of a special variability of the spathidiids is caused by observations of a few species in pure cultures, where variability is indeed high – not only in spathidiids but also in most other ciliates. Malformed specimens can survive and sometimes even reproduce in pure cultures due to the optimal food supply, while they usually die in nature. The number of ciliary rows usually varies only slightly, that is, between 5–10% and is thus one of the most important features for species recognition. Most other characteristics vary between 10% and 20%, while body length:width ratio and the number of macronucleus nodules in multinucleate species have high coefficients of variation between 20 and 40%. The morphometric data give important information about the stability of features and their significance for species recognition. Thus, all descriptions should be accompanied by morphometrics of at least the main features.

1·2 Nuclear apparatus (Fig. 4)

Spathidiids have a single macronucleus and micronucleus or many macronucleus nodules and several micronuclei. The shape of the macronucleus ranges from globular to a long, tortuous strand, which may be band-like flattened, a curious feature described only recently (FOISSNER et al. 2002). The micronuclei may be globular, ellipsoidal, lenticular, narrowly ovate, or bluntly fusiform. Altogether, we have distinguished 16 macronuclear and 10 micronuclear configurations (Fig. 4). This high diversity makes the nuclear apparatus to one of the most important features for species recognition. The patterns are as stable or as variable as those of other ciliates and are sometimes obscured by post-divisional, post-conjugational, or ontogenetic reorganization processes (MOORE 1924a, b, XU & FOISSNER 2004). **When in doubt, look at very early dividers which invariably show the "real" nucleus pattern.** So far, chromatin extrusion has not been described.

The macronucleus contains globular, oblong, or irregular dense masses about 1–5 μm in size. Usually, they are recognizable in vivo and impregnate deeply with protargol. There is some indication that these inclusions represent nucleoli: (i) the central mass of the macronucleus of *Colpoda steinii* and *Dileptus* sp. deeply impregnates with protargol and represents a compound nucleolus, according to cytochemical and electronmicroscopical investigations (FOISSNER 1993, RAIKOV 1982); (ii) chromatin bodies are usually smaller than 1 μm and numerous, while nucleoli are often larger than 1 μm and comparatively rare (RAIKOV 1982). These two features apply to the macronuclear structures impregnating with various protargol methods. Thus, we designate these

spherical to
globular with
globular MI
attached

reniform with
lenticular MI
and globular
to oblong
nucleoli

oblong
(> 2:1–6:1)

cylindroidal
(6:1–12:1) with
globular MI

curved-
cylindroidal and
bimicronucleate

ellipsoidal with
globuar MI in
indentation of MA

dumbbell-shaped

binucleate with
MI in between

semicircular with
ellipsoidal MI

scattered macronucleus
nodules and multimic-
ronucleate

rod-shaped
(12:1–20:1)
with discoi-
dal MI

tortuous (filiform)
strand (> 20:1)
with MI near and
attached to MA

± distinctly nodulated
(moniliform), tortuous
(filiform) strand

flattened, tortuous
(filiform) strand

spiralized
(filiform)
strand

± moniliform
= nodules in
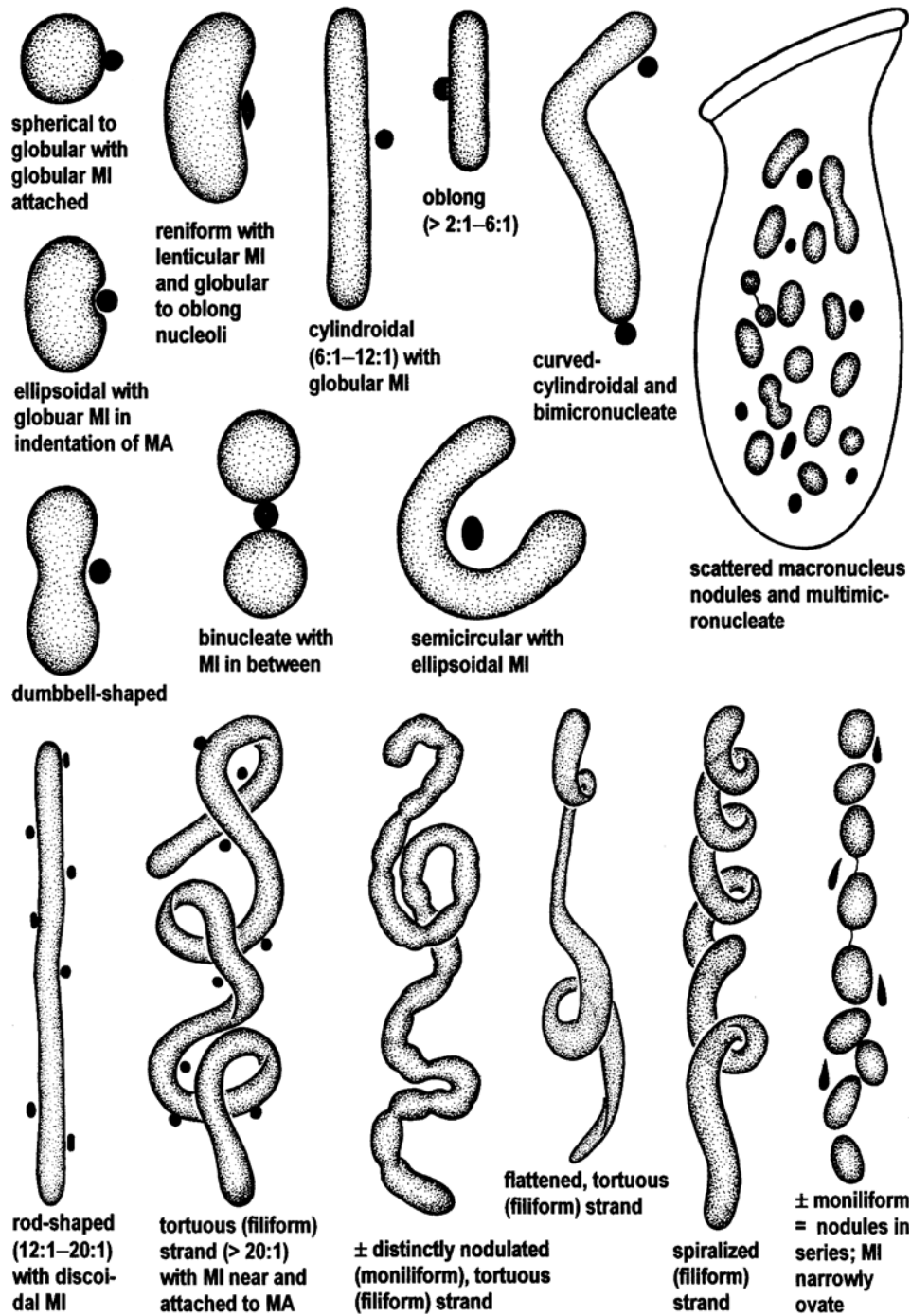series; MI
narrowly
ovate

Fig. 4 Shapes of macronucleus and micronucleus in spathidiid ciliates. Nucleoli shown only in the reniform type. MA – macronucleus, MI – micronucleus.

structures as "nucleoli", being aware that this needs confirmation by cytochemical investigations. See BOHATIER et al. (1978) for a brief electronmicroscopical description of the nuclear apparatus of *Epispathidium amphoriforme*.

1·3 Contractile vacuole and cytopyge (Fig. 5)

Most spathidiids have a single contractile vacuole in posterior body end; few have a second contractile vacuole with distinct excretory pores underneath the dorsal brush, that is, above mid-body, for instance *Arcuospathidium bulli*; and some have several contractile vacuoles, each with their own excretory pores, in a dorsal and/or ventral row (Fig. 5). The bivacuolate pattern evolved independently in *Spathidium* and *Arcuospathidium*, while the multivacuolate pattern is diagnostic of the genus *Supraspathidium* (FOISSNER 2003c). Usually, the vacuole is a simple blister surrounded by smaller collecting vesicles during the diastole. Some species of doubtful classification have a more or less conspicuous collecting canal which supplies the main vacuole, for instance, *Spathidium latissimum* and *Supraspathidium teres* (Vol. II).

