Protist, Vol. 164, 842–860, xx 2013
http://www.elsevier.de/protis
Published online date xxx

Protist

PROTIST TOOLS

A Short Guide to Common Heterotrophic Flagellates of Freshwater Habitats Based on the Morphology of Living Organisms

Alexandra Jeuck, and Hartmut Arndt[1]

Department of General Ecology, Zoological Institute, Cologne Biocenter, University of Cologne, Zülpicher Straße 47b, D-50674 Cologne, Germany

Introduction

Heterotrophic flagellates (HF) are very likely the most abundant eukaryotes on Earth, hundreds of specimens occur in each droplet of water even in groundwater and the deep sea. As the main feeders on bacteria they play an essential role in aquatic and terrestrial food webs (Arndt et al. 2000; Azam et al. 1983; Bonkowski 2004). In addition, they can act as important herbivores (Arndt and Mathes 1991; Nauwerck 1963; Sherr and Sherr 1994), detritivores (Scherwass et al. 2005) and osmotrophs (Christoffersen et al. 1997; Sanders et al. 1989; Sherr 1988) as well as mixotrophs (Bird and Kalff 1986; Sanders 1991). The relative contributions to these different modes of feeding can vary within taxonomic groups and even within one and the same organism, e.g. *Ochromonas* sp. (Jones 2000; Wilken et al. 2013). Furthermore, bacterial communities are not only grazed by protozoans but are also structured by protistan grazers (e.g. Boehme et al. 2009; Güde 1979; Jürgens and Matz 2002; Pernthaler 2005).

HF are a very heterogenous group with an enormous size range between 1-450 μm (some authors refer to the species smaller than 15 μm as heterotrophic nanoflagellates "HNF", Arndt et al. 2000). High tolerances to changes in salinity allow several species to live both in marine as well as in freshwater habitats, though several phylogenetic studies have also indicated clearly separated marine and freshwater clades (e.g. Kim and Archibald 2013). In contrast, the dominant taxonomic groups within different marine and freshwater pelagic communities (e.g. stramenopile taxa, dinoflagellates, choanoflagellates, kathablepharids) and benthic communities (e.g. euglenids, free-living kinetoplastids, cercozoans) seem to be surprisingly similar (Arndt et al. 2000). Their modes of movement as important taxonomic characteristics comprise gliding or free-swimming forms or they may be temporarily or permanently attached to a substrate (Fenchel 1987). Feeding modes include true filter-feeding (e.g. choanoflagellates), direct interception feeding (e.g. chrysomonads) or raptorial feeding (e.g. most benthic forms) (Boenigk and Arndt 2002; Fenchel 1991).

One important prerequisite to estimate the role of HF for the flux of matter in ecosystems is the determination of their abundance. In the early days of HF quantification, the so called "HNF" were counted exclusively in fluorescently stained fixated samples (e.g. Porter and Feig 1980). However, fixation bears the problem that many species are disrupted upon the fixation process (Choi and Stoecker 1989; Sonntag et al. 2000) or are misidentified as small naked amoebae, zoospores, yeasts, or disrupted cells from a range of eukaryotes, since flagella are not always adequately preserved (Patterson and Larsen 1991). Especially benthic HF are affected by the above explained problems because they are additionally masked by sediment particles. Further, large HF can easily be overlooked on membrane filters due to their low contribution to total HF abundance and their special sensitivity to fixatives. This is crucial since they may contribute to about half of the HF biovolume (Arndt et al. 2000). Therefore, the

[1]Corresponding author; fax +49 221 470 5932
e-mail hartmut.arndt@uni-koeln.de (H. Arndt).

