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BIOINDICATION WITH PROTISTS IN THE ACTIVATED SLUDGE PROCESS: SOLUTION OF THE TAXONOMIC IMPEDIMENT

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Summary

Historically, one might recognize three principal periods in using protists as indicators in the activated sludge process. The Age of Discovery and Exploitation may be set between 1914 and 1950 when Arden & Lockett (1914) created the term “activated sludge” and several scientists recognized the importance of protists in the cleaning process. The Age of Bloom may be set between 1950 and 2000. It commenced with the revision of the saprobic system (Liebmann 1951, 1958) and the studies of Curds & Cockburn (1970a, b). Now the field developed rapidly because several protistological groups dealt with activated sludge and practical indices were developed classifying the performance of sewage plants (Madoni 1994). Since the turn of the century, we are in a Period of Decline mainly because most of the young biologists don't like identification and taxonomy of microscopic organisms although very useful identification literature is available (Berger & Foissner 2003) and shown in my lecture and in a practical demonstration. Further, I provide a Table showing what is indicated by certain organism species and communities.

Introduction

Historically, one might recognize three principal periods in using protists as indicators in waste water purification. The periods are connected with technical innovations in the water works and the increasing concern of the society about the heavy pollution of many rivers and lakes by organic and inorganic wastes in the industrialized countries.

The Age of Discovery and Exploitation may be set between 1914 and 1950 when Arden & Lockett (1914) created the term “activated sludge” and researchers recognized the importance of protists in cleaning the waste water during the activated sludge process (Barker 1942, 1943, Liebmann 1936).

The Age of Bloom may be set between 1950 and 2000. It started with the revision of the saprobic system by Liebmann (1951, 1958), who recognized the usefulness of protists as indicators of water pollution when combined with metazoans and some physico-chemical parameters. These and other data were used by Curds (1966) and Curds & Cockburn (1970a, b) to update bioindication in waste water treatments plants. Their faunistic and experimental studies lay the ground for a scientific treatment of the field. They showed which ciliate species dominate in sewage plants and demonstrated their significance for a clear effluent (Fig. 1).

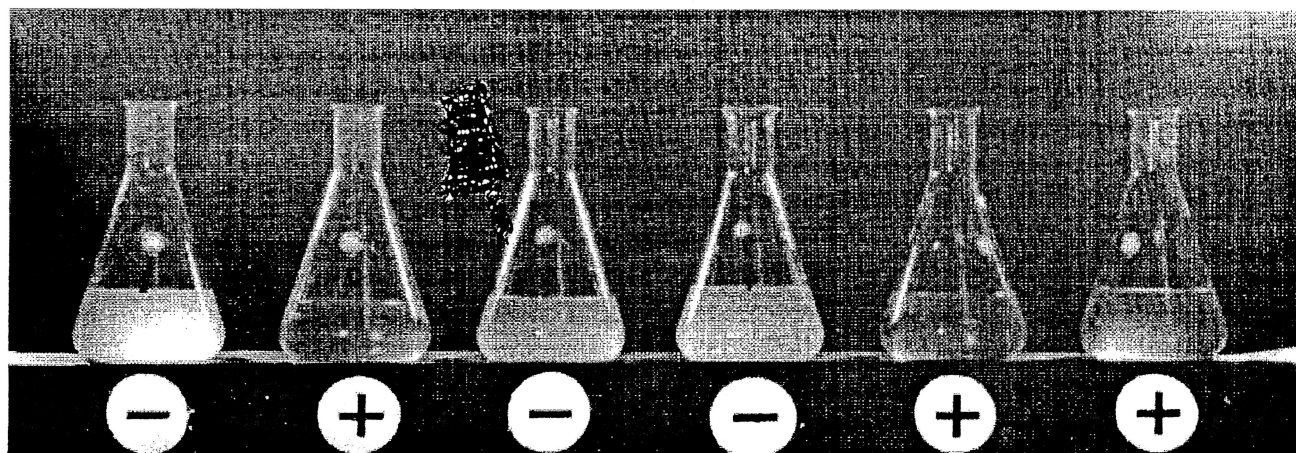


Fig. 6.3. Photograph of effluents issuing from laboratory-scale pilot plants operating in the presence (+) and absence (-) of ciliated protozoa (from Curds 1992)

The increasing concern of the society on river and lake pollution caused stronger government regulations and thus a massive increase in the number of sewage-treatment works. Concomitantly, problems with the performance of sewage plants increased, such as sludge bulking and poor effluent contaminating the rivers. This stimulated much protistological research on activated sludge, viz., by Curds in England, Fernández-Galiano in Spain, Bick in Germany, Madoni in Italy, Jenkins in the U.S.A., and my group in Austria. Soon, the number of students

and postdocs increased and hundreds of faunistic, ecological, and taxonomical papers were published and finally collected in excellent reviews: Berger & Foissner (2003), Buck & Buck (1980), Curds (1982, 1992), Eikelboom & van Buijsen (1992), Foissner & Berger (1996), Foissner *et al.* (1991, 1992, 1994, 1995, 1999), Ganner *et al.* (2002), Jenkins *et al.* (2004), Kinner (1984), Madoni (1991, 1994), Mudrak & Kunst (1994), Pauli *et al.* (2001), Schleypen & Gschlössl (1992), Scherb (1968), and Sládeček (1973).

During this period practical tools were developed, i. e., methods and indices classifying the performance of sewage plants (Al-Shahwani & Horan 1991, Curds & Cockburn 1970b, Madoni 1994, Schleypen & Gschlössl 1992) and specific identification keys were published (Berger & Foissner, 2003, Berger *et al.*, 1997, Fernández-Galiano *et al.* 1996, Foissner & Berger 1996, Foissner *et al.* 1991, 1992, 1994, 1995, 1999). Thus, all was prepared for a successful development of the field. But things turned out differently.

The Age of Decline commenced around the turn of the century and continues. It is characterized by a distinct decrease in the number of scientific papers and loss of taxonomic knowledge, partially due to a massive change in the society but also due to a saturation of the field. Presently, most students and young scientists are unwilling to identify microscopic organisms and to do taxonomic work – in spite of the good identification literature and unemployment – because it has low social reputation and of only vague interest in the matter. Indeed, I observed several times that colleagues who became employed in river pollution classification soon stopped identification work and allocated it to technical assistants!

Thus, my lecture has two goals: first, to show how simple it is for an academic biologist to identify the few organisms occurring frequently in activated sludge and second to provide a Table showing what is indicated by certain organismic communities and species.

Release from the taxonomic impediment

Whatever you want identify, plants, microscopic organisms, or beer mats, it needs good basic knowledge and some enthusiasm, as any work. The protists are a bit uncomfortable because they are so small that a microscope is needed. However, when you agree that a sewage biologist should be an academic then the person should be able to overcome this peculiarity, even in the time of molecular biology where classic morphology is usually strongly neglected in the university education. Of course, you need a good identification key, i. e., flow charts where the organisms are shown.

The electronic key you get is the most recent one and guides to 357 common ciliate species, of course including those frequently occurring in activated sludge. The guide, which is in English language and has 160 pages solves not only the taxonomic impediment but informs you on many other things, such as biomass and indicator value of the individual species as well as on ciliate morphology and terminology (Berger & Foissner 2003). This I shall show you now on 17 slides using peritrichs as an example because there are many misidentifications in the literature.

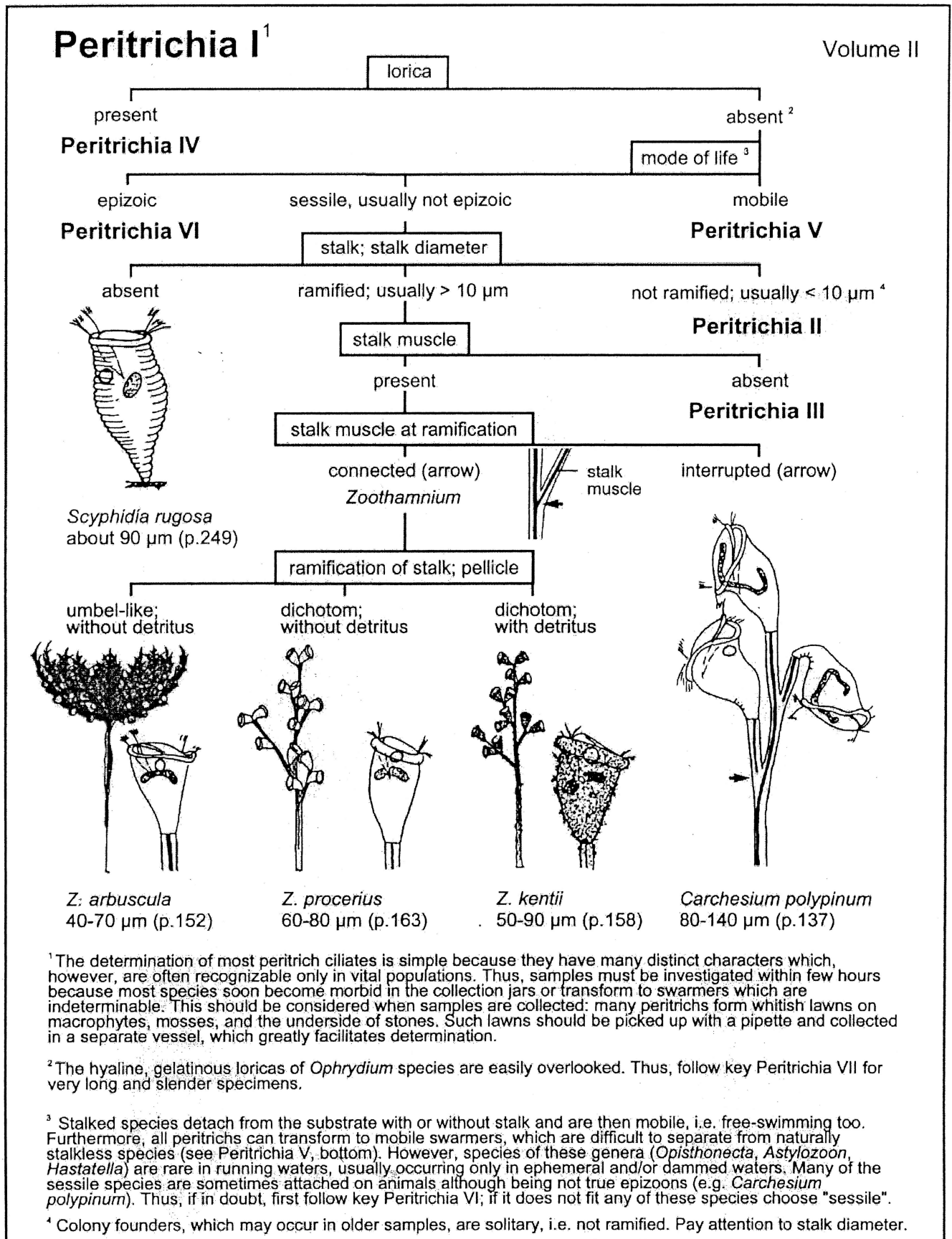
Possibly, I should mention that a reliable method for molecular identification of sewage ciliate species is not available but the first trial is promising (Marsh *et al.* 1998).