The fluid collected by the contractile vacuole is expelled via one or several ordinary excretory pores. Usually, the pores are scattered in the posterior pole area, sometimes they are slightly subterminal on the ventral or dorsal side, and in a few tailed species they are far subterminal, for instance, in *Spathidium apospathidiforme* and *Apospathidium atypicum* (Vol. II).

The cytopyge is known in only few species, where it is in the posterior area, that is, near the excretory pores of the contractile vacuole. In some species, for instance, *Rhinotrix porculus*, the fecal mass traverses the contractile vacuole when it is expelled.

1·4 Extrusomes (Fig. 5–7)

Shape, size, and arrangement of the extrusomes are highly diverse and are thus a main diagnostic feature of the individual species (Fig. 5–7). This was already emphasized by KAHL (1930a, b). Accordingly, the extrusomes must be carefully studied in vivo because they often become distorted in silver preparations or do not impregnate at all. Indeed, the extrusome features are so important that species cannot be recognized without this information.

Most or even all spathidiids are predators. Thus, they have toxicysts, except of some curious species which lack toxicysts at all. About one third of the species has two shape and/or size types of toxicysts, and some even have three kinds, for instance, *Rhinothrix porculus*. Further, (likely) all spathidiids have at least one kind of mucocysts, appearing as "cortical granules"(Fig. 7).

The toxicysts are studded in the oral bulge and scattered in the cytoplasm; additionally, they may be attached to the somatic cortex. For determining the shape, size, and arrangement of the toxicysts only fully developed (mature) toxicysts may be used, that is, those which are anchored to the oral bulge or somatic cortex (Fig. 5, 6); cytoplasmic toxicysts are frequently not fully developed. This is evident, inter alia, from their impregnation capacity: anchored toxicysts usually do not impregnate with protargol, while various cytoplasmic developmental stages often impregnate rather deeply.

Shape and arrangement of the mucocysts are much less diverse, at least in the light
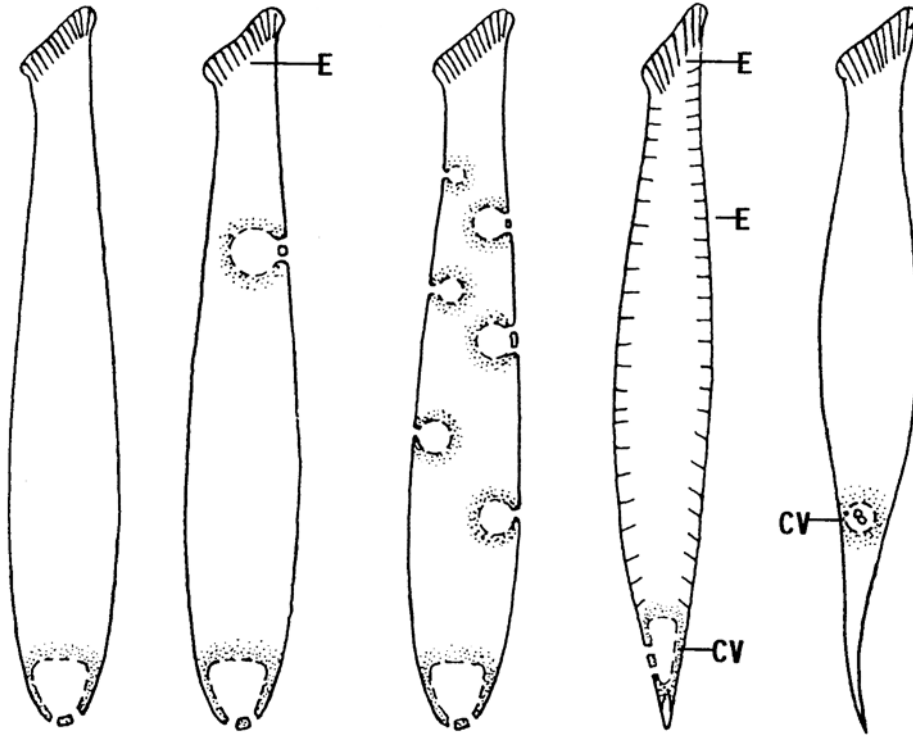
single CV in posterior end; toxicysts in oral bulge

bivacuolate, that is, a CV each in anterior half and rear end

multivacuolate, that is, a dorsal and/or ventral row of CVs each with excretory pores

posterior end acute; CV slightly subterminal; oral and somatic toxicysts

posterior end tail-like narrowed; CV distinctly subterminal
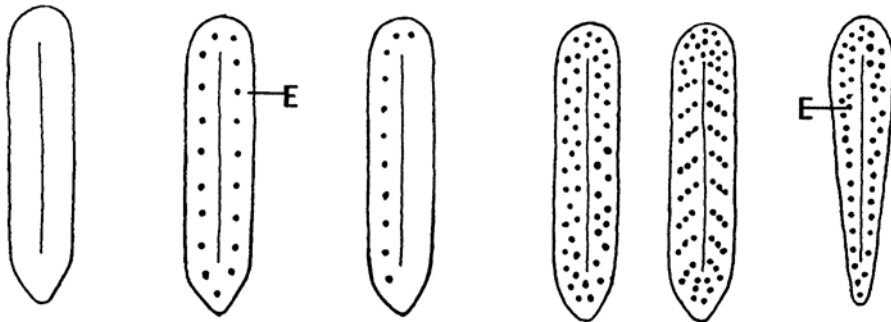
TOXICYSTS

lacking

in single row each in right and left half of oral bulge

a row in right or left half of oral bulge

scattered

in short, oblique rows

scattered dorsally and in rows ventally

Fig. 5 Contractile vacuole (CV) and toxicyst (E) patterns and rare body shapes in spathidiid ciliates.

very narrowly ellipsoidal (6:1)
narrowly (elongate) ellipsoidal (3:1)
ellipsoidal (2:1)
broadly ellipsoidal (3:2)

very narrowly
ovate / cuneate
(6:1–12:1)

narrowly
ovate / cuneate
(3:1–5:1)

ovate / cuneate
(2:1)

ampulliform

broadly ovate / cuneate (3:2)
acicular (> 12:1)

symmetrically / asymmetrically
obclavate

oblong / with conical
anterior end (< 6:1)

rod-shaped / with
rounded or conical
ends (6:1–20:1)

filiform, straight
or curved (> 20:1)

fusiform

bluntly or broadly
fusiform (transverse
section circular);
lenticular (transverse
section flattened)

Fig. 6 Shapes of extrusomes in spathidiid ciliates: toxicysts. Length about 1–50 μm, often 5–20 μm.

shapes   **loose**   **ordinary**   **dense**   **two size-types**

Fig. 7 Shapes and arrangement of extrusomes in spathidiid ciliates: mucocysts and related structures. Size about 0.2–2 μm.

microscope (Fig. 7). They are globular, broadly ellipsoidal, or ellipsoidal and ≤ 2 μm long. In the light microscope, the mucocysts appear granular ("cortical granules") and are arranged in rows following the slightly oblique course of the postciliary microtubular ribbons (FOISSNER et al. 2002, WILLIAMS et al. 1981; Fig. 7). When densely arranged, the mucocysts form an opaque sheet in the cortex, obscuring more or less completely the ciliary pattern, depending on their affin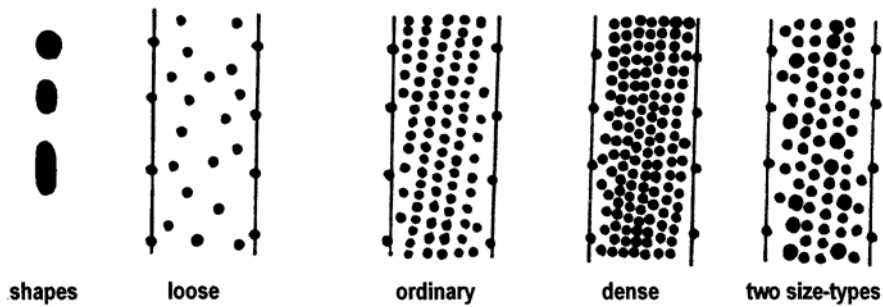ity to protargol. Rarely occur two types of mucocysts or mucocyst-like organelles, differing in size and/or refractivity and/or fine structure (FOISSNER et al. 2002, WILLIAMS et al. 1981). The mucocysts are not easily released. Methyl green-pyronin, which causes mucocyst extrusion in many ciliates (FOISSNER 1991), is usually ineffective. By chance, we were successful in obtaining some good SEM micrographs showing the release in several spathidiids (see volume II). These observations show that the "cortical granules" are indeed mucocysts and are extruded like those of other ciliates (HAUSMANN et al. 2003).