http://dx.doi.org/10.1016/j.protis.2013.08.003

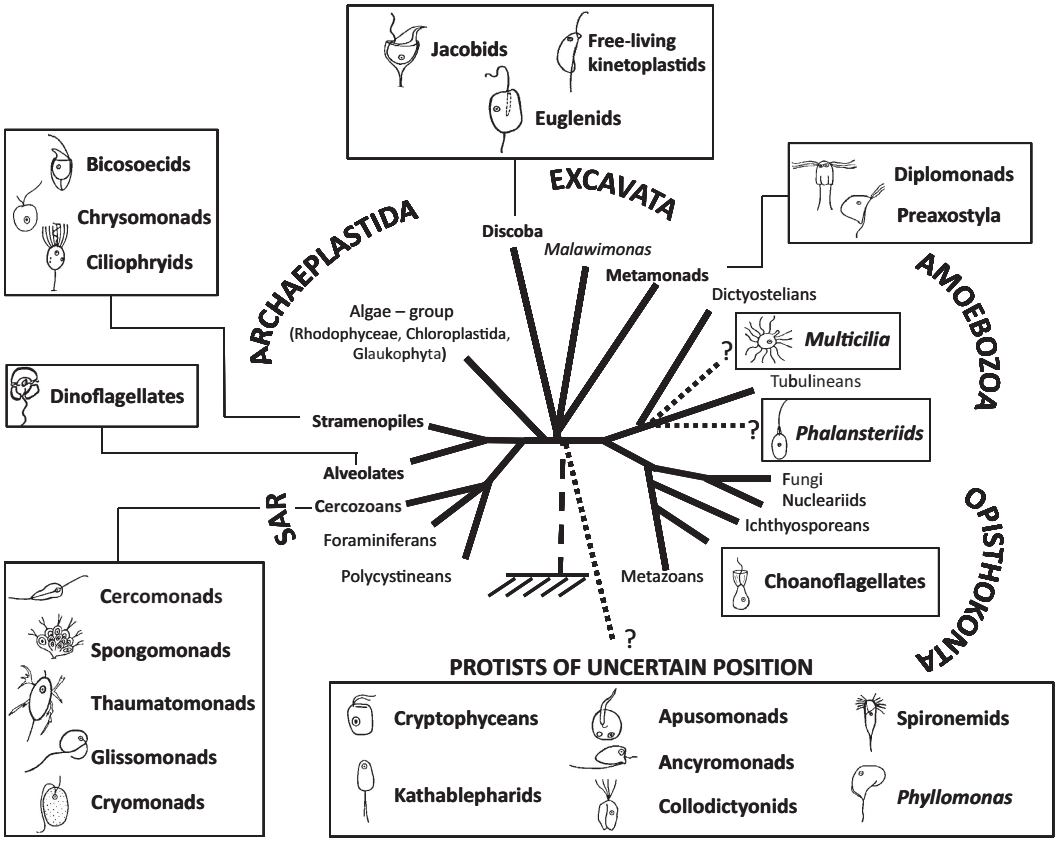


Figure 1. Selective overview of the systematic position (according to Adl et al. (2012) of flagellate groups considered in the guide (see Charts 1–15).

taxonomic resolution of fixation processes is often very low, most quantitative studies even ignored the variety of HF.

In contrast, live counting is an essential and reliable method for determining HF abundances. It allows a combination of taxonomic and quantitative studies for understanding the role of HF in microbial food webs (Leadbeater and Green 2000; Patterson and Larsen 1991). Unfortunately, the taxonomic resolution of HF studies is hampered by the fact that the literature is often focused on only a few taxonomic groups. Therefore, we designed a (hopefully) user-friendly guide for ecologists to unravel the "HNF" black box. In contrast to the few already existing taxonomic keys/reviews (e.g. Bass et al. 2009; Cavalier-Smith and Chao 2010; Lee et al. 2000; Patterson and Hedley 1992; Patterson and Larsen 1991; Zhukov 1993), the present guide is compact and focuses on the dominant and common forms of HF occurring in pelagic and benthic freshwater habitats. Following the idea of Foissner and Berger (1996) with regard to ciliates, we designed a guide which should be useful also for biologists not specialized in the study of heterotrophic freshwater flagellates due to its polytomous organization, the help of drawings and video sequences (Supplementary Material). According to our experience in quantitative studies of morphotypes, about 80-90 per cent of the dominant heterotrophic flagellates found in freshwater habitats should be identifiable at least to rough taxonomic categories (and functional guilds). The term morphotype is used here for taxa that can be differentiated by means of their morphology when only analysed by light microscopy. It means that e.g. genotypes may be hidden among certain morphotypes. A typical example is the species complex of *Neobodo designis* (Scheckenbach et al. 2006). The guide comprises all the main groups, so that a large number of species morphotypes can be determined at least to the level of genus. Special attention was given to the most interesting and ecologically relevant forms. The taxonomic categories were used according to the recently revised classifications by Adl et al. (2012).