Sewage plant performance by bioindication

Although the compilation in Table 1 is far from being complete, it shows that sewage plant performance by bioindication is possible to a certain extent but it is not the philosopher's stone. It is a valuable, integrative method that gives information on plant performance some weeks before and after the investigation. I think progress is possible, especially when the identification of the organisms is improved and a combined view is applied, i. e., when the plant type, the structure of the flocs, the bacterial community, and the usual physico-chemical parameters are taken into account.

Fig. 1 Illustrated guide and ecological notes to ciliate indicator species (Protozoa, Ciliophora) in running waters, lakes, and sewage plants

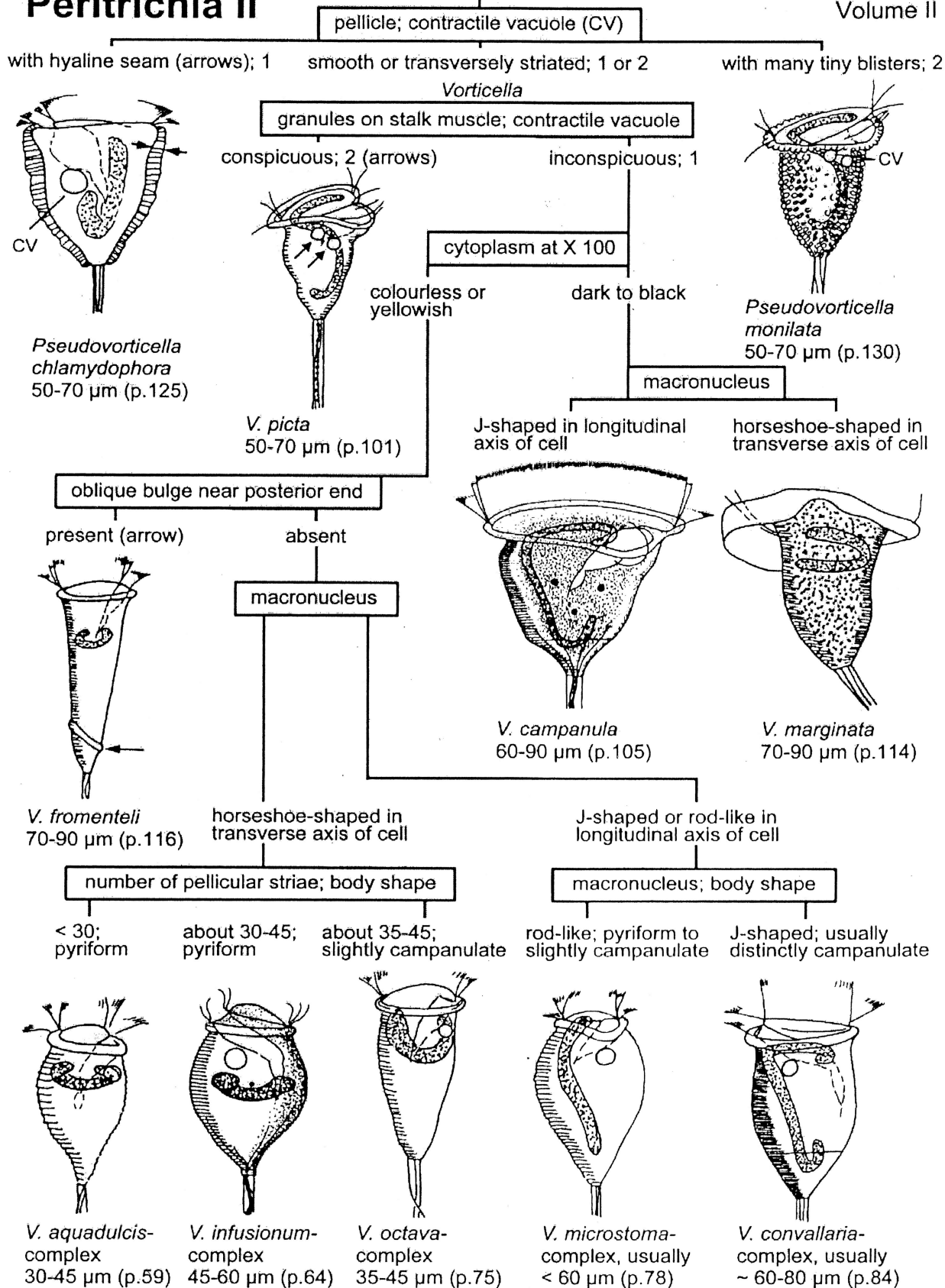
H. BERGER AND W. FOISSNER



Peritrichia II

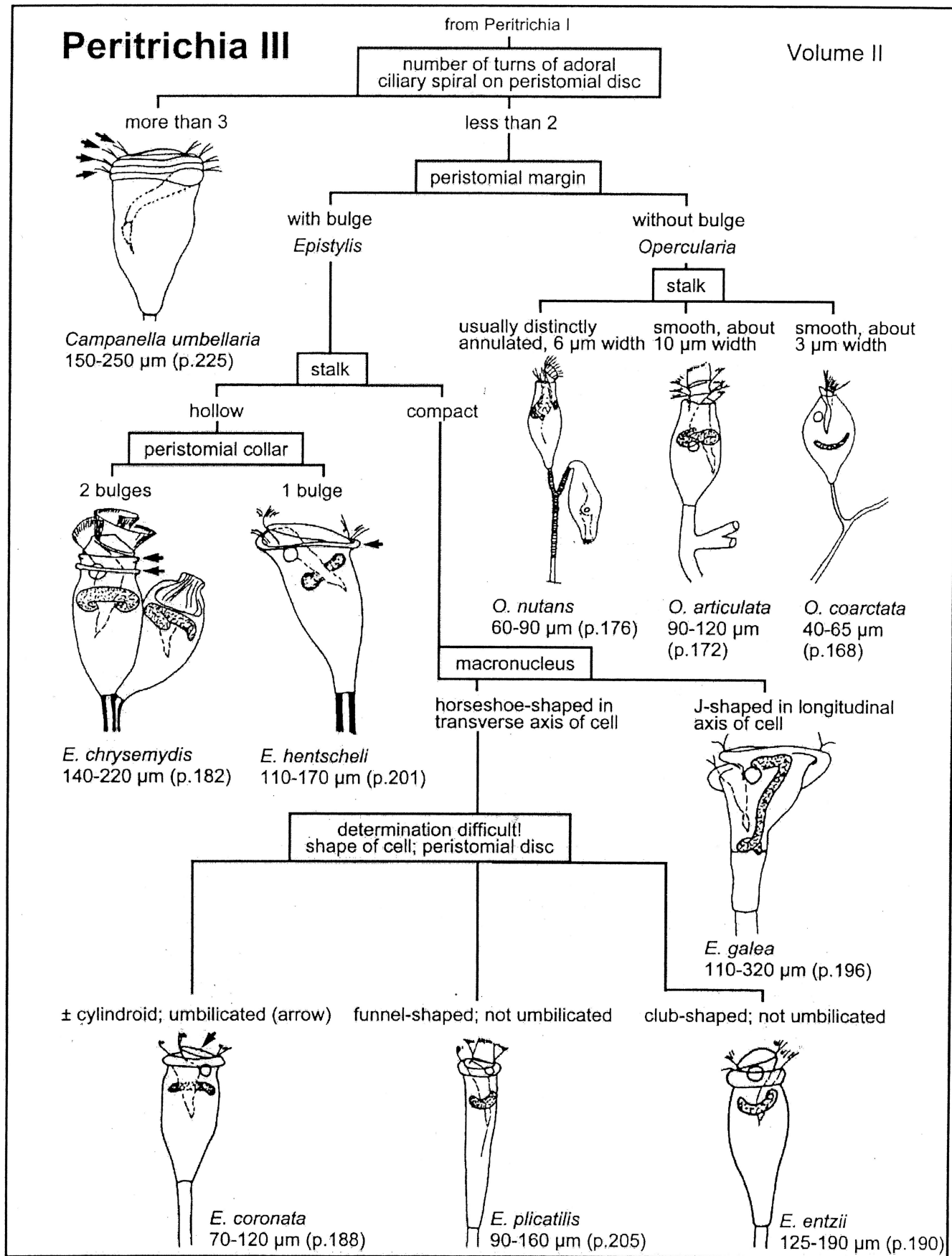
from Peritrichia I

Volume II



Peritrichia III

Volume II

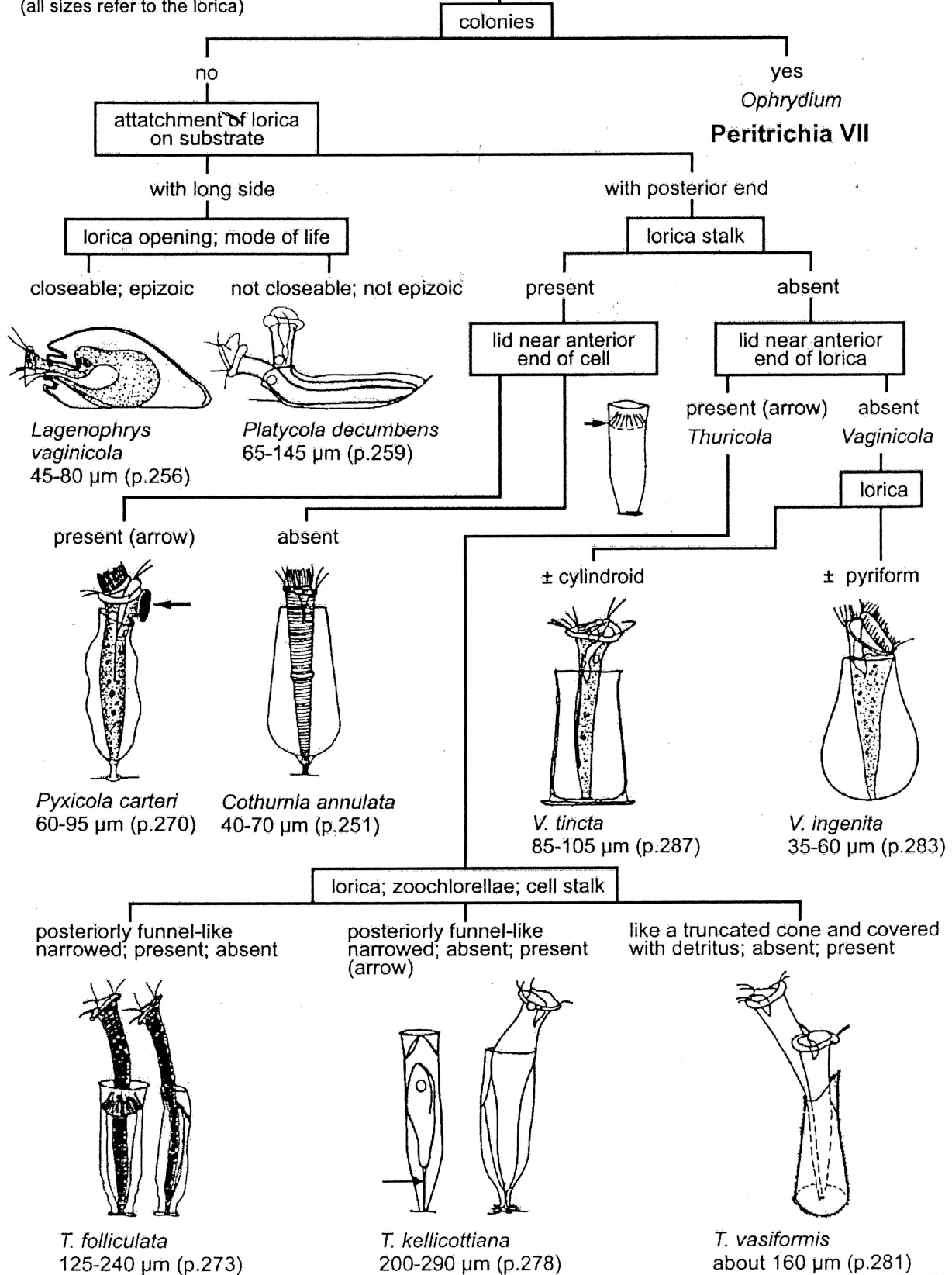


Peritrichia IV

(all sizes refer to the lorica)

