A General Section

1 Morphology, Biology, and Terminology

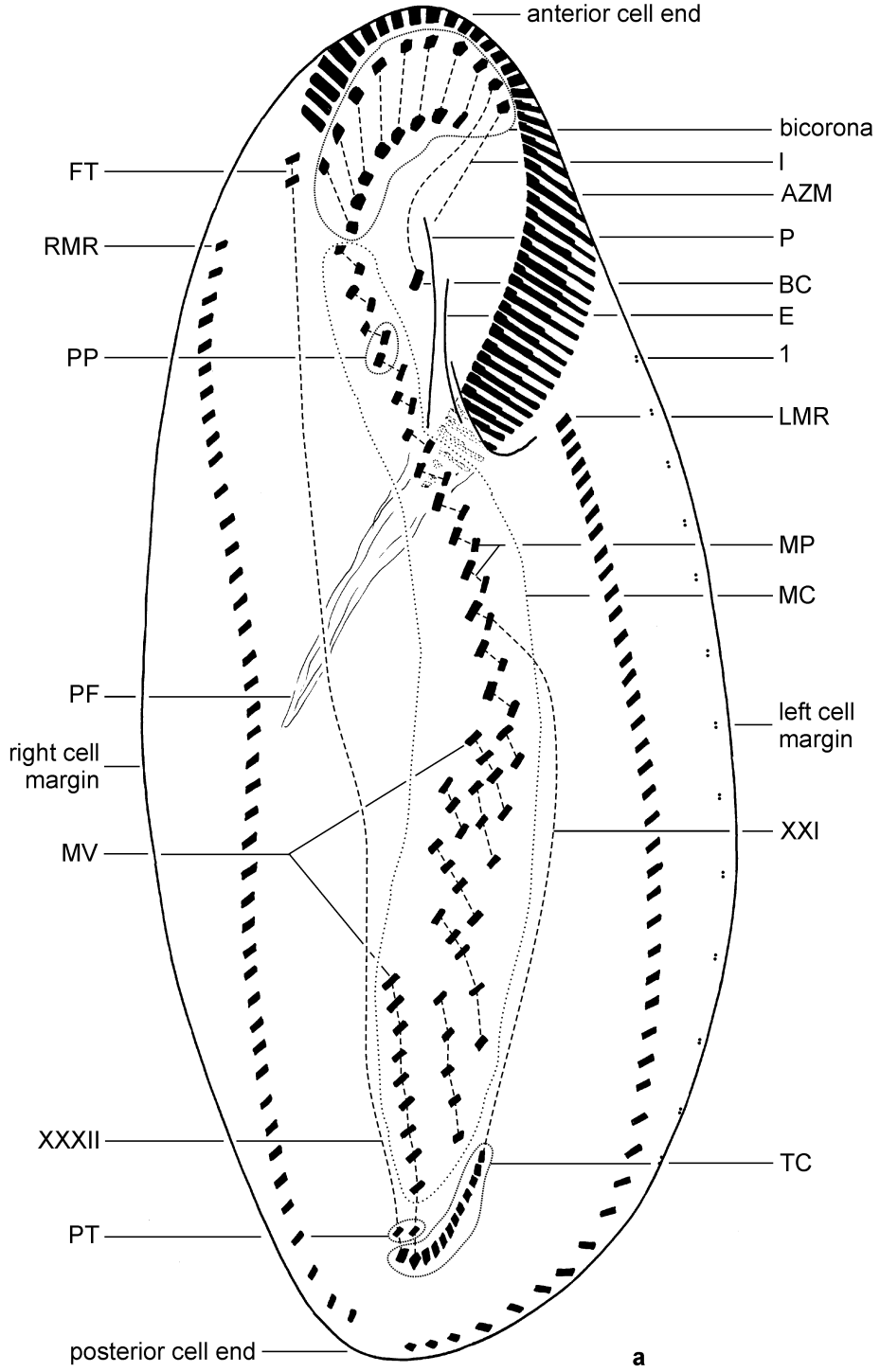
In the following chapters the general external and internal morphology of the Urostyloidea (= urostyloids)[1] and terms specific to this group are described and explained (Fig. 1a–g). For explanation of other terms, see Corliss (1979), Corliss & Lom (1985, 2002), Lynn & Corliss (1991), Hausmann & Hülsmann (1996), and Hausmann et al. (2003). Moreover, some other topics (e.g., parasitism, ecology and distribution) are briefly discussed. For discussion of the ground pattern of the Urostyloidea see the systematic section.

1.1 Size and Shape

The body length of urostyloid ciliates ranges from about 50 µm (e.g., small specimens of *Holosticha pullaster*) to ca. 850 µm (*Urostyla gigas*); the majority is between 100 µm and 300 µm long. The body length:width ratio ranges from about 3:1 or less (e.g., some *Urostyla* species) to about 10:1 in some *Anteholosticha* species, for example, *Anteholosticha fasciola*. Consequently, the body outline of urostyloids is basically either broadly elliptical, elongate elliptical, or almost vermiform. The ventral side is, as in most other hypotrichs, usually flat, the dorsal side more or less distinctly vaulted (Fig. 1d, f). The dorsoventral flattening given in the descriptions is the (usually roughly) estimated ratio of body width to body height (Fig. 1d). For example, a specimen with a body width of 30 µm and a body height of 10 µm is flattened 3:1 dorsoventrally.

Urostyloid hypotrichs are flexible (supple), and almost acontractile to distinctly (up to about 30%) contractile. So far no urostyloid with a rigid body is reliably described. A rigid body/cortex in the Hypotricha is only known from the Stylonychinae (Fig. 14a). Very likely this conspicuous feature evolved convergently in the euplotids and stylonychines (Berger 1999). The adoral zone of membranelles ("oral apparatus") is, as is usual, in the left anterior portion of the cell, and usually less than 40% of body length, in most species around 30%. Hypotricha-species with a longer adoral zone (more than 40%) are either immature postdividers or, if their body is inflexible, stylonychines for which a relative length of 40% or more is characteristic. Moreover, some stylonychines, for example, *Pattersoniella vitiphila* (for review see Berger 1999, p. 766), have a cirral pattern very similar to the midventral pattern of the urostyloids. The biomass of urostyloids ranges from about 12 mg (e.g., *Holosticha pullaster*) to about 8000 mg for the huge *Urostyla gigas* which is nearly 1 mm long.

[1] For names of higher taxa used in the present book see Figs. 13a, 14a and Table 1.

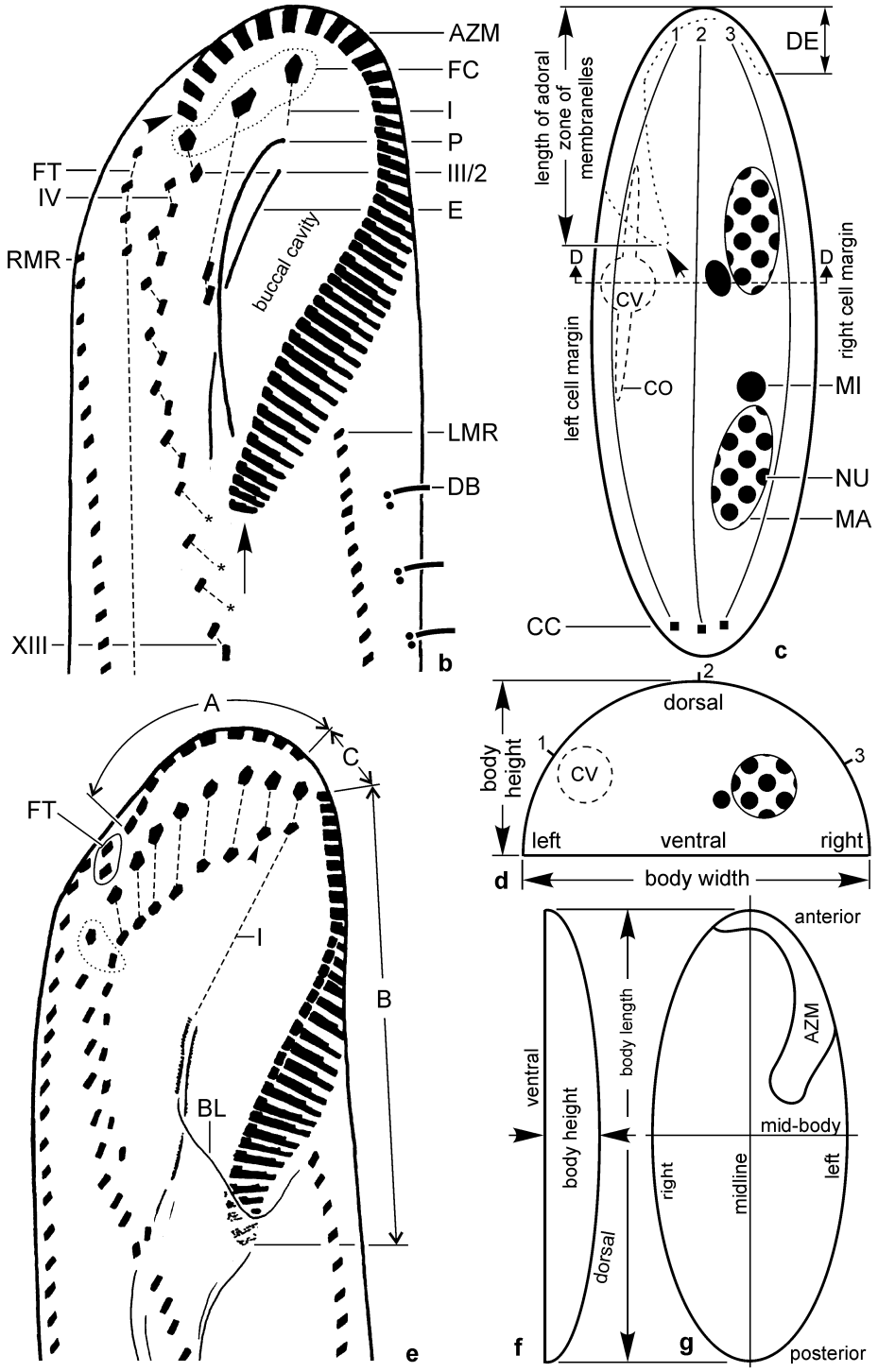

anterior cell end
bicorona
I
AZM
P
BC
E
1
LMR
MP
MC
left cell margin
XXI
TC
posterior cell end
PT
XXXII
MV
right cell margin
PF
PP
RMR
FT
a

1.2 Nuclear Apparatus

Urostyloids have – like most ciliates – a homomerous, polyploid macronucleus (for reviews see Raikov 1969, 1982 and Prescott 1994, 1998). It is composed either of two relatively large nodules (e.g., *Holosticha pullaster*; Fig. 28a); several, more or less scattered nodules (e.g., *Caudiholosticha islandica*; Fig. 48d); more or less moniliformly arranged nodules (e.g., *Anteholosticha monilata*; Fig. 57c); or very many scattered, rather small nodules (e.g., *Urostyla grandis*; Fig. 208h). Species with two macronuclear nodules have either one or more micronuclei attached to each nodule (e.g., *Caudiholosticha stueberi*; Fig. 44e), or a single micronucleus between the two nodules (e.g., *Caudiholosticha navicularum*; Fig. 51a). Fragmentation of the two macronuclear nodules into more than two pieces probably occurred several times independently within the urostyloids. In contrast to other hypotrich taxa, the urostyloids contain a very high number of species with many nodules, whereas the Oxytrichidae are dominated by species with only two macronuclear nodules (for review, see Berger 1999). As in other ciliates, the nuclear apparatus pattern is a very important feature for identification.

Suganuma & Inaba (1966, 1967) and Inaba & Suganuma (1966) studied the fine structure of the macronucleus of *Urostyla grandis*. The chromatin-material in the macronucleus forms a large, irregular network composed of threads of up to 500 nm across. The fundamental components of these threads are pairs of fine fibrils, each 10 nm thick. The nucleoli are about 1–2 µm across. They appear to be composed partly of fine fibrils about 10 µm across, and partly of granular appearing material. The nuclear envelope is double, about 21 nm thick, and there are many discontinuities (50 nm across), which may represent the pores. The micronuclei of *U. grandis* are bounded by a double, porous envelope similar to that of the macronuclear nodules. Chromatic material in the micronucleus forms a small network of comparatively thin threads, 60–80 µm thick. The macronuclear nodules of *Pseudokeronopsis carnea* appear moderately dense and homogeneous with few nucleoli, or with a single, central endosome, whereas other

← **Fig. 1a** Terminology of urostyloid ciliates (from Berger 2004b, supplemented). Infraciliature (after protargol impregnation) of ventral side of a species with a bicorona. Frontal-midventral-transverse cirri which originate from the same anlage are connected by a broken line (for the sake of clarity only the leftmost transverse cirrus, and the two rightmost transverse cirri and pretransverse ventral cirri are connected with the corresponding midventral pair, respectively, midventral rows). AZM = adoral zone of membranelles, BC = buccal cirrus, E = endoral (endoral plus paroral are the undulating membranes), FT = frontoterminal cirri (= migratory cirri), LMR = anterior end of left marginal row, MC = midventral complex (= midventral pairs plus midventral rows), MP = midventral pairs, MV = midventral rows, P = paroral (paroral plus endoral are the undulating membranes), PF = pharyngeal fibres (= cytopharynx), PP = pseudo-pair (composed of rear [= left] cirrus of an anlage and front [= right] cirrus of next anlage, that is, the cirri of a pseudopair do not originate from the same anlage), PT = pretransverse ventral cirri (= cirri ahead the two rightmost transverse cirri; = accessory transverse cirri according to Wicklow 1981), RMR = anterior end of right marginal row, I = first (= leftmost) frontal-(midventral-transverse) cirral anlage (forms always the leftmost frontal cirrus and the undulating membranes), TC = transverse cirri (form a pseudorow), XXI = 21. frontal-midventral-transverse cirral anlage (forms the leftmost [anteriormost] transverse cirrus in this specimen), XXXII = last (= rightmost; = 32. from left, respectively, from the front) frontal-midventral-transverse cirral anlage (number of anlagen varies among species and often within species), 1 = dorsal kinety 1 (= leftmost kinety).


AZM
FC
I
P
III/2
E
FT
IV
RMR
buccal cavity
LMR
DB
XIII
b
DE
length of adoral zone of membranelles
1
2
3
D
D
CV
left cell margin
right cell margin
CO
MI
NU
MA
CC
c
2
dorsal
body height
1
CV
3
left
ventral
right
d
body width
A
C
FT
I
B
BL
e
anterior
AZM
body length
ventral
body height
mid-body
dorsal
right
midline
left
f
g
posterior

nodules may be crammed with condensed chromatin (Wirnsberger & Hausmann 1988b). Micronuclei are spherical and very densely stained.

The macronuclear nodules of urostyloids posses a replication (or reorganisation) band, a feature which evolved in the stem-line of the spirotrichs (Fig. 2a–g). In this band, which is a clear disc that gradually moves through the whole macronuclear nodule, DNA is replicated (for reviews see Raikov 1982 and Prescott 1994). The replication band of *Urostyla grandis* is, like that of other hypotrichs, divided into two zones: the forward zone consists of fine, twisted fibrils 30 nm thick formed by pairs of parallel fibrils, each about 10 nm thick. The rear zone is composed of small chromatin bodies and thin threads, both about 80 nm across, consisting of fine fibrils 10 nm thick. Reorganised chromatin threads appear to be thin spiral threads 80–130 nm thick in striking contrast to the thick (100–500 nm in diameter) threads composing the large, irregular network observed in early interphase macronucleus (Suganuma & Ibana 1966, 1967, Ibana & Suganuma 1966; for review see Olins & Olins 1994, p. 150).

The development of the urostyloid nuclear apparatus during cell division is obviously the same as in the other hypotrichs, that is, the individual macronuclear nodules fuse to a single mass and divide again in the species-specific number. In *Urostyla grandis* the many macronucleus-nodules are small, about the size of micronuclei, and scattered throughout the cell. The fusion of the individual nodules of *U. grandis* into a single macronucleus within a matter of minutes is a rather impressive event (Fig. 3a–l). As cytokinesis begins, the composite macronucleus in various species quickly undergoes one or more rapid, successive amitotic divisions to produce the appropriate number of daughter macronuclei in each filial product (Prescott 1994). Nothing is known about the triggering of macronuclear fusion at the beginning of ontogenesis, or its mo-

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← **Fig. 1b–g** Terminology of urostyloid ciliates (b–d, from Berger 2004b, supplemented; e–g, originals). Frontal-midventral cirri which originate from the same anlage are connected by a broken line. **b:** Infraciliature (after protargol impregnation) of a species with three frontal cirri. Arrow marks proximal end, arrowhead distal end of adoral zone of membranelles. Asterisks mark anlagen, which eventually produce only a single midventral cirrus, that is, one cirrus (in the present case the left one) of a pair is resorbed in late dividers. **c:** Schematic illustration of infraciliature of dorsal side, nuclear apparatus, and contractile vacuole. **d:** Schematic cross section (about at level D-D of Fig. 1c) showing, inter alia, dorsoventral flattening and contractile vacuole. Arrow marks proximal end of adoral zone of membranelles. **e:** Infraciliature (after protargol impregnation) of a species with a gap in the adoral zone. First midventral pair encircled by dotted line. "Buccal cirrus" marked by arrowhead. **f, g:** Left lateral view and ventral view showing some terms used in the species descriptions. A = distal (= frontal) portion of adoral zone of membranelles, AZM = adoral zone of membranelles, B = proximal (= ventral) portion of adoral zone of membranelles, BL = buccal lip, C = gap in adoral zone of membranelles, CC = caudal cirri (at rear end of dorsal kineties), CO = collecting canal (of contractile vacuole), CV = contractile vacuole, DB = anteriormost dorsal bristle of kinety 1 (= leftmost kinety), DE = distance between anterior body end and distal end of adoral zone of membranelles (for DE-value see chapter 1.8), E = endoral, FC = frontal cirri (left = cirrus I/1; middle = homologous to cirrus II/3 of the 18-cirri oxytrichids; right = homologous to cirrus III/3), FT = frontoterminal cirri, LMR = left marginal row, MA = posterior macronuclear nodule, MI = micronucleus, NU = nucleolus, P = paroral, PC (= III/2) = parabuccal cirrus(i) (= cirrus behind right frontal cirrus), RMR = right marginal row, I, IV, XIII = cirri which originated from the first, fourth, and thirteenth frontal-midventral-transverse cirral anlage, III/2 (= PC) = cirrus behind right frontal cirrus (also designated parabuccal cirrus/cirri), 1–3 = dorsal kineties (kinety 1 is the leftmost kinety).

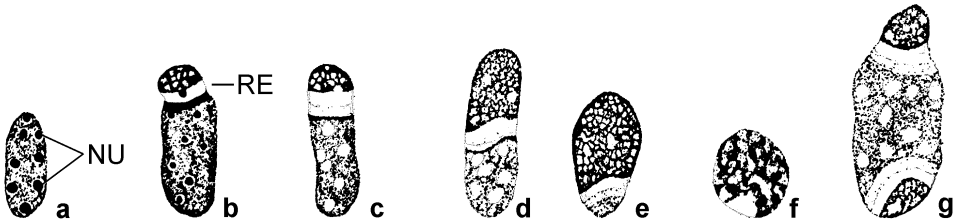


Fig. 2a–g Macronuclear nodules of *Urostyla grandis* during first stages of cell division (from Tittler 1935. a, c, fixed with Schaudinn's fluid; b, fixed with Flemming's fluid; d–g, fixed with Gilson Carnoy's fluid; a, b, stained with Heidenhain's iron haematoxylin; c, Feulgen stain; d–g, Mayer's haemalaun stain) **a:** Interphasic nodule, about 8 µm long (all other nodules drawn to same scale). **b–f:** Passing of replication band. **g:** Macronucleus nodules with two replication bands occur very rarely. NU = nucleoli, RE = replication (reorganisation) band.

lecular mechanism or what controls and accomplishes amiotic division at the end of cell division. Perhaps the cytoskeleton mediates these events (Prescott 1994).

The multiple micronuclei in a single cell are all genetically identical: they are all derived by mitosis from one original micronucleus formed by fertilisation at cell mating. Micronuclei divide mitotically during vegetative growth, but the form of mitosis is different from that of plant and animal cells (for details see Prescott 1994). Mitosis occurs intranuclearly, that is, without breakdown of the nuclear envelope, and individual chromosomes are not distinguishable. Rather, the mitotic micronucleus contains long strands of chromatin that distribute to produce two genetically equivalent daughter micronuclei (Fig. 4a–h). Details of the process are poorly understood (Prescott 1994).

Only in the pseudokeronopsines, which have many macronuclear nodules, does the macronuclear development differ from that in other hypotrichs, a feature reviewed by Raikov (1982, p. 348). For example, the macronuclear anlage of *Pseudokeronopsis rubra* contains paired filamentous chromosomes (possibly in a state of somatic conjugation), but they are not clearly polytenic (Ruthmann 1972). Neither the transverse fragmentation of chromosomes nor the "achromatic" phase in macronuclear development have been found. Ruthmann (1972) has shown by electron microscopy that the chromatin of the macronuclear anlage gradually condenses into compact bodies that are separated from the anlage into the cytoplasm and become small definitive macronuclei, each of which contains a paradiploid amount of DNA. Ruthmann (1972) believed that the chromatin bodies preformed in the anlage are diploid subnuclei that later become individual macronuclei. These numerous macronuclei divide without prior fusion to a single mass. Often, they divide into parts with an unequal DNA content. This means that the genome of the *Pseudokeronopsis* macronuclei fragments into subunits smaller than even the haploid genome (Ruthmann 1972). Consequently, the macronucleus apparatus of *Pseudokeronopsis* has features of both the subnuclear and the chromomeric types. This type of macronuclear division was already discovered by Gruber (1884a).

The just mentioned mode of macronucleus-development also occurs in *Uroleptopsis* (Mihailowitsch & Wilbert 1990, Berger 2004b) and therefore has to be considered as

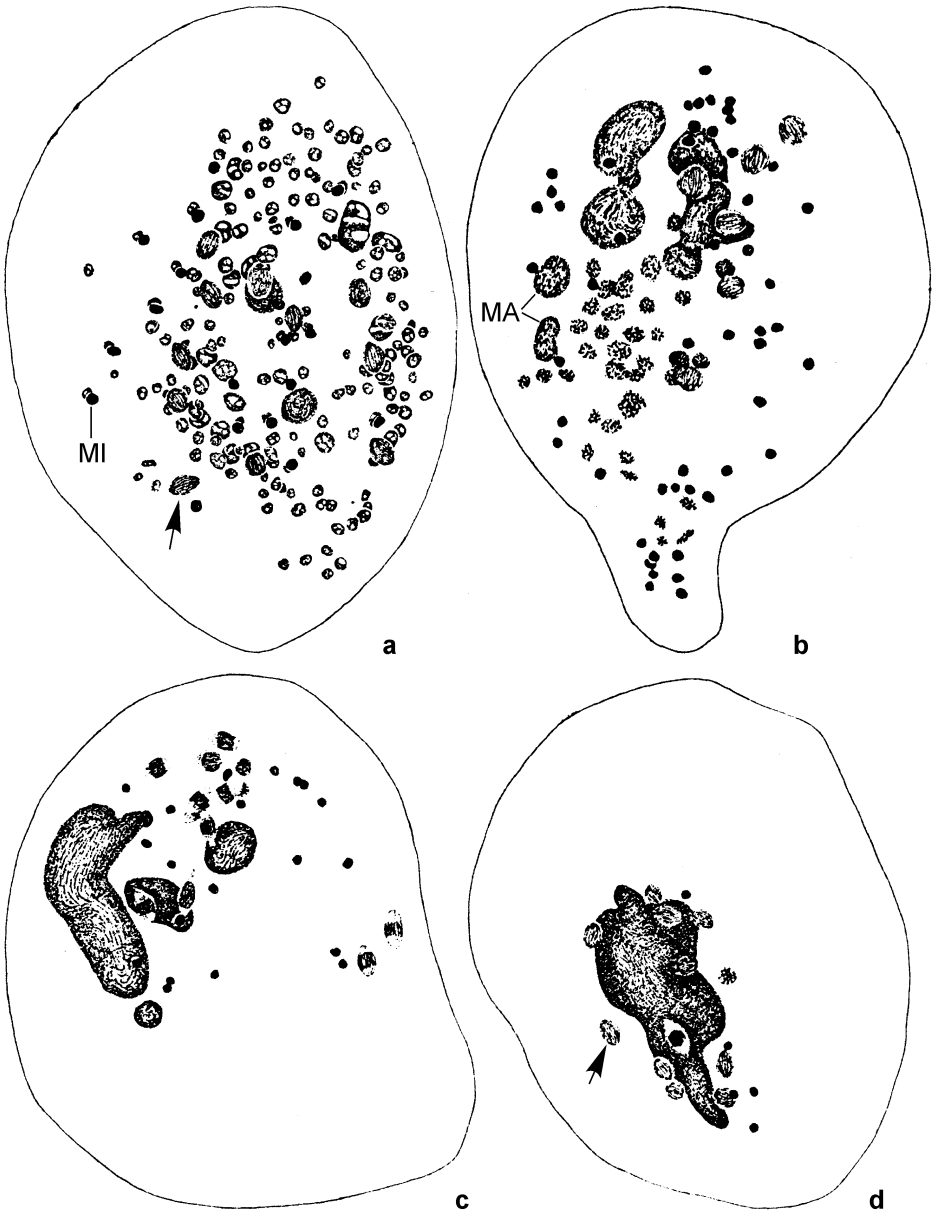


Fig. 3a–d Division of macronucleus in *Urostyla grandis* (from Tittler 1935. a, b, d, fixed with Gilson-Carnoy's fluid; c, fixed with Schaudinn's fluid; a–c, Mayer's haemalaun stain; d, Heidenhain's iron haematoxylin stain). The many individual macronuclear nodules present in specimen (a) fuse to a single mass (d). a = 200 µm long, b = 162 µm; c = 160 µm, d = 152 µm. Arrows in (a, d) mark dividing micronuclei. Following stages, see Fig. 3e–h. For details, see text. MA = non-dividing macronuclear nodules, MI = non-dividing micronucleus.

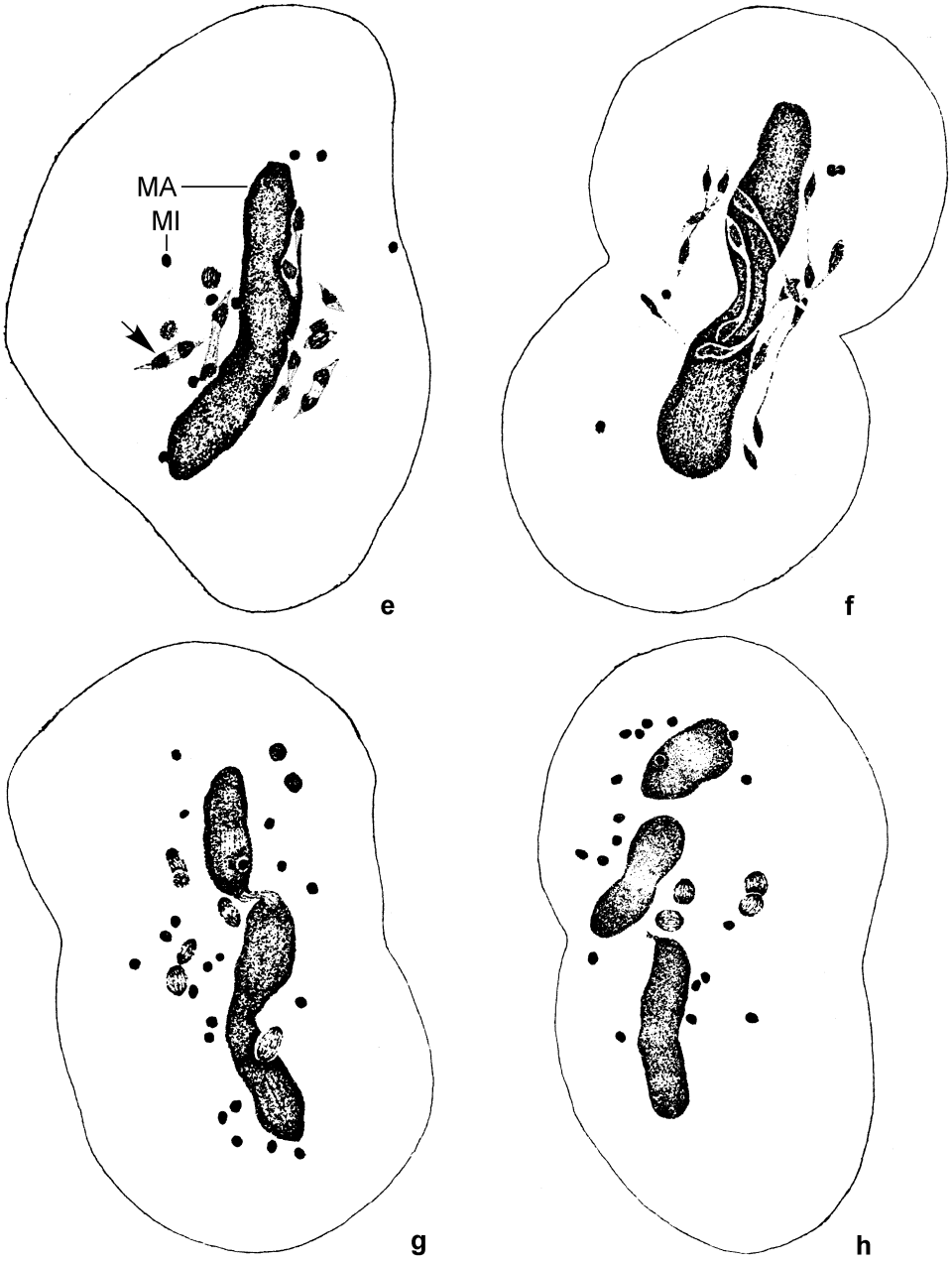


Fig. 3e–h Division of macronucleus in *Urostyla grandis* (from Tittler 1935. a, b, fixed with Schaudinn's fluid; c, fixed with Gilson-Carnoy's fluid; d, fixed with Bouin's fluid; a, c, d, Mayer's haemalaun stain; b, Feulgen stain). The fused macronucleus (e) begins to divide (f–h). Arrow in (e) marks dividing micronucleus. e = 170 µm long, f = 185 µm, g = 180 µm, h = 195 µm. For details, see text. MA = fused macronucleus, MI = non-dividing micronucleus.