1·5 Cytoplasm and colour

All spathidiids are colourless. However, when packed with highly refractive food and/or lipid droplets and/or crystal-like inclusions, they appear dark or black under low brightfield magnification, for instance, *Arcuospathidium cooperi* (Fig. 56a) and *Rhinothrix porculus* (Fig. 149a–i). Some species are green due to ingested or symbiotic algae, for instance, *Spathidium chlorelligerum*. Crystal-like inclusions are frequent in some species, for instance, in *Apertospathula inermis* (Fig. 146a, d–f); in others, such inclusions are possibly partially digested paramylon grains from euglenoid prey. The food vacuoles may be large because the prey is sometimes ingested as a whole; soon, such vacuoles dissociate into several smaller ones with granular contents.

1·6 Movement

Basically, spathidiids glide and creep slowly to rapidly on and among the mud particles of limnetic and terrestrial habitats. However, when approaching the free water, they swim by rotation about the main body axis, whereby the oral region may be curved laterally and, in the long and slender species, swings more distinctly than the posterior, making the cells looking like swimming cones. The large, voluminous species usually glide majesticly, while the long or slender species show a worm-like behaviour wriggling like nematodes and slender hypotrichs. With very few exceptions (genus *Spathidiodes*), spathidiids are very flexible and can squeeze through small spaces. Pronounced contractility does not occur, but many species may show some shortening of the body, especially under mild coverslip pressure.

1·7 Somatic ciliature

1·7·1 The basic ciliary patterns (Fig. 8)

Spathidiids are asymmetric. Thus, both a ventral and dorsal side as well as a right and a

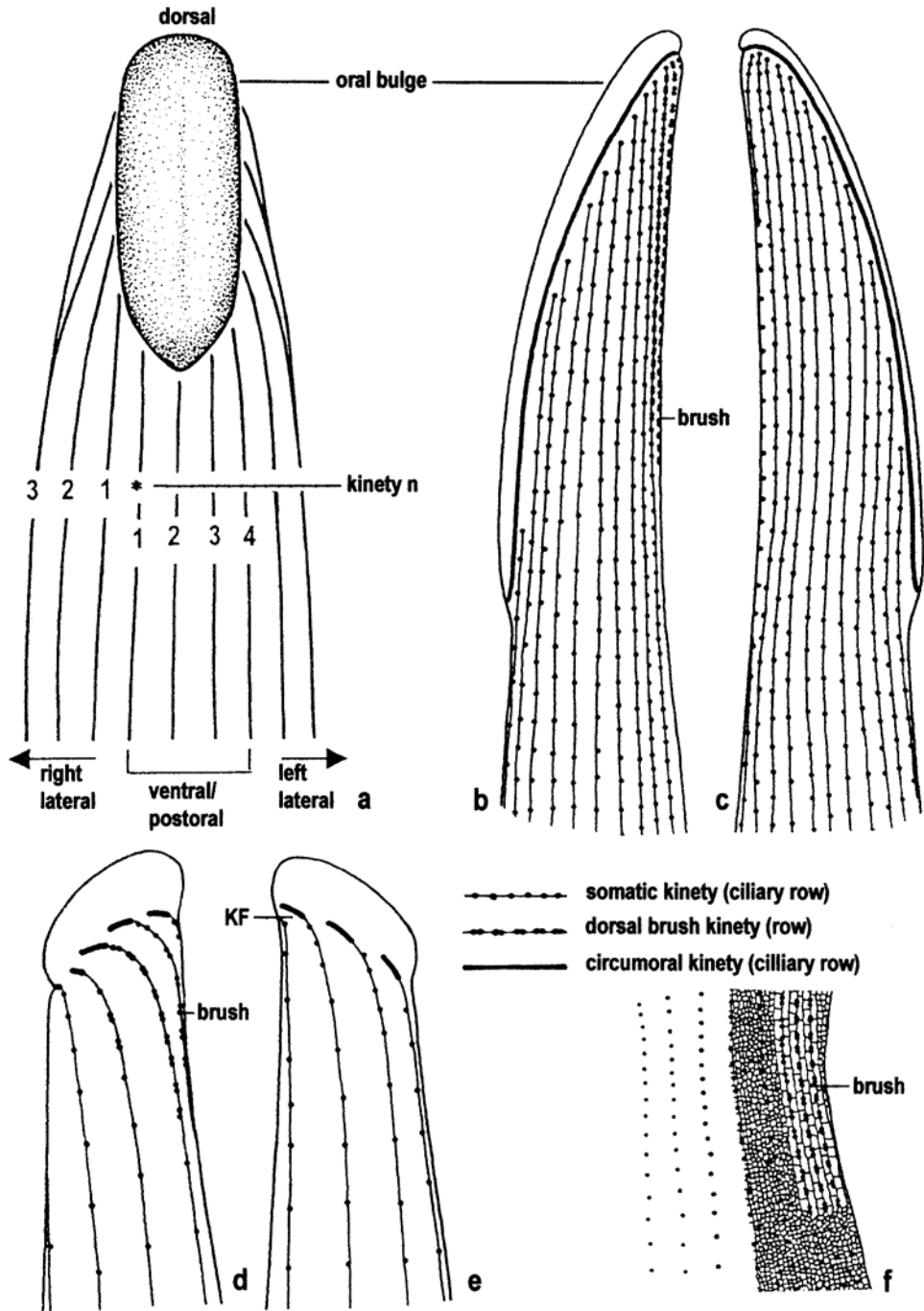**Fig. 8a–f** Designation of ciliary rows (a), *Arcuospathidium* ciliary pattern (b, c), *Protospathidium* ciliary pattern (d, e), and silverline pattern (f) of spathidiid ciliates. For details, see text of general section. From FOISSNER (1980b, 1984) and original (a).
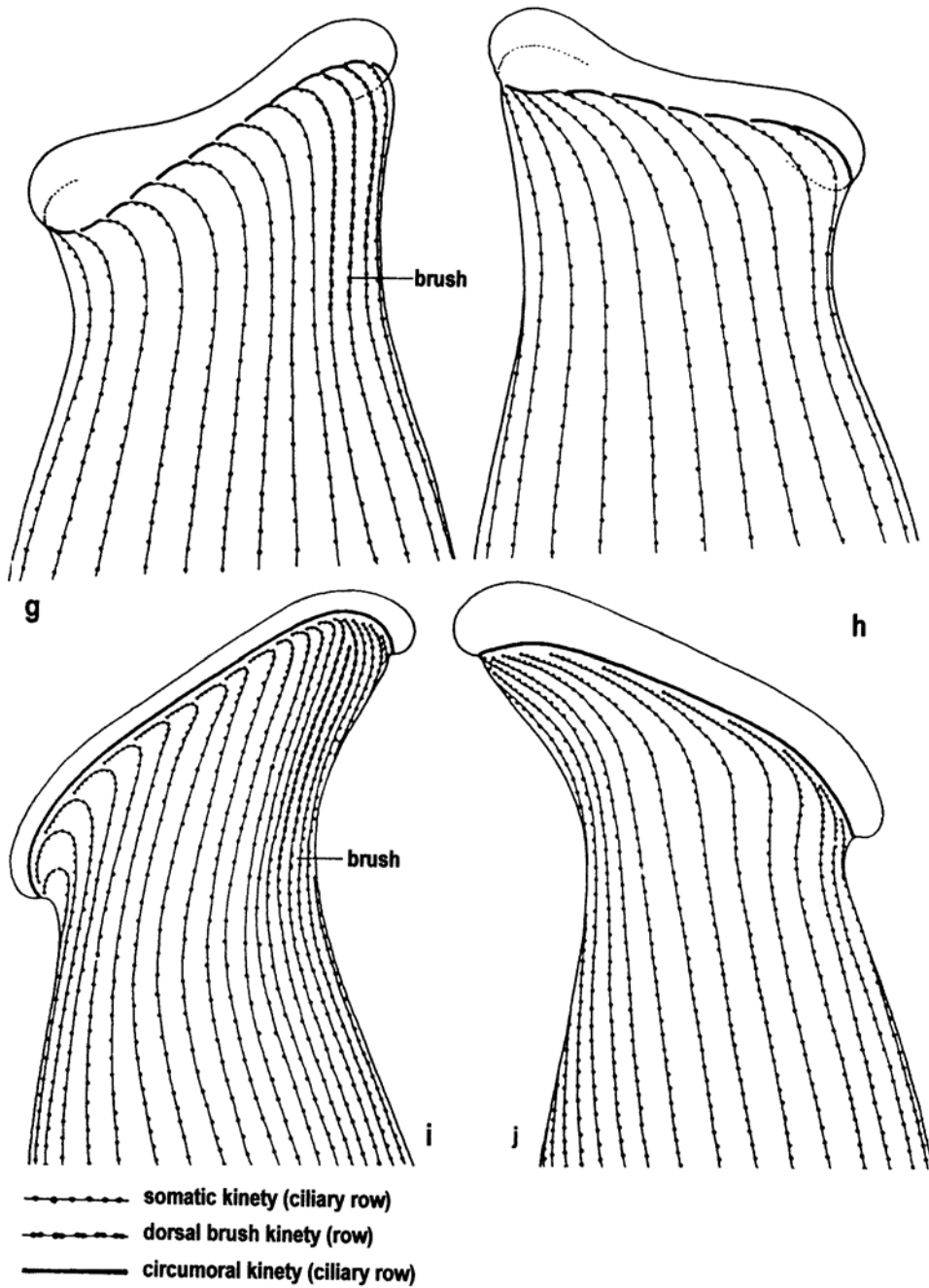
Fig. 8g–j *Spathidium* (g, h) and *Epispathidium* (i, j) ciliary pattern. For details, see text of general section. From FOISSNER (1984).