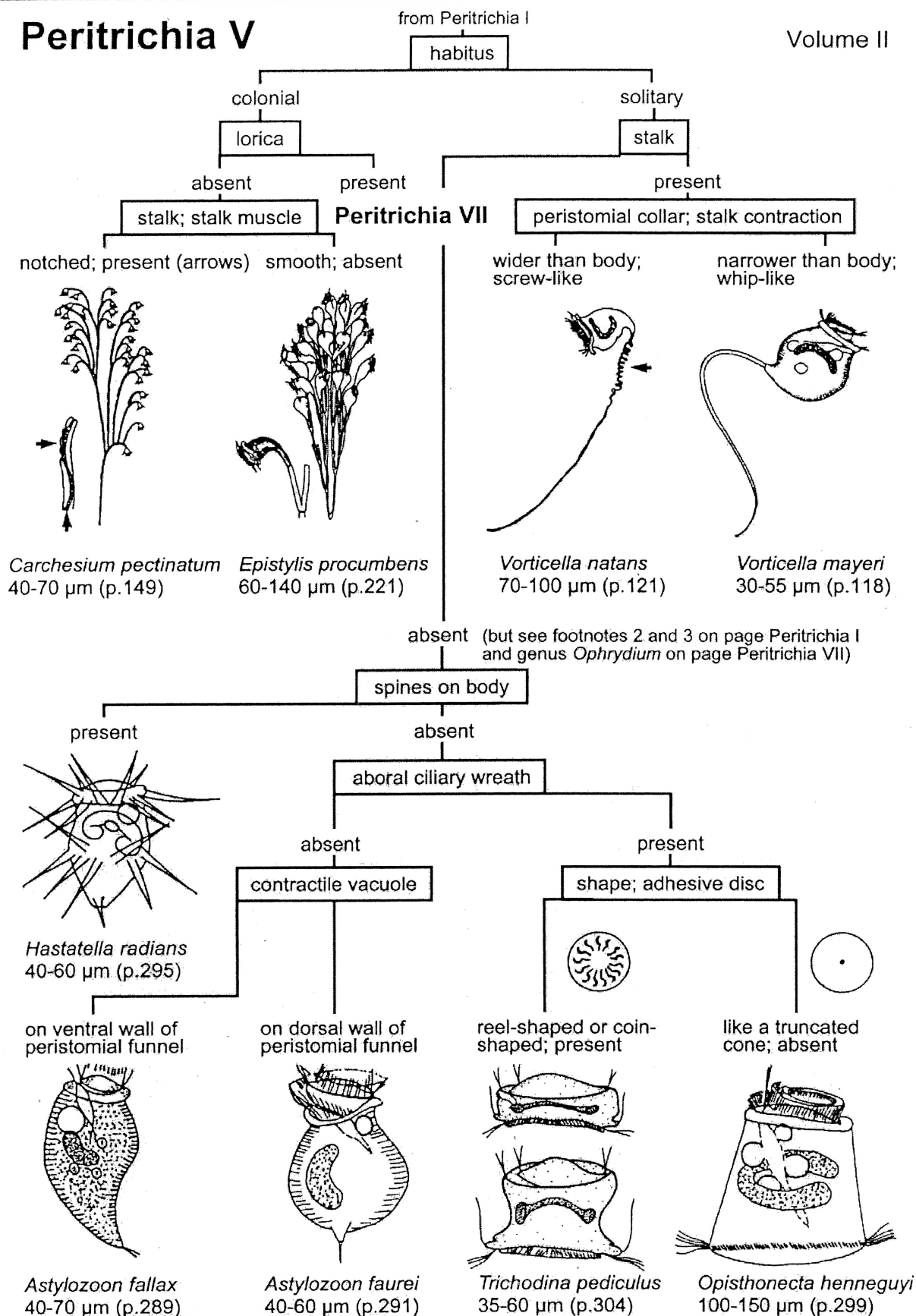
from Peritrichia I

Volume II



Peritrichia V

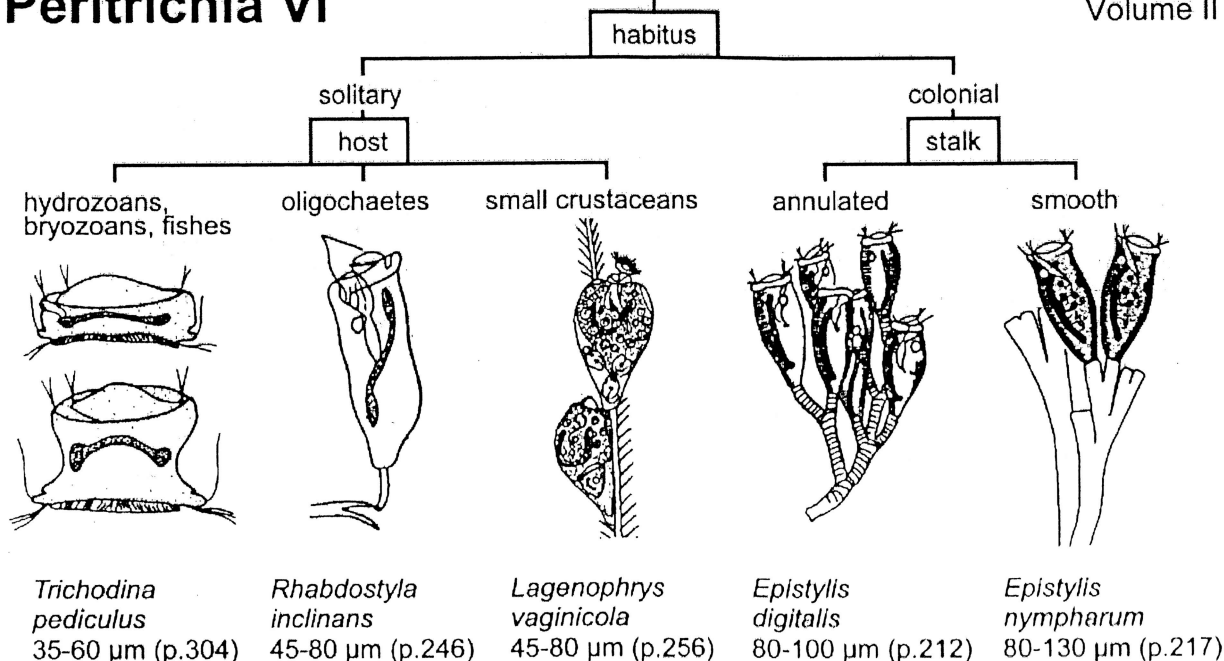
Volume II



Peritrichia VI

from Peritrichia I

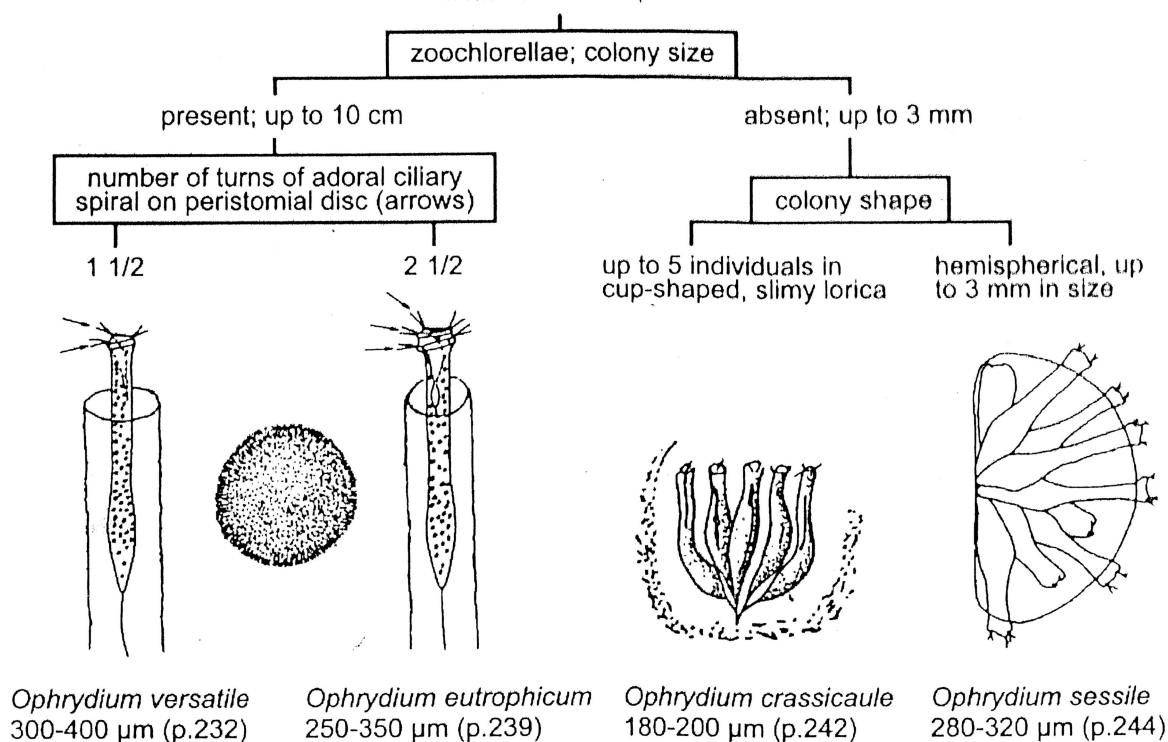
Volume II



Peritrichia VII¹

from Peritrichia IV, V

Volume II

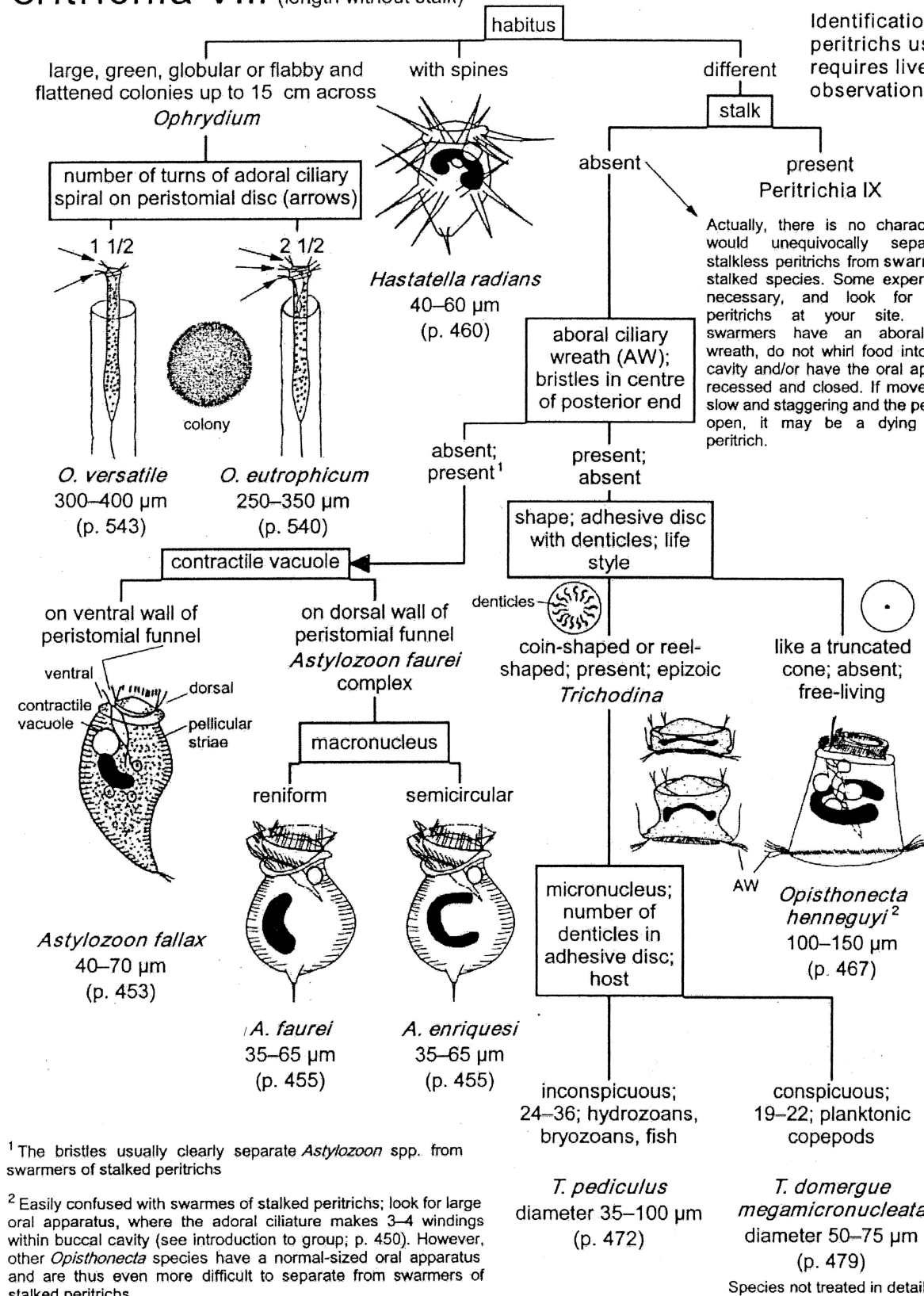


¹ Often only stalkless, loricaless solitary specimens in running waters and plankton; then difficult to separate from *Gerda* spp., which lacks a lorica!

Peritrichia VIII (length without stalk)

Plankton Ciliates

Identification of peritrichs usually requires live observation!



Peritrichia IX

(length without stalk)

Plankton Ciliates

from Peritrichia VIII

lorica

¹ Occasionally, epiplanktonic, colonial peritrichs detach with the stalk from the substrate. If in doubt, follow keys "Peritrichia X, XI"

present (usually hyaline or deserted and thus easily overlooked)

Ophrydium versatile or
O. eutrophicum (→ Peritrichia VIII)

There are rather many loricate epiphytoplanktonic peritrichs (*Vaginicola*, *Thunicola*, ...), which are poorly known and were thus excluded from the book (for determination, consult KAHL 1935, SOMMER 1951, STILLER 1940, 1971)

absent

mode of life

eiplanktonic, that is,
attached to other planktonic
organisms (or debris)
Peritrichia X

euplanktonic, that is,
not attached to other
plankton organisms or
debris¹

stalk

branched (colonial)

unbranched (solitary)

stalk; shape; zoochlorellae

shape; zoochlorellae;
stalk

contractile;