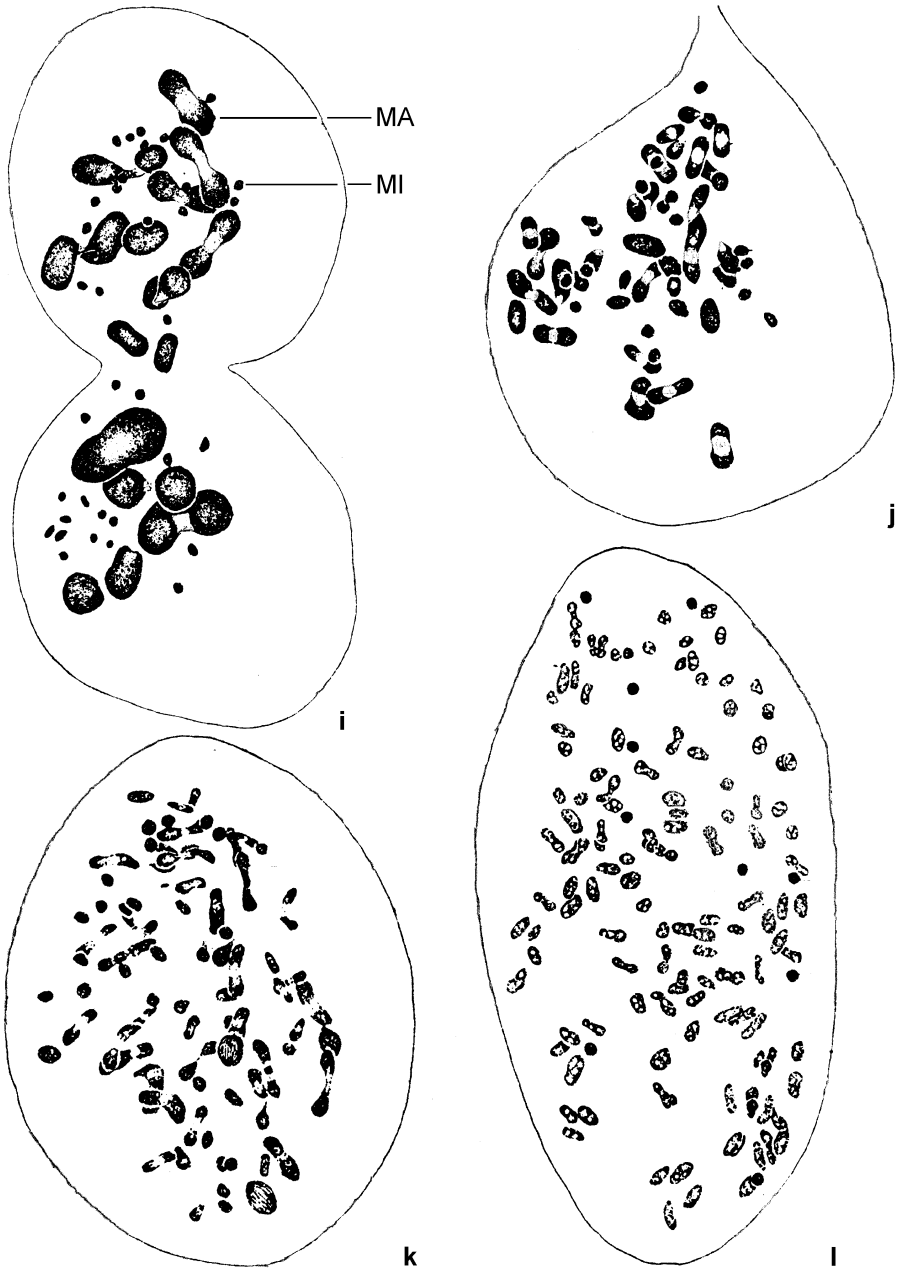


Fig. 3i–l Division of macronucleus in *Urostyla grandis* (from Tittler 1935. i–l, fixed with Schaudinn's fluid; i, Heidenhain's iron haematoxylin stain; j, k, Mayer's haemalaun stain; l, Feulgen stain). **i:** Late divider, 230 µm. **j:** Very late divider (proter not illustrated), 138 µm. **k:** Post-divider, 128 µm. **l:** Specimen with normal nucleus-apparatus, 188 µm. MA = dividing macronucleus, MI = non-dividing micronucleus.

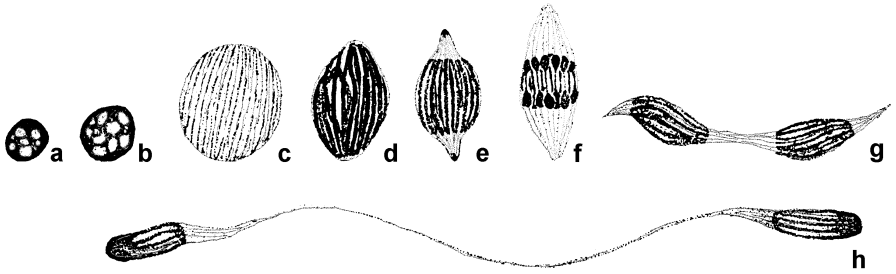


Fig. 4a–h Micronucleus of *Urostyla grandis* during division (from Tittler 1935. a, d–h, fixed with Schaudinn's fluid; b, fixed with Gilson Carnoy's fluid; c, fixed with Bouin's fluid; a, d, f–h, Feulgen stain; b, c, Mayer's haemalaun stain; e, Haindenhain's iron haematoxylin stain). **a:** Interphasic micronucleus, about 3.5 µm across (all other micronuclei drawn to same scale). **b:** Early prophase. **c, d:** Late and very late prophase. **e:** Metaphase. **f:** Early anaphase. **g:** Late anaphase. **h:** Telophase.

autapomorphy of the Pseudokeronopsinae (Fig. 167a, autapomorphy 3). *Thigmokeronopsis antarctica* and *T. crystallis* also have many macronuclear nodules, which do not fuse to a single mass, but to several parts (Petz 1995). This state can be interpreted as a transitional state between the total fusion, for example, in *Urostyla grandis*, and the specific mode described for the Pseudokeronopsinae. Berger (2004b) considered the *Thigmokeronopsis* type of macronuclear division as autapomorphy of the Pseudokeronopsidae (Fig. 167a, autapomorphy 1).

Maula et al. (1993) found prokaryotic endosymbionts in the macronucleus, but not in the micronuclei of *Pseudokeronopsis* sp.

1.3 Contractile Vacuole and Cytopyge

Many urostyloids have, like most other Hypotricha, a single contractile vacuole near the left cell margin about or slightly behind the level of the cytostome (Fig. 1c, d). The very common *Holosticha pullaster* has this organelle distinctly behind mid-body so that it is very easy to identify (Fig. 28f–i). Few species have more than one contractile vacuole (e.g., *Pseudokeronopsis sepetibensis*; Fig. 186a). For a relatively high number of marine species no contractile vacuole is described, possibly because it is lacking. In some marine species a vacuole is present, but contracts in rather long intervals. Very little is known about the excretory pore in the urostyloids. Probably it is, as in other Hypotricha, on the dorsal surface.

The cytopyge of the urostyloids is a little-known organelle which is usually – as in other hypotrichs (Berger 1999) – located in the posterior portion of the cell (Fig. 135b, 181c, 226n).

1.4 Cytoplasm, Cortex, and Colouring

The cytoplasm of many urostyloids is more or less colourless. Some species have, however, a yellow (e.g., *Anteholosticha xanthichroma*, *Uroleptopsis citrina*) or reddish (e.g., *Diaxonella pseudorubra*) coloured cytoplasm. Few species (e.g., *Caudiholosticha viridis*, *Urostyla viridis*) are green due to symbiotic algae. Symbiotic algae must not be confused with ingested algae, which are enclosed in usually distinctly recognisable food vacuoles. The size of the food vacuoles depends mainly on the size of the species and the size of the ingested diet. By contrast, symbiotic green algae usually occur in high numbers, are of same size (usually 4–6 µm across) and morphology, have a distinct membrane, but are not in vacuoles, and have a dark central or acentral globule (Foissner et al. 1999). In many (all?) pseudokeronopsids blood-cell-shaped structures occur underneath the cortex (see next chapter).

In *Pseudokeronopsis carnea* a typical plasma membrane covers flat alveoli that are frequently insignificant or invisible in main parts of the somatic region, but very distinct in the buccal area (Wirnsberger & Hausmann 1988b). Below the somatic pellicle is a single layer of longitudinal subpellicular microtubules. By contrast, the rigid stylonychines have several layers of subpellicular microtubules, which are arranged crosswise in *Stylonychia* (Calvo et al. 1986, Puytorac et al. 1976).

The plasma membrane of some (all?) Hypotricha (e.g., *Urostyla grandis*, *Pseudokeronopsis rubra*, *Pseudourostyla cristata*, *Uroleptus caudatus*, *Paraurostyla weissei*, *Oxytricha fallax*, *Stylonychia mytilus*, *Urosoma* sp.) and oligotrichs (e.g., *Strombidium*) is covered by an additional layer called perilemma (Bardele 1981, Grimes 1972, Laval 1971, Laval-Peuto 1975, Wasik & Mokolajczyk 1992). This outer coating also covers cilia, membranelles, and cirri. The perilemma is lacking in *Halteria* and the euplotids. Bardele (1981) assumed that the perilemma in hypotrichs is a temporary structure which is discarded quite often because numerous layers of the perilemma are usually seen in the buccal cavity. Unfortunately, nothing is known as to how the perilemma is derived or replenished. Lynn & Corliss (1991) supposed that it may be a special kind of fixation artefact of the glycocalyx, that is, the protein and glycoprotein layer of the plasma membrane.

The endoplasm of *Pseudokeronopsis carnea* is characterised by many mitochondria, reserve organelles such as paraglycogen granules and lithosomes, and the nuclear apparatus (Wirnsberger & Hausmann 1988b).

1.5 Cortical Granules

These organelles have various names in the urostyloid literature, for example, Öltröpfchen (= oil droplets; e.g., Stein 1859), protrichocysts (Kahl 1932), Perlen (pearls; e.g., Kahl 1932), subpellicular granules (e.g., Berger & Foissner 1987), or pigmentocysts (Wirnsberger & Hausmann 1988b). Cortical granules occur in many species of the Urostyloidea (e.g., Fig. 208r), but also in many species of the Oxytrichidae (for review see Berger 1999) and other ciliate groups (e.g., Foissner et al. 2002), that is, these or-

ganelles are an old feature (homology of these organelles assumed). Their absence in the Stylonychinae is successfully used to characterise this monophylum (Berger & Foissner 1997). Likely most of the granules in the Urostyloidea belong to the mucocyst type. Their colour, size, shape, and arrangement are very important features, which cannot usually be seen after protargol impregnation. Live observation is therefore absolutely necessary for reliable identification of urostyloids (e.g., Stein 1859, Kahl 1932, Berger & Foissner 1987a, Borror & Wicklow 1983).

Wirnsberger & Hausmann (1988b) studied the fine structure of *Pseudokeronopsis carnea*. The striking orange-red colour of this species is caused by two types of pigment structures, the pigment vacuoles and the pigmentocysts. The pigment vacuoles are not extrusive and are confined to a characteristic ectoplasmic zone, about 1.5–3 µm thick, where they form 2–5, but usually three layers. Only a few mitochondria can be found in this area. The pigment vacuoles show a loose, fluffy periphery and a central, more intensely stained part, which is elliptic with a sometimes lamellar appearance. The pigmentocysts are narrowly arranged around the ciliary organelles on the ventral and dorsal side of the cell. A few also occur in the endoplasm and between the ciliary organelles. Under the light microscope, the pigmentocysts appear darker red than the pigment vacuoles. They are globular to oviform and about 0.5–1.0 µm long. A short, electron-dense channel is oriented, to and connected with, the pellicular membranes. However, Wirnsberger & Hausmann (1988b) never observed the discharge of the pigmentocyst content.

Several pseudokeronopsid species have a distinct layer of curious organelles underneath the cell surface (e.g., Fig. 180c, 185l–n, 192e, f, i, j, 193b, c). They have the shape of the erythrocytes of mammals, are colourless and therefore sometimes difficult to recognise although about 2.0 µm across. However, these structures are also described for some non-pseudokeronopsid species, for example, *Anteholosticha warreni*. Their function is unknown, ultrastructure data are lacking.

1.6 Movement

There exist only very few detailed studies about the movement of urostyloids. Most urostyloids are, like the majority of the hypotrichs, thigmotactic, that is, they adhere more or less strongly to the substrate whenever the opportunity arises. They creep on their flattened ventral side by means of the cirri; usually the specimens move hastily to and fro, but sometimes they remain immobile for more or less long periods during feeding. Verworn (in Pütter 1900, p. 284) found that *Urostyla grandis* bends both left and right when unimpededly swimming. When it makes spontaneous reverse movements or when it is bumped, for example, due to shaking the slide, then it always changes direction rightwards.

All urostyloids have a flexible body which bends to varying degrees. Thus, if you see a rigid, freely motile hypotrich you can exclude that it is a urostyloid.

1.7 Somatic Ciliature and Ultrastructure

The somatic ciliature of the Urostyloidea is composed of rows and localised groups of cirri on the flattened ventral side, and several (about 3–10) rows of more or less widely spaced, usually short (2–5 μm) and stiff cilia (bristles) on the vaulted dorsal side (Fig. 1a–e).

The Urostyloidea are characterised by the midventral complex, which is usually composed of ventral cirral pairs forming a more or less distinct zigzag pattern (Fig. 1a). Although this pattern very likely evolved convergently in, for example, *Uroleptus*, *Territricha*, *Pattersoniella* (see the phylogeny chapter and Fig. 16a–o) the "midventral"-species treated in the present book form very likely a monophylum. The only exceptions are *Neokeronopsis spectabilis* and *Urostyloides sinensis*, which have a pronounced midventral pattern, but also dorsomarginal kineties and a fragmenting dorsal kinety (Fig. 242a–h, 243a–m). The dorsal ciliature features assign them unequivocally to the Oxytrichidae (Fig. 14a).

The arrangement of the cirri is a very important feature for urostyloid/hypotrich systematics. Therefore, an unambiguous terminology is necessary. Fig. 1a–g show many important features necessary for the understanding of the urostyloid morphology and phylogeny. In the following paragraphs the individual cirri and, respectively, cirral groups are discussed. Note that many cirri of the various taxa of the Hypotricha (e.g., Urostyloidea, Oxytrichidae) can be homologised and therefore some of them have, of course, the same designation in these taxa (for discussion of the confusing terminology of some cirri see Berger 1999). As in volume I of the revision of Hypotricha (Berger 1999), I use the well-established numbering system introduced by Wallengren (1900a). Note that cirral groups/rows can be true rows (e.g., marginal rows) or pseudorows (e.g., transverse cirri). The cirri of a true row originate from the same anlage, whereas the cirri of a pseudorow originate from different anlagen. For details on the homology see the phylogeny chapter. The oral apparatus is described in the next chapter.

Frontal cirri (FC). These are the cirri near the anterior end of the cell (Fig. 1b). Many urostyloid species have, likely most oxytrichids (Berger 1999), three more or less distinctly enlarged frontal cirri. They are homologous in all groups (Fig. 16). The left frontal cirrus (= cirrus I/1) is usually ahead of the anterior end of the paroral. During cell division it originates from the same anlage (= anlage I) as the undulating membranes. The middle frontal cirrus is homologous to cirrus II/3 of the 18-cirri oxytrichids. During morphogenesis it originates, like the buccal cirrus, from anlage II. The right frontal cirrus is homologous to cirrus III/3 of the 18-cirri oxytrichids. Usually this cirrus is arranged close to the distal end of the adoral zone of membranelles. *Biholosticha* obviously has only two frontal cirri, many other urostyloid taxa, however, have an increased number of frontal cirri. The increase is due to the insertion of additional cirral anlagen, which produce – like those of the midventral complex – usually only two cirri. This results in the formation of a so-called bicorona (Fig. 1a). Usually, anlage I produces only the leftmost frontal cirrus. However, in *Uroleptopsis citrina* it forms two cirri (Fig. 192v–x). If more than two cirri per anlage are produced, then a tricorona (*Tricoronella*; Fig. 147f, h) or a (more or less regular) multicorona (e.g., *Uro-*

styla grandis, *Epiclintes*, *Eschaneustyla*) is formed. Live, it is rather easy to recognise whether a specimen has three enlarged frontal cirri or, for example, a bicorona.

Buccal cirrus (BC). This term is used for the cirrus immediately right of the paroral (Fig. 1a). It is homologous with the buccal cirrus (= cirrus II/2) of the other hypoptrichs (e.g., Berger 1999). For a discussion of the confusing terminology, see Berger (1999). Borror & Wicklow (1983) introduced the term malar cirrus in their revision on the urostyloids. Most urostyloid species have, like the majority of the Hypotricha, a single buccal cirrus, which is often slightly to distinctly behind the anterior end of the paroral (Fig. 1a). Some taxa, for example, *Paragastrostyla* have lost the buccal cirrus, some species have two (Fig. 1b) or more such cirri (e.g., *Anteholosticha adami*; Fig. 74b, i). In *Uroleptopsis citrina* the buccal cirrus is not right of the paroral, but forms a part of the bicorona (Fig. 1e, arrowhead). In life, the buccal cirrus is sometimes difficult to recognise because it is often fine and therefore easily misinterpreted as paroral cilia.

Parabuccal cirrus (PC). This is cirrus III/2 according to Wallengren's (1900) terminology. Most species with three frontal cirri have a single parabuccal cirrus (Fig. 1b). A taxon with more than one such cirrus is *Bakuella*.

Frontoterminal cirri (FT). This term was introduced by Hemberger (1982, p. 11). Most species have two such cirri which are homologous to the frontoventral cirri VI/3 and VI/4 of the 18-cirri oxytrichids (Fig. 16b; for review see Berger 1999). Borror & Wicklow (1983) introduced the term migratory cirri because of the conspicuous migration from posterior to near the distal end of the adoral zone of membranelles during late stages of cell division (Fig. 1a). They always originate from the rightmost (= rearmost) frontal-midventral-transverse cirral anlage. In some species they possibly occur from the two rightmost anlagen (e.g., *Bakuella edaphoni*); however, these data should be checked again. As already mentioned, most species have, like many Oxytrichidae, two frontoterminal cirri. Unfortunately, the frontoterminal cirri are very difficult to recognise in life, and even in protargol preparations they are sometimes hardly recognisable. In some cases ontogenetic stages are needed to be certain whether or not this cirral group is present. Some taxa, for example, *Holostichides* and *Keronella*, have more than two frontoterminal cirri (Fig. 1b, 201l–s, 202a). Few taxa, for example, *Urostyla grandis* and *Australothrix* and *Parabirojimia* lack frontoterminal cirri.

Midventral complex (MC). The terminology for the autapomorphy of the urostyloids, the midventral cirri, was rather confusing since the term midventral row has not been used uniformly. Thus I introduced the term "midventral complex" (Berger 2004b; Fig. 1a). The expression midventral cirri was introduced by Borror (1972) as follows: "Between the right and left marginal cirri in members of the Holostichidae is a double row of cirri that often is arranged in a zigzag position. The midventral cirri arise from a longitudinal series of transverse streaks in *Urostyla cristata*, ...". However, this term was not used in all subsequent papers on urostyloid hypotrichs. For example, Buitkamp (1977) designated the two rows formed by the zigzagging cirri as ventral rows (note that these two rows are pseudorows!). Hemberger (1982) and Foissner (1982) basically accepted Borror's expression and designated the two pseudorows as right and left midventral row. In several urostyloid taxa (e.g., *Bakuella*, *Keronella*) not only cirral pairs,

but also more or less long rows are formed by the midventral anlagen. Wiackowski (1985) summarised both the cirral pairs and the cirral rows under the term midventral cirri. By contrast, Song et al. (1992) confined the expression midventral row to the zigzagging cirral pairs and designated the cirral rows in the posterior body portion as ventral rows. In 1994, Eigner introduced two terms for these cirral rows in the posterior body portion of some taxa, namely (i) short midventral row composed of 3–4 cirri, and (ii) long midventral row composed of more than four cirri. According to Eigner's terminology, for example, a *Bakuella* species has (i) a "midventral row" (composed of zigzagging cirral pairs), (ii) one or more "short midventral rows", and (iii) one or more "long midventral rows". Since the midventral row mentioned under (i) can also be either short or long, the terms introduced by Eigner are somewhat misleading. In addition, the left cirrus of several cirral pairs is lacking in non-dividers of *Uroleptopsis citrina* further complicating the terminology (see below). To overcome these terminological problems, the various structures are designated as shown in Figs. 1a, b, e. The generic term is "midventral complex", which can be composed of various structures. For example, in *Holosticha* species the midventral complex consists of midventral pairs only, whereas in *Bakuella* it is composed of midventral pairs and midventral rows. In *Epiclintes* and *Eschaneustyla* the midventral complex is composed of midventral rows only, that is, midventral pairs and therefore the characteristic urostyloid zigzag pattern is lacking. In species with three enlarged frontal cirri, the distinction between the frontal cirri and the midventral complex is straightforward (Fig. 1b). In taxa with a bicorona – for example, *Kerononella* and *Uroleptopsis* – it is sometimes difficult to define the beginning of the midventral complex (Fig. 1a, e). However, usually the cirri of the anterior corona and even those of the posterior are slightly to distinctly larger than the midventral cirri and often at least slightly set off from them.

The right cirrus of a midventral pair is often larger than the left cirrus. Likely this is due to the fact that the right cirri are homonomous to the anterior cirri of a bicorona (Fig. 1a), respectively, the enlarged frontal cirri (Fig. 1b), which are more or less distinctly larger then the other cirri (e.g., buccal cirrus, cirrus III/2) of the same anlage. However, the enlargement is sometimes indistinct and in many cases such details are neither mentioned nor illustrated in the individual descriptions. Only in *Uroleptus*, which is very likely not a urostyloid, is the difference usually very distinct (Fig. 16j).

Pretransverse ventral cirri (PT). This term was introduced by Berger & Foissner (1997) for two, often inconspicuous cirri immediately ahead of the transverse cirri (Fig. 1a). Unfortunately, we overlooked the older term accessory transverse cirri introduced by Wicklow (1981, p. 348). According to Wallengren's (1900) numbering system they have the designation V/2 and VI/2, that is, they originate from the two rightmost frontal-ventral-transverse cirral anlagen. Interestingly, these two cirri are also present in some urostyloids, for example, *Anteholosticha australis* and *A. mancoidea*. Of course, in these species they do not originate from the anlagen V and VI, but from the two rightmost anlagen, which, however, are homologous with the anlagen V and VI of the 18-cirri oxytrichids and the amphisiellids (Berger 2004a). In many urostyloid species pretransverse cirri are lacking, in others they have likely been subsumed under the term transverse cirri.

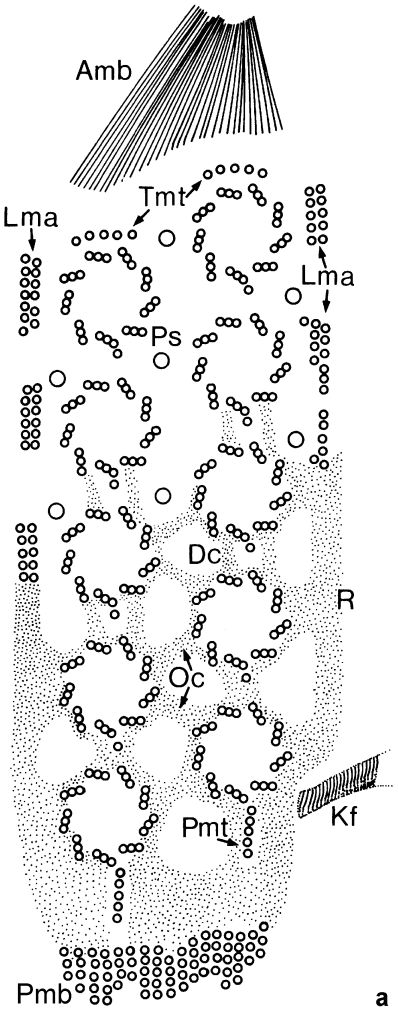


Fig. 5a *Pseudokeronopsis carnea* (from Wirnsberger & Hausmann 1988b). Scheme of the fine structure of a left marginal cirrus illustrating the positions of the various fibres. The top part of the figure represents a distal level of section, whereas the lower parts correspond to proximal sections. Amb = anterior microtubular bundles, DC = double connections, Kf = kinetodesmal fibre, Lma = linear microtubular arrays, Oc = oblique connections, Pmb = posterior microtubular bundles, Pmt = postciliary microtubules, Ps = parasomal sacs, R = rampart, Tmt = transverse microtubulus.

Transverse cirri (TC). This cirral group, which is a pseudorow, is usually in the posterior quarter of the cell (Fig. 1a). Transverse cirri are present in most hypotrichs. A transverse cirrus is, per definition, the rearmost cirrus produced by a frontal-(mid)ventral-transverse cirral anlage. It forms – usually together with other rearmost cirri – a "transverse" pseudorow (typically it is more or less obliquely arranged). In the Urostyloidea the transverse cirri are usually not or only slightly larger than, for example, the midventral cirri. By contrast, in many Stylonychinae they are very large and therefore prominent (Berger 1999). In most urostyloids only the rearmost cirral anlagen produce a transverse cirrus. Only in few taxa, for example, *Holosticha* and *Pseudoamphisiella*, does each anlage (except the anteriormost anlagen) produce a transverse cirrus resulting in a rather uncommon cirral pattern. Other taxa (e.g., *Holostichides*) lack this cirral group.

Marginal cirri (LMR, RMR). These cirri run along the left and right body margin. Many urostyloids have one left and one right marginal row (Fig. 1a, 5a). Some taxa, for example, *Pseudourostyla*, *Urostyla*, or *Diaxonella* have more than two marginal rows. However, the increase in number certainly occurred several times independently, as indicated by the rather different morphogenetic pattern (see the cell division chapter). Usually the marginal rows are more or less distinctly separated posteriorly. However, the gap is often difficult to recognise because it is seemingly occupied by the caudal cirri, which, however, insert on the dorsal surface (Fig. 1a, c).

Dorsal cilia (DB; 1, 2, 3, ...). The dorsal side of all hypotrichs and euplotids is covered with a more or less high number of kineties, which are therefore named dorsal kineties or dorsal bristle rows (e.g., Fig. 101g). Many urostyloids have three kineties, but species with up to 10 bristle rows are known. The kineties of the urostyloids are basically bipolar, that is, they

extend from near the anterior end to the rear body end. Dorsomarginal kineties (originating from/near the right marginal primordium; Fig. 243j, l) and fragmenting kineties (one, usually kinety 3, or more kineties fragments into an anterior and posterior portion; Fig. 243k, m) are lacking. The dorsomarginal kineties are the morphological apomorphy of the Dorsomarginalia, the fragmentation the apomorphy of the Oxytrichidae (Fig. 14a). Thus, *Neokeronopsis* and *Urostyloides* belong to the Oxytrichidae (p. 1190, 1205).