left side can be distinguished. However, the boundaries are fluent because all ciliary rows are alike. We suggest to designate the kineties as shown in figure 8a.

The spathidiids contained in this monograph have a simple, holotrichous (~ complete) ciliature composed of serially arranged monokinetids (= single, ciliated basal bodies). The monokinetids usually form equidistant rows extending from the circumoral kinety to the posterior body end; the rows may be slightly shortened both anteriorly and/or posteriorly and frequently contain some irregularities, such as small breaks or overlapping pieces; further, often some rows are strongly shortened anteriorly and/or posteriorly. Depending on row distance, we distinguish narrowly spaced rows ($\leq$ 4 µm), ordinarily spaced rows (4–8 µm), and widely spaced rows ($\geq$ 8 µm), as shown in figure 9.

The cilia within the rows are densely, ordinarily, or loosely spaced (Fig. 9). Usually, they are more densely spaced orally than postorally; rarely, vice versa. Frequently, the kineties contain some dikinetid-like kinetids, especially in the middle third. The posterior basal body of these pairs is ciliated, while the anterior is bare. Likely, the anterior kinetids are a reservoir for growing and/or dividing cells.

Based on FOISSNER (1984), we distinguish four basic ciliary patterns which, however, show some transitions and even may be obscured when the ciliary rows are distinctly shortened anteriorly and/or the species has less than 10 ciliary rows, for instance, in *Arcuospathidium namibiense*. Frequently, such species cannot be classified properly at the present state of knowledge. Originally, the patterns were used to define genera (FOISSNER 1984). In our system, the patterns define families, except of the *Epispathidium* pattern. The rank elevation will be discussed in the phylogenetic section of volume II. Briefly, it is due the discovery of new evolutionary lines (genera) having the same pattern but differing in other important features, for instance, the location of the dorsal brush in *Arcuospathidium* and *Cultellothrix* (XU & FOISSNER 2003).

Protospathidium pattern (Fig. 8d, e): The kinetofragments composing the circumoral kinety are separated by gaps one to four dikinetids wide and are connected to the ciliary rows. Thus, the circumoral kinety is discontinuous. The anterior region of the right side kineties is directed dorsally, while the anterior region of the left side kineties is directed ventrally.

Arcuospathidium pattern (Fig. 8b, c): The oral kinetofragments are aligned to a continuous circumoral kinety distinctly separate from the ciliary rows. The anterior end of the lateral somatic kineties is directed dorsally on **both** sides of the cell.

Spathidium pattern (Fig. 8g, h): The oral kinetofragments are usually connected to the ciliary rows from which they originated. Thus, minute discontinuities occur in the circumoral kinety. The anterior region of the right side kineties is directed dorsally, while the anterior region of the left side kineties is directed ventrally. The *Spathidium* pattern comprises several subtypes shown and discussed in the characterization of the genus.

Epispathidium pattern (Fig. 8i, j): The oral kinetofragments are aligned to a continuous circumoral kinety distinctly separate from the ciliary rows. The anterior region of the somatic kineties is densely ciliated and usually so distinctly curved dorsally (right side) or ventrally (left side) that the circumoral kinety is seemingly doubled, that is, the anterior region of the ciliary rows parallels the circumoral kinety.

SK

spacing of ciliary rows (body diameter : number of ciliary rows):

very narrowly to narrowly spaced (≤ 4 μm);
ordinarily spaced (4–8 μm);
widely to very widely spaced (≥ 8 μm)

cilia spaced

densely   ordinarily   loosely

average ciliary distance

≤ 2 μm   2–4 μm   ≥ 4–8 μm

DK

brush dikinetids spaced

densely   ordinarily   widely (loosely)

interkinetidal distance
(brush row length : number of dikinetids)

≤ 0.7 μm   0.7–1.5 μm   ≥ 1.5 μm

B1 2 3

dorsal brush

isostichad   heterostichad   distinctly heterostichad

row length difference

≤ 20%   20–50%   ≥ 50%

CK

SM

DK

SM

tailless

dorsal brush

isomorphic   heteromorphic

dorsal brush
monokinetids with or
without specialized
bristles/cilia anteriorly
and/or posteriorly

Fig. 9 Terminology of the somatic and dorsal brush ciliature. B1-3 – dorsal brush rows, CK – circumoral kinety, DK – brush dikinetids, SK – somatic ciliary rows, SM – specialized monokinetids.

1·7·2 The dorsal brush (Fig. 9)

The anterior region of some dorsal and/or left lateral ciliary rows is modified to the so-called "dorsal brush" or, simply, "brush". The area consists of specialized, narrowly spaced mono- and dikinetids with bristle-like cilia usually distinctly shorter than ordinary somatic cilia. The function of the brush is not known. The dorsal brush shows a considerable diversity and is thus of high significance in genus and species taxonomy.