campanulate; absent

acontractile;
campanulate;
absent

acontractile;
vase-shaped (globular
when contracted);
present

vase-shaped;
present;
acontractile

campanulate or
pyriform; absent;
contractile

peristomial collar;
stalk contraction

wider than body;
screw-like (arrow)

narrower than
body; whipe-like

*Epicarchesium
pectinatum*
40–70 µm
(p. 508)

*Epistylis
procumbens*
60–140 µm
(p. 527)

*Ophrydium
naumanni*
solitary or in
small colonies
40–50 µm
(p. 551)

*Pelagovorticella
natans*
70–100 µm
(p. 482)

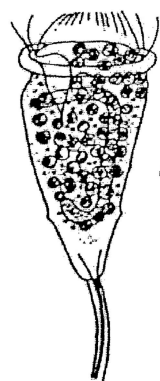
*Pelagovorticella
mayeri*
30–55 µm
(p. 480)

Peritrichia X

(length without stalk)

from Peritrichia IX

Plankton Ciliates



present
Vorticella chlorellata
44–64 μm
(p. 491)

unbranched (solitary)

branched (colonial)

Epistylis
Peritrichia XI

zoochlorellae

absent

stalk muscle

present

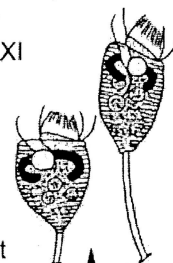
stalk contraction; stalk length

zigzag (sinuous);
usually $\leq$ body length
Pseudohaplocaulus
(usually attached to
Anabaena)

helical; usually $\geq$ body
length
Vorticella and
Pseudovorticella

absent

Epistylis pygmaeum
22–50 μm
epibiotic on planktonic
rotifers and crustaceans
(p. 535)
or *Rhabdostyla* species



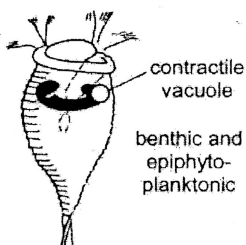
size; shape; surface; granules on stalk muscle

15–55 μm , usually
35 μm ; pyriform;
coarse transverse
striae; very
inconspicuous

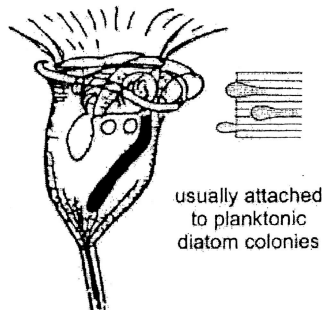
40–56 μm ; campanulate;
fine transverse striae, few
to numerous blisters;
inconspicuous