As in other hypotrichs, the dorsal kineties of the urostyloids consist of basal body pairs. The bristle originates from the anterior basal body, is usually short (about 2–5 µm), and more or less stiff. The number of bristle rows is difficult to recognise in life, that is, usually protargol preparations are needed to know the number. However, in species with distinct (usually coloured) cortical granules the number of kineties corresponds with the number of stripes formed by the cortical granules. The fine structure is likely identical to that of the Oxytrichidae (see Berger 1999 for review). The function of the dorsal bristles is not known. Likely they are remnants of the ciliature of an early ancestor. The dorsal kinety which is closest to the left marginal row is designated as kinety 1 (Fig. 1a–d).

Caudal cirri (CC). These cirri originate at the rear end of the bipolar dorsal kineties (Fig. 1c). Dorsomarginal kineties and the anterior portion of a fragmenting kinety are never associated with a caudal cirrus (Berger 1999). Usually they are inserted at the rear tip of the cell, often above the gap formed by the rear end of the marginal rows. Thus, study your slides (in vivo and protargol!) carefully and do not misinterprete caudal cirri as marginal cirri! Some species produce more than one caudal cirrus per dorsal kinety. On the other hand, several urostyloids which lack these cirri exist. Very likely, the loss occurred several times independently. In one case this feature is characteristic for a relatively large group, which is therefore named Acaudalia (Fig. 144a). The caudal cirri of the urostyloids are usually inconspicuous, that is, neither very long and/or strong. By contrast, the caudal cirri of some oxytrichids (e.g., *Stylonychia*) are rather long and therefore very conspicuous (Berger 1999).

Fine structure of cirri and membranelles. There exist only few data on the fine structure of urostyloids (Yasuzumi et al. 1972, Wicklow 1981, Carey & Tatchell 1983, Wirnsberger & Hausmann 1988b, Wicklow & Borror 1990). In *Pseudokeronopsis carnea* the marginal, frontoterminal, and midventral cirri have the same microtubular and microfibrillar associates (Fig. 5a). The anterior and posterior microtubular bundles of several cirri overlap and accompany the single layer of subpellicular microtubules. The pairs of midventral cirri are very closely set; thus, each kinetodesmal fibre of the left midventral cirrus is in contact with the margin of the right midventral cirrus (Wirnsberger & Hausmann 1988b).

Linear microtubular arrays which characteristically comprise two rows of 5–7 serially arranged microtubules border the longer sides of the cirral bases and extend toward the pellicle, probably contributing to the single layer of subpellicular microtubules. Likewise, they occur to the right and to the left of each adoral membranelle as well as to the right of the paroral and to the left of the endoral. The left microtubular arrays of the membranelles may contribute to the postmembranellar fibre, and the right ones probably line the buccal cavity and build the highly ordered structure to the left of the

endoral. Between the groups of three sheets of 7–8 microtubules, there are prominent vacuoles and alveoli beneath the pellicle. Thus, the whole system resembles oral ribs (Wirnsberger & Hausmann 1988b).

Wirnsberger & Hausmann (1988b) discussed some fine structural features that may unify urostyloid hypotrichs based on the data about *Thigmokeronopsis jahodai* and *Pseudokeronopsis carnea*. Unfortunately, Wicklow (1981) gave sparse details concerning the fine structure of the buccal region; therefore, Wirnsberger & Hausmann's findings are difficult to compare and no definite taxonomic conclusion could be derived. They found that both taxa share some ultrastructural characters that are perhaps restricted to urostyloids. (i) At present, the additional linear microtubular arrays bordering the cirri are unique for urostyloid taxa. This microtubular system reminds one of that found in the heterotrich ciliate, *Plagiotoma lumbrici* (Wicklow 1981). (ii) In *Pseudokeronopsis carnea* these linear microtubular arrays are also present beside the left and the right buccal organelles. Although Wicklow (1981) described them to the right of the membranelles only, they are also visible in the paroral of *T. jahodai*. (iii) Wicklow (1981) considered the urostyloid midventral cirral pairs to be linked in a ladder-like array owing to the anterior microtubular bundles joining them in *Thigmokeronopsis*. This is obviously not the case in *Pseudokeronopsis*, but the pairs of midventral cirri are in fact very closely set and seem to be "linked" by the kinetodesmal fibre of the left midventral cirrus. (iv) In contrast to oxytrichid taxa, the anterior frontal cirri have not been found to be linked with the frontal adoral membranelles in urostyloids. For details on the fine structure of *Epiclintes auricularis*, see species description.

1.8 Oral Apparatus

The oral apparatus of the Urostyloidea is composed, as in the remaining Hypotricha, of an adoral zone of membranelles, two undulating membranes (paroral and endoral), the buccal cavity (buccal field, oral field), and associated fibres including the cytopharynx (e.g., Fig. 1a, b, e, 151g, 208j–l, n, o). For details, see Foissner & AL-Rasheid (2006).

The adoral zone of membranelles, the most prominent part of the oral apparatus, extends from the anterior body end along the left anterior body margin to near midline of the cell and usually terminates at about 25–35% of body length. Usually, it is roughly the shape of a question mark. In some taxa the distal (= frontal) portion extends far onto the right body margin. This feature was used by Wicklow (1981) to characterise the Keronopsidae (now Pseudokeronopsidae). Wiackowski (1988) quantified this character in that he divided the distance between the anterior body end and the distal end of adoral zone by the distance between the anterior body end and the proximal end of the adoral zone (Fig. 1c). He distinguished four ranges: less than 0.11 (designated as plesiomorph by Wiackowski); 0.11–0.20; 0.21–27; and 0.28 or more (most derived). Whether a low or high value is apomorph is not yet certain because we do not know the state in the last common ancestor of the Hypotricha. Preliminarily, I accept Wiackowski's assumption that high values are derived. For the sake of simplicity this quotient introduced by Wiackowski (1988) is named "DE-value" (for *D*istal *E*nd of adoral zone).

High DE-values occur not only in the pseudokeronopsids, but also in the pseudourostylids, the Epiclintidae, *Pseudoamphisiella*, and some taxa outside the Urostyloidea (e.g., *Amphisiella namibiensis*, *Pseudouroleptus caudatus*; Foissner et al. 2002), indicating that this feature occurred, like many others, convergently.

Some species, for example, *Holosticha* spp., *Uroleptopsis citrina* or *Afrothrix* spp. have a more or less distinct gap (break) in the zone (Fig. 31b, 104a, f, 105a, f, 192k). The proximal (= ventral) portion is sometimes distinctly spoon-shaped. In some *Holosticha*-species the proximalmost membranelles are slightly to distinctly wider than the remaining membranelles (Fig. 29b, 34b, f).

Species of the Hypotricha are characterised by two undulating membranes, the paroral and the endoral (Fig. 1a, b, e, 61t, 151g). For a detailed discussion of the rather bewildering terminology of these structures, see Berger & Foissner (1997) and Berger (1999). In general, the paroral extends between two usually inconspicuous cytoplasmic lips at the right outer margin of the buccal cavity, that is, on the cell surface, while the endoral is on the bottom and right wall of the cavity. This means that the membranes extend at different levels. However, if the cell is viewed from the ventral side, they appear to lie side by side (e.g., *Pseudokeronopsis*, *Uroleptopsis*; Fig. 192k) or to intersect (e.g., *Urostyla*; 208h, j, o), depending on their shape and arrangement.

In the Oxytrichidae, the shape and arrangement of the adoral zone and especially the undulating membranes is often used to recognise subgroups (for reviews see Kahl 1932, Berger & Foissner 1997, and Berger 1999). This is also possibly within the Urostyloidea, however, to a distinctly smaller extent. Especially the undulating membranes show a lower diversity than in the Oxytrichidae, where rather curious patterns occur (e.g., *Steinia* pattern with a fragmented endoral; Berger & Foissner 1997). However, there exist urostyloid groups with a characteristic undulating membrane pattern, for example, the pseudokeronopsids where membranes are rather short and arranged more or less parallel. Very likely some further patterns can be recognised (distuingished) when more detailed data become available.

The buccal cavity is also different in shape and size and usually described by the terms flat or deep and wide and narrow. Flat means that the cavity is only slightly hollowed, whereas a deep cavity extends to near the dorsal side of the cell, making the oral field conspicuously bright. In species with a wide cavity, the right margin of the cavity is in the midline of the cell, whereas in a narrow cavity it is arranged close to the right margin of the adoral zone (further details see Berger 1999 and Foissner & Al-Rasheid 2006).

Wirnsberger & Hausmann (1988b) studied the fine structure of the oral apparatus of *Pseudokeronopsis carnea*. The basic features resemble those of other hypotrichs. Therefore they described only details that are peculiar to the urostyloid *P. carnea*. The cilia of the endoral are connected by a microfibrillar material (Fig. 6a). These peculiar connections have not been found between the cilia of the paroral. Proximally, the basal bodies of the endoral are linked by amorphous connectives, which join the triplets 7, 8 of the anterior basal body with the triplets 2, 3 of the posterior one. About five transverse microtubules are associated with the triplets 9, 1, and 2; two postciliary microtubules are oriented to the right, and parasomal sacs are situated to the left of the endoral. The two

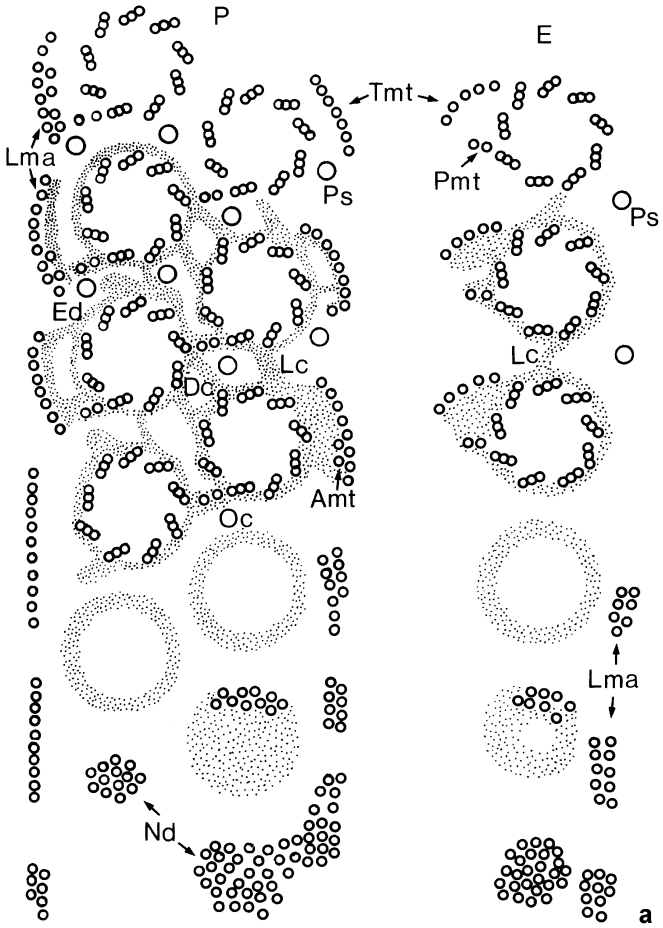


Fig. 6a *Pseudokeronopsis carnea* (from Wirnsberger & Hausmann 1988b). Scheme of the fine structure of the paroral (P) and endoral (E). The top part of the figures represent a distal level of section, whereas the lower parts correspond to proximal sections. The basal bodies are embedded in electron-dense material (Ed) which forms longitudinal (Lc), oblique (Oc), and double connections (Dc). Two postciliary microtubules (Pmt) are associated with basal bodies. The transverse microtubules (Tmt) of both membranes are differently oriented, the paroral ones to the left and the endoral ones to the right. Sometimes additional microtubules (Amt) occur in a second row of the paroral transverse microtubules. The characteristic linear microtubular arrays (Lma) are associated with the right of the paroral and with the left endoral. Ps = parasomal sac, Nd = nematodesmata.

rows of paroral basal bodies are proximally linked by electron-dense material at four different locations: two of them connect both neighbouring basal bodies, which are reminiscent of dikinetids; in addition, longitudinal linkages join the basal bodies within one row and oblique ones connect the neighbouring "pairs". About 7–9 transverse microtubules originate beside the left row of paroral basal bodies and a second sheet of microtubules may appear at a more proximal level, possibly corresponding to nematodesmata. Two postciliary microtubules appear at the right of each paroral basal body, and parasomal sacs are situated to the right and to the left. Nematodesmata emerge from the proximal part of all endoral and paroral basal bodies, contributing to the pharyngeal basket (Wirnsberger & Hausmann 1988b).

1.9 Silverline System

The silverline system of urostyloids is, like that of the Oxytrichidae (for review see Berger 1999), composed of small (1–2 µm) polygonal meshes (Fig. 103b; Foissner 1980a, 1982). It has no systematic value in the hypotrichs, whereas it is successfully used to characterise euplotids (e.g., Borror & Hill 1995).

1.10 Life Cycle

The Urostyloidea have, like most other Hypotricha, a normal life cycle, that is, the theronts feed, become trophonts and divide, encyst, or conjugate. There is much less specific literature available about these topics than for the Oxytrichidae (for review of this group see Berger 1999).

1.10.1 Cell Division

Urostyloid ciliates divide by isotomic transverse fission, like many other ciliates (Foissner 1996c; e.g., Fig. 7a–t). The anterior filial product is the proter, the posterior the opisthe. Early in division, a replication (= reorganisation) band traverses each macronuclear nodule. In species with many tiny nodules, this feature is often difficult to recognise (Fig. 2a–g). The two to very many nodules fuse to a single mass during early and middle stages of cell division. The macronucleus divides amitotically just before cytokinesis (Fig. 3a–l). By contrast, the micronuclei(eus) divide(s) mitotically (Fig. 4a–h). Only the pseudokeronopsids show a deviating pattern in that the many macronuclear nodule divide individually (e.g., Fig. 192r).

The changes of the ciliature during cell division are known from a relatively low number of species. Morphogenetic data allowed homologising the individual cirri of the urostyloid with those of the 18-cirri oxytrichids. For example, the frontoterminal cirri of the urostyloid are certainly homologous with the frontoventral cirri VI/3 and VI/4 of the 18-cirri oxytrichids, because in both cases these are the two anteriormost cirri (of a total of four) of the rightmost (= rearmost) frontal-(mid)ventral-transverse cirral anlage (e.g., Hemberger 1982, Wirnsberger 1987, Berger 1999). Moreover, in both groups these two cirri migrate anteriorly in the area between the distal end of the adoral zone and the anterior end of the right marginal row (Fig. 1a, 192w, x).

As in other hypotrichs, the parental ventral and dorsal somatic ciliature of the urostyloids is completely renewed during cell division. The parental oral apparatus is either retained after a more or less distinct reorganisation, or it is completely renewed as, for example, in the pseudokeronopsids.

In the urostyloids, the ventral somatic ciliature develops from more than six, more or less obliquely arranged frontal-midventral-transverse cirral anlagen (Fig. 7a–e, k–o). Usually these anlagen are numbered from I to n (I = leftmost anlage forming cirrus I/1 and undulating membranes; anlage n = rightmost anlage usually forming the frontoter-

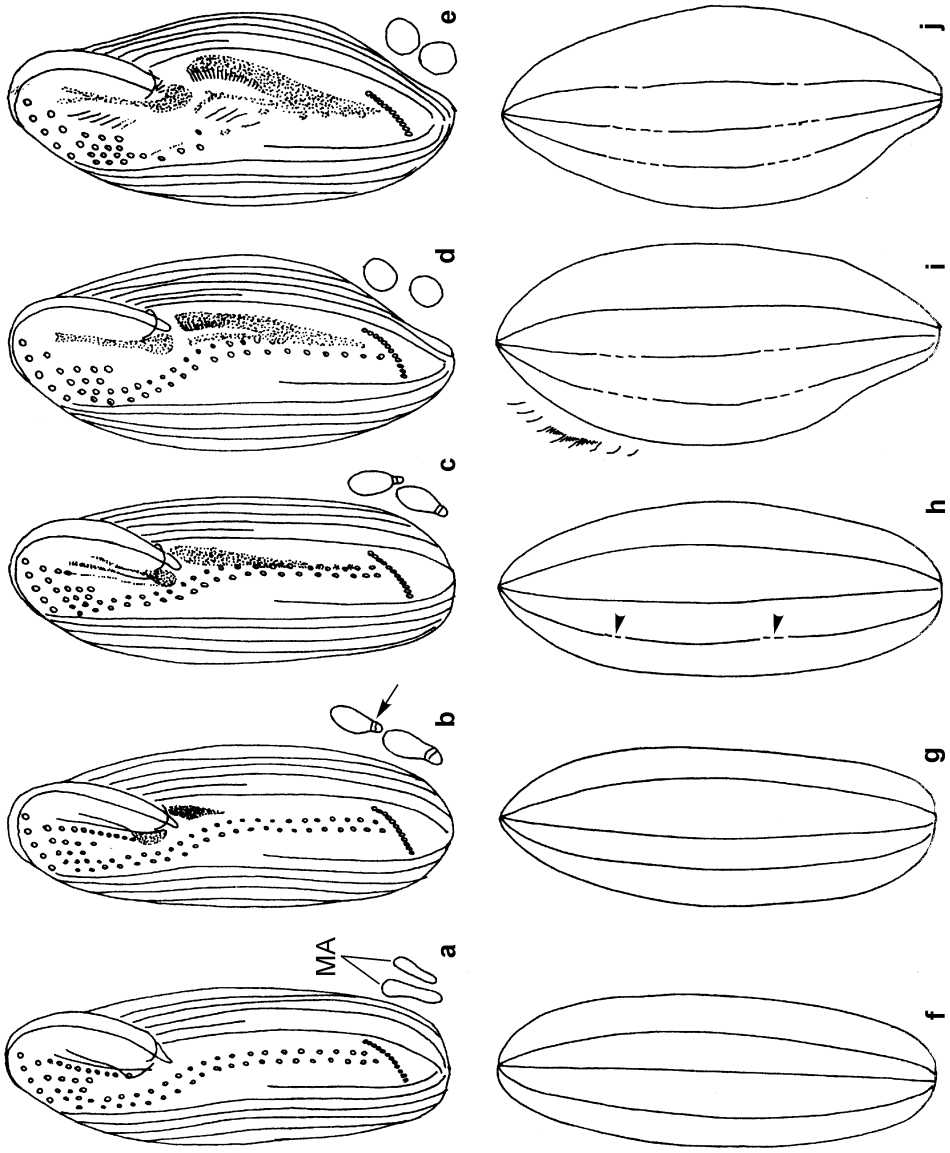


Fig. 7a–j *Urostyla grandis* (from Jerka-Dziadosz 1972. After protargol impregnation). Schematic illustrations of an interphasic specimen (a, f) and early to middle stages of cell division in ventral (b–e) and dorsal (g–j) view. Arrowheads in (h) mark the beginning of the intrakinetal formation of the dorsal kinety primordia. Arrow in (b) marks replication band. Note that in *U. grandis*, many other urostyloid species, and all remaining hypotrichs the macronuclear nodules fuse to a single mass prior to cell division. For details see text. MA = two of many macronuclear nodules.

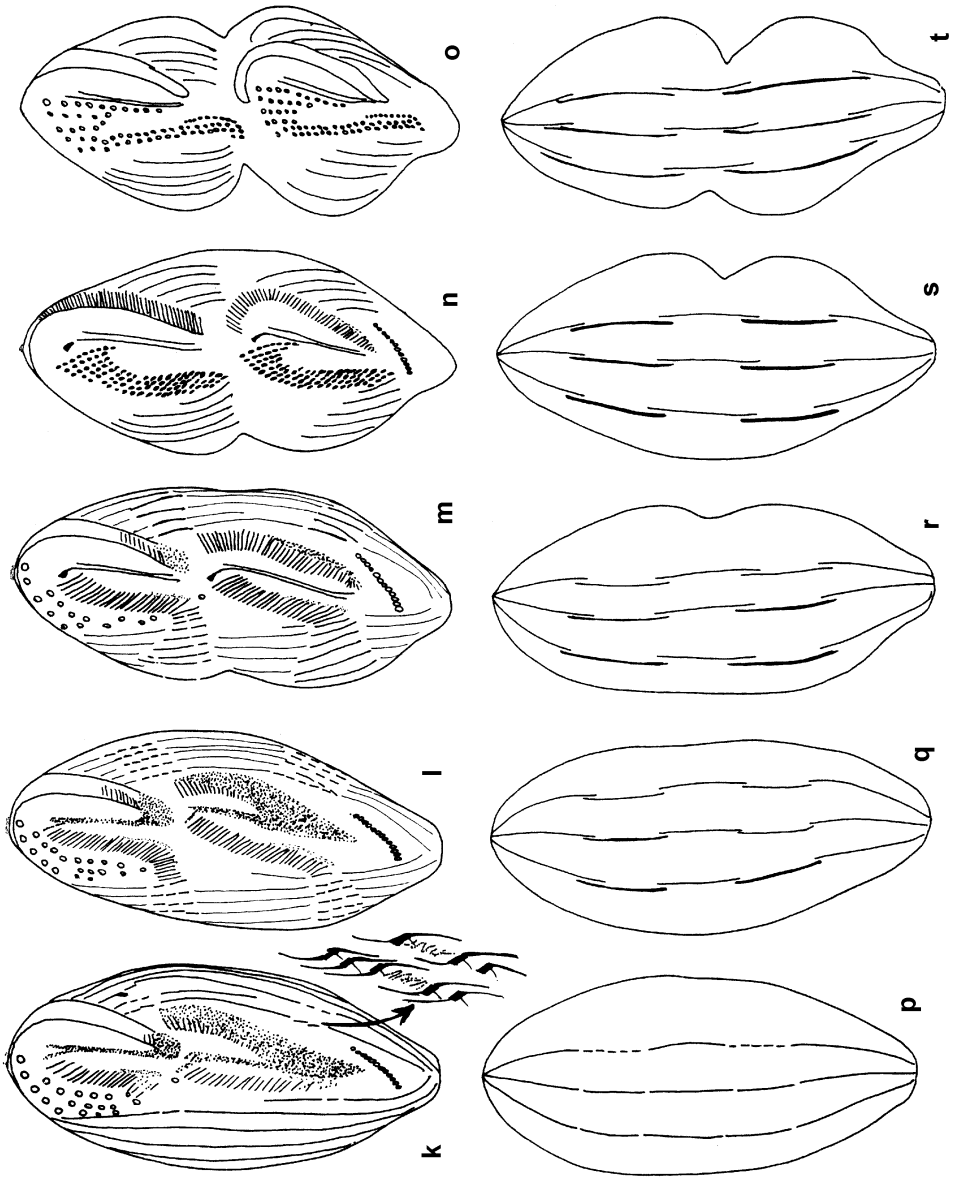


Fig. 7k–t *Urostyla grandis* (from Jerka-Dziadosz 1972. After protargol impregnation). Schematic illustrations of late to very late stages of cell division in ventral (k–o) and dorsal (p–t) view. Note that the frontal-midventral-transverse cirral pattern of urostyloid hypotrichs originates from (usually) many obliquely arranged cirral anlagen. The many marginal rows of *Urostyla grandis* divide, like the dorsal kineties, individually. By contrast, in *Pseudourostyla* the marginal rows of each side are formed from a common anlage. For details see text.

minal cirri, the right pretransverse ventral cirrus, and the rightmost transverse cirrus; Fig. 1a)[1]. The cirral pattern of the urostyloids is therefore much more variable than that of the 18-cirri oxytrichids, which usually have, as indicated by the designation, 18 frontal-ventral-transverse cirri. In the urostyloids, the number of frontal-midventral-transverse cirri varies not only among the species, but also within them because the number of anlagen forming the midventral pattern is usually more or less variable. The supposed relationships of the hypotrichs with the euplotids require the assumption that the urostyloids evolved from an ancestor which had six anlagen (Fig. 12, 13a, 14a). This hypothesis can also explain the fact that the so-called midventral pattern, that is, the zigzag pattern formed by the ventral cirri evolved several times independently in rather different groups of hypotrichs (see chapter phylogeny).

The frontal-midventral-transverse cirral anlagen of the urostyloids arise (i) from parental ciliature, (ii) new, and (iii) from the oral primordium. However, it is often rather difficult to recognise how an anlage originates. The conspicuous zigzag cirri-pattern of the urostyloid ciliates is formed from anlagen, which produce only two cirri. Only the posterior anlagen form a pair plus a transverse cirrus. In a relatively high number of species not only cirral pairs but also more or less long rows are formed per midventral anlage (Fig. 1a). However, this feature conflicts with the frontal ciliature.

Berger & Foissner (1997) and Berger (1999) used several morphogenetic peculiarities of the 18-cirri oxytrichids to elucidate the phylogeny of this group. This is not yet possible in such a big way for the urostyloids because their cirral pattern is much more variable, and much less relevant data are available. In spite of this, cell division data are useful markers to elucidate the evolutionary relationships among the Urostyloidea. For example, in *Holosticha* species the midventral cirral anlagen originate largely right of the parental midventral complex, a feature well supporting other morphological traits characterising seven species as a monophyletic group (Berger 2003). Ontogenetic data are also useful to understand deviating cirral patterns. *Uroleptopsis citrina* has a curious midventral complex, that is, the zigzag pattern is lacking in the central portion of the complex. Cell division data showed that this is due to the resorption of the left cirrus of some pairs (Fig. 192v, w; Berger 2004b).

Dorsal morphogenesis proceeds simply in the urostyloids because each dorsal kinety forms one anlage each in the proter and the opisthe by intrakinetal proliferation of basal bodies (Fig. 7f–j, p–t; Foissner & Adam 1983). Dorsomarginal kineties and fragmentation of dorsal kineties – characteristic features for most non-urostyloid hypotrichs, respectively, the Oxytrichidae (for review see Berger 1999) – are lacking in the urostyloids. Only in very few urostyloids (e.g., *Holosticha bradburyae*) did a more complex dorsal morphogenesis evolve. Caudal cirri originate at the rear end of a dorsal kinety anlage. As stated above, proliferation of basal bodies begins at two levels within parental dorsal kineties (Fig. 7h–j). These two regions correspond to the same levels within which the marginal cirri proliferate on the ventral surface (Fig. 7l, q). Several urostyloid

[1] This numbering system has the disadvantage that the two rightmost (= rearmost) anlagen, which produce, inter alia, the two pretransverse ventral cirri, do not have the same Roman numbers in the urostyloids and oxytrichids.

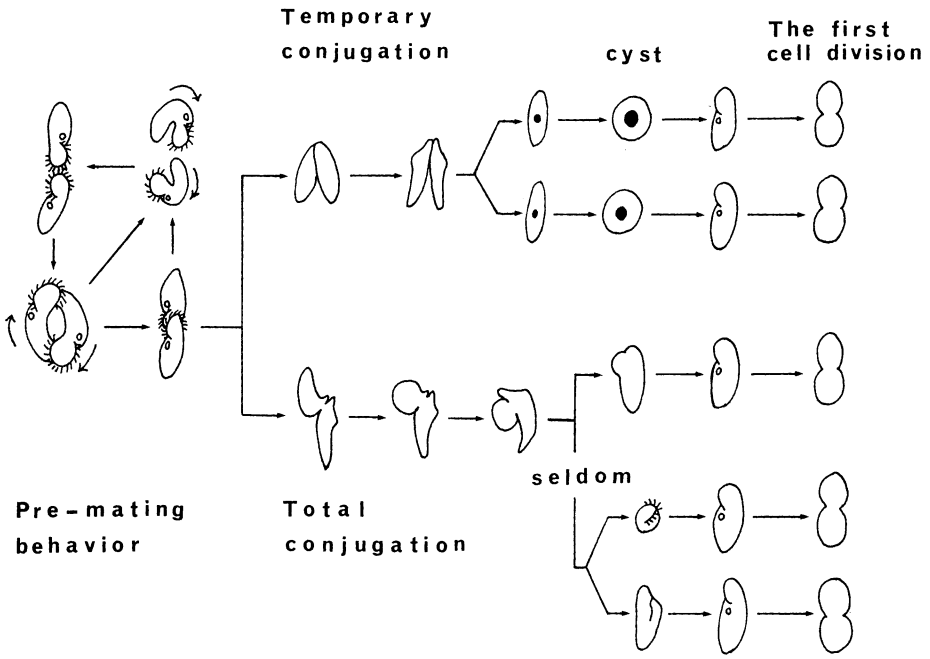


Fig. 8a Schematic illustration of temporary and total conjugation in *Pseudourostyla levis* (from Takahashi 1973). For details see text.

taxa with more than two marginal rows are known. In *Urostyla grandis* the marginal rows divide individually (Fig. 7l). By contrast, in *Pseudourostyla cristata* all marginal rows of a side originate from a common anlage (Fig. 149f–h).