Two features of the brush are of generic significance because they are not caused by simple space constraints. The first feature is the number of brush rows, which is highly stable: usually, there are three, but some species groups have only two (*Schmidinger-ophrya*) or more than three (*Semibryophyllum*). The second feature is the location of the brush: usually, the rows extend dorsally or dorsolaterally, while they are entirely or almost entirely displaced left laterally in *Cultellothrix* and *Latispathidium*.

A variety of brush features is used to characterize and distinguish species. Most details are shown in figure 9, and thus will be mentioned only briefly. The dorsal brush can be short (longest row ≤ 15% of body length in protargol preparations), ordinary (15–35%), or long (≥ 35% of body length). The individual brush rows may be of similar length (isostichad), of different length (heterostichad), or of very different length (distinctly heterostichad). Usually, the dorsal brush is isomorphic, that is, composed of bristles throughout; rarely, it is heteromorphic, that is, mixed with ordinary cilia (Fig. 9). The individual brush rows may commence and end with dikinetidal bristles. This is the tailless condition, which is rarely found, for instance, in most *Cultellothrix* species. Usually, the brush rows commence underneath the circumoral kinety with a short "anterior tail", that is, with a few ordinarily ciliated monokinetids. The posterior end of the brush rows is either dikinetidal, heteromorphic or, in the leftmost row 3, modified to an inconspicuous "posterior tail" composed of rather narrowly spaced monokinetids with up to 5 μm long bristles. The posterior tail of brush row 3 usually extends to near mid-body, rarely it is absent (some protospathidiids) or extends to near rear body end (some *Spathidium* species). Underneath the brush, the rows continue as ordinary somatic kineties and extend to the posterior end of the cell (Fig. 8b, d). Finally, the brush dikinetids may be densely, ordinarily, or widely spaced (Fig. 9).

During cell division, the brush bristles are generated as shown in figure 131f: a new bristle each is formed anteriorly of the parental cilia which gradually shorten to posterior brush bristles.

1·8 Oral apparatus

1·8·1 Structure (Fig. 10–12)

The location and structure of the spathidiid oral apparatus are basically as in other haptorid ciliates. However, details are sufficiently different to base two families on the oral apparatus, viz., the *Apertospathulidae* and the *Pharyngospathidiidae* (volume II). Likewise, the shape of the oral bulge varies considerably and is thus an important feature for species recognition (Fig. 10). Detailed electronmicroscopical investigations on the spathidiid oral bulge are lacking. However, the basic organization was described by
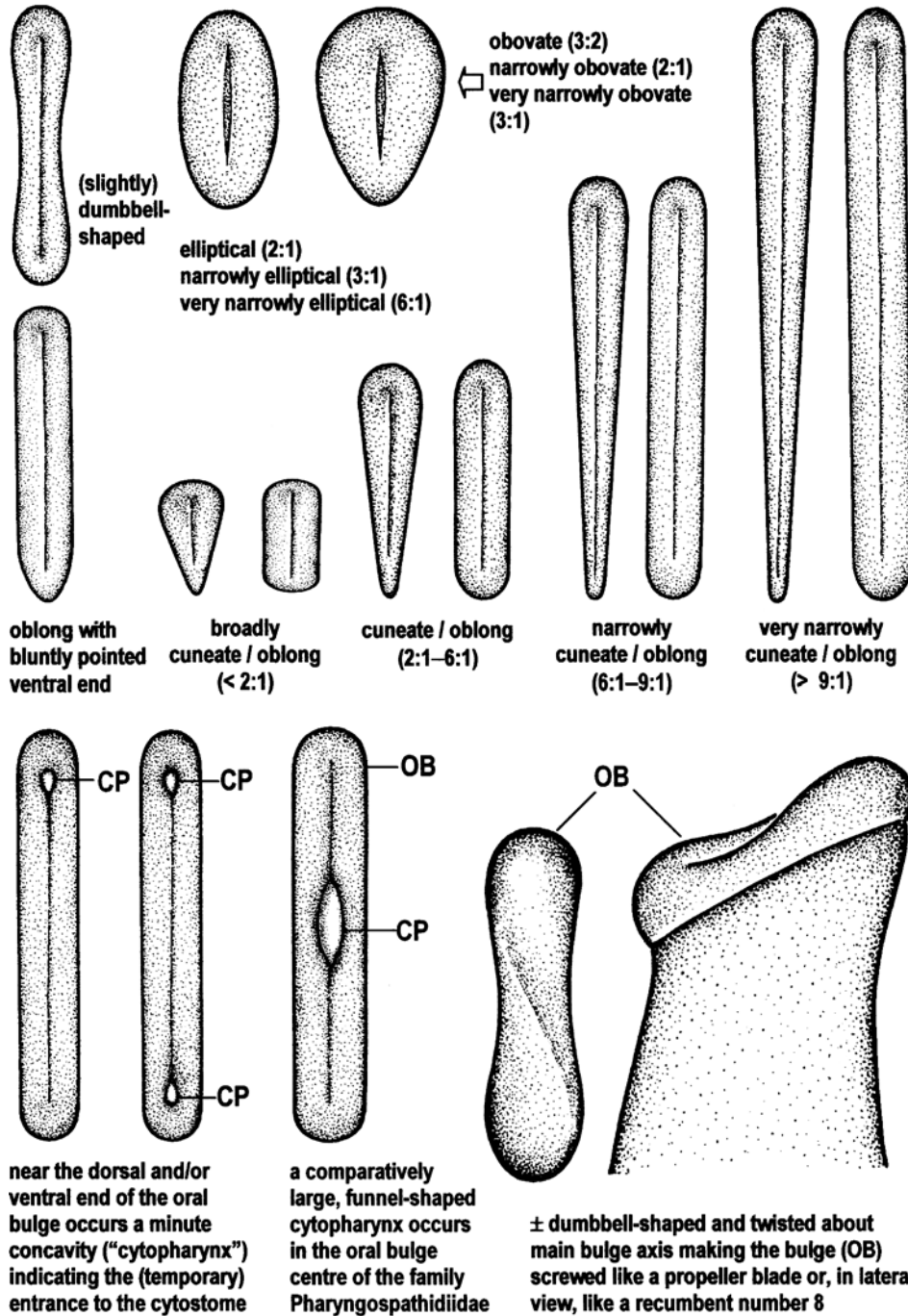
(slightly) dumbbell-shaped

elliptical (2:1)
narrowly elliptical (3:1)
very narrowly elliptical (6:1)

obovate (3:2)
narrowly obovate (2:1)
very narrowly obovate (3:1)

oblong with bluntly pointed ventral end

broadly cuneate / oblong (< 2:1)

cuneate / oblong (2:1–6:1)

narrowly cuneate / oblong (6:1–9:1)

very narrowly cuneate / oblong (> 9:1)

CP — CP — OB — OB

CP

CP

near the dorsal and/or ventral end of the oral bulge occurs a minute concavity ("cytopharynx") indicating the (temporary) entrance to the cytostome

a comparatively large, funnel-shaped cytopharynx occurs in the oral bulge centre of the family Pharyngospathidiidae

± dumbbell-shaped and twisted about main bulge axis making the bulge (OB) screwed like a propeller blade or, in lateral view, like a recumbent number 8

Fig. 10 Shapes of oral bulge and mouth patterns in spathidiid ciliates. CP – cytopharyngeal opening, OB – oral bulge.

BOHATIER et al. (1978). These findings and the light microscopical data collected during the preparation of the present monograph were used for the scheme shown in figure 12. Invariably, the spathidiid oral apparatus ("mouth") occupies the entire or almost the entire anterior body end. It consists of the oral bulge including the cytostome and the toxicysts; the circumoral kinety (ciliary row); and the oral basket.