50–70 μm ;
campanulate; fine
transverse striae;
conspicuous

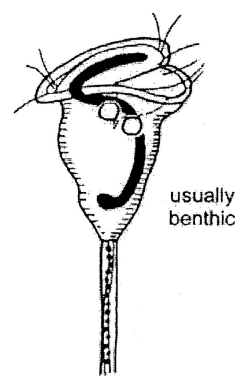
50–70 μm ;
campanulate; vesicular;
inconspicuous



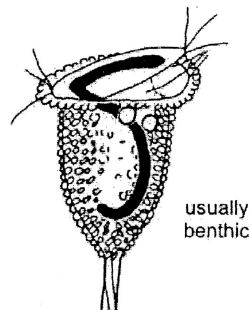
Vorticella aquadulcis complex
(p. 486)



Vorticella vernalis
(p. 494)



Vorticella picta



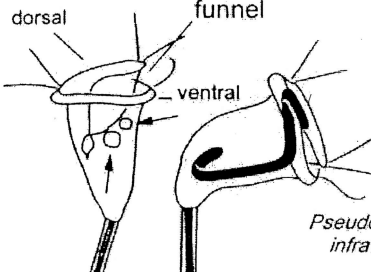
Pseudovorticella monilata

see Peritrichia II

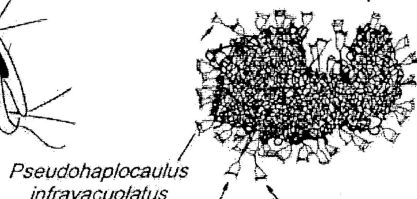
location of contractile vacuoles (arrows)

both on ventral wall of peristomial
funnel

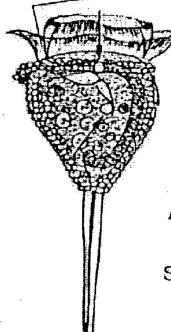
one on ventral, the other on dorsal wall of
peristomial funnel



P. infravacuolatus
47–67 μm
(p. 501)



Pseudohaplocaulus infravacuolatus
arrows mark *Vorticella chlorellata*

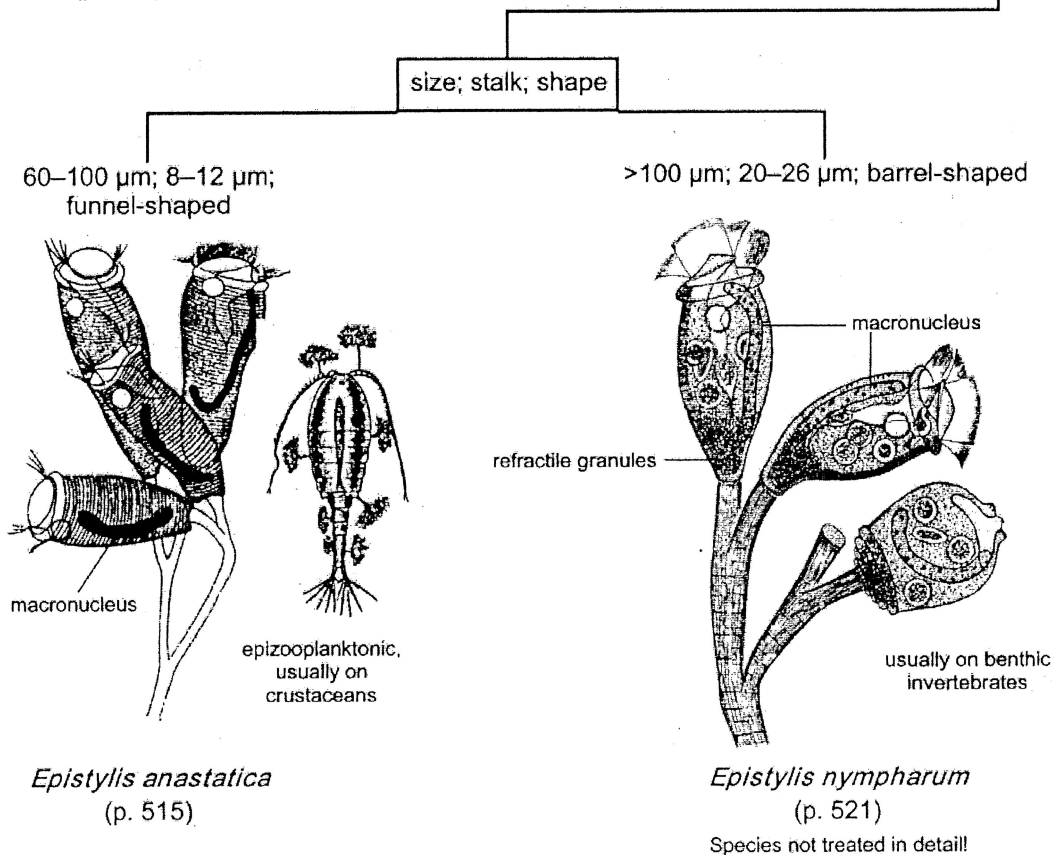
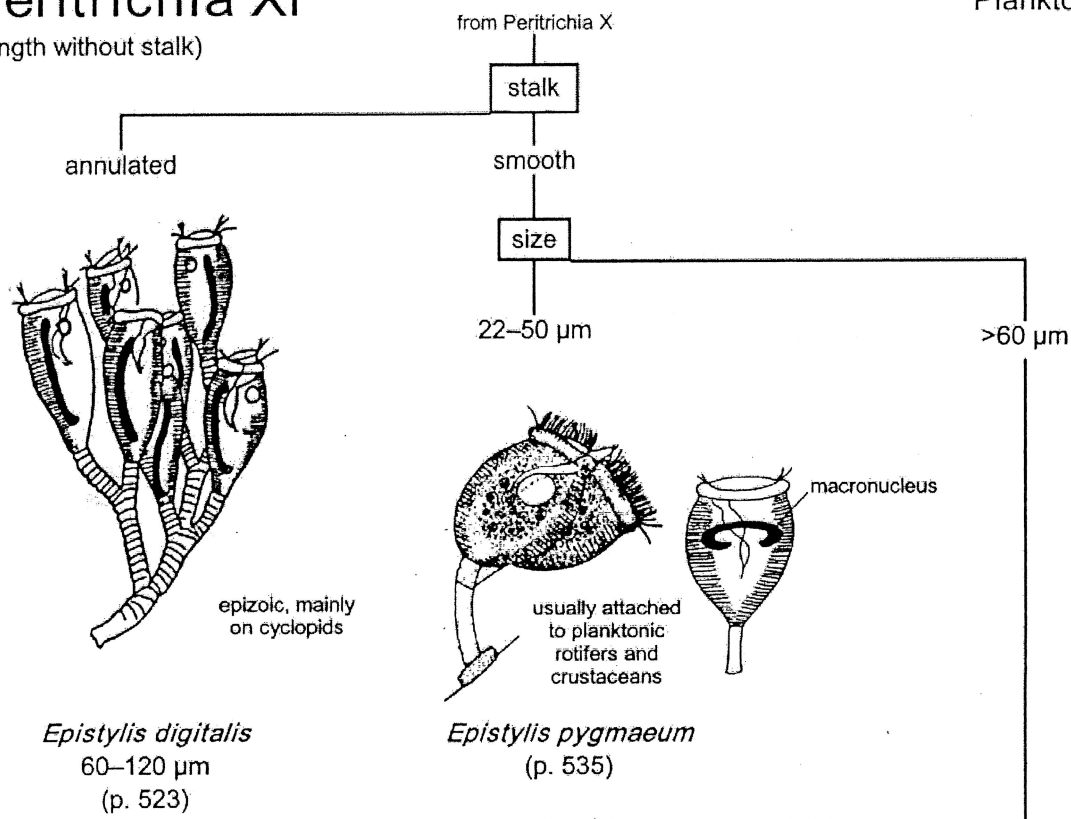


P. anabaenae
40–50 μm
Species not treated
in detail!

Peritrichia XI

(length without stalk)

Plankton Ciliates



paroral membrane: → undulating membrane.
 pellicle: "skin" of the ciliates, sensu lato identical with → cortex, sensu stricto with cell membrane and pellicular → alveoli.
 peristome: ± → oral apparatus.
 pharynx: throat; nonciliated tubular passeway leading from the → cytostome proper into the inner cytoplasm; typically, → food vacuoles are formed at its inner end; often strengthened by oral basket rods (nematodesmata, → oral basket) made of microtubules.
 postoral: behind (underneath) of → oral apparatus.
 preoral: in front (above) of → oral apparatus.
 protargol: silver-protein-compound (→ silver impregnation).
 reorganization band: special section in the → macronucleus of some ciliates (→ hypotrichs, → oligotrichs), involved in DNA replication and histone synthesis.
 replication band: → reorganization band.
 rhabdos: → oral basket.
 scopula: stalk forming and attachment → organelle at posterior pole of → peritrichs. The analogous structure of stalked → suctorians is named scopuloid.
 scutica: usually a small group of barren → basal bodies underneath the → oral apparatus; during → stomatogenesis, a part of the new → mouth is formed by this structure, which occurs only in the scuticociliates (for example, → *Histiobalantium*), a group of the → hymenostomes.
 silver impregnation: cytological methods where silver ions (e.g. → protargol, silver nitrate) deposit onto argyrophilic sites (especially the → basal bodies) and which are then visible in the light microscope. For a detailed description of these methods, see FOISSNER et al. (1999).
 silverline system: striated or reticulate cortical structure, which can be stained (impregnated) with silver nitrate (→ silver impregnation); usually composed of → fibres, and possibly involved in → morphogenetic processes and/or conduction of stimuli.
 somatic: belonging to the body; as opposed to oral.
 stomatogenesis: formation of the new → oral apparatus during cell division (→ morphogenesis).
 suture: the linear space left between the ends of converging ciliary rows (for example, in front of and behind the → oral apparatus of → *Frontonia*).
 swarmer: freely motile disperse stage in the life cycle of a number of sessile ciliates, for example, of → peritrichs and → suctorians.
 symbiotic algae: single-celled algae living symbiotic in the ciliate cytoplasm (→ zoochlorellae).
 toxicysts: → extrusomes filled with poison and used for capture of prey.
 trichocysts: needle-shaped → extrusomes used for defence.
 type species: species on which a genus is based; the sole species which cannot be removed from the genus.
 undulating membrane (paroral membrane): one or several rows of narrowly spaced cilia, which usually adhere together at the right margin of the → oral apparatus; see also → endoral membrane.
 vacuole: vesicle; → contractile vacuole and → food vacuole.
 vestibulum: → buccal cavity.
 zoochlorellae: → symbiotic algae of the genus *Chlorella*.
 zooids: the individuals of → peritrichs with branched stalk.