1.10.2 Conjugation

Relatively little is known about this part of the urostyloid life cycle. Takahashi (1973) found two types of conjugation in *Pseudourostyla levis*, namely temporary conjugation and total conjugation (Fig. 8a). In a mixture of two opposite mating types, cells gave no sign of mating during at least five or more hours (a refractory period), but then showed characteristic pre-mating behaviour before forming conjugating pairs. In pre-mating behaviour, two specimens came closer by creeping on the bottom of the container and came in contact. A cell attached with its anterior end to the posterior part of the other specimen. The contacted cells revolved clockwise for several minutes, and then united with their mouths in straight fashion through further complicated behaviour. During this process, the united cells were suddenly separated by interference of another cell, but the disjoined cells again made contact with each other. The reunited head-to-head pair remained as it was for about 20 min, and then changed into a typical pair with side-to-side contact (Fig. 8a).

The conjugating pair underwent meiosis of one micronucleus, which lay near the posterior end of the cytostome, exchanged migratory pronuclei, and formed a synkaryon in each conjugant within 20 h of the onset of conjugation. The old macronuclei of the pair decreased gradually in number as a result of absorption into cytoplasm during this period. Each exconjugant derived from the pair contained a macronuclear primordium, two new micronuclei, and several old macronuclei. Thereafter the exconjugant fell into encystment without cell division two or three days after the separation. The cyst formed was about 50–100 µm across, had no cyst wall, and the macronuclear primordium developed into a new elongate macronucleus 5–8 days after the encystment. The cyst excysted and produced a swimming cell, which bore a new nodulated macronucleus, two new micronuclei, and several old macronuclear nodules. The swimming specimen underwent the first cell division within 48 h after excystment. The nuclear processes during conjugation are described in detail by Takahashi (1974).

For a brief description of the cortical reorganisation during conjugation see Fig. 149s–z.

1.10.3 Cyst

Knowledge about this stage of the life cycle is modest compared to the Oxytrichidae (for review of this group see Berger 1999). Resting cysts are described only for a few urostyloids (e.g., 44f–i, 206c, d, 208d). Reproductive cysts are not known in this group. Factors that induce encystment are, inter alia, desiccation (especially in soil species) and deficiency of food (e.g., Corliss & Esser 1974, Gutiérrez & Martin-González 2002).

The classification of resting cysts is based on a system proposed by Walker & Maugel (1980), who designated the cysts of the euplotids as NKR (non-kinetosome-resorbing) and those of the oxytrichids as KR (kinetosome-resorbing; for review on oxytrichids cyst literature see Berger 1999). Matsusaka et al. (1989) studied the resting

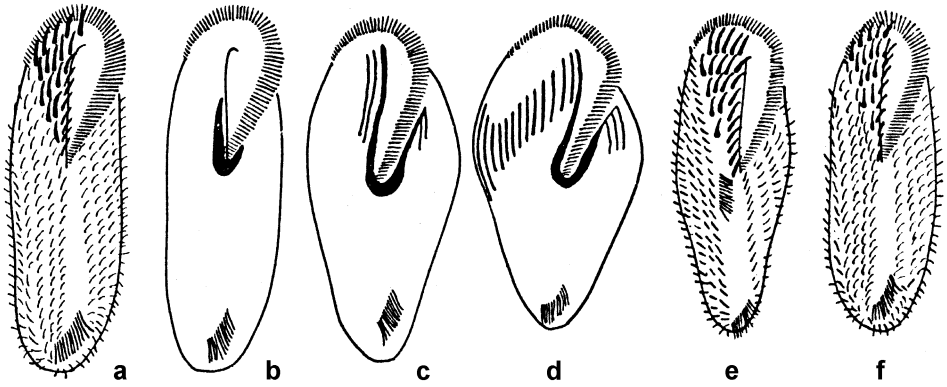


Fig. 9a–f Schematic illustrations of physiological reorganisation in *Urostyla grandis* (from Jerka-Dziadosz 1963). For details see text.

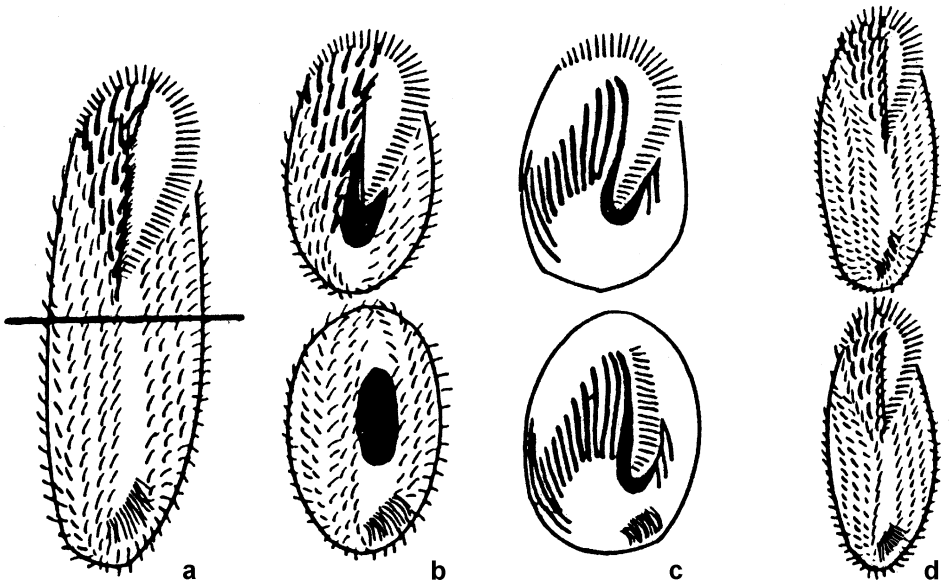


Fig. 10a–d Schematic illustrations of posttraumatic regeneration in *Urostyla grandis* (from Jerka-Dziadosz 1963). For details see text.

cysts of *Anteholosticha adami*, *Pseudourostyla levis*, and *Gonostomum affine* and found that they produce an intermediate type. They therefore distinguished an *Oxytricha*-type cyst (= KR-type), a *Urostyla*-type cyst, and a *Euplotes*-type cyst (NKR-type). Martin-González et al. (1991, 1992) proposed a modification of Walker & Maugel's system in that they named the *Urostyla*-type PKR-cysts (partial-kinetosome-resorbing).

The review by Gutiérrez et al. (2003) indicates that oxytrichids have four cyst wall layers (including the granular layer), whereas the urostyloids have only three. More data on the fine structure (number of cyst wall layers; state of macronuclear nodules, that is, fused or not fused; degree of ciliature resorption) of resting cysts will very likely increase our insights into the phylogeny of the hypotrichs.

1.10.4 Reorganisation, Regeneration, Doublets

Like other hypotrichs, the Urostyloidea produce ciliature not only during cell division or other normal parts of the life cycle (conjugation, excystment), but also during physiological reorganisation and post-traumatic regeneration.

Physiological reorganisation. This part of the life cycle is defined as morphogenesis which re-establishes a complete set of ciliary structures in an intact morphostatic (non-dividing) cell (Grimes & Adler 1978). Usually, this process is a response to an altered nutritional status induced by unfavourable culture conditions (e.g., starvation) or other more subtle changes in the environment. For example, Wirnsberger (1987)

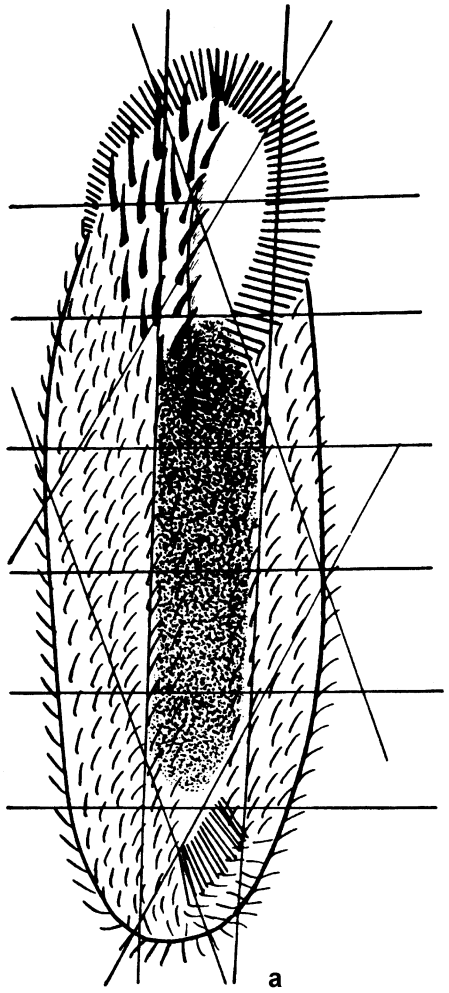


Fig. 11a Presumptive organisation area (dotted) in *Urostyla grandis* (from Jerka-Dziadosz 1964). For details see text.

studied reorganisational morphogenesis in *Pseudokeronopsis rubra* (Fig. 179q; see species description for details). As in other hypotrichs, the morphogenetic processes occurring during physiological reorganisation are rather similar to those during cell division (Fig. 9a–f). Thus it is sometimes difficult to distinguish early stages of these two processes.

Regeneration. Fauré-Fremiet (1910b; 1948, p. 46) made merotomy experiments on cell division by cutting middle dividers at various sites. Jerka-Dziadosz (1967), who also made microsurgical experiments, observed that an individual continued to divide normally when the section line ran somewhat farther from the division furrow. Jerka-Dziadosz (1963, 1964, 1965) found that in *Urostyla grandis* the postoral region is the morphogenetically most active region and thus named it the presumptive organisation area (Fig. 10a–d, 11a). This area is able to develop the primordia of ciliature in division and regeneration. Jerka-Dziadosz (1974) studied, inter alia, the formation of primordia in right fragments. In the right fragments obtained after longitudinal section along the central meridian of the ventral side, in which the wound repair occurs in situ, the primordium of the adoral zone is formed near the wounded margin. The primordium first appears as a small group of basal bodies located near the postoral part of the ventral cirri. In later stages of regeneration it can be seen that such fragments are able to form all of the kinds of ventral and dorsal primordia. For review on this topic, see Frankel (1974; 1989, p. 119).

Doublets. There is little information available about urostyloid doublets as compared to the oxytrichids (for review see Berger 1999). Altmann & Ruthmann (1979) studied doublet formation in *Urostyla grandis*. Accordingly, the formation of homopolar doublets can be induced by the action of antibiotics, which inhibit the growth of cytoplasmic bacterial symbionts whose cell cycle appears to be controlled by the host. At 50 μg ml^{-1} the rate of doublet formation, expressed as a percentage of the total number

of cells, was 0.43%, at 100 µg ml^{-1} 0.61%, and at 200 µg ml^{-1} 1.3%. The symbionts multiply during the macronuclear S-phase of the ciliate, and are enclosed in vesicles and largely destroyed just before cell division is completed. Since doublet formation is due to incomplete cell division, and because experimental disturbances at the cell cortex of dividing ciliates also led to doublets, the symbionts are thought to contribute some factor which is essential for normal cytokinesis of *Urostyla grandis* (Altmann & Ruthmann 1979). Homopolar doublets of *Urostyla trichogaster*, a synonym of *Urostyla grandis*, were analysed by Fauré-Fremiet (1945a, b; 1948, p. 49). The illustrations in Fauré-Fremiet (1967, p. 264) do not show *Urostyla trichogaster*, as mistakenly indicated in the legend, but *Paraurostyla weissei* (see Fig. 3, 5, 6 in Fauré-Fremiet 1945b).

2 Phylogeny

2.1 Notes on the Spirotricha Bütschli, 1889 [1]

The urostyloids are part of the spirotrichs, a large group likely comprising 2000 or more extant species. The main (sole?) "morphological" apomorphy of the Spirotricha is the replication band where DNA is replicated locally and sequentially along the macronucleus (Raikov 1982).[2] Whether the more or less well-developed adoral zone of membranelles is a further apomorphy or a plesiomorphy is not yet clear. The perilemma, considered as still doubtfull apomorphy of the spirotrichs by Petz & Foissner (1992), is possibly lacking in the euplotids (Bardele 1981, Agatha 2004).

All authorities agree that the oligotrichs, the euplotids, and the hypotrichs are the three major components of the spirotrichs. Moreover, the monophyly of each of these three groups is widely accepted[3]. There are three ways to arrange these taxa (Fig. 12a–c),

[1] For names of higher taxa (see Fig. 13a, 14a and Table 1).

[2] Very likely *Phacodinium* does not have a replication band (Lynn & Small 2002, p. 420, 421). If we assume that this feature is primarily lacking in *Phacodinium*, then it does not belong to the spirotrichs. If the replication band was lost during evolution in *Phacodinium*, then it has to be included in the Spirotricha (if we use the replication band to limit the group). Molecular data about *Phacodinium* are rather contradictory. According to Shin et al. (2000), it clusters between the oligotrichs and the euplotids. Bernhard et al. (2001), Petroni et al. (2002), and Johnson et al. (2004) found that it is the sistergroup of the unit formed by the three major taxa of the spirotrichs, and according to Strüder-Kypke & Lynn (2003) it is the sistergroup of the euplotids. By contrast, *Protocruzia* with its highly interesting nuclear apparatus (Ammermann 1968) is the adelphotaxon to the unit formed by all taxa mentioned above in all studies using small subunit rRNA gene sequences (Shin et al. 2000, Bernhard et al. 2001, Petroni et al. 2002). However, phylogeny derived from histone H4 analyses shows a quite different position for *Protocruzia* (Bernhard & Schlegel 1998).

[3] The fact that the halterids are assigned either to the oligotrichs (morphologists; e.g., Foissner et al. 1999, Lynn & Small 2002) or to the hypotrichs near *Oxytricha* (molecular biologists; e.g., Strüder-Kypke & Lynn 2003, Dalby & Prescott 2004, Adl et al. 2005) has no influence on the monophyly of the oligotrichs. In the first case the halterids are a subgroup of the oligotrichs, in the second the halterids are a subgroup of the oxytrichids. For a discussion of the "*Halteria*-problem" see Foissner et al. (2004a).

Interestingly, some molecular trees indicate that the euplotids (e.g., *Euplotes*, *Uronychia*, *Diophrys*) do not

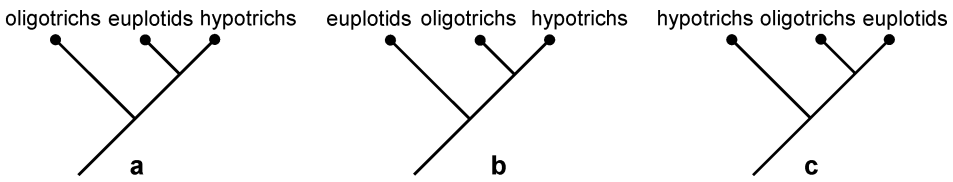


Fig. 12a–c The three possibilities to arrange the three major taxa of the spirotrichs (original). *Protocruzia* and *Phacodinium*, each composed of only one or very few species, are not considered. For explanation see text.

and if the assumptions just mentioned are correct, only one of the trees reflects the truth. However, each of these three trees has potential, depending on the features used. The arrangement shown in Fig. 12a is mainly suggested by morphologists, with the presence of cirri as main apomorphy for the unit euplotids + hypotrichs (e.g., Petz & Foissner 1992, Agatha 2004). By contrast, many molecular studies indicate that the oligotrichs and the hypotrichs are adelphotaxa (e.g., Fleury et al. 1995, Shin et al. 2000, Bernhard et al. 2001, Petroni et al. 2002, Strüder-Kypke & Lynn 2003, Agatha et al. 2004, Johnson et al. 2004; Fig. 12b). Less often, molecular data suggest a common ancestor for euplotids and oligotrichs (e.g., Snoeyenbos-West et al. 2004, Foissner et al. 2004a; Fig. 12c, 15). To decide which of the three hypothesis is correct, further data (e.g., fate of the somatic ciliature in cysts, different molecular markers) on more species are likely needed.

Although it is rather a nomenclatural than a taxonomic problem, the naming of the spirotrich taxa has to be discussed. For a long time (1859–1985) the name Hypotricha (or its derived forms Hypotrichea, Hypotrichia, Hypotrichida, Hypotrichina, depending on the category assigned; for review see Berger 2001) was used in a rather uniform way, that is, for a group comprising the euplotids, the oxytrichids, the urostyloids, etc. Based on features of the dorsal somatic kinetids, Small & Lynn (1985) suggested that the hypotrichs be divided between the Postciliodesmatophora and the Cyrtophorea. Their monotypic and therefore redundant subclass Hypotrichia contained the order Euplotida. The (non-euplotid) hypotrichs were assigned to the likewise monotypic (and therefore redundant) subclass Stichotrichia Small & Lynn, 1985 with the order Stichotrichina Fauré-Fremiet, 1961 (Table 1).

Lynn & Sogin (1988) analysed the 16S-like ribosomal RNA and found that the classification of euplotids and stichotrichids in different higher taxa proposed by Small & Lynn (1985) was incorrect (for review see Lynn 1991). However, they retained Small & Lynn's subclasses Hypotrichia and Stichotrichia and suggested the introduction of the class Hypotrichea to include the Hypotrichia (in their sense), the Stichotrichia, and the Oligotrichia. However, for this group the name Spirotricha (originally incorrectly

form a monophyletic group (e.g., Baroin-Tourancheau et al. 1992, Chen & Song 2002, Lynn 2003). Moreover, *Prodiscocephalus* and related taxa very likely do not belong to the Euplota as suggested by Lynn & Small (2002), but to the hypotrichs, as, inter alia, indicated by the presence of two undulating membranes (Lin et al. 2004).

Table 1 Comparison of names of some higher spirotrich taxa used in four recent books and in the present review

Present book [a]	Corliss 1979	Small & Lynn 1985	Tuffrau & Fleury 1994	Lynn & Small 2002
Spirotricha Bütschli, 1889 (spirotrichs)	Polyhymenophora Jankowski, 1967 [b]	Spirotrichea Bütschli, 1889	Spirotricha Bütschli, 1889	Spirotrichea Bütschli, 1889
Euplota Ehrenberg, 1830 (euplotids)	Euplotidae Ehrenberg, 1838; Aspidiscidae Ehrenberg, 1838; Gastrocirrhidae Fauré-Fremiet, 1961	Hypotrichia Stein, 1859 [d]	Euplotia author? [f]	Hypotrichia Stein, 1859
Oligotricha Bütschli, 1889 (oligotrichs)	Oligotrichida Bütschli, 1889	Choreotrichia n. subclass.	Oligotrichea Bütschli, 1887 [g]	Choreotrichia Small & Lynn, 1985; Oligotrichia Bütschli, 1889
Hypotricha Stein, 1859 (hypotrichs)	Hypotrichida Stein, 1859 [c]	Stichotrichia n. subclass. [e]	Oxytrichia author? [f]	Stichotrichia Small & Lynn, 1985
Urostyloidea Bütschli, 1889 (urostyloids)	Urostylidae Bütschli, 1889; Holostichidae Fauré-Fremiet, 1961	Urostylina Jankowski, 1979	Urostylida Jankowski, 1979	Urostylida Jankowski, 1979

[a] Note that in the present book the names are usually used as originally introduced (see Berger 2001). Vernacular names in brackets.

[b] With the single subgroup Spirotricha Bütschli, 1889 (for a brief discussion of the redundancy of the name Polyhymenophora, see Berger & Foissner 1992).

[c] Also includes the Euplotidae, the Aspidiscidae, and the Gastrocirrhidae.

[d] With the single subgroup Euplotida n. ord.

[e] With the single subgroup Stichotrichida Fauré-Fremiet, 1961.

[f] According to Berger (2001) the authors of this subclass are Tuffrau & Fleury (1994).

[g] Incorrect year.

also including the heterotrichs and peritrichs) was introduced by Bütschli (1889, p. 1719), a name now generally used for the monophylum formed by the oligotrichs, the euplotids, the hypotrichs, *Phacodinium*, and *Protocruzia* (e.g., Foissner et al. 1999, Lynn & Small 2002, Hausmann et al. 2003, Strüder-Kypke & Lynn 2003; Fig. 13a). By contrast, the name Hypotricha (or one of its derived forms) is not used uniformly since the publication of Small & Lynn's (1985) paper, that is, either only for the euplotids (mainly by molecular biologists) or non-euplotid hypotrichs (mainly morphologists; e.g., Lemullois et al. 2004).

The ordername Stichotrichina was introduced by Fauré-Fremiet (1961) for hypotrichs with a frontoventral ciliature mainly composed of distinct cirral rows (e.g., *Urostyla*, *Holosticha*, *Strongylidium*). Simultaneously, Faurè-Fremiet (1961) introduced the

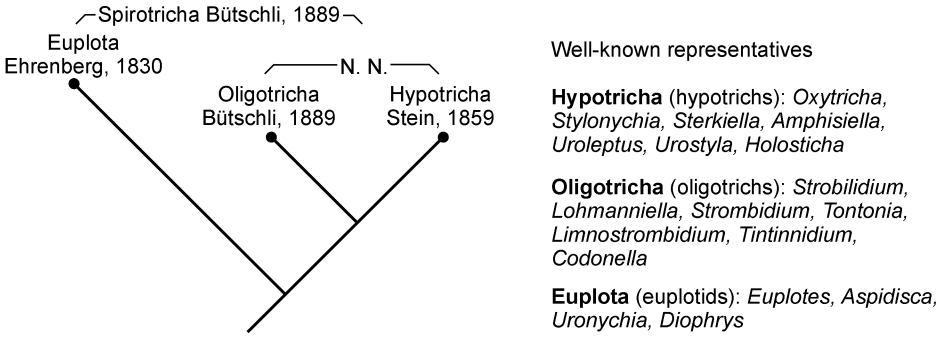


Fig. 13a Names of the three major taxa of the Spirotricha as used in the present book (original). Note that (i) I usually use the same spellings as in the original descriptions, and (ii) I do not use categories (e.g., family, order), simply because they do not exist in nature. N. N. = Nomen nominandum. This term was introduced by Ax (1999, p. 18) for a supposed monophylum, which is not yet well founded. In the present case several morphological features indicate that the euplotids, and not the oligotrichs, are the sister group of the hypotrichs (Fig. 12a). Further details see text.

suborder Sporadotrichina for taxa with "sporadically" distributed frontoventral cirri originating from six anlagen (e.g., *Steinia*, *Gastrostyla*, *Euplotes*). Thus, the name Stichotrichia – although established as new category by Small & Lynn (1985) – for all non-euplotid hypotrichs is somewhat misleading. I do not use the name Stichotrichina (or one of its derived forms) because there are enough older names available to handle the situation (Fig. 14a). However, a discussion about this topic is difficult because the nomenclature above the "familylevel" is not regulated by the ICZN.[1]

2.2 The Hypotricha Stein, 1859

The name Hypotricha was introduced by Stein (1859, p. 72, 73) to include the "Oxytrichinen Ehrenberg" (e.g., *Oxytricha*, *Stylonychia*, *Urostyla*, *Holosticha*, *Uroleptus*), the "Euplotinen Ehrenberg" (e.g., *Euplotes*), the "Aspidiscinen Ehrenberg" (e.g., *Aspidisca*), and the "Chlamydodonten Stein" (e.g., *Chlamydodon*, *Trochilia*). The misclassification of the chlamydodonts in the hypotrichs was recognised very early and therefore the Hypotricha consisted of the oxytrichids, the euplotids, and the aspidiscids over a long period (e.g., Kahl 1932, Corliss 1961). In the present book I confine the name Hypotricha to the non-euplotid hypotrichs because there is some evidence, especially from molecular data, that not the euplotids, but the oligotrichs are the sistergroup of the hy-

[1] The examples hypotrichs and stichotrichs show very impressively how different the authorship of higher taxa is handled. Stein (1859) established the Hypotricha as order, and no author dared to add his own name when he lowered or raised the rank (see, for example, Small & Lynn 1985, Lynn & Small 2002, and Tuffrau & Fleury 1994 for classifications including authorships). In the case of the suborder Stichotrichina Fauré-Fremiet, 1961 the situation is different. Small & Lynn (1985) introduced the monotypic subclass Stichotrichia, which is now generally assigned to Small & Lynn (1985).

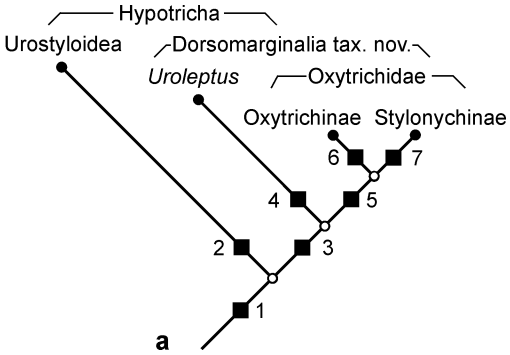


Fig. 14a Diagram of phylogenetic relationships within the Hypotricha (original). This arrangement is roughly supported by a tree based on rDNA (Hewitt et al. 2003), but also supports the CEUU-hypothesis proposed by Foissner et al. (2004a). For characterisation of the taxon Dorsomarginalia see chapter 2.4. For details see chapter 2 of the general section and ground pattern of the Urostyloidea (systematic section). Autapomorphies (black squares 1–7): **1** – oral primordium on cell surface; two macronuclear nodules; three dorsal kineties; 18 frontal-ventral-transverse cirri; somatic ciliature new; somatic ciliature largely lost in cyst (PKR-cyst; see chapter 1.10.3); endoral present (that is, two undulating membranes); one left and one right marginal row; high agreement in SSU rRNA gene sequences. **2** – more than six frontal-ventral-transverse cirral anlagen produce a distinct zigzag pattern of ventral cirri (that is, midventral complex composed of pairs only); more than five transverse cirri. **3** – dorsomarginal kineties present; micronuclear DNA polymerase alpha genes scrambled. **4** – more than 6 frontal-ventral-transverse cirri anlagen; body slender and tailed; actin I gene scrambling type *Uroleptus*. **5** – fragmentation of dorsal kinety 3; 4-layered cyst wall; actin I gene scrambling type *Oxytricha*; 2 or more macronuclear molecules encode histone H4. **6** – no apomorphy known, therefore likely a paraphyletic group. **7** – body rigid; adoral zone of membranelles ≥40% of body length; cortical granules lacking.

potrichs (Fig. 12b, 13a). Likely it would be more fair to use the older name Oxytrichina Ehrenberg, 1830 (or one of its derivatives; see Berger 2001) for this group instead of Hypotricha. However, at present it seems wise to retain the name Hypotricha and to use the name Oxytrichina, respectively its derived form Oxytrichidae, for a subgroup of the Hypotricha.

As just mentioned, the Hypotricha comprise all non-euplotid hypotrichs (Fig. 14a). Most morphologists agree that oxytrichids and urostyloids are two monophyletic lineages (e.g., Borror 1972, Corliss 1979, Borror & Wicklow 1983, Lynn & Small 1997, 2002, Shi et al. 1999). In contrast, Eigner (1997) proposed a non-monophyly of the oxytrichids, that is, he assumed that the characteristic "18 frontal-ventral-transverse cirral pattern" of this group and the specific ontogenetic processes producing this pattern evolved several times. However, the pattern and the ontogenesis are too complex to assume a convergent evolution. On the other hand, Eigner (2001) also supports a monophyly of the urostyloids. For further details on the this taxon see the next chapter.

The monophyly of the Oxytrichidae is, from the morphological point of view, mainly based on the fragmentation of dorsal kinety 3 (Fig. 14a; apomorphies 5).[1] The

[1] A fragmentation is also known from the "urostyloid" *Neokeronopsis spectabilis* (Fig. 243k, m). This indicates that *Neokeronopsis* is not a urostyloid, but an oxytrichid which convergently produced a zigzagging cirral pattern feigning a urostyloid origin.

18 frontal-ventral-transverse cirri, proposed as apomorphy by Berger & Foissner (1997) and Berger (1999), are likely a plesiomorphy at this level. Probably this pattern occurred for the first time in the last common ancestor of the Hypotricha (Fig. 14a, apomorphies 1). The dorsal kinety fragmentation is rather curious and therefore a convergent evolution is very unlikely. Of course, this pattern was transformed in various ways, for example, from simple to multiple fragmentation in *Pattersoniella* (for review see Berger 1999). Berger & Foissner (1997) proposed a rather distinct separation within the Oxytrichidae in the Oxytrichinae and the Stylonychinae. The Stylonychinae are characterised by at least three apomorphies, namely a rigid body, a long (more than 40% of body length) adoral zone of membranelles, and the lack of cortical granules (Fig. 14a, apomorphies 7). This group is supported by almost all molecular studies (e.g., Bernhard et al. 2001, Chen & Song 2002, Agatha et al. 2004, Foissner et al. 2004a), strongly indicating that the Stylonychinae are indeed a monophylum. Berger (1999) recognised from morphological data that *Pattersoniella*, *Laurentiella*, and *Onychodromus* are also members of the Stylonychinae. This was confirmed by molecular data some years later (Bernhard et al. 2001, Foissner et al. 2004a).