Within the next decade, next generation sequencing of field samples will give more detailed insights into the structure of HF field communities

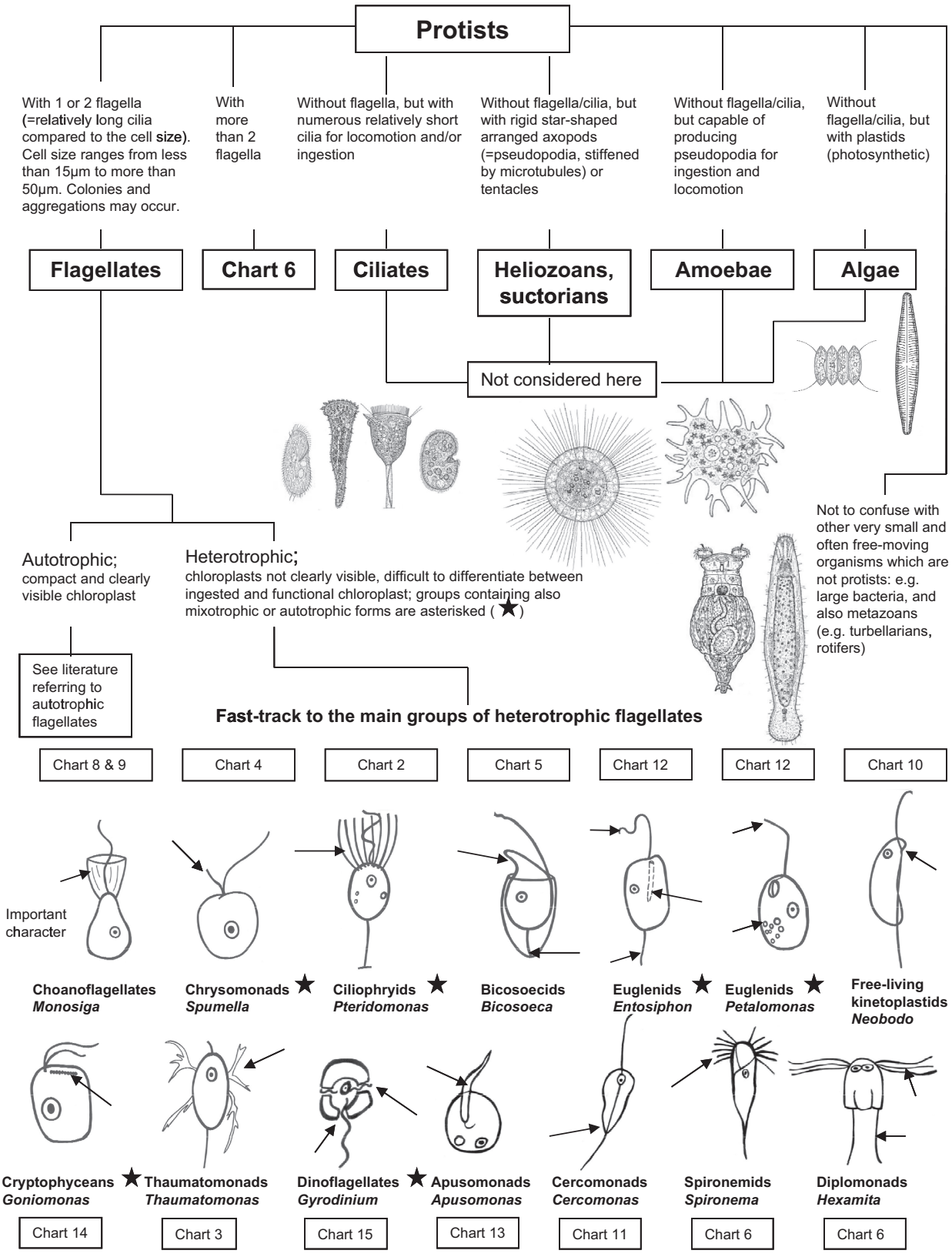


Chart 1. User-friendly guide to common heterotrophic freshwater flagellates. All scale bars in the guide indicate 10 µm.