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13 Sytematic Index

The index contains all scientific names mentioned in the flow charts. It is two-sided, that is, species appear both with the generic name first (if one knows only the genus name) and, more importantly, with the species-group name first (if one knows the species-group name but not the newest generic combination). Furthermore, all pages where a certain species is mentioned are indexed, which provides some sort of cross-referencing showing where the same species may be separately arrived at. Generic (for example, *Carchesium*) and species names (for example, *Carchesium polypinum*) appear in *italics*; suprageneric taxa (main groups, for example, Peritrichia) are given in boldface; communities (for example, Carchesium polypinae) are written in ordinary roman type.

A

acanthocryptum, *Ctedoctema* 50, 89, 131
acarus, *Mesodinium* 74, 78
Acineria 56, 128
Acineria incurvata 117, 139
Acineria uncinata 117, 138
Acineta 37, 125
Acineta flava 125
Acineta grandis 125
Acineta tuberosa 125
acrostomia, *Askenasia* 79
Actinobolina 8, 20, 25, 30, 41, 54
Actinobolina radians 68, 74, 78
Actinobolina smalli 61, 66, 78
Actinobolina vorax 68, 74, 78
Actinobolina wenrichii 66, 67, 78
aculeata, *Stichotricha* 91
acuminata, *Frontonia* 84, 87, 131
acuminata, *Lagynophrya* 18, 19, 30, 44, 54, 76, 77
acuminatus, *Tropidoatractus* 47, 82
aediculatus, *Euplotes* 92
affine, *Gonostomum* 135
affinis, *Euplotes* 47, 92, 129, 131, 149
affinis, *Euplotopsis* 149
africana, *Teuthophrys trisulca* 77
agilis, *Pseudomicrothorax* 37, 47, 49, 100
agilis, *Urotricha* 41, 119, 122
algivora, *Pseudochilodonopsis* 32, 73
alpestris, *Chlamydonella* 52, 73
alpestris, *Litonotus* 117, 131, 133
alpestris, *Odontochlamys* 42, 73, 133
alveolata, *Pelagothrix* 120
alveolatum, *Monodinium* 79
ambiguum, *Spirostomum* 81, 129

8 Ecological summary of the species contained (Table)

Table 1 is a compilation of tables contained in FOISSNER et al. (1995, 1999). It orientates about live biomass (and thus much more useful than many literature data based on pre-

served and stored specimens), food (many species likely have a broader range), salinity tolerance, preferred water and habitat type, community allocation, and saprobic classification (re-assessed in the monographs cited and thus sometimes different from that contained in the key works by SLÁDEČEK (1973) and SLÁDEČEK et al. (1981).

Table 1: Ecological characterization of the species described in the Ciliate Atlas (FOISSNER et al. 1991, 1992, 1994, 1995) and the plankton book (FOISSNER et al. 1999). a = alphamesosaprobic, A = Aufwuchs (periphyton), Al = algae (except of diatoms, but inclusive autotrophic flagellates), b = betamesosaprobic, B = benthal, Ba = bacteria, Bo = terrestrial (mostly wetland) soils, BOD = influence of soil and/or moss, C = ciliates, CAR = Carchesietosum polypinae, COL = Colpidietum colpodae, Cy = cyanobacteria, CYR = Cyrtophoretea, e = euryaprobic, EP = eupelagic, ECP = epiplanktonic on cyanobacteria, EPP = epiphytoplanktonic, EZB = epizobenthic, EZP = epizooplanktonic, F = flowing waters, Fl = heterotrophic flagellates, Fs = anaerobic mud (and anaerobic zones in the pelagial), h = histophagous, HBE = high-load and/or oxygen deficient activated sludge, he = holo-euryhaline, i = isosaprobic, K = sewage-treatment works (activated sludge plants), Ki = diatoms, m = metasaprobic, M = mixotrophic, that is, autotrophic (due to symbiotic algae or sequestered chloroplasts) and heterotrophic, MAR = Marynetum, MET = Metopetum, MOO = mire influence, mpe = meso- to poly-euryhaline, mps = meso- to poly-stenohaline, NBE = normal activated sludge, O = omnivorous (feeds on autotrophic and heterotrophic protists, sometimes even on small metazoans), o = oligosaprobic, oe = oligo-euryhaline, OLI = Oligotrichetea (lake influence), ome = oligo- to meso-euryhaline, oms = oligo- to meso-stenohaline, os = oligo-stenohaline, p = polysaprobic, P = pelagial, pe = poly-euryhaline, PLE = Pleuronemetum coronatae, ps = poly-stenohaline, R = predacious (feeds on protozoa, mostly ciliates, some species even ingest small metazoans), Ro = rotifera, S = stagnant waters, Sb = sulphur bacteria, STE = Stentoretum, T = epizoic, TRI = Trithigmostometum cucullulae, x = xenosaprobic.

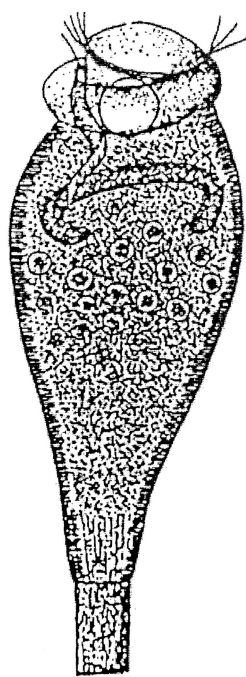
Species	Biomass (mg) of 10 ⁴ ind. ^(a)	Main food	Salinity tolerance ^(b)	Occurrence			
				Preferred water type	Preferred habitat	Community ^(c)	Saprobity ^(d)
<i>Acinertia incurvata</i>	55	R	he	F, S, K	A, B	COL, HBE	p-i
<i>Acinertia uncinata</i>	10	R	os	F, S, K	A, B	COL, NBE	a-p
<i>Acineta flava</i>	30	R	oe?	F, S	A, T		b
<i>Acineta grandis</i>	150	R	oe?	F, S	A, T		b-o
<i>Acineta tuberosa</i>	20	R	he	S, F, K	A, T		a-b
<i>Actinobolina radians</i>	125	R	oe?	S, F	P, A, B	OLI	b
<i>Actinobolina smalli</i>	11	M, R	os	S	P, B	OLI	
<i>Actinobolina vorax</i>	250	R	oms?	S	EP, Bo	OLI	o
<i>Actinobolina wenrichii</i>	90	M, R	os	S	EP?	OLI	
<i>Amphileptus carchesii</i>	200	R	os	S, F	A	CAR	a
<i>Amphileptus clapedii</i>	60	R	he?	S, F	A	CAR	a
<i>Amphileptus pleurosigma</i>	150	R	oms	S, F	A, B	STE	b-a
<i>Amphileptus procerus</i>	160-1500	R	os	S, F	B		b-a
<i>Amphileptus punctatus</i>	80	R	os	S, F	A, B		a
<i>Askenasia acrostomia</i>	32	Al, C	os	S	EP	OLI	
<i>Askenasia chlorelligera</i>	20	M	os	S	EP	OLI	
<i>Askenasia volvox</i>	35	Al, Ki	oe?	S, F	P, B	OLI	b
<i>Aspidisca cicada</i>	10	Ba	he?	F, S, K	B, A	TRI, CYR, NBE	a-b
<i>Aspidisca lynceus</i>	17	Ba	ome?	F, S, K	B, A	TRI, CYR, NBE	b-a
<i>Aspidisca turrita</i>	7	Ba	he	F, S, K	B, A	NBE	a-b
<i>Astylozoon fallax</i>	30	Ba	os	S	EP	MAR, OLI	b-a
<i>Astylozoon faurei</i>	50	Ba	oms?	S, F	EP	MAR, OLI	b-a
<i>Astylozoon faurei-complex</i>	50	Ba	oms?	S, F	EP	MAR, OLI	b-a
<i>Balanion planctonicum</i>	0.3-3.6	Al, Ba	os	S	EP	OLI	o
<i>Balantidium pellucidum</i>	60	O	os	S	EP	OLI	
<i>Belonophrya pelagica</i>	11	Ro	os	S	EP	OLI	
<i>Blepharisma coeruleum</i>	250	Al (O)	os	S, F	B		b
<i>Blepharisma lateritium</i>	250	Ba, Al	os	S	B, P		b

Table 1. Microfaunal species and communities as indicators of sludge plant performance.