In contrast to the Stylonychinae, the Oxytrichinae are less well defined, both from the morphological and molecular point of view, strongly indicating the Oxytrichinae sensu Berger & Foissner (1997) and Berger (1999) are paraphyletic (Schmidt et al. 2004a, b). Various molecular markers indicate that almost all hypotrich species which do not belong to the urostyloids, to *Uroleptus*, or to the Stylonychinae, cluster somewhere inside the oxytrichids. This means that taxa like *Amphisiella* (dorsomarginal kineties and fragmenting dorsal kinety lacking; Wicklow 1982, Berger 2004a), *Kahliella* and *Parakahliella* (dorsomarginal kineties present, kinety fragmentation lacking; Berger et al. 1985, Berger & Foissner 1989b, Eigner 1995), *Engelmanniella* (dorsomarginal kineties and kinety fragmentation lacking; Wirnsberger-Aescht et al. 1989), *Paraurostyla* (dorsomarginal kineties and kinety fragmentation present; Wirnsberger et al. 1985, for review see Berger 1999) are more or less strongly modified 18-cirri oxytrichids. For example, the cirral pattern of *Amphisiella* species can be rather easily derived from the pattern of 18-cirri oxytrichids by a more or less distinct increase of cirri produced per anlage (Berger 2004a). However, since we do not know whether the lack of dorsomarginal kineties in *Amphisiella* is primary or secondary, we cannot estimate its position in the phylogenetic system; that is, we do not know whether or not it belongs to the Dorsomarginalia (Fig. 14a). Unfortunately, the position of other "difficult" taxa (e.g., *Engelmanniella*, *Gonostomum*) is rather different in various molecular trees.

2.3 The Urostyloidea Bütschli, 1889

The separation of the urostyloids from the oxytrichids dates back to Bütschli (1889), who established it as subfamily of the Oxytrichina family. Originally, the Urostylinae included *Trichogaster*, *Urostyla*, *Kerona*, *Epiclintes*, *Stichotricha*, *Strongylidium*, *Holosticha*, *Amphisia*, *Uroleptus*, and *Sparotricha*. Simultaneously, Bütschli established the

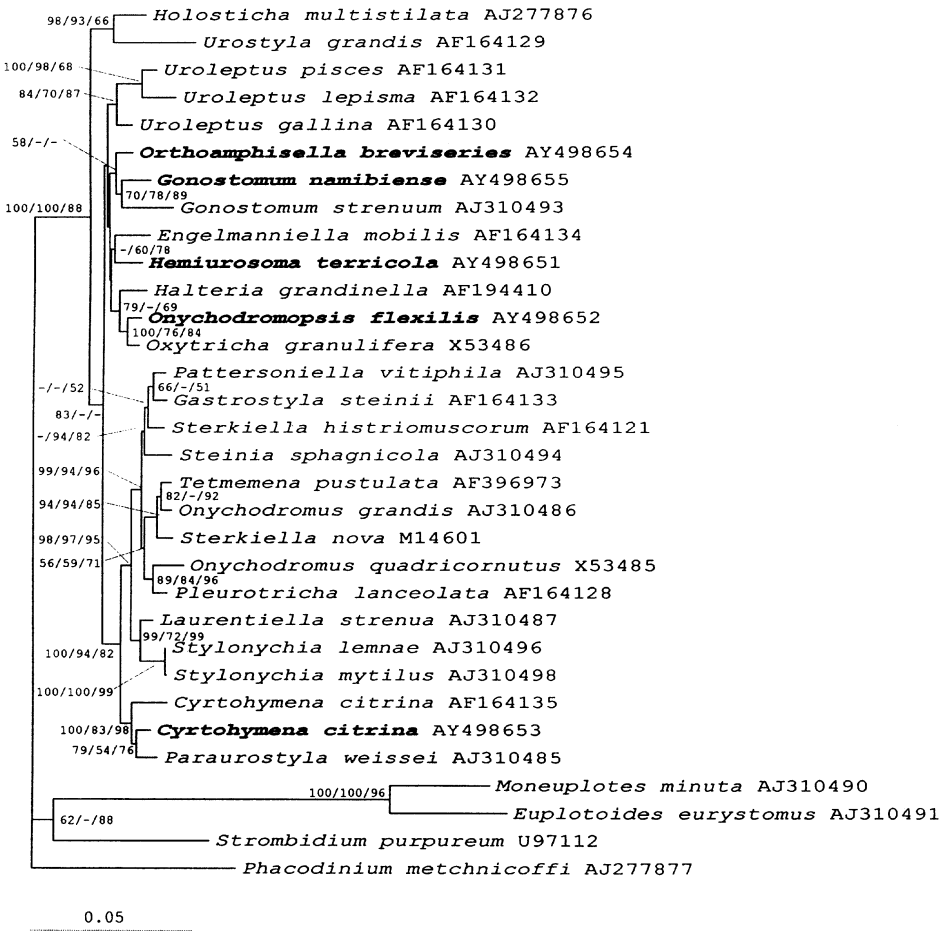


Fig. 15a Neighbour-joining tree of spirotrichs (mainly urostyloid and oxytrichid hypotrichs) based in 18S rRNA gene sequences (from Foissner et al. 2004a). The codes following the names are the GenBank Accession Numbers. The numbers at nodes represent the neighbour-joining and maximum-parsimony bootstrap percentages from 100 replicates (values below 50% not shown) and the quartet puzzle support values obtained with 10000 puzzling steps, respectively. The scale bar corresponds to a distance of 5 substitutions per 100 nucleotide positions. For details see text. *Holosticha multistilata* = *Anteholosticha intermedia* in present book.

subfamilies Pleurotrichina (e.g., *Pleurotricha*, *Oxytricha*, *Histrio*) and Psilotrichina (*Psilotricha* and *Balladina*).

Kahl (1932), the next revisor, ignored Bütschli's Urostylinae and again divided the hypotrichs in the Oxytrichidae, the Euplotidae, and the Aspidiscidae, that is, he included all non-euplotid hypotrichs in the Oxytrichidae without further division. Corliss (1961) accepted Kahl's scheme. By contrast, Faurè-Fremiet (1961) divided the hypotrichs into two new taxa, the Stichotrichina and the Sporadotrichina. The first group comprised the Urostylidae, which he incorrectly assigned to Calkins, the Keronidae, the

Holostichidae, and the Strongylidae. The Sporadotrichina contained the Pleurotrichidae, the Euplotidae, the Gastrocirrhidae, and the Aspidiscidae. We now know that Fauré-Fremiet's classification was rather artificial.

Based on the observations on *Pseudourostyla cristata* by Jerka-Dziadosz (1964) and own data, Borror (1972) refined terminology by introducing the term midventral cirri (see chapter 1.7 for details), which are paired and form a highly characteristic zigzag pattern (Fig. 1a). He also recognised the importance of the special origin of this pattern from rather many oblique cirral anlagen. Within the hypotrichs (euplotids and non-euplotids) he distinguished six families including the Urostylidae and the Holostichidae (Table 3). Some years later, he came to the conclusion that the Holostichidae are a junior synonym of the Urostylidae because both groups are characterised by midventral cirri (Borror 1978, 1979).

Borror & Wicklow (1983) made the last revision of the urostyloids. They provided a key to all species and list of synonyms, but neither detailed discussions nor descriptions. They introduced the Pseudokeronopsidae comprising the two subgroups Pseudokeronopsinae and Thigmokeronopsinae (Table 8).

The Urostyloidea are a large group of hypotrichs; however, their phylogenetic position in the hypotrichs is not yet certain. Molecular data about the origin of the urostyloids are rather contradictory. In some trees they are classified within the 18-cirri oxytrichids (e.g., Shin et al. 2000, Bernhard et al. 2001, Snoeyenbos-West et al. 2002 [their Fig. 2], Strüder-Kypke & Lynn 2003), in others they cluster outside the oxytrichids (e.g., Lozupone et al. 2001, Snoeyenbos-West et al. 2002 [their Fig. 1], Hewitt et al. 2003, Croft et al. 2003, Foissner et al. 2004a, Dalby & Prescott 2004, Coleman 2005; Fig. 15a).

The neighbour-joining and parsimony analyses by Foissner et al. (2004a) support the morphological and morphogenetical data on the monophyly of the *Urostyla/Anteholosticha* clade, which is the sister group of all other hypotrichs (Fig. 15a). However, the three *Uroleptus* species do not cluster with the urostyloids, but with the oxytrichids, although they look like typical urostyloids differing from *Anteholosticha* only in body shape (tailed vs. untailed) and the presence/absence of dorsomarginal kineties (Borror 1972, Martin et al. 1981, Hemberger 1982, Borror & Wicklow 1983, Eigner 2001). A similar result was obtained by Dalby & Prescott (2004) using *Urostyla grandis* and *Holosticha polystylata* (= *Diaxonella pseudorubra* in the present book) as representatives of the urostyloids. This suggests that *Uroleptus*-species are not urostyloids, a hypothesis supported by the rather similar trees by Snoeyenbos-West et al. (2002) and Hewitt et al. (2003). In contrast, the trees by Shin et al. (2000), Bernhard et al. (2001), and Chen & Song (2002) cluster "*Holosticha*" (= *Anteholosticha* in present book) very near to *Oxytricha granulifera*. Likely, this is because they did not include *Urostyla*. This indicates that such profound differences in treestructures are caused by insufficient taxa sampling. Sequences are available from only about 40 of the more than 300 known oxytrichids (Berger 1999) and urostyloids (present book). Further, more than 50% of the ciliate species are probably undescribed (for review see Foissner et al. 2002). Accordingly, molecular trees contain less than 5% of the hypotrich species that probably exist. Nonetheless, differences in alignment, outgroup, phylogenetic algorithm, and

clustering method may also contribute to the differences in the molecular trees available. Lastly, the low sequence divergence among the hypotrichs hampers phylogenetic analysis and makes SSU-rRNA gene analyses very sensitive to undersampling (Foissner et al. 2004a). However, the morphological situation is not much better because the many conflicting features prevent the construction of a convincing tree.

For some comments on the phylogenetic relationships within the Urostyloidea see the systematic section.

2.4 Is *Uroleptus* a Subgroup of the Urostyloidea?

Uroleptus is generally assigned to the urostylids because it has – like, for example, *Holosticha* or *Urostyla* – zigzagging ventral cirri (e.g., Bütschli 1889, Borror 1972, Borror & Wicklow 1983, Tables 2–8, 10). Only rarely is it assigned to other higher taxa, for example, the Kahliellidae (Tuffrau & Fleury 1994; p. 137).

However, several molecular studies suggest that the inclusion of *Uroleptus* in the urostyloids is incorrect (Snoeyenbos-West et al. 2002, Hewitt et al. 2003, Croft et al. 2003; Foissner et al. 2004a, Fig. 15a; Dalby & Prescott 2004). According to these molecular data, *Uroleptus* is more closely related to the oxytrichids than to the urostyloids. This requires the assumption that the conspicuous zigzag-pattern formed by the ventral cirri evolved convergently in the Urostyloidea and in *Uroleptus* (if we assume that the last common ancestor of the Hypotricha had six frontal-ventral-transverse cirral anlagen; see below). Thus, we proposed the CEUU (*C*onvergent *E*volution of *U*rostylids and *U*roleptids) hypothesis, which tries to combine morphological and molecular data (Foissner et al. 2004a). Traditionally, 18-cirri[1] oxytrichids and euplotids – that is, spirotrichs with relatively few and "sporadically" arranged cirri – are regarded as derived from a *Urostyla*-like ancestor which had many cirral rows (e.g., Kahl 1932, Borror 1972, Wirnsberger 1987). The CEUU hypothesis, however, tries to explain the opposite; that is, an euplotid-like ancestor because the euplotids have an "*Oxytricha*-like" cirral pattern originating from six anlagen (or vice versa; Wallengren 1900) and are the adelphotaxon of the group formed by the oligotrichs and the hypotrichs in many molecular trees (Fig. 12b; e.g., Eisler et al. 1995, Shin et al. 2000, Bernhard et al. 2001, Petroni et al. 2002, Modeo et al. 2003, Strüder-Kypke & Lynn 2003). Moreover, the hypothesis proposes that cirri- and anlagen-multiplication are not necessarily correlated with the production of a midventral complex and occurred several times in the Oxytrichidae (e.g., *Paraurostyla*, *Laurentiella*, *Styxophrya*), including midventral complex-like arrangements as, for example, in *Pattersoniella* and *Territricha* (for review see Berger 1999). Indeed, *Pattersoniella* and *Territricha* have been united in the Pattersoniellidae and included in the urostylids by Shi et al. (1999; Table 10). However, detailed morphological and molecular studies clearly show that *Pattersoniella* and *Territricha* belong to the Oxytrichidae (see above; Berger 1999, Bernhard et al. 2001, Foissner et al. 2004a).

[1] The term "18-cirri oxytrichids" is an abbreviation for oxytrichids with 18 frontal-ventral-transverse cirri arranged in the highly characteristic pattern discussed in detail by Berger (1999).

Fig. 16 shows the proposed homology of the euplotid, urostyloid, and oxytrichid cirri. The homology of the euplotid and oxytrichid cirral pattern was already explained in detail by Wallengren (1900). If we assume that the last common ancestor of the euplotids and hypotrichs (Fig. 12a), respectively, the spirotrichs (Fig. 12b), had six cirral anlagen then we have to assume that the urostyloid midventral cirral pattern originated by inserting additional anlagen, each producing a pair of cirri, among the basic six anlagen (I–VI). If the tree shown in Fig. 14a is basically correct then this process must have occurred at least twice. The first separation process caused the common ancestor to split in a urostyloid and oxytrichid (including *Uroleptus*) lineage. Later, a similar zigzag pattern evolved again either outside (Fig. 14a) or within the Oxytrichidae (Foissner et al. 2004a) to form the *Uroleptus* lineage. Although cirri and anlagen multiplication are obviously not correlated with the generation of a midventral pattern, the scenario above is quite likely, considering the many cirral patterns found in the oxytrichids (for review see Berger 1999). Possibly, the second separation event was driven by ecological constraints because all *Uroleptus* species are, per definition, more or less distinctly tailed (Foissner et al. 2004a).

Although the CEUU hypothesis is reasonable and in accordance with several molecular trees as well as with the high diversity of the oxytrichid cirral pattern in general, Foissner et al. (2004a) did not have a specific morphological proof. The tree proposed in Fig. 14a is basically in accordance with the molecular tree presented by Hewitt et al. (2003)[1], especially in that *Uroleptus* clusters outside the oxytrichids. For this hypothesis the morphology can provide a very good apomorphy for the group *Uroleptus* + Oxytrichidae (Fig. 14a, apomorphies 3), namely, the presence of dorsomarginal kineties. These kineties, which are never associated with caudal cirri, originate from/very near the right marginal row primordium and occur only in *Uroleptus* species (e.g., Martin et al. 1981, Foissner et al. 1991, Eigner 2001), very many oxytrichids (for review see Berger 1999; Fig. 243j, l, m), and some other taxa, for example, *Kahliella*, *Parakahliella, Nudiamphisiella* (Berger et al. 1985, Eigner 1995, Foissner et al. 2002). This feature is rather conspicuous and therefore has to be interpreted as synapomorphy implying that *Uroleptus* is more closely related to the oxytrichids than to the urostyloids.[2] On the other hand, fragmentation of dorsal kineties is lacking in *Uroleptus*, which is a distinct hint that it splits off outside the Oxytrichidae. The close relationship of *Uroleptus* and the Oxytrichidae is not only indicated by the dorsomarginal row, but also by molecular features. Thus, the monophylum composed of *Uroleptus* and Oxytrichidae is rather certain and therefore named Dorsomarginalia taxon novum[3]: Hypotricha with dorsomarginal kineties and scrambled micronuclear DNA polymerase alpha genes (Fig. 14a, apomorphies 3). For a discussion of the features, see the ground pattern chapter of the Urostyloidea. *Amphisiella* and some other taxa lack a midventral pattern and dorsomarginal

[1] Unfortunately, the three *Uroleptus* species used in this paper do not form a monophylum.

[2] Hemberger (1982, p. 89) described, but did not illustrate (!), the de novo origin of a dorsal kinety beside the right marginal row in *Holosticha pullaster*. Whether or not this feature is homologous to the dorsomarginal kineties is not known.

[3] The name refers to the main (sole?) morphological autapomorphy of this group, the dorsomarginal kineties, which are formed at/near the right marginal row primordium.

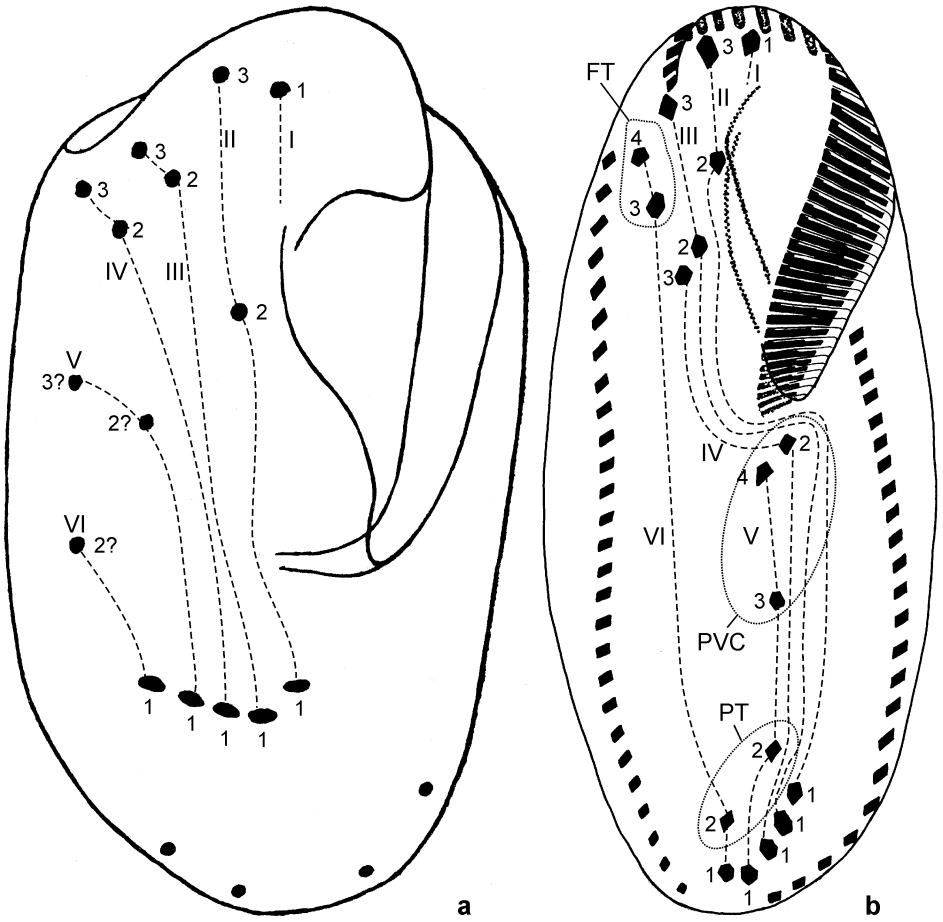


Fig. 16a, b Homology of cirri in a euplotid (a, *Euplotes harpa*; from Wallengren 1900) and an 18-cirri oxytrichid (b, *Sterkiella histriomuscorum*; from Augustin & Foissner 1992), as representative of the hypotrichs (further hypotrichs see Fig. 16c–o). Numbering of frontal-ventral-transverse cirral anlagen (I–VI) and cirri (1–4) according to Wallengren (1900). Cirri originating from the same anlage are connected by a broken line. The rearmost cirrus (1) of each anlage is the so-called transverse cirrus; these cirri form the transverse cirral row which is a pseudorow. The main difference between *Euplotes* and *Sterkiella* is the different number of cirri formed by the anlagen V and VI: *Euplotes* forms only three, respectively, two cirri, whereas *Sterkiella* produces each four cirri. Unfortunately, we are unable to say which cirri are lacking in *Euplotes*, respectively, supernumerary in *Sterkiella*; thus the question marks in *Euplotes*. Anyhow, the similarities between the cirral patterns and their origin in these two representatives are too high to be explained by chance, that is, we have to assume that the last common ancestor of the euplotids and hypotrichs produced a relatively low number of distinct cirri from six (I–VI) anlagen. Thus, six frontal-ventral-transverse cirri anlagen is obviously a plesiomorphy in the stem-lineage of the Hypotricha (Fig. 14a). FT = frontoterminal cirri, PT = pretransverse ventral cirri, PVC = postoral ventral cirri, I–VI = frontal-midventral-transverse cirral anlagen (primordia, streaks), 1–4 = cirri formed within an anlage (the rearmost cirrus has the number 1).

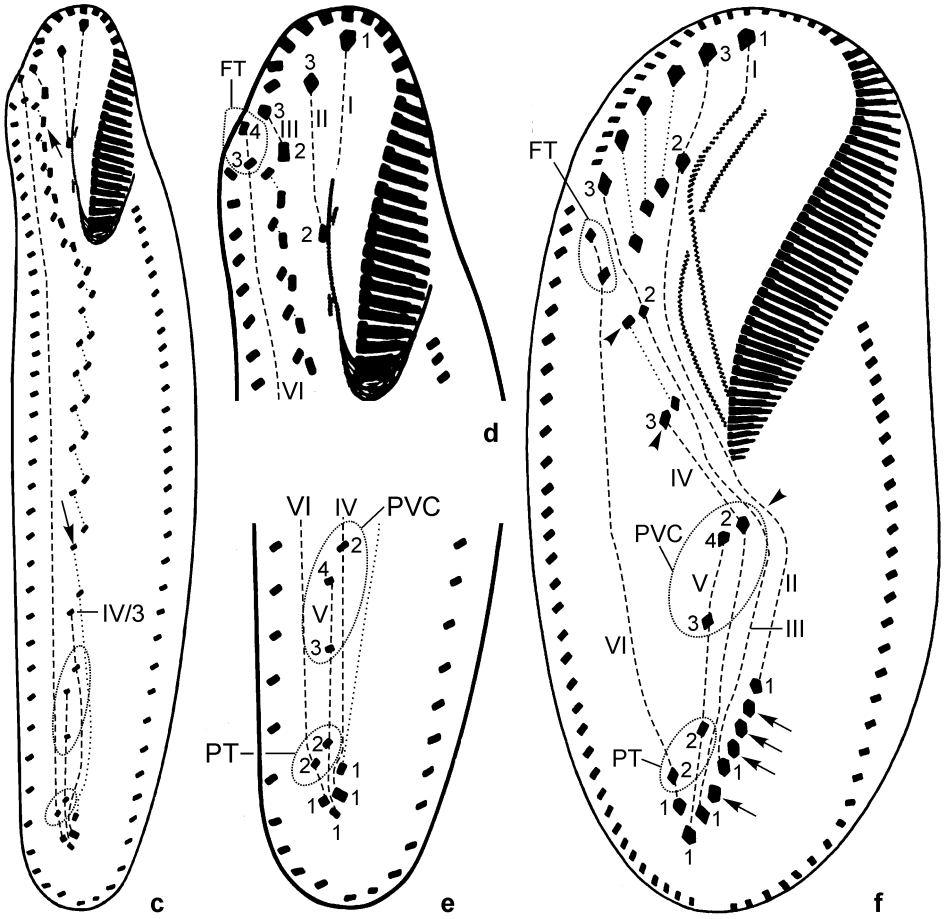


Fig. 16c–f Homology of cirri in a urostyloid (c–e; *Anteholosticha australis*) and a stylonychine (f; *Pattersoniella vitiphila*, from Foissner 1987b) hypotrich (after Foissner et al. 2004a, supplemented; see also Fig. 16a, b, g–o). Urostyloid hypotrichs likely evolved from an 18-cirri ancestor by inserting additional anlagen generating cirral pairs, which produce the highly characteristic zigzagging midventral pattern (first and last additional cirral pair marked by an arrow each in (c). Cirri of each additional pair connected by dotted line; for zigzag-pattern see Fig. 16l). Numbering of frontal-ventral-transverse cirral anlagen (I–VI) and cirri (1–4), which are homologous to those in euplotids and 18-cirri oxytrichids (Fig. 16a, b) according to Wallengren (1900). Note that the insertion of additional anlagen did not occur only in the urostyloids, but also in oxytrichids as indicated in (f) which shows *Pattersoniella*, a stylonychine oxytrichid "feigning" a bicorona and a urostyloid midventral pattern (pseudopairs marked by arrowheads). Arrows denote the additional cirral anlagen. FT = frontoterminal cirri, PT = pretransverse ventral cirri, PVC = postoral ventral cirri (in urostyloids distinctly dislocated posteriorly), I–VI = frontal-midventral-transverse cirral anlagen (primordia, streaks), 1–4 = cirri formed within an anlage (the rearmost cirrus has the number 1).

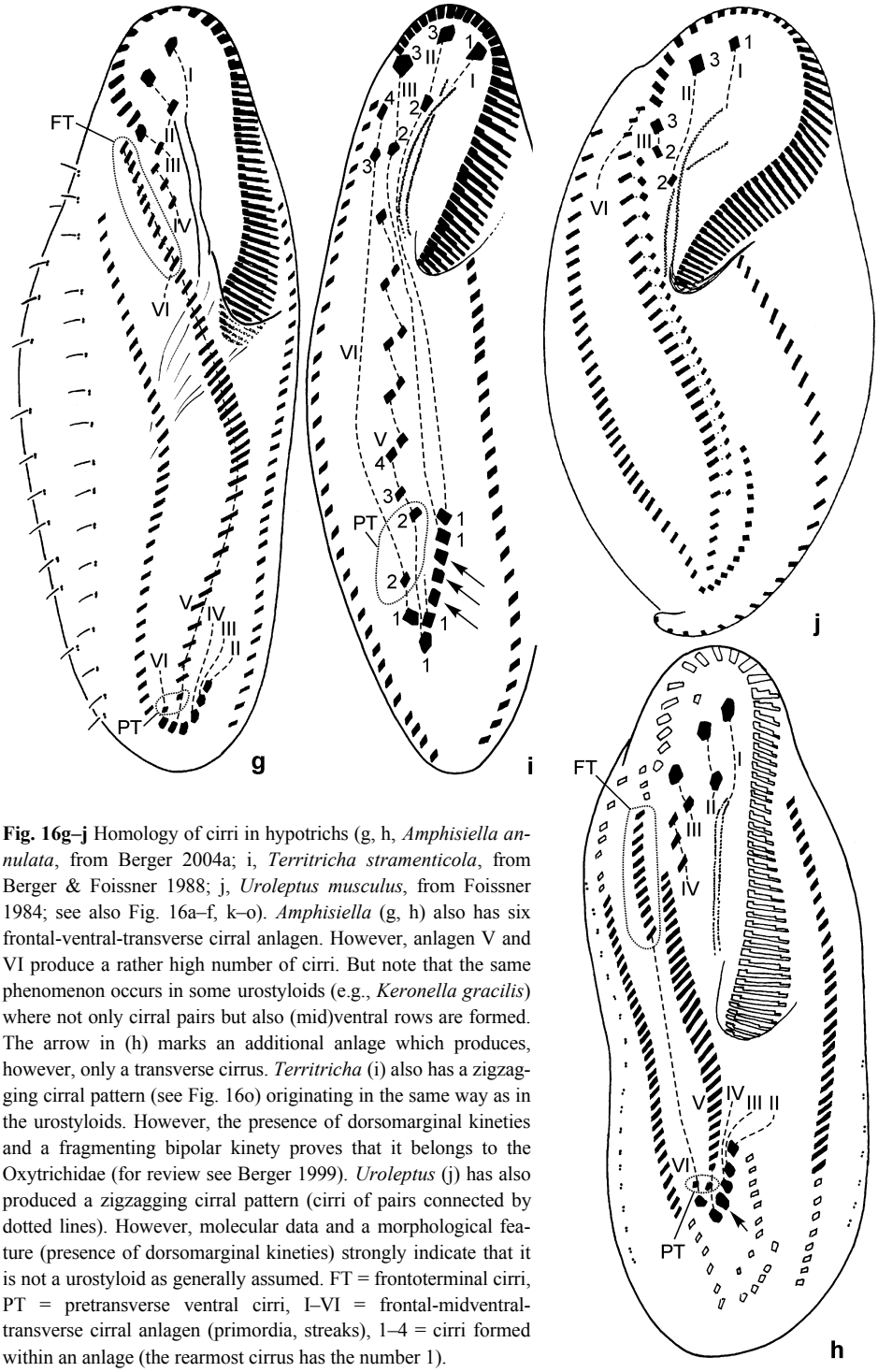


Fig. 16g–j Homology of cirri in hypotrichs (g, h, *Amphisiella annulata*, from Berger 2004a; i, *Territricha stramenticola*, from Berger & Foissner 1988; j, *Uroleptus musculus*, from Foissner 1984; see also Fig. 16a–f, k–o). *Amphisiella* (g, h) also has six frontal-ventral-transverse cirral anlagen. However, anlagen V and VI produce a rather high number of cirri. But note that the same phenomenon occurs in some urostyloids (e.g., *Keronella gracilis*) where not only cirral pairs but also (mid)ventral rows are formed. The arrow in (h) marks an additional anlage which produces, however, only a transverse cirrus. *Territricha* (i) also has a zigzagging cirral pattern (see Fig. 16o) originating in the same way as in the urostyloids. However, the presence of dorsomarginal kineties and a fragmenting bipolar kinety proves that it belongs to the Oxytrichidae (for review see Berger 1999). *Uroleptus* (j) has also produced a zigzagging cirral pattern (cirri of pairs connected by dotted lines). However, molecular data and a morphological feature (presence of dorsomarginal kineties) strongly indicate that it is not a urostyloid as generally assumed. FT = frontoterminal cirri, PT = pretransverse ventral cirri, I–VI = frontal-midventral-transverse cirral anlagen (primordia, streaks), 1–4 = cirri formed within an anlage (the rearmost cirrus has the number 1).