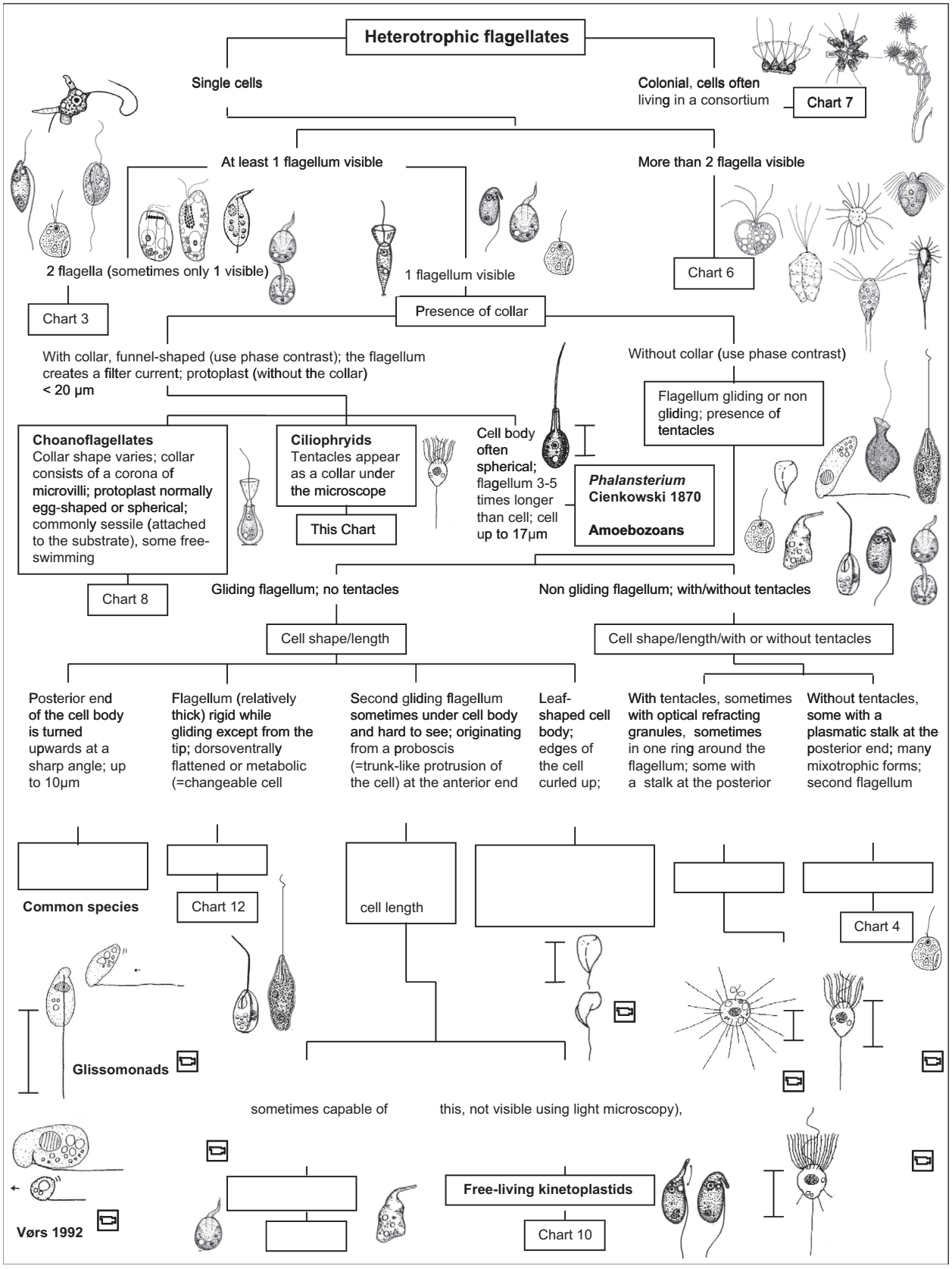

Heterotrophic flagellates
Single cells
Colonial, cells often living in a consortium
Chart 7
At least 1 flagellum visible
More than 2 flagella visible
2 flagella (sometimes only 1 visible)
1 flagellum visible
Chart 6
Chart 3
Presence of collar
With collar, funnel-shaped (use phase contrast); the flagellum creates a filter current; protoplast (without the collar) < 20 µm
Without collar (use phase contrast)
Flagellum gliding or non gliding; presence of tentacles
Choanoflagellates
Collar shape varies; collar consists of a corona of microvilli; protoplast normally egg-shaped or spherical; commonly sessile (attached to the substrate), some free-swimming
Ciliophryids
Tentacles appear as a collar under the microscope
This Chart
Cell body often spherical; flagellum 3-5 times longer than cell; cell up to 17µm
Phalansterium Cienkowski 1870
Amoebozoans
Chart 8
Gliding flagellum; no tentacles
Non gliding flagellum; with/without tentacles
Cell shape/length
Cell shape/length/with or without tentacles
Posterior end of the cell body is turned upwards at a sharp angle; up to 10µm
Flagellum (relatively thick) rigid while gliding except from the tip; dorsoventrally flattened or metabolic (=changeable cell
Second gliding flagellum sometimes under cell body and hard to see; originating from a proboscis (=trunk-like protrusion of the cell) at the anterior end
Leaf-shaped cell body; edges of the cell curled up;
With tentacles, sometimes with optical refracting granules, sometimes in one ring around the flagellum; some with a stalk at the posterior
Without tentacles, some with a plasmatic stalk at the posterior end; many mixotrophic forms; second flagellum
Common species
Chart 12
cell length
Chart 4
Glissomonads
sometimes capable of
this, not visible using light microscopy),
Vørs 1992
Free-living kinetoplastids
Chart 10

Chart 2.