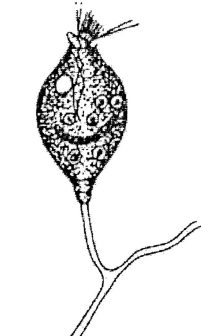
Organisms	Performance	Remarks	Literature ^{a)}
Species (usually when subdominant at least)			
<i>Aspidisca cicada</i> (Fig. 2)	Good	Stable plant conditions	1
<i>Coleps hirtus</i>	Good	Effective nitrification with good removal of ammoniacal-N in effluent	1
<i>Enchelyomorpha vermicularis</i> (Fig. 3)	Poor	Microaerobic; overloading; hydraulic problems	2, 3
<i>Euplotes patella</i> (Fig. 2)	Mediocre	When abundant and in connection with many rotifers indicative for an increasing sludge volume index; otherwise underload	2, 6
<i>Holophrya discolor</i>	Mediocre	Microaerobic; intermittent and very low oxygenation; high N-reduction	4
<i>Litonotus lamella</i>	Poor	Deficient sludge setting	
<i>Litonotus obtusus</i>	Poor	Poor sludge setting	16
<i>Metopus</i> spp. (Fig. 4)	Poor	Anaerobic conditions; overloading; hydraulic problems	1, 2, 5, 9
<i>Plagiocampa rouxi</i>	Mediocre	Microaerobic; intermittent and very low oxygenation; high N-reduction	4
<i>Spirostomum teres</i> (Fig. 3)	Mediocre	Microaerobic; intermittent and very low oxygenation; high N-reduction	4
<i>Trimyema compressum</i> (Fig. 3)	Poor	Microaerobic; overloading; hydraulic problems	2, 3
<i>Vorticella campanula</i>	Good	High effluent quality; underload	2, 10
<i>Vorticella convallaria</i> (Fig. 2)	Mediocre	Lack of nitrification	1
<i>Vorticella convallaria</i> and <i>Arcella hemisphaerica</i>	Good	High sludge retention time; underload	2, 16, 17
<i>Vorticella microstoma/infusioformis</i> (Fig. 3) and <i>Opercularia</i> sp. (Fig. 3)	Poor	Low clearing efficiency, especially when connected with high flagellate abundance; anaerobic; high sludge load and sludge volume index	7, 8, 9, 17
<i>Vorticella striata</i>	Poor	Poor effluent quality	1
Communities (when dominant or subdominant)			
Small flagellates	Poor	Oxygen depletion; overloading; sludge maturation period; onset of nitrification	8, 18
Small naked amoebae and flagellates	Poor	Very high load; not easily degradable material; sludge maturation	8
Small flagellates, naked amoebae, swimmers of peritrich ciliates; many dispersed bacteria	Poor	Unstable sludge; sludge maturation; toxic influences	2, 9
Testate amoeba	Good	Underloading, high sludge retention time; usually found in N-removal plants	8
Testate amoebae; crawling ciliates; attached peritrich ciliates with width peristome; nematods; rotifers (Fig. 2)	Good	Healthy, low-loaded, sufficiently aerated and well-flocculated sludge with high effluent quality	12
<i>Glaucoma</i> , <i>Dexiostoma campylum</i> (Fig. 3), <i>Vorticella microstoma</i> and peritrich swimmers, flagellates and naked amoebae	Poor	Insufficient oxygenation; many dispersed bacteria; poor effluent	2, 9
<i>Vorticella infusioformis</i> (Fig. 3); <i>Opercularia coarctata</i> ; <i>Acineria uncinata</i> (Fig. 2); small flagellates	Poor	High-loaded with insufficient oxygen; shock-load; high ammonia; many dispersed bacteria	12
Heterotrich ciliates and many flagellates	Poor	Poor operation of RBC system	5
<i>Epistylis</i> , large naked amoebae, rotifers	Good	When in last stage of RBC system	13

continued

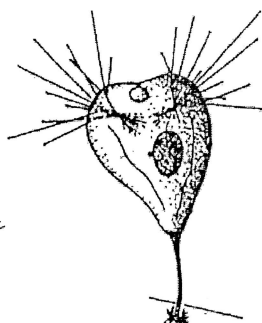
HEALTHY ACTIVATED SLUDGE



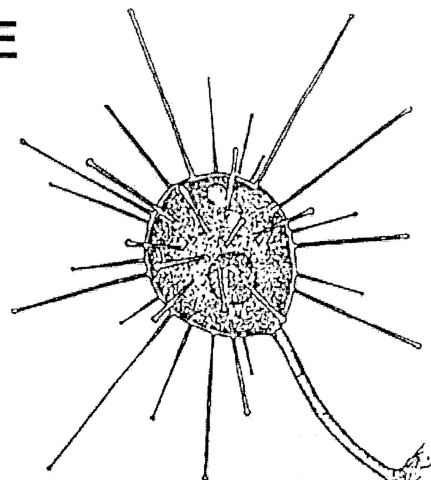
Epistylis spp.



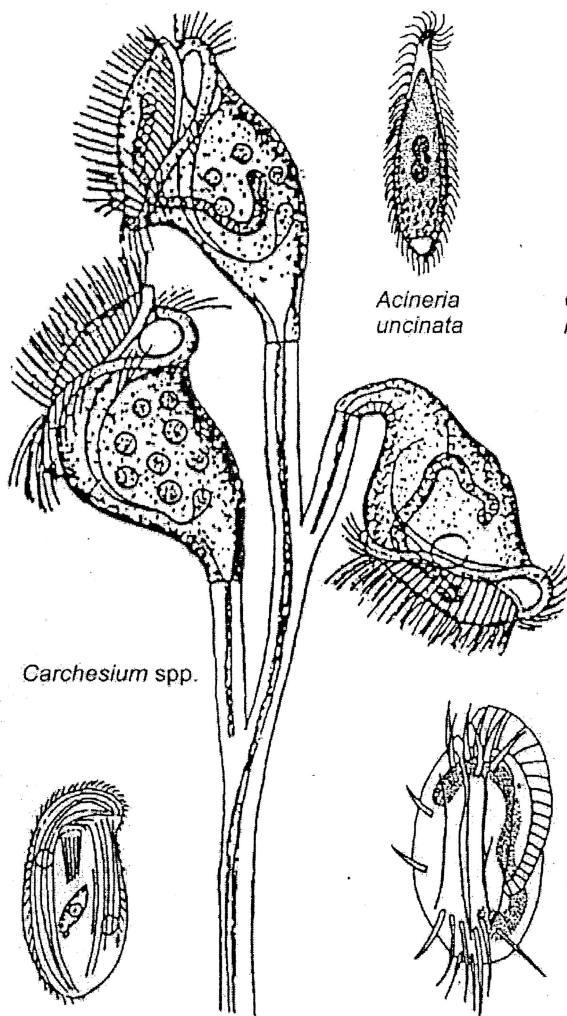
Opercularia spp.



Tokophrya spp.



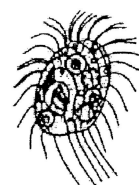
Prodiscophrya collini
Podophrya spp.



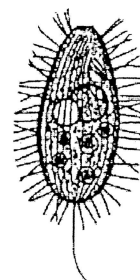
Carchesium spp.



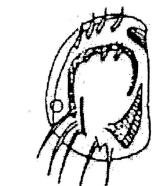
Acineria
uncinata



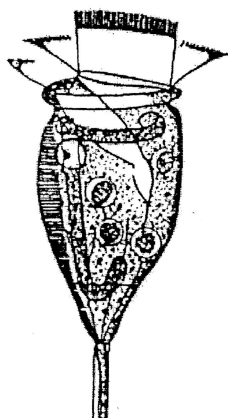
Cinetochilum
margaritaceum



Dextiotricha spp.



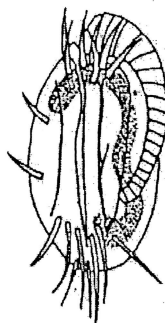
Aspidisca spp.



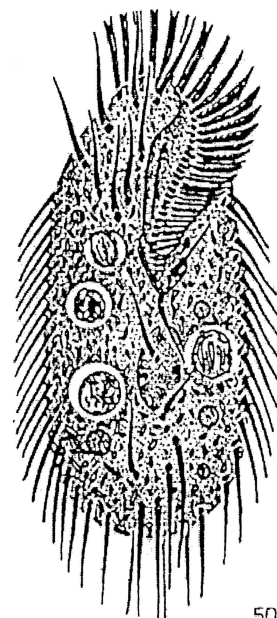
Vorticella
convallaria



Chilodonella
uncinata



Euplotes spp.