The oral bulge is a more or less distinct, unciliated structure proximally bordered by the circumoral kinety. Frequently, the bulge is oblique (30°–60°) increasing the size of the oral area, especially in several arcuospathidiids, where it extends to or near to midbody. We assume that this elongation is a derived feature. The shape (outline) of the oral bulge varies from elliptical to very narrowly cuneate/oblong (Fig. 10) and is roughly correlated with the familial classification: most protospathidiids have an elliptical or obovate bulge; in the spathidiids and pharyngospathidiids, the bulge is frequently oblong and twisted about the main axis making the bulge screwed like a propeller blade or, in lateral view, like a recumbent number 8; most arcuospathidiids have a cuneate to very narrowly cuneate oral bulge. The bulge cortex contains mucocysts ("cortical granules") and a fibre system sometimes distinctly impregnating with protargol and well recognizable in scanning electron micrographs (Fig. 119m, n, 135a–e, 138w, x). The fibre system consists of ribbons of microtubules corresponding to the transverse microtubule ribbons of the somatic basal bodies (Fig. 12). Thus, spathidiids have a rhabdostype oral apparatus (CORLISS 1979). The microtubule ribbons originate from the bare basal bodies of the circumoral dikinetids and support/extend to the cytostome wall; depending on shape and size of the oral bulge, the fibres form a spiral (small, ± obovate bulge) or arrowhead-like (large, oblong bulge) pattern in the distal bulge surface.

The frontal (distal) surface of the oral bulge contains a flat groove which, basically, represents the cytostome, that is, the groove opens when prey is ingested. This is the simplest cytostome type and found in many species of various spathidiid families (Fig. 11a). The second cytostome type has a minute, obconical depression, which is lined by the transverse microtubule ribbons, near the dorsal (rarely also ventral) bulge end (Fig. 11b, 12). When feeding commences, the obconical depression opens, for instance, in *Arcuospathidium muscorum* (Fig. 14b). We call this minute depression "temporary cytostome" because it is open only during feeding. The temporary cytostome can be recognized in vivo, in the scanning electron microscope, and in protargol preparations (Fig. 60a, b, 114i, 118d, 119h, i, 139a, d). A third cytostome type is found in the new family Pharyngospathidiidae, described in the second volume. Here, the cytostome is a comparatively large, elliptical, permanent opening in the bulge centre. The wall of the permanent cytostome is lined by many transverse microtubule ribbons forming a conspicuous "inner oral basket" (Fig. 11c–e, 12).

The circumoral kinety marks the proximal margin of the oral bulge. Usually, it has the same shape as the oral bulge, rarely it is slightly different, for instance, an oblong, ventrally rounded oral bulge may be associated with a cuneate, ventrally tapering circumoral kinety. The circumoral kinety is ring-like closed in most genera. However, in the Apertospathulidae the ventral end is open because the left branch of the kinety is slightly to distinctly shortened. The circumoral kinety is composed of pairs of basal bodies (dikinetids) with gradually increasing distances from dorsal to ventral bulge end. The two basal bodies are arranged in a certain angle, and only the more posteriorly located one is ciliated (Fig. 12). Thus, the circumoral kinety consists of dikinetids forming a single ciliary row (Fig. 11a). The anterior basal body of the dikinetids is bare, but
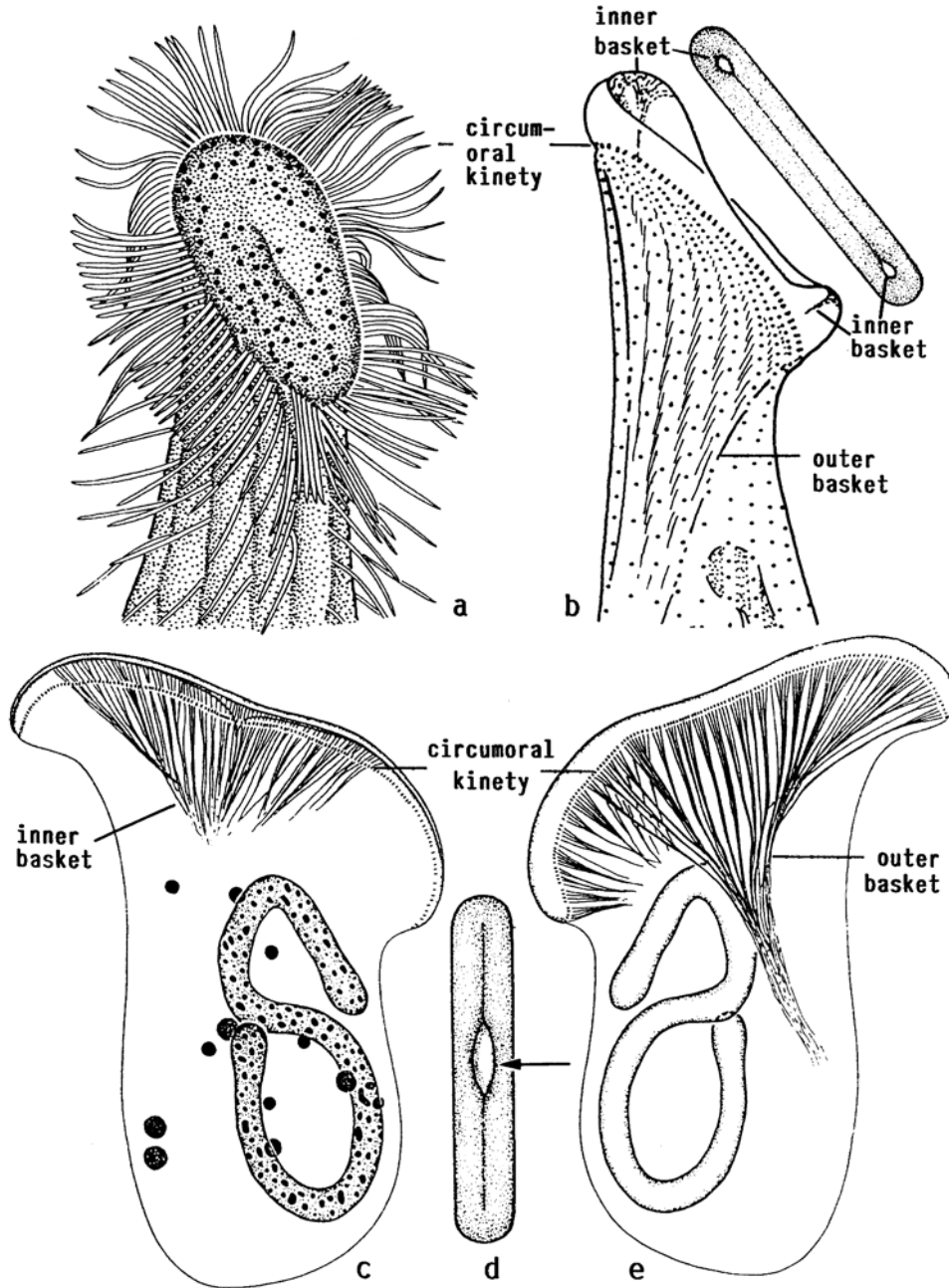
Fig. 11a–e Oral apparates of spathidiid ciliates. For details, see section 1.8 and next figure. **a)** Ventrolateral view of *Spathidium namibicola* (from FOISSNER et al. 2002). **b)** Right side and frontal view of *S. stammeri* (from FOISSNER 1987c). **c–e)** *Pharyngospathidium longichilum longichilum* (described in Vol. II) has a permanent cytostome (arrow) and a strongly developed inner and outer oral basket.
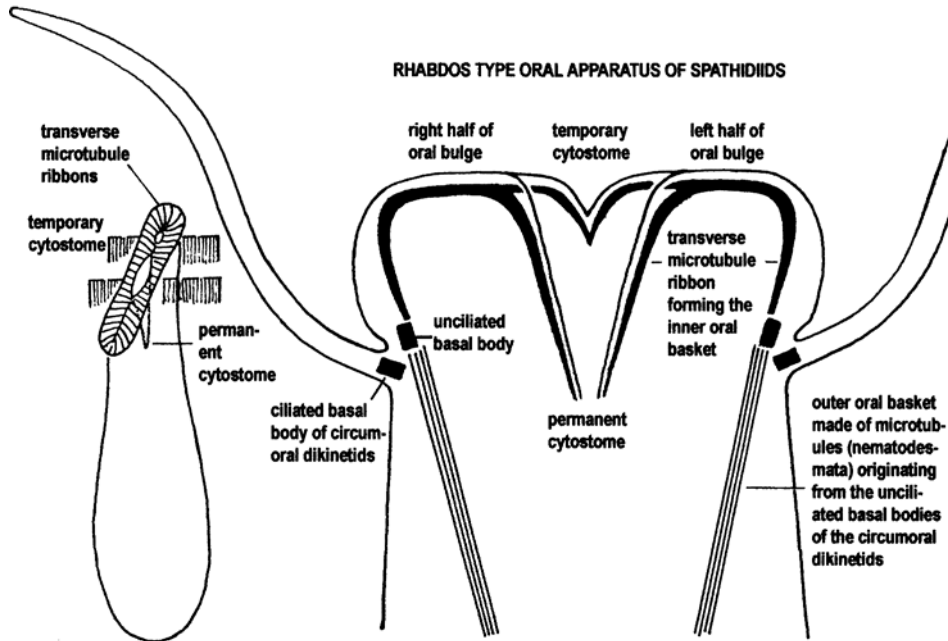
RHABDOS TYPE ORAL APPARATUS OF SPATHIDIIDS