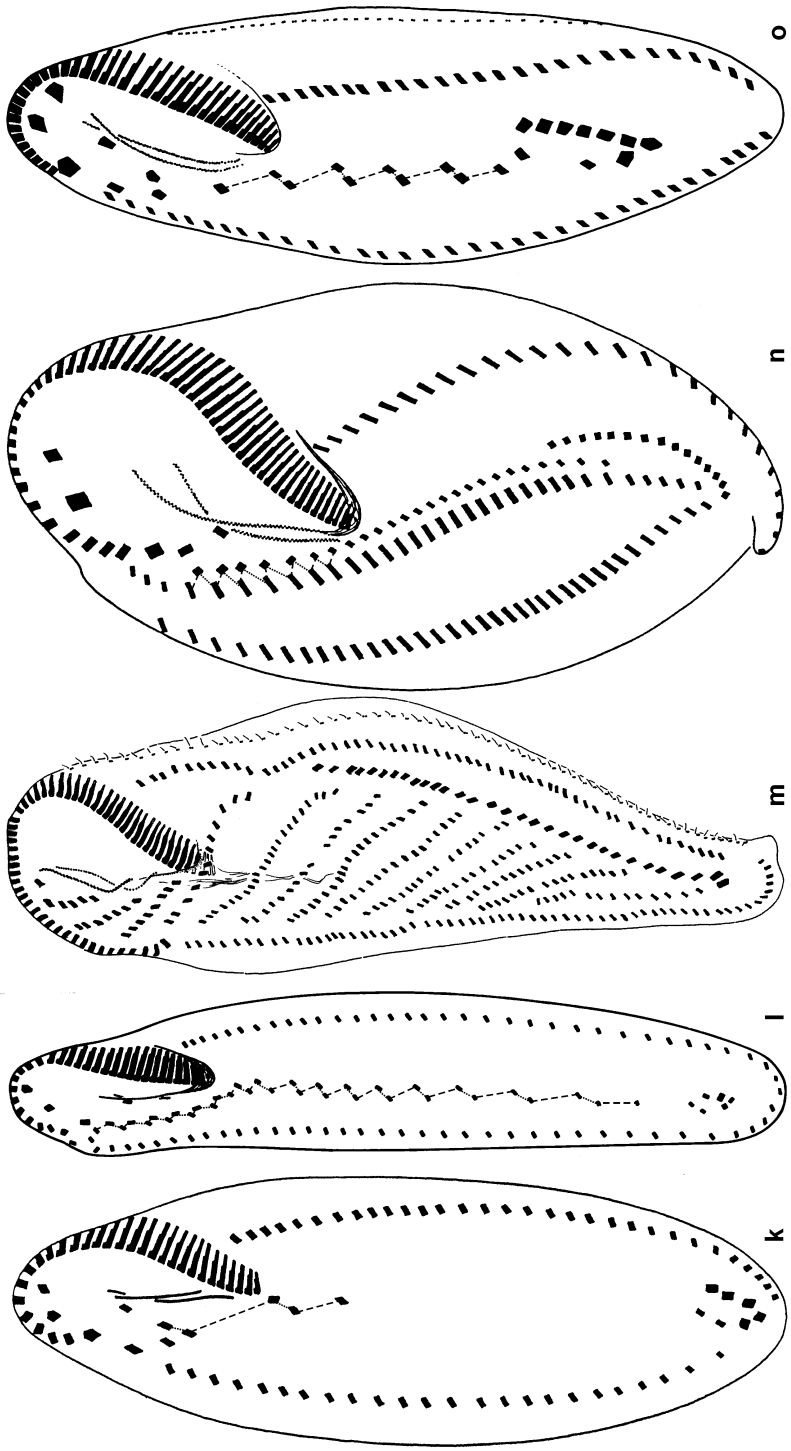


Fig. 16k–o "Zigzag" pattern in *Oxytricha lanceolata* (k; from Foissner 1996a), *Anteholosticha australis* (l), *Uroleptus musculus* (n, from Foissner 1984), and *Territricha stramenticola* (o, from Berger & Foissner 1988; see also Fig. 16a–j). Cirri of a true pair are connected by a broken line, cirri of a pseudopair (see Fig. 1a) are connected by a dotted line. The higher the number of cirral pairs, the better becomes recognisable the zigzag pattern. *Epiclintes auricularis* (m), a urostyloid, has lost the characteristic zigzagging midventral pattern because the anlagen do not produce cirral pairs, but cirral rows.

kineties (Wicklow 1982, Berger 2004a). Molecular data are needed to understand whether dorsomarginal kineties are primarily or secondarily lacking in these groups.

Kelminson et al. (2002) found that in *Uroleptus* sp. only one macronuclear molecule encodes histone H4, whereas in oxytrichids (e.g., *Sterkiella*, *Stylonychia*, *Pleurotricha*, *Oxytricha*) two or more molecules encode histone H4. Harper & Jahn (1989) detected only a single histone H4 gene in *Moneuplotes crassus*, indicating that this is the plesiomorphic state within the spirotrichs. This would suggest that (i) *Uroleptus* split off outside the Oxytrichidae (Fig. 14a), and not inside as suggested by Foissner et al. (2004a), and (ii) the increased number of histone H4 encoding macronuclear molecules are an apomorphy of the Oxytrichidae. Possibly, the four-layered cyst wall is a further apomorphy of the Oxytrichidae (for review on cyst wall data, see Gutiérrez et al. 2003).

Anyhow, at the present state of knowledge there is strong evidence that *Uroleptus* is a distinct group more closely related to the Oxytrichidae than to the Urostyloidea. Thus, it is not treated in the present review.

3 Previous Classifications and Revisions

Several classifications of urostyloids exist. The original classification by Bütschli (1889) and some modern classification schemes are shown in Tables 2–11. I did not change the original presentation, for example, original spelling of names; moreover, authors are partially not included in my reference list. Kahl (1932) included all non-euplotid hypotrichs in the Oxytrichidae without further subdivision. Thus, his classification is not presented.

Kahl (1932) provided the last detailed revision of urostyloid taxa; that is, his paper included a key to all species and a description and illustration of each species. Later reviews contained either only a list of genera and species (e.g., Borror 1972), or descriptions (Hemberger 1982), or a key and list of synonyms (Borror & Wicklow 1983). Thus, it was not too early to review the data on the Urostyloidea thoroughly.

For a brief discussion of the various schemes presented below, see the systematic section. Several taxa (e.g., *Amphisiella*, *Gonostomum*, *Pattersoniella*, *Uroleptoides*) are not considered in the present book, although classified by some authors in the urostyloids or holostichids. For an explanation of the exclusion, see the chapter "Taxa not considered" at the end of the book.

Table 2 Classification of urostyloid ciliates according to Bütschli (1889)

Family Oxytrichina (Ehrbg) Stein, 1859
Subfamily Urostylinae Bütschli
Trichogaster Sterki, 1878
Urostyla Ehrenberg, 1830
Kerona Müller, 1786
Epiclintes Stein, 1862
Stichotricha Perty, 1849
Strongylidium Sterki, 1878

Table 2 Continued

Holosticha Wrzesniowski, 1877
Amphisia Sterki, 1876
Uroleptus Ehrenberg, 1831
Sparotricha Entz, 1879

Table 3 Classification of urostyloid ciliates according to Borror (1972)

Family Urostylidae Bütschli, 1889
Urostyla Ehrenberg, 1830
Amphisiella Gourret & Roeser, 1887
Balladyna Kowalewski, 1882
Banyulsella Dragesco, 1953
Epiclintes Stein, 1862
Kahliella Corliss, 1960
Kerona Ehrenberg, 1835
Lacazea Dragesco, 1960
Paraurostyla n. g.
Family Holostichidae Fauré-Fremiet, 1961
Holosticha Wrzesniowski, 1877
Keronopsis Penard, 1922
Paraholosticha Kahl, 1932
Pseudourostyla n. g.
Trichotaxis Stokes, 1891
Uroleptopsis Kahl, 1932
Uroleptus Ehrenberg, 1831

Table 4 Classification of urostyloid ciliates according to Borror (1979)

Family Urostylidae Bütschli, 1889
Urostyla Ehrenberg, 1830
Bakuella Agamaliev & Alekperov, 1976
Holosticha Wrzesniowski, 1877
Keronopsis Penard, 1922
Pseudourostyla Borror, 1972
Uroleptus Ehrenberg, 1831

Table 5 Classification of urostyloid ciliates according to Corliss (1979)

Family Urostylidae Bütschli, 1889
Banyulsella Dragesco, 1953
Hemicycliostyla Stokes, 1886
Isosticha Kiesselbach, 1936
Paraholosticha Kahl, 1932
Paraurostyla Borror, 1972
Urostyla Ehrenberg, 1838
Family Holostichidae Fauré-Fremiet, 1961
Amphisiella Gourret & Roeser, 1888
Bakuella Agamaliev & Alekperov, 1976
Balladyna Kowalewski, 1882
Balladynella Stiller, 1974

Table 5 Continued

Gonostomum Sterki, 1878
Holosticha Wrzesniowski, 1877
Keronopsis Penard, 1922
Lamtostyla Buitkamp, 1977
Laurentiella Dragesco & Njiné, 1971
Paruroleptus Kahl, 1932
Parurosoma von Gelei, 1954
Psammomitra Borror, 1972
Pseudourostyla Borror, 1972
Trachelochaeta Sramek-Husek, 1954
Trachelostyla Kahl, 1932
Trichotaxis Stokes, 1891
Uncinata Bullington, 1940
Uroleptoides Wenzel, 1953
Uroleptus Ehrenberg, 1831
Wallackia Foissner, 1977

Table 6 Classification of urostyloid ciliates according to Wicklow (1981)

Suborder Urostylina Jankowski, 1979
- Superfamily Urostyloidea Bütschli, 1889
  - Family Urostylidae Bütschli, 1889
    - Subfamily Holostichinae (n. subfam.)
      - *Holosticha*
      - *Bakuella*
      - *Uroleptus*
    - Subfamily Urostylinae (n. subfam.)
      - *Urostyla*
  - Family Keronopsidae Jankowski, 1979
    - Subfamily Keronopsinae (n. subfam.)
      - *Keronopsis*
    - Subfamily Thigmokeronopsinae (n. subfam.)
      - *Thigmokeronopsis*
- Superfamily Pseudourostyloidea (n. superfam.)
  - Family Pseudourostylidae Jankowski, 1979
    - *Pseudourostyla*

Table 7 Classification of urostyloid ciliates according to Hemberger (1982)

Family Urostylidae Bütschli, 1889
- *Urostyla* Ehrenberg, 1838
- *Bakuella* Agamaliev & Alekperov, 1976
- *Holosticha* Wrzesniowski, 1877
- *Periholosticha* n. gen.
- *Trichototaxis* Stokes, 1891
- *Uroleptopsis* Kahl, 1932
- *Uroleptus* Ehrenberg, 1831

Table 8 Classification of urostyloid ciliates according to Borror & Wicklow (1983)

Urostylina Jankowski, 1979
- 1. Urostyloidea Bütschli, 1889
  - 1. Urostylidae Bütschli, 1889
    - 1. Urostylinae Bütschli, 1889
      - 1. *Urostyla* Ehrenberg, 1838
    - 2. Holostichinae Fauré-Fremiet, 1961
      - 1. *Holosticha* Wrzesniowski, 1877
      - 2. *Bakuella* Agamaliev & Alekperov, 1976
      - 3. *Uroleptus* Ehrenberg, 1831
  - 2. Pseudokeronopsidae fam. nov.
    - 1. Pseudokeronopsinae subfam. nov.
      - 1. *Pseudokeronopsis* gen. nov.
    - 2. Thigmokeronopsinae Wicklow, 1981
      - 1. *Thigmokeronopsis* Wicklow, 1981
- 2. Pseudourostyloidea Jankowski, 1979
  - 1. Pseudourostylidae Jankowski, 1979
    - 1. *Pseudourostyla* Borror, 1972

Table 9 Classification of urostyloid ciliates according to Tuffrau & Fleury (1994)

Order Urostylida Jankowski, 1979
- Family Urostylidae Bütschli, 1889
  - *Bakuella* Agamaliev & Alekperov, 1976
  - *Isosticha* Kiesselbach, 1936
  - *Urostyla* Ehrenberg, 1830
- Family Pseudourostylidae Jankowski, 1979
  - *Pseudourostyla* Borror, 1972
- Family Holostichidae Fauré-Fremiet, 1961
  - *Holosticha* Wrzesniowski, 1877
  - *Paruroleptus* Kahl, 1932
  - *Periholosticha* Hemberger, 1982
  - *Trichotaxis* Stokes, 1891
- Family Pseudokeronopsidae Borror & Wicklow, 1983
  - *Keronella* Wiackowski, 1985
  - *Pseudokeronopsis* Borror & Wicklow, 1983
  - *Thigmokeronopsis* Wicklow, 1981
  - *Uroleptopsis* Kahl, 1932

Table 10 Classification of urostyloid ciliates according to Shi et al. (1999)

Suborder Urostylina Jankowski, 1979
- Family Urostylidae Bütschli, 1889
  - *Urostyla* Ehrenberg, 1830
  - *Metabakuella* Alekperov, 1989
  - *Pseudourostyla* Borror, 1972
  - *Metaurostylopsis* Song & Petz, in press
  - *Australothrix* Blatterer & Foissner, 1988
  - *Birojimia* Berger & Foissner, 1989
  - *Thigmokeronopsis* Wicklow, 1981
- Family Holostichidae Fauré-Fremiet, 1961
  - *Keronella* Wiackowski, 1985

Table 10 Continued

Bakuella Agamaliev & Alekperov, 1976
Parabakuella Song & Wilbert, 1987
Pseudokeronopsis Borror & Wicklow, 1983
Tricoronella Blatterer & Foissner, 1988
Holosticha Wrzesniowski, 1877
Uroleptus Ehrenberg, 1831
Periholosticha Hemberger, 1985
Notocephalus Petz et al., 1995
Family Pseudoamphisiellidae Song et al., 1997
Pseudoamphisiella Song, 1996
Family Pattersoniellidae Foissner, 1987
Pattersoniella Foissner, 1987
Territricha Berger & Foissner, 1988

Table 11 Classification of urostyloid ciliates according to Lynn & Small (2002)[a]

Order Urostylida Jankowski, 1979
Family Urostylidae Bütschli, 1889
Notocephalus Petz, Song & Wilbert, 1995
Eschaneustyla Stokes, 1886
Birojima Berger & Foissner, 1989
*Urostyla*Ehrenberg, 1830
Australothrix Blatterer & Foissner, 1988
Paruroleptus Kahl, 1932
Parabakuella Song & Wilbert, 1988
Bakuella Agamaliev & Alekperov, 1976
Holosticha Wrzesniowski, 1877
Territricha Berger & Foissner, 1988
Family Pseudourostylidae Jankowski, 1979
Pseudourostyla Borror, 1972
Family Pseudokeronopsidae Borror & Wicklow, 1983
Thigmokeronopsis Wicklow, 1981
Keronella Wiackowski, 1985
Tricoronella Blatterer & Foissner, 1988
Bicoronella Foissner, 1995
Pseudokeronopsis Borror & Wicklow, 1983

[a] Lynn & Small (2002) listed only representative genera.

For the classification used in the present book see table of content.

4 Parasitism

Urostyla grandis is sometimes attacked or infected by the suctorian *Podophrya urostylae* (Maupas, 1881) Jankowski, 1963 (basionym *Sphaerophrya urostylae*)[1]. The first record of this parasite was likely provided by Cohn (1851, p. 277, Tafel VII, Fig. 11, 12), who found *Urostyla*-cells packed with black-grey globules. Cohn, Lachmann (1856, p.

[1] According to Dovgal (2002, p. 245) the original combination, *Sphaerophrya urostylae*, is correct.

386), and Stein (1859) mistakenly interpreted the globules as embryos (embryonal hypothesis) of *Urostyla grandis*. But even Balbiani (1858, 1860), Engelmann (1876), and Bütschli (1876) recognised the parasitic nature of the suctorians, whose life cycle was described in detail by Stein (1859, Fig. 17a–y) and Jankowski (1963).

Adult specimens of the suctorian ciliate are about 35 µm across (Matthes 1988, p. 165). The swarmer has seven ciliary wreathes and tentacles with which it adheres to the host. It loses the cilia and causes an invagination at the host, which it penetrates and starts to suck with the tentacles. The invagination produced by the suctorian ciliate is not closed during the development to a globular, adult suctorian which has one or two contractile vacuoles, a spherical macronucleus, and one micronucleus. The swarmer is formed by external budding (Fig. 18a). Adult specimens can also be found outside the host. They are stalked or unstalked and have tentacles of ordinary length (Fig. 18b). *Podophrya urostylae* forms stalked resting cysts, which deviate distinctly from the *Podophrya*-type (Fig. 18c).

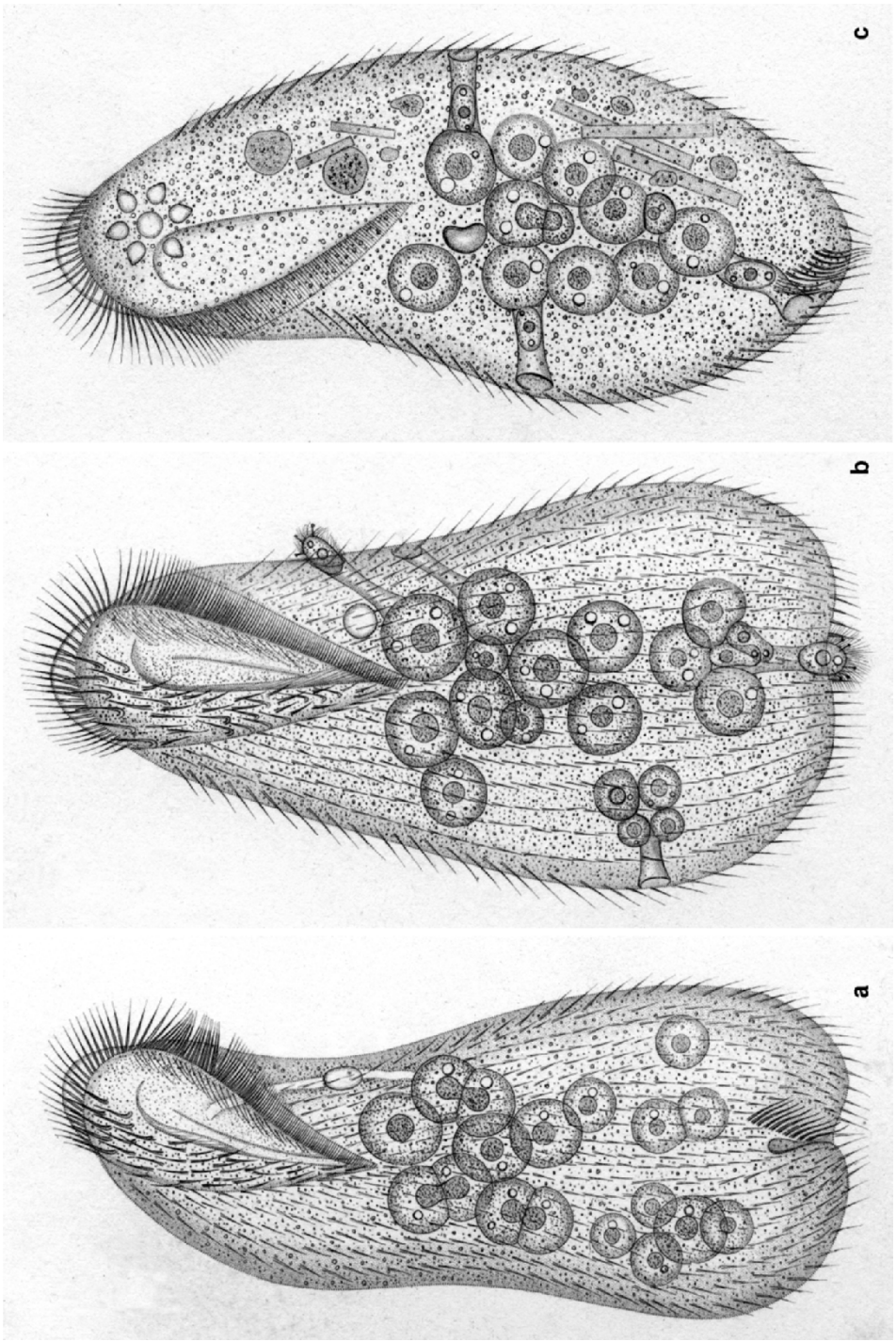
5 Ecology, Occurrence, and Geographic Distribution

Urostyloids live, throughout the year, in almost all biotopes, for example, freshwater (brooks, rivers, lakes, ponds), brackish water, sea, semiterrestrial habitats, and soil (e.g., Borror & Wicklow 1983, Foissner 1987a, 1998, Foissner et al. 1995, 1995a, Kahl 1932, Patterson et al. 1989, Petz & Leitner 2003). No symbiotic or parasitic species is known.

Very likely all limnetic and marine species are, as in most other hypotrichs, bottom-dwellers creeping on, for example, detritus, stones, or macrophytes. No species is obligatorily pelagic, however, several species can be occasionally found in the plankton community of large rivers, lakes, ponds, and the sea (for review see Foissner et al. 1999 and Petz 1999).

Many species are confined to one of the three major habitats, freshwater, sea, or soil. Only few species are reliably recorded from two habitats. For example, *Holosticha pullaster* is very common in limnetic and marine habitats, and *Anteholosticha intermedia* (= *Holosticha multistilata* of earlier papers) is present both in soil and freshwater. However, *Holosticha pullaster* was never reliably recorded from terrestrial habitats, and the large limnetic *Urostyla grandis* obviously does not occur in the sea or the soil. Possibly, the populations of a species inhabiting different habitats (e.g., freshwater and sea) are sibling species because gene flow among these populations is hampered (not existent?). Interestingly, a rather high percentage of urostyloid species occurs in marine habitats. Some groups are confined to the sea (*Thigmokeronopsis*), or at least most included species occur exclusively in this habitat (*Pseudokeronopsis*, *Holosticha*).

Fig. 17a–c *Urostyla grandis* parasitised by *Podophrya urostylae* (from Stein 1859). Stein and some other → workers misinterpreted the parasitisation as embryonic reproduction. The suctors are usually scattered throughout the cytoplasm. Three suctors in the *Urostyla* specimen shown in (a) divide; some adult suctors have formed swarmers, which leave the host (b, c). Further details see text and Stein's (1859) exhaustive description.


c
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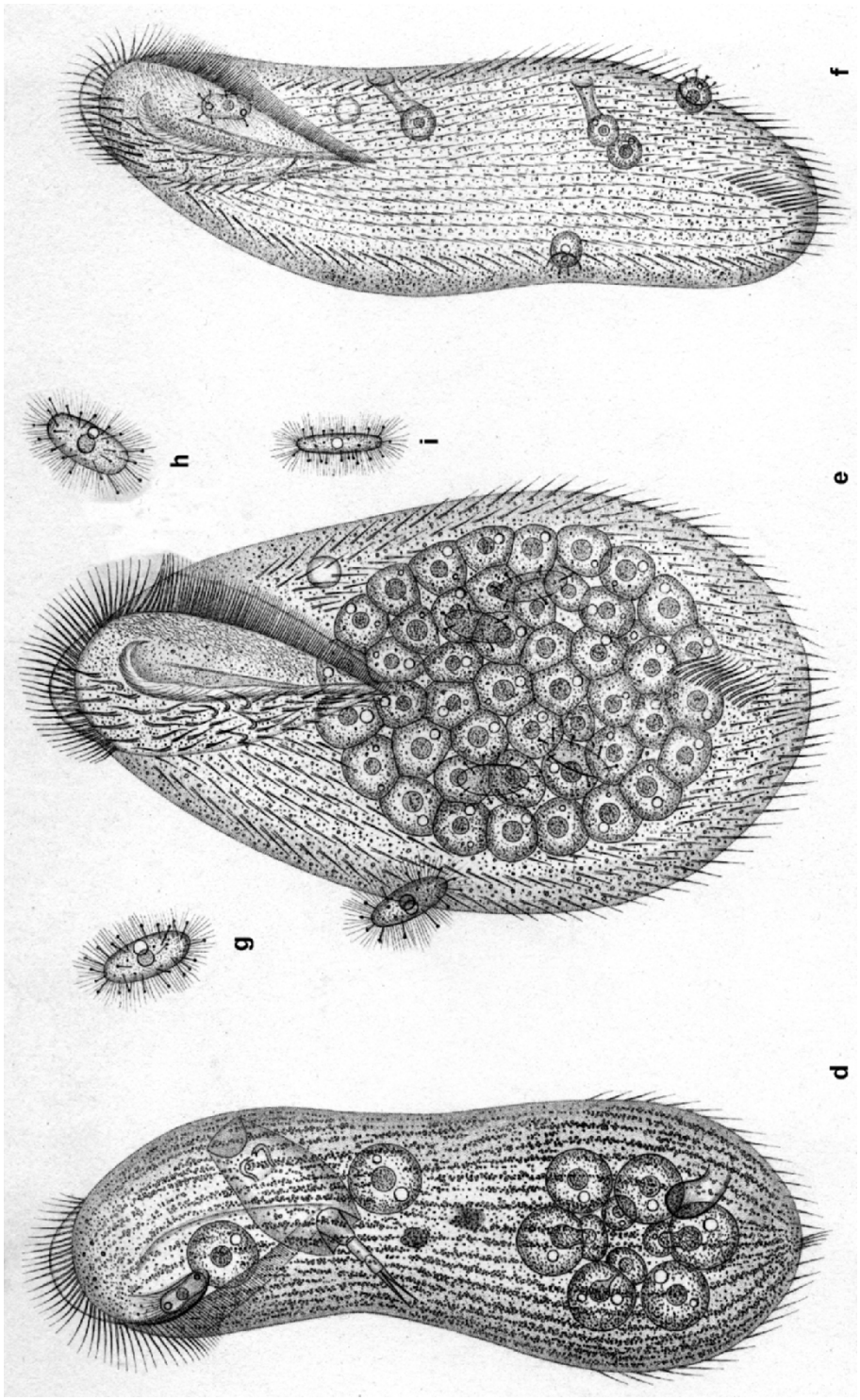
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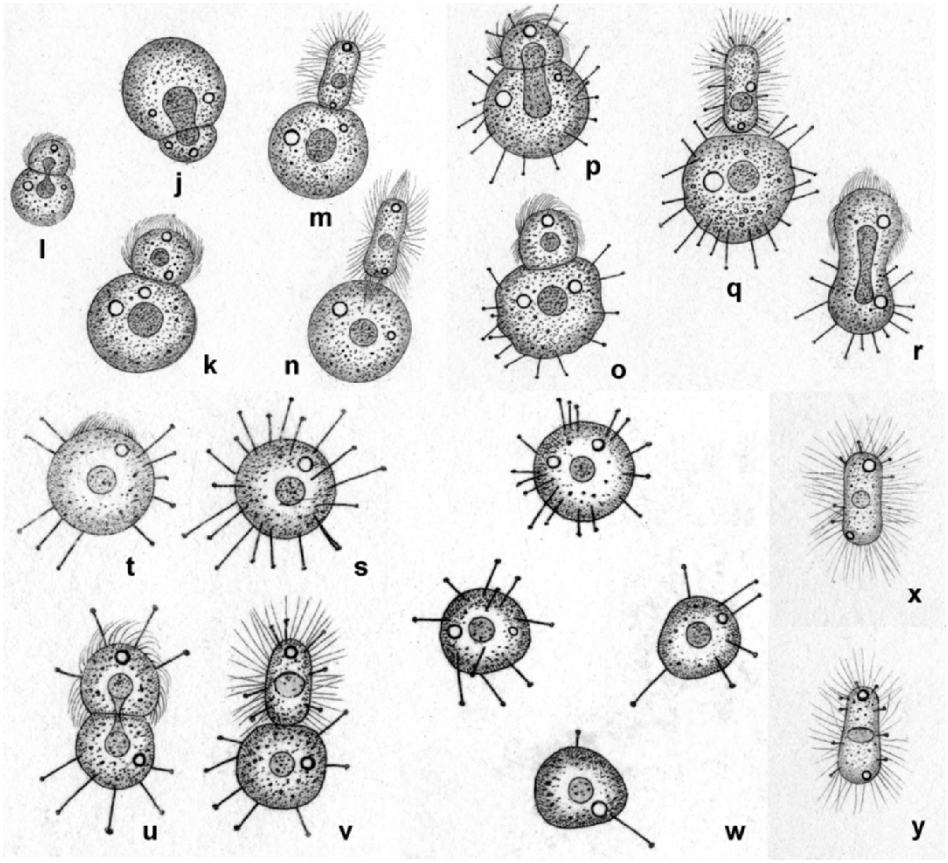

Fig. 17j–y *Podophrya urostylae*, a suctorian parasite of *Urostyla grandis* (from Stein 1859; see also Figs. 17a–i). The swarmers of the parasite are formed by external budding. Further details see text and Stein's (1859) detailed description.