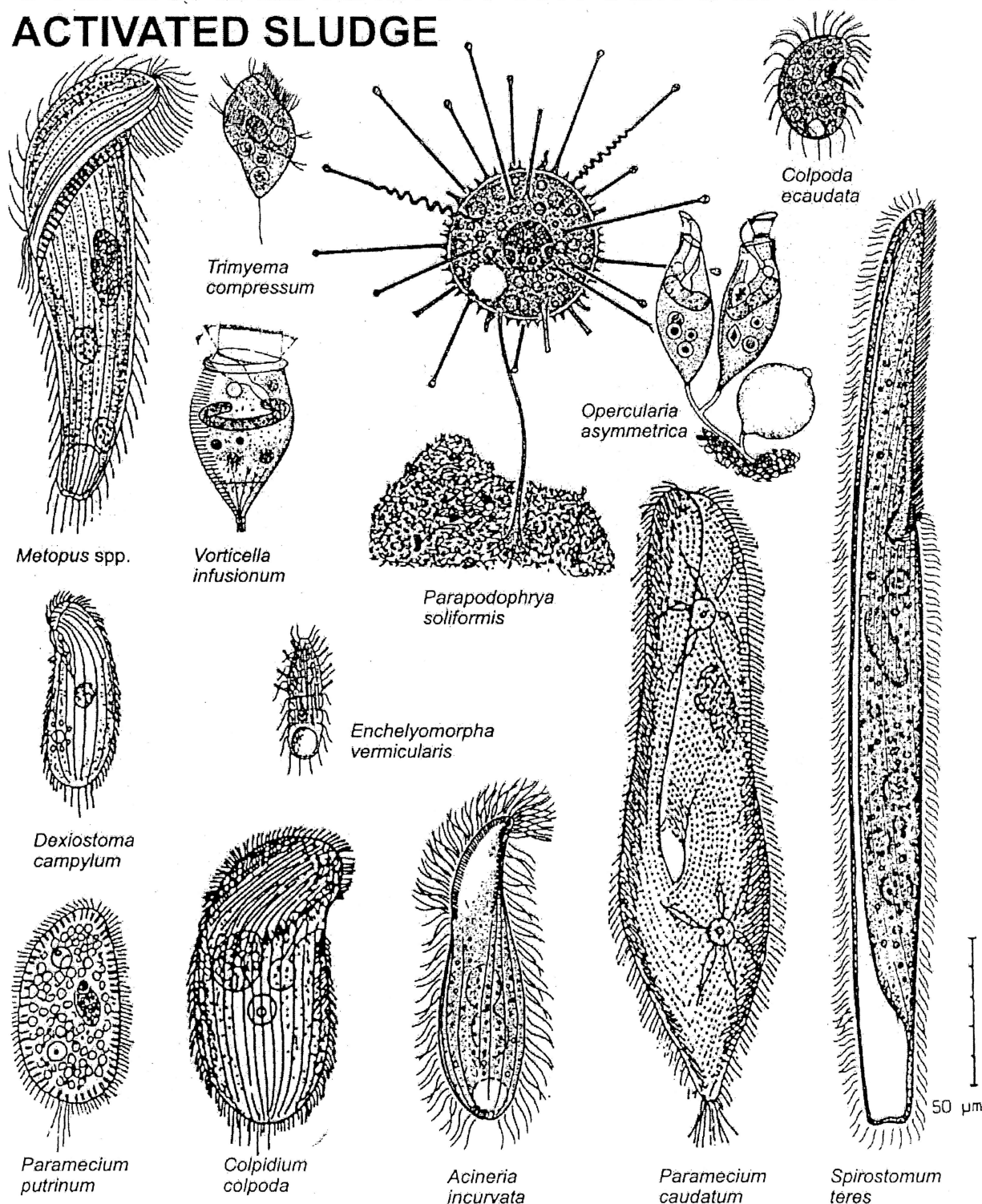


Sterkiella
histriomuscorum

50 μ m

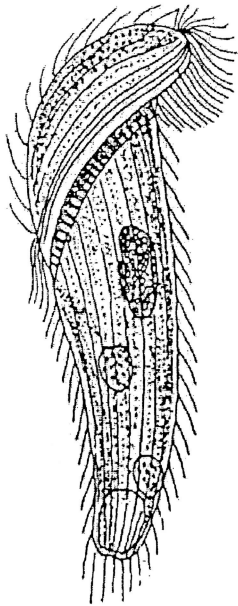
Ciliate community of healthy ("normal") activated sludge. An assortment of species usually occurring in moderately and heavily polluted (alpha-mesosaprobic to beta-mesosaprobic, alpha-mesosaprobic) running waters is found in "normal" activated sludge. The species of this community indicate sufficient oxygen supply and appropriate load. Often, ciliates achieve high abundances (> 10000 individuals / ml) and feed on bacteria, thereby reducing the turbidity of the effluent (Curds 1992). See Schleyen & Gschlössl (1992) for detailed advice on activated sludge investigation. Scale bar division 10 μ m.

OVERLOADED AND/OR OXYGEN DEFICIENT ACTIVATED SLUDGE

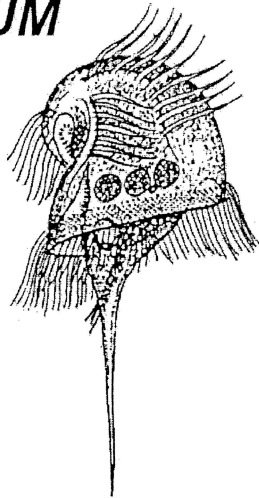


Ciliate community of overloaded and/or oxygen deficient activated sludge. An assortment of species usually occurring in heavily and very heavily polluted (alpha-mesosaprobic to polysaprobic, polysaprobic) running waters is found in overloaded and/or oxygen deficient activated sludge. The species of this community indicate insufficient oxygen supply (*Vorticella infusionum*-complex, *Dexiostoma*), anaerobic conditions (e.g., *Metopus*, *Trimyema*) or overload (e.g., *Colpidium*, *Dexiostoma*, *Paramecium*). The effluent is often turbid because free bacteria are insufficiently eliminated. See Schleyen & Gschlössl (1992) for detailed advice on activated sludge investigation. Scale bar division 10 µm.

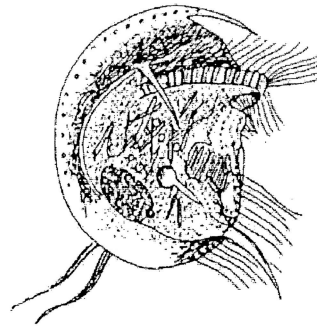
METOPETUM



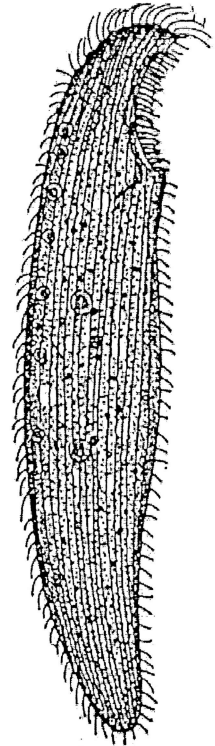
Metopus spp.



Caenomorpha spp.



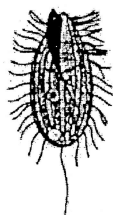
Discomorphella
pectinata



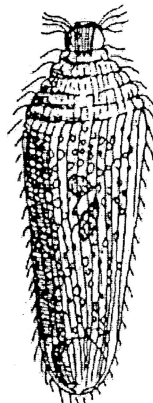
Loxodes spp.



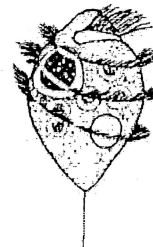
Enchelyomorpha
vermicularis



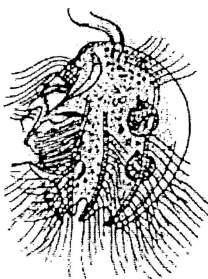
Pseudocohnilembus
pusillus



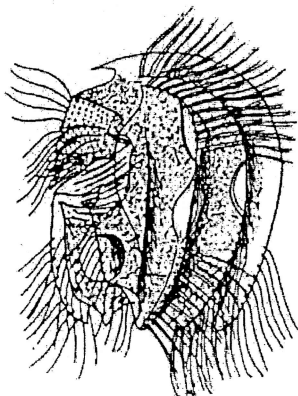
Lagynus
elegans



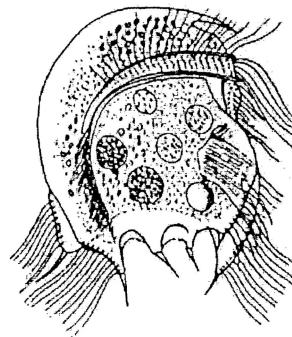
Trimyema
compressum



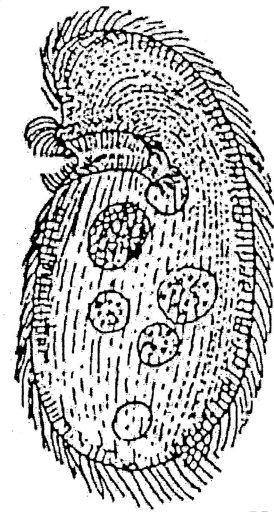
Pelodinium
reniforme



Epalxella spp.



Saprodinium spp.



Plagiopyla
nasuta

50 µm

Ciliate community of anaerobic mud (*Metopetum*). Indicator species are members of the genus *Metopus* (s.l.) and certain heterotrichs (all described in Vol. II). Most of the species belonging to the *Metopetum* are strictly bound to anaerobic conditions, i.e. oxygen is poisonous for them; they do not have mitochondria but hydrogenosomes and tolerate the richly occurring H_2S without damage. This community is often poor in species and individuals and feeds mainly on (sulphur) bacteria. The occurrence of one or several of these species in a sample is an unfailing indication of microaerobic or anaerobic conditions. Scale bar division 10 µm.

Table 1. (continued)

Organisms	Performance	Remarks	Literature ^{a)}
Green algae on plant wall	Good	Underload since a long time	9
Small swimming ciliates	Mediocre	Too short sewage retention time; insufficient oxygenation	8
Large swimming ciliates (Fig. 3)	Mediocre	Overloading; insufficient oxygenation	8
Crawling ciliates (abundance > 2000/ml)	Good	Sludge volume index < 200	8
Sessile and crawling ciliates	Good		8
Crawling and attached ciliates	Good	High ratio indicates good effluent	15
Sessile ciliates	Decreasing	Transient phenomena, such as recent sludge extraction, discontinuous load	8
Sessile ciliates	Good		1
Ciliates	Good	When abundance is 10 ⁶ /l or more	8
Ciliates	—	Abundance < 10 ⁴ (poor), 10 ⁴ –10 ⁶ (mediocre), > 10 ⁶ (good)	11, 17
<i>Metopetum</i> (Fig. 4)	Poor	Anaerobic conditions; overload; hydraulic problems; putrefaction	2, 9
Swimming and attached ciliates	Mediocre	When highly diverse indicative for stable sludge but insufficient effluent quality	2, 9
Swimming ciliates	Mediocre	Often dominate in plants with short retention time; effluent mediocre; disappear after pH-shock	1, 18
<i>Vorticella microstoma</i> and <i>V. campanula</i>	Good	Well-setting sludge	10
Cyrtophorids, hypotrichs, scuticociliates, pleurostomatids (Fig. 2)	Good	Good operation of RBC system (Rotation Biological Contactor)	5
<i>Opercularia</i> , <i>Uronema</i> , nematods	Poor	Indicate overloading when in last stage of RBC system	13
Carnivorous ciliates, e. g., <i>Litonotus lamella</i> , <i>Amphileptus</i>	Poor	Poor-setting sludge	10
<i>Aspidisca cicada</i> , <i>Chilodonella</i> spp., <i>Vorticella striata</i> (Fig. 2)	—	High sludge retention time	10
<i>Epistylis plicatilis</i> and <i>Vorticella striata</i>	Decreasing	Indicate beginning sludge bulking when their abundances distinctly increase; high sludge volume index (SVI)	14

^{a)} 1 = MARTIN-CERECEDA et al. (1996), 2 = FOISSNER et al. (1995), 3 = PEREZ-UZ et al. (1998), 4 = GANNER et al. (2002), 5 = MARTIN-CERECEDA et al. (2001), 6 = CINGOLANI et al. (1991), 7 = GORI et al. (1991), 8 = MADONI (1994), 9 = SCHLEYPEN & GSCHLÖSSL (1992), 10 = LEE et al. (2004), 11 = DE MARCO et al. (1991), 12 = DRZEWICKI & KULIKOWSKA (2011); 13 = BERRI & CASASCHI (1991), 14 = HU et al. (2013); 15 = BEDOGNI et al. (1991), 16 = ZHOU et al. (2006), 17 = TOMAN (2002); 18 = CYBIS & HORAN (1997).

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