Fig. 12 Scheme of the rhabdos type oral apparatus of spathidiids. Based on our light microscopical data and the electronmicroscopical investigation of BOHATIER et al. (1978).

is associated with two important microtubule bundles: the oral basket rods (nematodesmata) originate from the proximal end of the basal bodies and constitute the (outer) oral basket, while the laterally originating transverse microtubule ribbons support the oral bulge and, if present, the pharyngeal opening, forming the inner oral basket (Fig. 11, 12).

1·8·2 Feeding (Fig. 13, 14)

Feeding of *Spathidium spathula* was described in detail by WOODRUFF & SPENCER (1921b, 1922). Unfortunately, these observations were overlooked by reviewers and disappeared from the modern literature (e.g. CALKINS & SUMMERS 1941, VERNI & GUALTIERI 1997). Thus, we provide an extended summary of their observations (Fig. 13a–h).

(i) *Spathidium spathula* readily paralyzes and swallows almost any small ciliate (e.g., *Colpidium*) with which it comes in contact, though flagellates of various kinds are immune.

(ii) *Spathidium* comes in contact with its prey solely through chance. They easily miss their prey. Nothing happens unless the quarry strikes the anterior end of the *Spathidium* nearly or quite in the center. Touching the edges produces no effect on either animal.

(iii) When the contact is made, the prey usually instantly becomes motionless and quickly shows vacuolization of the cytoplasm and disintegration of cilia. The stimulus afforded by the contact immediately stops the forward movements of the *Spathidium*, which gives a few rapidly repeated avoiding reactions that tend to keep it at or near the spot the capture was made. Meanwhile, the prey is moved around, apparently as a result of combined activity of the longer oral cilia and the gradually expanding edges of the oral bulge, until it may be conveniently be encircled. When the process of mouthing is successfully accomplished, nothing further is visible except the gradual sinking of the prey through the greatly expanded cytostome of the captor into the cytoplasm (Fig. 13a–h).

(iv) The whole process from contact to complete ingestion takes place in about thirty seconds, and the *Spathidium*, which usually sinks to the bottom during the process, within a few seconds more resumes its active foraging. Three such captures have been observed to occur within eight minutes and five over about an hour (Fig. 13a–h).

(v) Prey which has been paralyzed and has become removed from the oral region of the *Spathidium* is recovered in a majority of instances by a complex series of successively modified reactions, indicating "sensing at a distance". The factor involved in sensing is apparently a substance secreted by the *Spathidium* when the prey is paralyzed (authors: likely, these are the toxicysts which may form a long, reticulate mass).

Later, BALTES & WENZEL (1966) and WENZEL & BALTES (1967) investigated feeding and "sensing at a distance" in *Spathidium stammeri*. As concerns feeding in general, the data of WENZEL & BALTES (1967) largely match those of WOODRUFF & SPENCER (1922), while distance sensing was experimentally proven to depend on an organic acid – still effective after heating to 200°C – released by the prey. WENZEL & BALTES (1967) showed that *Spathidium* engulfes even Al_2O_3-particles, if they are impregnated with aqueous extracts of *Tetrahymena pyriformis* or meat extract.

Our own observations basically match those of WOODRUFF & SPENCER (1922) and those of related ciliates, e.g., *Homalozoon vermiculare* (KUHLMANN et al. 1980). Thus, we provide here only a brief overview (Fig. 14a–g) and refer to the individual species descriptions for details. Spathidiids are rapacious carnivores feeding on other protists, preferably ciliates and flagellates; some of the smaller species and those lacking extrusomes likely feed on bacteria, while the largest species may ingest small and middle-sized testate amoebae and small metazoans, mainly rotifers (Fig. 13i). The toxicysts play an important role in prey capture, as indicated by their great diversity (Fig. 6). The prey is ingested within a few minutes either whole (Fig. 14a, b, f, g) or as a mash when it is lysed outside (likely) by the toxicysts (Fig. 14e). The whole oral area is very extensible and thus can become funnel-shaped during the uptake of large prey (Fig. 14c), while the body usually becomes stouter and more or less deformed (Fig. 14b, e). When the prey touches the oral bulge, a bursiform or tube-like vacuole is generated at the site of the temporary or permanent cytostome (Fig. 14e–g). Then the opening widens more or less distinctly, depending on prey size, and the victim glides into the cell where a large vacuole has formed (Fig. 14b, e). WILLIAMS (1958) showed that axenic media (e.g., Osterhout's solution) and the ciliate *Colpidium* are insufficient for growth of *Spathidium spathula*, which grows only in lettuce infusion with up to 3.2 divisions/day.
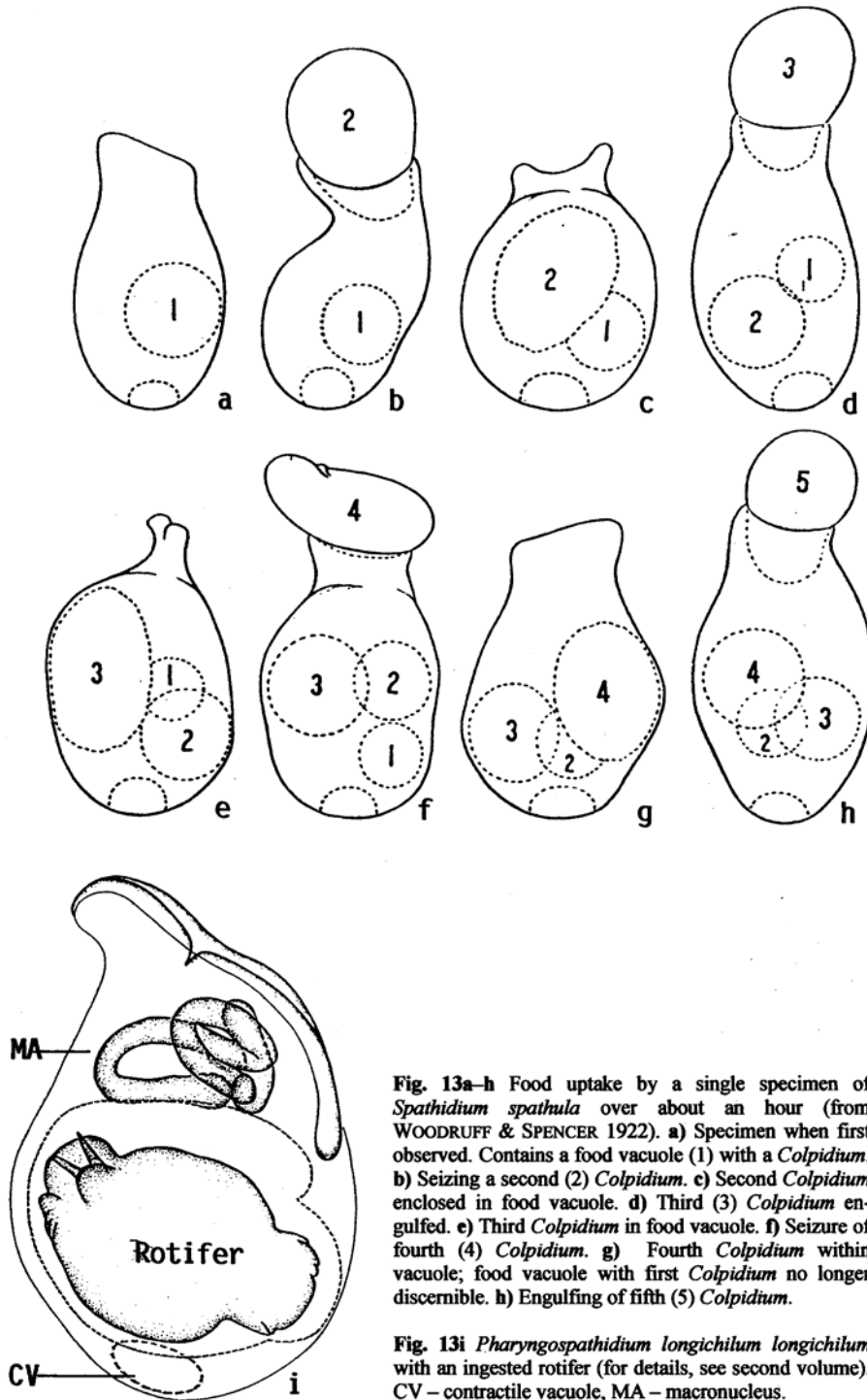
Fig. 13a–h Food uptake by a single specimen of *Spathidium spathula* over about an hour (from WOODRUFF & SPENCER 1922). **a)** Specimen when first observed. Contains a food vacuole (1) with a *Colpidium*. **b)** Seizing a second (2) *Colpidium*. **c)** Second *Colpidium* enclosed in food vacuole. **d)** Third (3) *Colpidium* engulfed. **e)** Third *Colpidium* in food vacuole. **f)** Seizure of fourth (4) *Colpidium*. **g)** Fourth *Colpidium* within vacuole; food vacuole with first *Colpidium* no longer discernible. **h)** Engulfing of fifth (5) *Colpidium*.