Urostyloid hypotrichs have about the same food spectrum as other hypotrichs (Berger 1999), that is, they feed on bacteria, cyanobacteria, algae (including diatoms), hyphae and spores of fungi, auto- and heterotrophic flagellates, other ciliates, and small metazoans, for example, rotifers (Fig. 138a, 206b). The spectrum of the individual species is of course usually much narrower.

Very little is known about the geographic distribution of urostyloids. The group as such is likely distributed world-wide. However, we are unable to say whether individual

← **Fig. 17d–i** *Urostyla grandis* parasitised by *Podophrya urostylae* (from Stein 1859). Various stages of parasitisation. The host shown in (e) contains about 50 parasites. The body outline of the swarmers varies from slender to broad elliptical (g–i). Further details see text and Stein's (1859) exhaustive description.

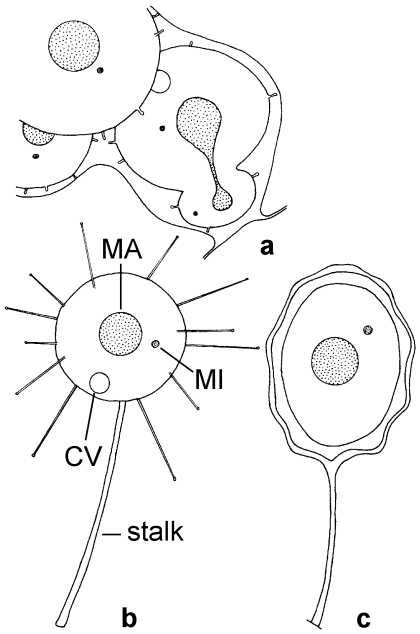

Fig. 18a–c *Podophrya urostylae*, a suctor parasitising *Urostyla grandis* (after Jankowski 1963 from Matthes 1988). **a:** Parasitic stage with beginning external budding in *Urostyla*. **b:** Stalked, free-living (adult) specimen. **c:** Resting cyst. CV = contractile vacuole, MA = macronucleus, MI = micronucleus.

species or subgroups are confined to certain biogeographic regions or not, simply because too few reliable data are available. In the descriptions of the individual species I mention all published records I know from all over the world. There is no doubt that several determinations are incorrect. Thus, records which are not substantiated by *serious* morphological data should be used with caution (or better not at all) for biogeographical interpretations. Certainly, many more urostyloid species than reviewed in the present book exist because little is known about ciliates outside Europe. Moreover, the sea likely harbours a considerable number of not yet known species (e.g., Wanick & Silva-Neto 2004).

Six urostyloids are used as indicators of water quality (Table 12). By contrast, 24 oxytrichid species, four *Uroleptus* species, and seven euplotids are included in relevant lists (Foissner et al. 1991, Berger 1999, Berger & Foissner 2003). A detailed description of the morphology and ecology of these hypotrichs, euplotids, and other species is given in our "ciliate atlas" (Foissner et al. 1991, 1992b, 1994, 1995). Keys and revised lists with the saprobic classification can also be found in Foissner et al. (1995a), Foissner & Berger (1996), Berger et al. (1997), and Berger & Foissner (2003). Note that *Holosticha gibba* is marine and *Urostyla viridis* is little known. *Holosticha pullaster* is very common in freshwater, but unfortunately euryoecious and therefore has the lowest indicative weight. *Anteholosticha intermedia*, *A. monilata*, and *Urostyla grandis* occur regularly in running waters, but usually with low abundance.

Only few urostyloids (*Anteholosticha monilata*, *Diaxonella pseudorubra*, *Pseudourostyla cristata*) are reliably recorded from activated sludge plants (Augustin & Foissner 1992, Oberschmidleitner & Aescht 1996). *Holosticha pullaster*, although rather common in stagnant and running waters, was never reliably recorded from activated sludge.

Species found in the marine interstitial are summarised by Carey (1992) and Patterson et al. (1989). Likely no species is obligatorily anaerobic, although *Anteholosticha fasciola* can be maintained in anaerobic cultures (Fenchel & Finlay 1991, 1995; identification uncertain).

Table 12 Saprobic classification of urostyloid ciliates (from Foissner et al. 1991)[a]

Species[b]	S	Valency				I	SI	Page
		o	b	a	p			
Anteholosticha intermedia (Bergh, 1889) comb. nov.[c]	a–b	-	4	5	1	2	2.7	317
Anteholosticha monilata (Kahl, 1928) Berger, 2003	a–b	-	3	6	1	3	2.8	297
Holosticha gibba (Müller, 1786) Wrześniowski, 1877	a–b	-	4	5	1	2	2.7	99
Holosticha pullaster (Müller, 1773) Foissner, Blatterer, Berger & Kohmann, 1991	b–a	1	4	4	1	1	2.5	128
Urostyla grandis Ehrenberg, 1830	a	-	3	7	-	4	2.7	1048
Urostyla viridis Stein, 1859	b–a	-	5	5	-	3	2.5	1106

[a] S = indication of saprobity by simple letter, o = oligosaprobic, b = betamesosaprobic, a = alphamesosaprobic, p = polysaprobic, I = indicative weight (1, 2, 3, 4, or 5) of species, SI = saprobic index (ranging from 1 to 4 in the limnosaprobic area).

[b] Some species have a different name in the present book and in Foissner et al. (1991): *Anteholosticha intermedia* = *Holosticha multistilata* in Foissner et al. (1991); *Anteholosticha monilata* = *Holosticha monilata*; *Holosticha gibba* = *Holosticha kessleri*; *Urostyla viridis* = *Paraurostyla viridis*.

[c] See description for combination.

6 Collecting, Culturing, Observing, and Staining of Urostyloid Ciliates

A detailed description of these topics for all ciliates is given by Foissner (1991, 1993) and Foissner et al. (1991, 1999, 2002).

6.1 Collecting and Culturing

Urostyloids occur in terrestrial (litters, humic and mineral soil horizons), semiterrestrial (e.g., astatic puddles, mosses, flood plains), limnetic (e.g., ponds, lakes, brooks, rivers, sewage treatment plants), and brackish water biotops. Furthermore, a rather high number of marine species is described.

There are two principle techniques available for collecting protozoans from waters: either direct sampling of natural substrates, or artificial substrate sampling. Urostyloids – and hypotrichs in general – can be sampled from natural substrates by collecting algae masses, mud, debris, macrophytes, small stones, and leaves, and by brushing off the aufwuchs from stones, twigs, and vegetation (e.g., Berger et al. 1997, Blatterer 1995, Foissner et al. 1991, 1992a, Heuss 1976, Liebmann 1962). Plankton samples (mesh size ≤10 µm) should be fixed with saturated, aqueous mercuric chloride (formalin destroys all [?] urostyloids) or studied in life (Foissner et al. 1999). For a detailed description of foam sampling, see Cairns & Henebry (1982) and Pratt & Kepner (1992). Samples should be collected in at least 0.5–1.0 l wide-necked bottles and transported to the laboratory in a cooler. The investigation should be done within 24 h after collecting because

the ciliate biocoenosis changes very rapidly. Occasionally some urostyloids thrive in older, slightly fouling cultures.

Water samples can be studied by the so-called cover glass method, a simple but very effective technique (for review see Berger & Foissner 2003). The bottles containing the collected material are opened in the laboratory. Place a cover glass on the water surface of the bottle and remove it with a cover glass forceps after 30 min or more. Put the cover glass on an ordinary microscope slide and look for the numbers and kinds of ciliates present. Ciliates accumulate on the cover glass due to oxygen depletion in the deeper zones of the bottle and because of their life style, that is, many are aufwuchs inhabitants and therefore attach to solid surfaces, that is, the cover glass. The ciliate community obtained in this way is very clean and rich. Do not distribute the material collected in a large Petri dish! This would slow down oxygen depletion and ciliate attachment to the cover glass. It was just this mistake why Krieg (in Tümpling & Friedrich 1999) did not succeed with the cover glass method. Finally, take some drops from the bottle's sediment surface and investigate it for bottom-dwellers, which usually do not, or with low abundance, attach to the cover glass. For detailed water quality assessment follow the method described by Blatterer (1995; see also Berger et al. 1997 and Moog et al. 1999), which is now even available as Austrian Standard (ÖNORM M6118).

Activated sludge samples can be analysed as follows (for review see Berger & Foissner 2003): use fresh sludge, which is taken from the plant with a trowel, put into a 500 ml bottle, and transported to the laboratory under cool conditions. Take care for anaerobic zones, which must be sampled and assessed separately. For investigation, shake the bottle, take a small drop (about 0.1 ml) with an ordinary pipette, put it on a microscope slide, and cover the preparation with a cover glass. Three replications should be investigated to obtain reliable data on the species present. Usually, semiquantitative investigation with a rating scale will be sufficient. However, quantitative investigation is also possible and easily performed with the method described by Augustin et al. (1989) and Augustin & Foissner (1992a). Sludge quality can be assessed with the sludge biotic index (SBI) of Madoni (1994) or the method by Großmann et al. (1999).

The most effective means for collecting and culturing urostyloids and other ciliates from soils and mosses is the non-flooded petri dish method as described by Foissner (1987a; see also Foissner 1993 and Foissner et al. 2002). Here, 10–200 g of fresh or air-dried soil or litter sample are placed in a petri dish (10–20 cm across) and saturated, but not flooded, with distilled water. A ciliate, flagellate, and naked amoeba fauna, often very rich, develops within a few days. Inspection of the cultures on days 2, 4, 6, 10, 14, and 20 usually suffices. Subsequent inspections reveal only few species due to the effects of ciliatostasis (Lüftenegger et al. 1987). *Paraholosticha* and *Keronopsis* species usually occur after few hours, the very common *Gonostomum affine* can be found also in old cultures. Several conditions influence the outcome of the method: (i) air-dried soil often yields more individuals and species than fresh soil, perhaps due to reduced microbiostasis; (ii) the sample should contain ample litter and plant debris and must be spread over the bottom of the petri dish in an at least 1 cm thick layer; (iii) the soil may not be flooded. Water should be added to the sample until 5–20 ml drains off when the

petri dish is tilted and the soil is gently pressed with a finger. This run-off contains the protozoa and can be used for further preparations such as silver staining.

The methods for culturing hypotrichous ciliates are treated only briefly here as detailed culturing methods – if available – are provided in the species descriptions. Furthermore, the general procedures as described, for instance by Dragesco & Dragesco-Kernéis (1986), Finlay et al. (1988), Foissner et al. (1991, 2002), Galtsoff et al. (1959), Lee et al. (1985), Mayer (1981), and Provasoli et al. (1958) apply also to the hypotrichs.

Some of the bacteriovorous urostyloids thrive on various media (e.g., diluted lettuce and/or hay extracts, table waters [e.g., Volvic], tap water) enriched with a little dried yolk, rice grains, or crushed wheat grains to promote bacterial growth. Some predatory species grow well with small ciliates (e.g., species of the *Tetrahymena pyriformis* complex, *Glaucoma scintillans*) as food. For marine species, artificial sea water (e.g., the supersoluble seasalt Biosal by Aqualine Buschke, Berg, Germany) can be used.

6.2 Observing Living Hypotrichs

Many physical and chemical methods have been described for retarding the movement of ciliates in order to observe structural details (for literature see Foissner 1991). Chemical immobilisation – for example, by nickel sulfate – or physical slowing down by increasing the viscosity of the medium (e.g., methyl cellulose) are rarely helpful. These procedures often change the shape of the cell or cause pre-mortal alterations of various cell structures. The following simple method is therefore preferable: place about 0.5 ml of the raw sample on a slide and pick out (collect) the desired specimens with a micropipette under a compound microscope with low magnification (for example, objective 4:1, ocular 10 ×). If specimens are large enough, they can be picked out from a petri dish under a dissecting microscope. Working with micro-pipettes, the diameter of which must be adjusted to the size of the specimens, requires some training. Transfer the collected specimens, which are now in a very small drop of fluid, onto a slide. Apply small dabs of Vaseline (Petroleum jelly) to each of the four corners of a small cover glass (Fig. 19a; the four dabs can be also applied to the slide); it is useful to apply the jelly by an ordinary syringe with a thick needle. Place the cover glass on the droplet containing the ciliates. Press on the vaselined corners with a mounted needle until ciliates are held firmly between slide and cover glass (Fig. 19b–d). As the pressure is increased the ciliates gradually become less mobile and more transparent. Hence, first the location of the main cell organelles (e.g., nuclear and oral apparatus, contractile vacuole) and then details (e.g., cortical granules, micronucleus) can easily be observed under low (100–400 ×) and high (1000 ×; oil immersion objective) magnification. The colour of the cortical granules and/or the cytoplasm must be studied with well-adjusted bright field.

The shape of the cells is of course altered by this procedure. Therefore, specimens taken directly from the raw culture with a large-bore (opening about 1 mm) Pasteur pi-

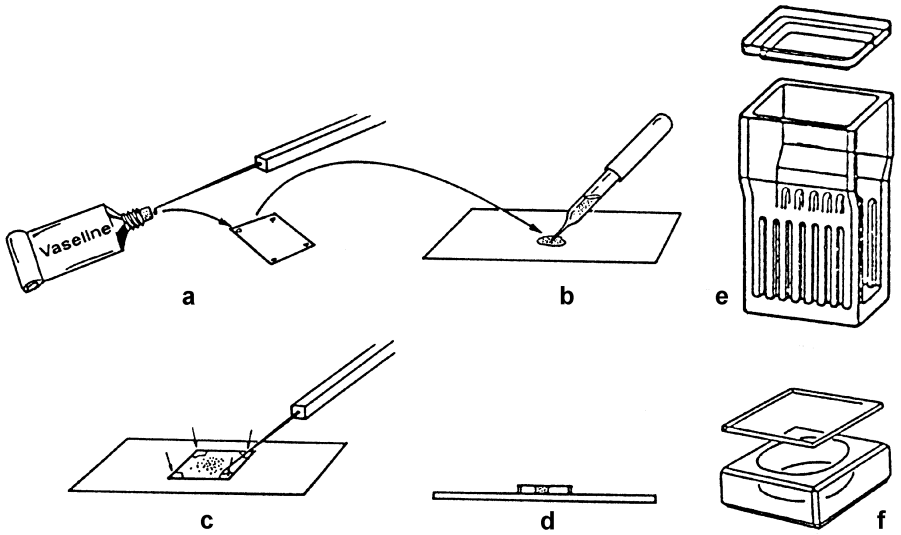

Fig. 19a–f Live observation and staining of urostyloid ciliates (from Foissner 1991). **a–d:** Preparation of slides for observing living ciliates. **e:** Staining jar for 8 and 16 (back to back) slides, respectively. **f:** Watch-glass for protargol procedure according to Wilbert.

pette must first be investigated under low magnification (100–400 ×), that is, without cover glass. Some species are too fragile to withstand handling with micro-pipette and cover glass trapping without deterioration. Investigation with low magnification also requires some experience, but it guarantees that the outline of undamaged cells are recorded. Video-microscopy is very useful at this point of investigation, especially for the registration of the swimming behaviour.

A compound microscope equipped with Normarski differential interference contrast optics is best for discerning the arrangement of the cirri and dorsal cilia in living hypotrichs. If not available, use bright-field. The nuclear apparatus is well-recognisable with differential interference contrast or phase-contrast when specimens are strongly squeezed.

Species that were not observed in life often cannot be identified after silver impregnation with certainty because important characters (e.g., shape, colour of cortical granules, colour of cytoplasm) are not known. As already mentioned above, the correct colour can only be seen with a well-adjusted bright field illumination.

6.3 Staining Procedures

There are many methods for staining ciliates, but only protargol silver impregnation yields (usually) good results in urostyloid hypotrichs. Thus, familiarity with this method is an absolute prerequisite for the description of urostyloids. It is thus described

in detail. Simple, namely molecular, formulae are given for the chemicals used, since usually only these are found in the catalogues of the suppliers (e.g., Merck). Other silver impregnation methods (dry silver nitrate method, wet silver nitrate method, silver carbonate method), detailed literature, and some general instructions are to be found in the reviews by Foissner (1991, 1993) and Foissner et al. (1991, 1999).

Apart from silver impregnation, some other staining techniques are useful for taxonomic work with ciliates, especially the Feulgen nuclear reaction and supravital staining with methyl green-pyronin in order to reveal the nuclear apparatus and, respectively, the extrusomes.

6.3.1 Feulgen Nuclear Reaction

Descriptions of this method are to be found, for example, in Dragesco & Dragesco-Kernéis (1986) and Lee et al. (1985). The Feulgen reaction reveals the nuclear apparatus very distinctively, but, because these organelles usually stain well with protargol, it is seldom used for hypotrichs.

6.3.2 Supravital Staining with Methyl Green-Pyronin

This simple method was described by Foissner (1979a). It is an excellent technique for revealing the mucocysts of most ciliates. Mucocysts are stained deeply and very distinctively blue or red, and can be observed in various stages of explosion because the cells are not killed instantly. The nuclear apparatus is also stained.

Procedure (after Foissner 1991)

1. Pick out desired ciliates with a micro-pipette and place the small drop of fluid in the centre of a slide.

2. Add an equally sized drop of methyl green-pyronin and mix the two drops gently by swivelling the slide.
Remarks: If ciliates were already mounted under the coverslip, add a drop of the dye at one edge of the coverslip and pass it through the preparation with a piece of filter paper placed at the other end of the coverslip.

3. Place a coverslip with vaselined corners on the preparation.
Remarks: Observe immediately. Cells die in the stain within 2 min. Mucocysts stain very quickly and many can be observed at various stages of explosion. To reveal the nuclear apparatus, cells should be fairly strongly squashed (= flattened). The preparation is temporary. After 5–10 min the cytoplasm often becomes heavily stained and obscures other details.

Reagents

1 g methyl green-pyronin (Chroma-Gesellschaft, Schmid GmbH and Co., Köngen/N., Germany)
add 100 ml distilled water
This solution is stable and can be used for years.

6.3.3 Protargol Methods

Protargol methods are indispensable for descriptive research on urostyloids and hypotrichs in general. The first procedures were provided by Kirby (1945), Moskowitz (1950), Dragesco (1962), and Tuffrau (1964, 1967), and many more modifications were subsequently proposed (see Foissner 1991 for references). Here, two variations which produce good results are described. These procedures work well with most ciliate species, but require at least 20 specimens. A single specimen cannot usually be handled successfully. Depending on the procedure used, protargol can reveal many cortical and internal structures, such as basal bodies, fibrillary systems, nuclear apparatus. The silverlines (which have no systematic value in the urostyloids), however, never impregnate. The shape of the cells is usually well preserved in permanent slides, which is an advantage for the investigation, but makes photographic documentation more difficult. However, pictures as clear as those taken from wet silver carbonate impregnations can be obtained with the Wilbert modification (procedure B) if the cells are photographed prior to embedding in the albumen-glycerol.

Procedure A (after Foissner 1991)

The quality of the slides is usually adequate but frequently not as good as with the Wilbert modification. The latter demands more material and experience; inexperienced workers may easily lose all the material. As in all protargol methods, the procedure is rather time-consuming and complicated because subject to many factors. A centrifuge may be used for step 2; staining jars (Fig. 19e) are necessary for steps 6–16.

1. Fix organisms in Bouin's or Stieve's fluid for 10–30 min.
Remarks: The fixation time has little influence on the quality of the preparation within the limits given. Ratio fixative to sample fluid should be at least 2:1. Pour ciliates into fixative using a wide-necked flask in order to bring organisms in contact with the fixative as quickly as possible. Both fixatives work well but may provide different results with certain organisms. Stieve's fluid may be supplemented with some drops of 2 % osmium tetroxide for better fixation of very fragile hypotrichs, for example, *Pseudoamphisiella*. This increases the stability of the cells but usually reduces their impregnability.

2. Concentrate by centrifugation and wash organisms 3 to 4 times in distilled water.
Remarks: There are now 2 choices: either to continue with step 3, or to transfer the material through 30–50–70 % alcohol into 70 % alcohol (ethanol), where it remains stable for several years. Transfer preserved material back through the graded alcohol series into distilled water prior to continuing with the next step. Impregnability of preserved material may be slightly different.

3. Clean 8 ordinary slides (or less if material is very scarce) per sample. The slides must be grease-free (clean with alcohol and flame).
Remarks: Insufficiently cleaned slides may cause the albumen to detach. Mark slides on back if several samples are prepared together. Alternatively you can use SuperFrost slides, which are ready to use. In addition, these slides have a field enabling simple labelling with a pencil. Use staining jars with 8 sections so that you can work with 16 slides simultaneously by putting them back to back (Fig. 19e).

4. Put 1 drop each of albumen-glycerol and concentrated organisms in the centre of a slide. Mix drops with a mounted needle and spread over the middle third.
Remarks: Use about equally sized drops of albumen-glycerol and suspended (in distilled water) organisms to facilitate spreading. The size of the drops should be adjusted so that the middle third of the slide is covered after spreading. Now remove sand, grains, etc. The thickness of the albumen layer should be equal to that of the organisms. Some thicker and thinner slides should, however, also be prepared because the thickness of the albumen layer greatly influences the quality of the preparation. Cells may dry out and/or shrink if the albumen layer is too thin; if it is too thick, it may detach, or the cells may become impossible to study with the oil immersion objective.

5. Allow slides to dry for at least 2 h at room temperature.
Remarks: We usually dry slides overnight, that is, for about 12 h. However, slides may be allowed to dry for up 24 h, but no longer if quality is to be maintained. Oven-dried (2 h at 60 °C) slides are usually also of poorer quality.

6. Place slides in a staining jar (Fig. 19e) filled with 95 % alcohol (ethanol) for 20 to 30 min. Place a staining jar with protargol solution into an oven (60° C).
Remarks: Slides should not be transferred through an alcohol series into concentrated alcohol as this causes the albumen layer to detach! Decrease hardening time to 20 min if albumen is already rather old and/or not very sticky.

7. Rehydrate slides through 70 % alcohol and 2 distilled water steps for 5 min each.

8. Place slides in 0.2 % potassium permanganate solution. Remove first slide (or pair of slides) after 60 s and the rest at 15 s intervals. Collect slides in a staining jar filled with distilled water.
Remarks: Bleaching is by permanganate and oxalic acid (step 9). The procedure described above is necessary because each species has its optimum bleaching time. The sequence in which slides are treated should be recorded because the immersion time in

oxalic acid must be proportional to that in the permanganate solution. The albumen layer containing the organisms should swell slightly in the permanganate solution and the surface should become uneven. If it remains smooth, the albumen is too sticky and this could decrease the quality of the impregnation. If the albumen swells strongly, it is possibly too weak (old) and liable to detach. Use fresh $KMnO_4$ solution for each series.

9. Quickly transfer slides to 2.5 % oxalic acid. Remove first slide (or pair of slides) after 160 s, the others at 20 s intervals. Collect slides in a staining jar filled with distilled water.
Remarks: Same as for step 8! Albumen layer becomes smooth in oxalic acid.

10. Wash slides three times in distilled water for 3 min each.

11. Place slides in warm (60° C) protargol solution and impregnate for 10–15 min at 60° C.
Remarks: Protargol solution can be used only once.

12. Remove staining jar with the slides from the oven and allow to cool for 10 min at room temperature.
Remarks: In the meantime organise six staining jars for developing the slides: distilled water – distilled water – fixative (sodium thiosulfate) – distilled water – 70 % alcohol – 100 % alcohol (ethanol).

13. Remove the first slide from the protargol solution and drop some developer on the albumen layer. Move slide gently to spread developer evenly. As soon as the albumen turns yellowish, pour off the developer, dip slide in the first 2 distilled water steps for about 2 s each and stop development by submerging the slide in the fixative (sodium thiosulfate), where it can be left for 1–5 min.
Remarks: Now control impregnation with the compound microscope. The impregnation intensity is sufficient if the infraciliature is just recognisable. The permanent slide will be too dark if the infraciliature is distinct at this stage of the procedure! The intensity of the impregnation can be controlled by the concentration of the developer and the time of development. 5–10 s usually suffice for the diluted developer! Development time increases with bleaching time. Therefore commence development with those slides, which were in the bleaching solutions for 60 and 120 s, respectively. The thinner the albumen layer, the quicker the development.

14. Collect slides in the fixative (sodium thiosulfate) and transfer to distilled water for up to 5 min.
Remarks: Do not wash too long; the albumen layer is very fragile and detaches rather easily!

15. Transfer slides to 70 % – 100 % – 100 % alcohol for 5 min each.

16. Clear by two 10 min transfers through xylene.

17. Mount in synthetic neutral mounting medium.
Remarks: Do not dry slides between steps 16 and 17! Mounting medium should be rather viscous to avoid air-bubbles being formed when solvent evaporates during drying. If air-bubbles develop in the mounted and hardened slide, re-immerse in xylene for some days until the coverslip drops off. Remount using a more viscous medium and remove possible sand grains protruding from the gelatine. Usually, some air-bubbles are found immediately after mounting; these can be pushed to the edge of the coverslip with a finger or mounted needle. The preparation is stable.