Fig. 13i *Pharyngospathidium longichilum longichilum* with an ingested rotifer (for details, see second volume). CV – contractile vacuole, MA – macronucleus.
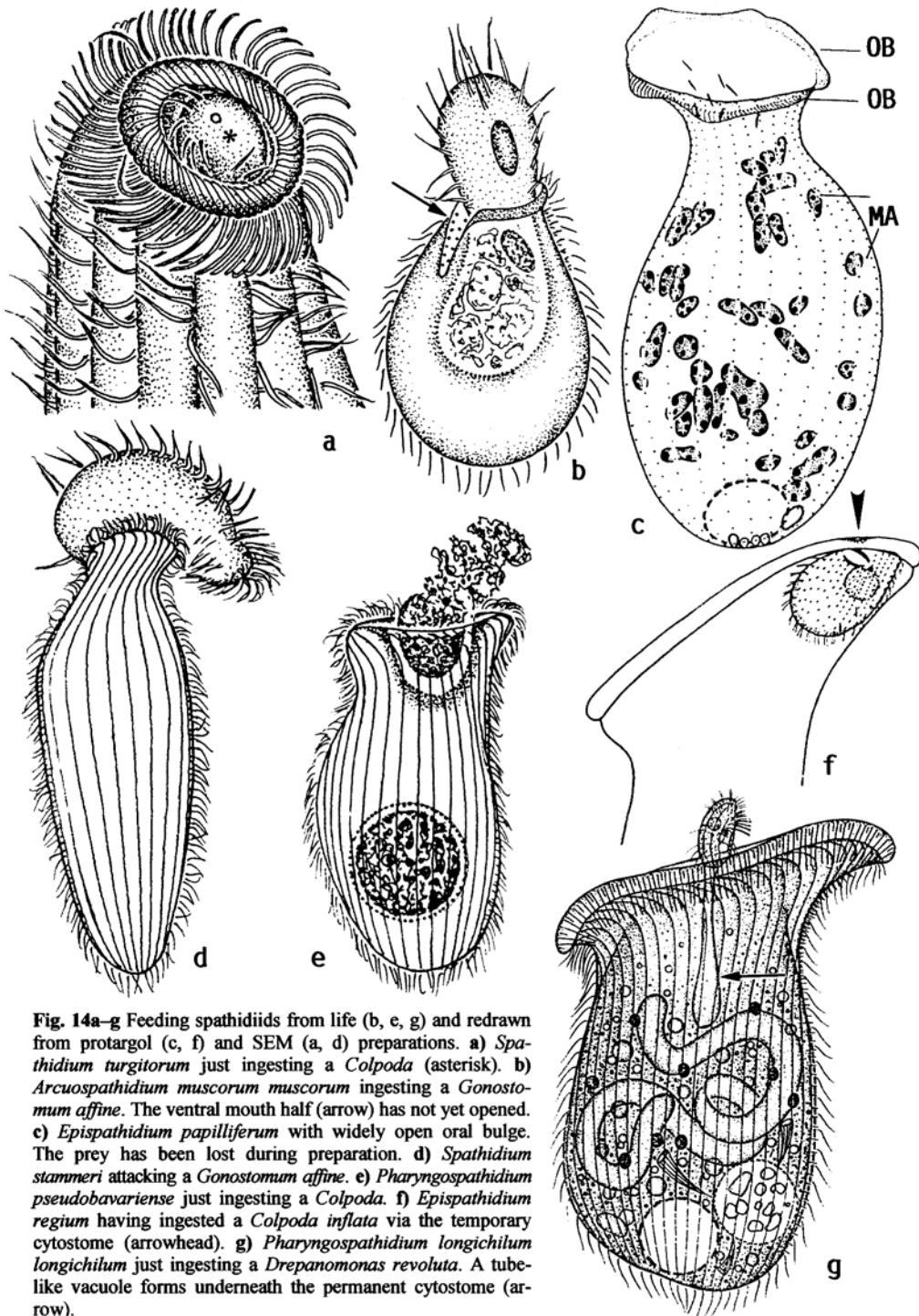
Fig. 14a–g Feeding spathidiids from life (b, e, g) and redrawn from protargol (c, f) and SEM (a, d) preparations. **a)** *Spathidium turgitorum* just ingesting a *Colpoda* (asterisk). **b)** *Arcuospathidium muscorum muscorum* ingesting a *Gonostomum affine*. The ventral mouth half (arrow) has not yet opened. **c)** *Epispathidium papilliferum* with widely open oral bulge. The prey has been lost during preparation. **d)** *Spathidium stammeri* attacking a *Gonostomum affine*. **e)** *Pharyngospathidium pseudobavariense* just ingesting a *Colpoda*. **f)** *Epispathidium regium* having ingested a *Colpoda inflata* via the temporary cytostome (arrowhead). **g)** *Pharyngospathidium longichilum longichilum* just ingesting a *Drepanomonas revoluta*. A tube-like vacuole forms underneath the permanent cytostome (arrow).