Reagents

a) Bouin's fluid (prepare immediately before use; components can be stored)
15 parts saturated, aqueous picric acid ($C_6H_3N_3O_7$; preparation: add an excess of picric crystals to, for example, 1 litre of distilled water; shake solution several times within a week; some undissolved crystals should remain; filter before use)
5 parts formalin (HCHO; commercial concentration, about 37 %)
1 part glacial acetic acid (= concentrated acetic acid; $C_2H_4O_2$)

b) Stieve's fluid (slightly modified; prepare immediately before use; components can be stored)
38 ml saturated, aqueous mercuric chloride (dissolve 60 g $HgCl_2$ in 1 litre of boiling distilled water)
10 ml formalin (HCHO; commercial concentration, about 37 %)
3 ml glacial acetic acid (= concentrated acetic acid; $C_2H_4O_2$)

c) Albumen-glycerol (2–4 month stability)
15 ml egg albumen
15 ml concentrated (98–100 %) glycerol ($C_3H_8O_3$)

Pre-treatment of the egg albumen and preparation of the albumen-glycerol: separate the white carefully from the yolk and embryo of three eggs (free range eggs are preferable to those from battery chickens, whose egg white is less stable and sticky). Shake the white by hand (do not use a mixer!) for some minutes in a narrow-mouthed 250 ml Erlenmeyer flask until a stiff white foam is formed. Allow the flask to stand for about 1 min. Then pour the viscous rest of the egg white in a second Erlenmeyer flask and shake again until a stiff foam is formed. Repeat until most of the egg white is either stiff or becomes watery; usually 4–6 Erlenmeyer flasks of foam are obtained. Leave all flasks undisturbed for about 10 min and discard the watery albumen from the last flask. During this time a glycerol-like fluid percolates from the foam. This fluid is collected and used. Add an equal volume of concentrated glycerol and a small thymol crystal ($C_{10}H_{14}O$) for preservation to the mixture. Mix by shaking gently and pour mixture into a small flask. Leave undisturbed for two weeks. A whitish slime settles at the bottom of

the flask. Decant the clear portion, discard slime and thymol crystal. A "good" albumen-glycerol drags a short thread when touched with a needle. The albumen is too thin (not sticky enough) or too old if this thread is not formed. Fresh albumen which is too thin may be concentrated by leaving it open for some weeks so that water can evaporate. If the albumen is too sticky, which may cause only one side of the organisms to impregnate well, it is diluted with distilled water or old, less sticky albumen to the appropriate consistency. The preparation of the albumen-glycerol must be undertaken with great care because much depends on its quality. Unfortunately, all commercial products which have been tried detach during impregnation. A somewhat simpler method to produce the albumen-glycerol is described by Adam & Czihak (1964, p. 274): the white of one or two fresh chicken egg(s) and the same amount of concentrated glycerol are well stirred to a homogenous, thick fluid (a magnetic stirrer can be used). Then filter through cotton wool. Add a small thymol crystal to the filtrate. The albumen-glycerol can be used right away.

d) 0.2 % potassium permanganate solution (stable for about 1 d)
0.2 g potassium permanganate ($KMnO_4$) are dissolved in 100 ml distilled water

e) 2.5 % oxalic acid solution (stable for about 1 d)
2.5 g oxalic acid ($C_2H_2O_4 \cdot 2H_2O$) are dissolved in 100 ml distilled water

f) 0.4–0.8 % protargol solution (stable for about 1 d)
100 ml distilled water
add 0.4–0.8 g protargol
Remarks: Use light-brown "protargol for microscopy" (for example, Merck's "Albumosesilber für die Mikroskopie" or "Proteinate d'Argent", Roques, Paris, France). Some dark-brown, cheaper products do not work! Sprinkle powder on the surface of the water in the staining jar and allow to dissolve without stirring.

g) Developer (mix in sequence indicated; sodium sulphite must be dissolved before hydroquinone is added)
95 ml distilled water
5 g sodium sulphite (Na_2SO_3)
1 g hydroquinone ($C_6H_6O_2$)
Remarks: This recipe yields the stock solution which is stable for some weeks and should be used undiluted for certain ciliates (step 13). Usually, however, it must be diluted with tap water in a ratio of 1:20 to 1:50 to avoid too rapid development and one-sided impregnation of the organisms. Freshly prepared developer is usually inadequate (the albumen turns greenish instead of brownish). The developer should thus be prepared from equal parts of fresh and old (brown) stock solutions. Take great care with the developer as its quality contributes highly to that of the slides. If the developer has lost its activity (which is not always indicated by a brown colour!) the silver is not or only insufficiently reduced and the organisms stain too faintly. A fresh developer should therefore be prepared for each "impregnation week", and some old developer

kept. Fresh developer can be artificially aged by adding some sodium carbonate (Na_2CO_3). However, better results are obtained with air-aged solutions, that is, by a developer which has been kept uncovered for some days in a wide-mouthed bottle. It first turns yellowish, then light brown (most effective), and later dark brown and viscous (at this stage the developer has lost most of its activity, but is still suitable for artificial ageing of fresh developer = 1:1 mixture mentioned above).
During the last years, we obtained very good slides with the low-speed developer used by Fryd-Versavel (pers. comm. to W. Foissner). It is composed of 7 g boric acid, 1.5 g hydroquinone, 10 g sodium sulphite, and 75 ml acetone, all solved, one by one, in 420 ml distilled water. This developer is stable for some weeks and should be used only once. Pour developer into a staining jar and immerse slides, one by one, controlling impregnation intensity after 30–60 s. Usually, developing is finished within 1–5 min (if not, double protargol concentration because slides should not be too long in the developer, as the albumen may detach). The further procedure is as described above (steps 14–17).
In many cases commercial paper developers (for example, Ilford Multigrade) yield very good results.

h) Fixative for impregnation (stable for several years)
25 g sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) are dissolved in 1000 ml distilled water

Procedure B (after Wilbert 1975 and Foissner 1991)

This modification produces excellent results but demands much experience. Manipulate large cells with micropipettes in a watch-glass, whereas the centrifuge is used for steps 1–4, 7, 8 if cells are smaller than about 150 µm. The watch-glass method is used when there are only few specimens of larger cells; thus an attempt is worthwhile even if only 20 cells are available. The organisms are very soft after development and fixation, and are thus easily compressed between slide and coverslip, which greatly facilitates photographic documentation.

1. Fix organisms as described in protargol procedure A.

2. Wash and, if so desired, preserve organisms as described in protargol procedure A.
Remarks: Wash cells either in the centrifuge (small species) or in a watch-glass (Fig. 19f). To change fluids allow cells to settle on bottom of watch-glass and remove supernatant with a micro-pipette under the dissecting microscope; concentrate cells in the centre of the watch-glass by gentle swirling.

3. Leave organisms in a small amount of distilled water and add, drop by drop, diluted sodium hypochlorite (NaClO) and bleach for about 1–3 min under the dissecting microscope (for small specimens, various concentrations of NaClO can be applied in centrifuge tubes, keeping the reaction time constant, for example, 1 min).

Remarks: This is the critical step in this modification. If bleaching is too strong or too weak all is lost: cells either dissolve or do not impregnate well. Systematic investigations showed that not the bleaching time but the amount of active chloride in the sodium hypochlorite and the pre-treatment of the cells (fixation method, fresh or preserved material) are decisive for the quality of the preparation. Different species need different concentrations. Unfortunately, the concentration of active chloride in the commercial products varies (10–13 %) and is dependent on the age of the fluid. It is thus impossible to provide more than only a few guidelines: 100 ml distilled water + 0.2–0.4 ml NaClO (if product is fresh and cells were not stored in alcohol) or 100 ml distilled water + 0.5–1.6 ml NaClO (if product is older and cells were stored in alcohol). The transparency of the cells under the dissecting microscope may serve as a further indicator: fixed, unbleached cells appear dark and opaque, whereas accurately bleached cells are almost colourless and rather transparent (depends, however, also on size and thickness of the cell). Thus, increase the concentration of sodium hypochlorite stepwise if cells appear too dark with the recommended concentrations. We routinely start with 3 different hypochlorite concentrations if enough material is available.

4. Wash organisms at least 3 times with distilled water and finally once in the protargol solution.
Remarks: Wash thoroughly, especially when fluids are changed with micro-pipettes, because even the slightest traces of the sodium hypochlorite disturb impregnation.

5. Transfer to 1 % protargol solution and impregnate for 10–20 min at 60° C.
Remarks: This and the next step should be carried out in a watch-glass even for material which is otherwise manipulated with the centrifuge. The impregnation time depends on the kind of material and the degree of bleaching. Check the progress of impregnation every 3–4 min under the compound microscope by picking out a few cells with the micro-pipette under the dissecting microscope; add these to 1 drop of developer. Dilute developer and/or interrupt development of adding a little fixative (sodium thiosulfate) if impregnation is strong enough.

6. Remove most of the protargol solution with a micro-pipette and add some drops of developer to the remainder containing the organisms.
Remarks: Fresh, undiluted developer is usually used (but see step 5). Control development in compound or dissecting microscope. As soon as the infraciliature becomes faintly visible, development must be stopped by adding a few drops of sodium thiosulfate. Judging the right moment is a question of experience; the permanent slide will be too dark if the infraciliature is very distinct at this stage of the procedure! Generally: if bleaching was correct, specimens cannot be over-impregnated.

7. Stabilise the impregnation by 2, approximately 5-minute transfers through sodium thiosulfate.

Remarks: The developer need not be removed before fixation. For small species this and the next step can be carried out in a centrifuge. Larger species must be manipulated with micro-pipettes because cells become very fragile and would be damaged in a centrifuge. Cells are very soft at this stage and can thus be easily compressed and photographed. Transfer some of the more darkly impregnated specimens with a very small amount of the fixative onto a clean slide using a micro-pipette and cover with a coverslip. Organisms are usually flattened by the weight of the coverslip; excess fluid my be removed from the edge of the coverslip with a piece of filter paper. Frequently, even better micrographs are obtained if specimens are flattened before fixed with sodium thiosulphate; that is, together with some developer.

8. Wash very thoroughly in distilled water (3 times with the centrifuge; 7–10 times in watch-glass with micro-pipettes). Finally remove as much of the water as possible.
Remarks: Even the slightest traces of the fixative destroy the impregnation within a few days or weeks.

9. Smear a moderately thick layer of albumen-glycerol on a clean slide with a finger. Drop impregnated, washed cells on the albumised slide with a large-bore pipette (opening about 1 mm) and dry preparation for at least 2 h.
Remarks: The cells are too fragile to be spread with a needle. With much care it is possible to orientate cells using a mounted eyelash. Commercial albumen-glycerol can be used.

10. Harden albumen by two 10-minute transfers through concentrated alcohol (ethanol).
Remarks: This and the next step are best carried out in staining jars. The albumen layer turns milky and opaque.

11. Clear by two 5-minute transfers through xylene.
Remarks: The albumen layer turns transparent.

12. Mount in synthetic neutral mounting medium.
Remarks: Do not dry slides between steps 11 and 12! Mounting medium should be rather viscous to avoid air-bubbles being formed when solvent evaporates during drying. If air-bubbles develop in the mounted and hardened slide, re-immerse in xylene for some days until the coverslip drops off. Remount using a more viscous medium and remove possible sand grains protruding from the albumen. Usually, some air-bubbles are found immediately after mounting; these can be pushed to the edge of the coverslip with a finger or mounted needle. The preparation is stable.

Reagents

If not stated otherwise, the same reagents as in the protargol procedure A are to be used (see above).

6.4 Preparation for Scanning Electron Microscopy

Hypotrichs, and especially urostylids, cannot usually be identified solely by scanning electron microscopy because only a limited number of characters is revealed. However, this method is useful in that it allows a three-dimensional view of the object, as well as for documenting details which are difficult to reveal with other methods. For a detailed instruction of preparation for scanning electron microscopy, see Foissner (1991, 1993), Foissner et al. (1991, 1999), and other textbooks.

7 Species Concept and Nomenclature

7.1 Species Concept

The species/subspecies concept used in the present book is the same as described by Foissner et al. (2002). Briefly, we usually apply the "morphospecies" concept as basically defined by Nixon & Wheeler (1990): "A species is the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)." That is, I consider two populations as belonging to two different species if they differ from each other in at least one "important" morphological feature (e.g., number of macronuclear nodules; presence/absence of cortical granules). Of course, there is no strict consensus about the importance of various features and, unfortunately, for many species several features (e.g., presence/absence of cortical granules or caudal cirri; number of dorsal kineties; length of dorsal bristles; exact arrangement of cirri) are not known, making revisions rather difficult. Often it is a matter of taste whether or not two species are synonymised or not. To overcome these difficulties I have kept the descriptions (and the ecological data) of synonyms separate, especially when the descriptions did not fit in all important details.

The presence/absence of a certain cirral group (e.g., frontoterminal cirri, caudal cirri, transverse cirri) is generally considered as diagnostic character, that is, such features are usually used to characterise supraspecific taxa. However, features of the cirral pattern are certainly not the sole source to elucidate the phylogenetic relationships. In the Oxytrichidae the consistence of the cell (flexible vs. rigid), the presence/absence of cortical granules, and the relative length (i.e., a quantitative feature!) of the adoral zone have been successfully used to characterise the Stylonychinae (Berger & Foissner 1997, Berger 1999). Moreover, molecular markers will significantly increase our knowledge on the phylogeny of hypotrichs (Fig. 14a).

For a discussion of the advantages and disadvantages of various species concepts see textbooks on evolution (e.g., Ax 1984, Wägele 2001) and references cited by Foissner et al. (2002, p. 35).

7.2 Notes on Nomenclature

In the case of nomenclatural problems the ICZN (1964, 1985, 1999) have been consulted, depending on the date when the paper was published. For explanation of nomenclatural terms (e.g., nomen nudum, holotype) see the glossary of the ICZN (1999) or various textbooks (e.g., Lincoln et al. 1985, CBE 1996, Winston 1999).

I tried to explain the meaning and origin of the scientific names using, inter alia, the ICZN (1985, 1999), Werner (1972), Hentschel & Wagner (1996), and Latin/German dictionaries. Only in few cases (likely less than 5%) does the original description contain an etymology section. The gender of ciliate genus-group names can be found in the valuable catalogue by Aescht (2001). I did not consult a Latin/Greek linguist; thus, improprieties cannot be excluded.

Note that Kahl (1932, 1933) divided *Holosticha* into several subgenera, a fact very often overlooked. Consequently, many species names including the combining authorities have been written incorrectly in many post-Kahlian papers.

For authorship and date of non-urostyloid hypotrichs see Berger (1999, 2001). A permanently updated version of the "Catalogue of Ciliate Names. 1. Hypotrichs" is available at http://protozoology.com.

As in the first volume of the revision of hypotrichs (Berger 1999), higher taxa are not provided with categories (e.g., family, order), simply because categories do not contain information and cannot be defined objectively (e.g., Ax 1995, Westheide & Rieger 1996, Wägele 2001). For example, the taxon Hypotricha was established as order by Stein (1859). Since then it also attained the categories suborder, subclass, and even class (for review see Berger 2001). However, to avoid inflation of names I use those which are available. Therefore the "defined" endings (ICZN 1999, Article 29.2; e.g., -idae, -inae) have **no meaning** in the present book.

7.3 Summary of New Taxa and Nomenclatural Acts

Within the framework of the revision of the Urostyloidea, three books (Berger 2001, Berger & Foissner 2003, present book), five papers (Berger 2003, 2004a, b, Berger et al. 2001, Foissner et al. 2004a), and six abstracts (Berger 2001a, 2003a, b, Berger et al. 2004, Schmidt et al. 2004a, b) have been published. In these publications the nomenclatural acts listed below have been undertaken.

New species: *Anteholosticha antecirrata* (present book, p. 370).

New combinations: *Anteholosticha adami* (Foissner, 1982) Berger, 2003 (p. 377; basionym: *Holosticha adami*); *Anteholosticha alpestris* (Kahl, 1932) comb. nov. (present book, p. 403; basionym: *Holosticha (Keronopsis) alpestris*); *Anteholosticha arenicola* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha arenicola*); *Anteholosticha australis* (Blatterer & Foissner, 1988) Berger, 2003 (p. 377; basionym: *Holosticha australis*); *Anteholosticha azerbaijanica* (Alekperov & Asadullayeva, 1999) comb. nov.

(present book, p. 454; basionym: *Holosticha azerbaijanica*); *Anteholosticha bergeri* (Foissner, 1987) Berger, 2003 (p. 377; basionym *Holosticha bergeri*); *Anteholosticha brachysticha* (Foissner, Agatha & Berger, 2002) Berger, 2003 (p. 377; basionym: *Holosticha brachysticha*); *Anteholosticha brevis* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha brevis*); *Anteholosticha camerounensis* (Dragesco, 1970) Berger, 2003 (p. 377; basionym: *Holosticha camerounensis*); *Anteholosticha distyla* (Buitkamp, 1977) Berger, 2003 (p. 377; basionym: *Holosticha distyla*); *Anteholosticha estuarii* (Borror & Wicklow, 1983) Berger, 2003 (p. 377; basionym: *Holosticha estuarii*); *Anteholosticha extensa* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha extensa*); *Anteholosticha fasciola* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha fasciola*); *Anteholosticha gracilis* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha gracilis*); *Anteholosticha* grisea (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha grisea*); *Anteholosticha intermedia* (Bergh, 1889) comb. nov. (present book, p. 317; basionym: *Urostyla intermedia*); *Anteholosticha longissima* (Dragesco & Dragesco-Kernéis, 1986) comb. nov. (present book, p. 437; basionym: *Keronopsis longissima*); *Anteholosticha macrostoma* (Dragesco, 1970) comb. nov. (present book, p. 365; basionym: *Pleurotricha macrostoma*); *Anteholosticha manca* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha manca*); *Anteholosticha mancoidea* (Hemberger, 1985) Berger, 2003 (p. 377; basionym: *Holosticha mancoidea*); *Anteholosticha monilata* (Kahl, 1928) Berger, 2003 (p. 377; basionym: *Holosticha monilata*); *Anteholosticha multistilata* (Kahl, 1928) Berger, 2003 (p. 377; basionym: *Holosticha multistilata*); *Anteholosticha muscicola* (Gellért, 1956) Berger, 2003 (p. 377; basionym: *Holosticha muscicola*); *Anteholosticha muscorum* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha muscorum*); *Anteholosticha oculata* (Mereschkowsky, 1877) Berger, 2003 (p. 377; basionym: *Oxytricha oculata*); *Anteholosticha plurinucleata* (Gellért, 1956) comb. nov. (present book, p. 399; basionym: *Holosticha manca plurinucleata*); *Anteholosticha pulchra* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha pulchra*); *Anteholosticha randani* (Grolière, 1975) Berger, 2003 (p. 377; basionym: *Holosticha randani*); *Anteholosticha scutellum* (Cohn, 1866) Berger, 2003 (p. 377; basionym: *Oxytricha scutellum*); *Anteholosticha sigmoidea* (Foissner, 1982) Berger, 2003 (p. 377; basionym: *Holosticha sigmoidea*); *Anteholosticha sphagni* (Grolière, 1975) Berger, 2003 (p. 377; basionym: *Holosticha sphagni*); *Anteholosticha thononensis* (Dragesco, 1966) Berger, 2003 (p. 377; basionym: *Keronopsis thononensis*); *Anteholosticha violacea* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha violacea*); *Anteholosticha vuxgracilis* (Berger, 2005) comb. nov. (present book, p. 369; basionym: *Holosticha vuxgracilis* nom. nov., present book, p. 369); *Anteholosticha warreni* (Song & Wilbert, 1997) Berger, 2003 (p. 377; basionym: *Holosticha warreni*); *Anteholosticha xanthichroma* (Wirnsberger & Foissner, 1987) Berger, 2003 (p. 377; basionym: *Holosticha xanthichroma*); *Apoamphisiella vernalis* (Stokes, 1887) comb. nov. (present book, p. 98; basionym: *Holosticha vernalis*); *Biholosticha discocephalus* (Kahl, 1932) Berger, 2003 (p. 378; basionym: *Holosticha discocephalus*); *Biholosticha arenicola* (Dragesco, 1963) Berger, 2003 (p. 378; basionym: *Keronopsis arenicola*); *Caudiholosticha algivora* (Kahl, 1932) Berger, 2003 (p. 377; basionym: *Holosticha algivora*); *Caudiholosticha gracilis* (Foissner, 1982) comb.

nov. (present book, p. 266; basionym: *Perisincirra gracilis*); *Caudiholosticha interrupta* (Dragesco, 1966) Berger, 2003 (p. 377; basionym: *Holosticha interrupta*); *Caudiholosticha islandica* (Berger & Foissner, 1989) Berger, 2003 (p. 377, 378; basionym: *Holosticha islandica*); *Caudiholosticha multicaudicirrus* (Song & Wilbert, 1989) Berger, 2003 (p. 378; basionym: *Holosticha multicaudicirrus*); *Caudiholosticha navicularum* (Kahl, 1932) Berger, 2003 (p. 378; basionym: *Holosticha navicularum*); *Caudiholosticha notabilis* (Foissner, 1982) comb. nov. (present book, p. 260; basionym: *Paruroleptus notabilis*); *Caudiholosticha paranotabilis* (Foissner, Agatha & Berger, 2002) comb. nov. (present book, p. 254; *Uroleptus paranotabilis*); *Caudiholosticha setifera* (Kahl, 1932) Berger, 2003 (p. 378; basionym: *Holosticha setifera*); *Caudiholosticha stueberi* (Foissner, 1987) Berger, 2003 (p. 378; basionym: *Holosticha stueberi*); *Caudiholosticha sylvatica* (Foissner, 1982) Berger, 2003 (p. 378; basionym: *Holosticha sylvatica*); *Caudiholosticha tetracirrata* (Buitkamp & Wilbert, 1974) Berger, 2003 (p. 377; basionym: *Holosticha tetracirrata*); *Caudiholosticha viridis* (Kahl, 1932) Berger, 2003 (p. 378; basionym: *Holosticha viridis*); *Diaxonella pseudorubra* (Kaltenbach, 1960) comb. nov. (present book, p. 463; basionym: *Keronopsis pseudorubra*); *Diaxonella pseudorubra polystylata* (Borror & Wicklow, 1983) comb. nov. (present book, p. 479; basionym: *Holosticha polystylata*); *Diaxonella pseudorubra pulchra* (Borror, 1972) comb. nov. (present book, p. 483; basionym: *Trichotaxis pulchra*); *Hemisincirra gellerti* (Foissner, 1982) Foissner in Berger, 2001 (p. 71: basionym: *Perisincirra gellerti*); *Hemisincirra gracilis* (Foissner, 1982) Foissner in Berger, 2001 (p. 71; basionym: *Perisincirra gracilis*); *Hemisincirra interrupta* (Foissner; 1982) Foissner in Berger, 2001 (p. 72; basionym: *Perisincirra interrupta*); *Paragastrostyla terricola* (Foissner, 1988) comb. nov. (present book, p. 631; basionym: *Holostichides terricola*); *Paraholosticha ovata* (Horváth, 1933) Berger, 2001 (p. 68; basionym: *Paraholosticha ovata*); *Paraholosticha vitrea* (Vörösváry, 1950) Berger, 2001 (p. 68; basionym: *Paraholosticha vitrea*); *Pseudourostyla magna* (Alekperov, 1984) comb. nov. (present book, p. 809; basionym: *Metaurostyla magna*); *Pseudourostyla raikovi* (Alekperov, 1984) comb. nov. (present book, p. 807; basionym: *Metaurostyla raikovi*); *Tetmemena bifaria* (Stokes, 1887) Berger, 2001 (p. 52, 53; basionym: *Oxytricha bifaria*); *Thigmokeronopsis crassa* (Claparède & Lachmann, 1858) comb. nov. (present book, p. 873; basionym: *Oxytricha crassa*); *Trichototaxis multinucleatus* (Burkovsky, 1970) Berger, 2001 (p. 95, 96; basionym: *Trichotaxis multinucleatus*); *Uroleptopsis (Plesiouroleptopsis) ignea* (Mihailowitsch & Wilbert, 1990) Foissner, 1995 in Berger (2004b, p. 115); *Uroleptopsis (Uroleptopsis) citrina* Kahl, 1932 in Berger (2004b, p. 114); *Uroleptopsis (Uroleptopsis) roscoviana* (Maupas, 1883) Kahl, 1932 in Berger (2004b, p. 114); *Uroleptopsis tannaensis* (Shigematsu, 1953) Berger, 2004b and *Uroleptopsis (Uroleptopsis) tannaensis* (Shigematsu, 1953) Berger, 2004b (p. 111, 114; basionym: *Keronopsis tannaensis*); *Uroleptopsis (Uroleptopsis) viridis* (Pereyaslawzewa, 1886) Kahl, 1932 in Berger (2004b, p. 114); *Urostyla variabilis* (Borror & Wicklow, 1983) comb. nov. (present book, p. 1104; basionym: *Bakuella variabilis*).

New subgenus: *Uroleptopsis (Plesiouroleptopsis)* Berger, 2004b (p. 114).

New genera: *Anteholosticha* Berger, 2003 (p. 377); *Biholosticha* Berger, 2003 (p. 378); *Caudiholosticha* Berger, 2003 (p. 377); *Styxophrya* Foissner et al., 2004a (p. 279).

New higher taxa: Acaudalia (present book, p. 749); Dorsomarginalia (present book, p. 38); Retroextendia (present book, p. 732).

New ranks: *Anteholosticha plurinucleata* (Gellért, 1956) (species rank; present book, p. 399); *Bakuella (Pseudobakuella)* Alekperov, 1992 (subgenus rank; present book, p. 576); *Diaxonella pseudorubra polystylata* (Borror & Wicklow, 1983) (subspecies rank; present book, p. 479); *Diaxonella pseudorubra pseudorubra* (Kaltenbach, 1960) (subspecies rank; present book, p. 468); *Diaxonella pseudorubra pulchra* (Borror, 1972) (subspecies rank; present book, p. 483); *Uroleptopsis (Uroleptopsis)* Kahl, 1932 (subgenus rank in Berger 2004b, p. 114).

New names: *Holosticha holomilnei* Berger, 2001 (p. 35) for *Holosticha (Holosticha) milnei* Kahl, 1932; *Holosticha vuxgracilis* (present book, p. 369, for *Holosticha gracilis* Vuxanovici 1963).

Corrected names: Urostylididae Dallas, 1851 (Insecta, Heteroptera) in Berger et al. (2001, p. 301).

Neotypifications: *Amphisiella annulata* (Kahl, 1932) Borror, 1972 in Berger (2004a, p. 13); *Anteholosticha intermedia* (Bergh, 1889) comb. nov. (present book, p. 317); *Pseudourostyla levis* Takahashi, 1973 (present book, p. 778); *Uroleptopsis citrina* Kahl, 1932 in Berger (2004a, p. 109).

New synonyms (including supposed synonyms): *Bakuella kreuzkampii* Song, Wilbert & Berger, 1992 is synonymous with *Bakuella agamalievi* Borror & Wicklow, 1983 (present book, p. 541); *Bakuella muensterlandii* Alekperov, 1992 is synonymous with *Bakuella agamalievi* Borror & Wicklow, 1983 (present book, p. 541); *Diaxonella trimarginata* Jankowski, 1979 is synonymous with *Diaxonella pseudorubra* (Kaltenbach, 1960) comb. nov. (present book, p. 463); *Holosticha corlissi* Fernandez-Galiano & Calvo, 1992 is synonymous with *Anteholosticha monilata* (Kahl, 1928) Berger, 2003 (present book, p. 314); *Holosticha (Keronopsis) muscorum* Kahl, 1932 is synonymous with *Anteholosticha intermedia* (Berger, 1889) comb. nov. (present book, p. 318); *Holosticha manca mononucleata* Gellért, 1956 is synonymous with *Anteholosticha plurinucleata* (Gellèrt, 1956) comb. nov. (present book, p. 400); *Holosticha nagasakiensis* Hu & Sudzuki, 2004 is synonymous with *Anteholosticha gracilis* (Kahl, 1932) Berger, 2003 (present book, p. 426); *Keronopsis macrostoma* Reuter, 1963 is synonymous with *Anteholosticha intermedia* (Berger, 1889) comb. nov. (present book, p. 317); *Keronopsis multiplex* Ozaki & Yagiu, 1943 is synonymous with *Uroleptopsis roscoviana* (Maupas, 1883) Kahl, 1932 (Berger 2004b, p. 111); *Oxytricha kessleri* Wrzesniowski, 1877 (and its synonyms) is synonymous with *Holosticha gibba* (Müller, 1786)

Wrzesniowski, 1877 (Berger 2003b, p. 376); *Oxytricha pernix* Wrzesniowski, 1877 is synonymous with *Holosticha pullaster* (Müller, 1773) Foissner, Blatterer, Berger & Kohmann, 1991 (present book, p. 146); *Periholosticha wilberti* Song, 1990 is synonymous with *Paragastrostyla lanceolata* Hemberger, 1985 (present book, p. 618); *Pseudokeronopsis trinesestra* Dragesco & Dragesco-Kernéis, 1991 is synonymous with *Diaxonella pseudorubra* (Kaltenbach, 1960) comb. nov. (present book, p. 463); *Trichototaxis rubra* Plückebaum, Winkelhaus & Hauser, 1997 is synonymous with *Diaxonella pseudorubra* (Kaltenbach, 1960) comb. nov. (present book, p. 463); *Urostyla algivora* Gellért & Tamás, 1958 is synonymous with *Pseudourostyla urostyla* (Claparède & Lachmann, 1858) Borror, 1972 (present book, p. 806); *Urostyla chlorelligera* Foissner, 1980 is synonymous with *Urostyla grandis* Ehrenberg, 1830 (present book, p. 1086); *Urostyla pseudomuscorum* Wang, 1940 is synonymous with *Pseudourostyla urostyla* (Claparède & Lachmann, 1858) Borror, 1972 (present book, p. 804).

7.4 Deposition of Slides

If mentioned in the individual papers, the site where the slide(s) (holotype; paratype; neotype; voucher) has (have) been deposited, is given in the corresponding entry of the list of synonyms. For a detailed list of type specimens deposited in the collection "diverse invertebrates" (except insects) of the Biology Centre Linz (Upper Austria), see Aescht (2003; also available at http://www.biologiezentrum.at). Slides used for original observations are also deposited in this collection.