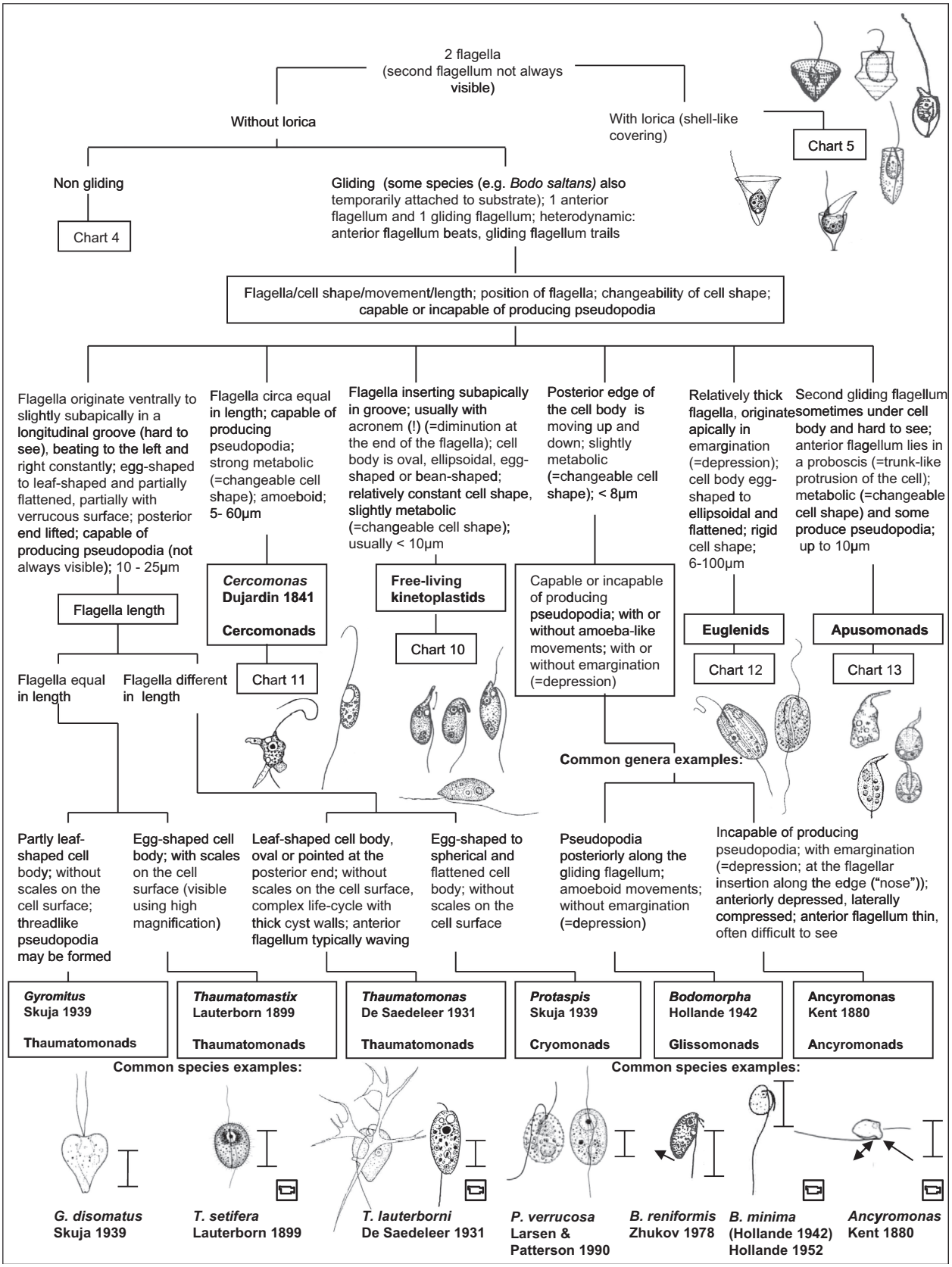

2 flagella (second flagellum not always visible)
Without lorica
With lorica (shell-like covering)
Chart 5
Non gliding
Chart 4
Gliding (some species (e.g. Bodo saltans) also temporarily attached to substrate); 1 anterior flagellum and 1 gliding flagellum; heterodynamic: anterior flagellum beats, gliding flagellum trails
Flagella/cell shape/movement/length; position of flagella; changeability of cell shape; capable or incapable of producing pseudopodia
Flagella originate ventrally to slightly subapically in a longitudinal groove (hard to see), beating to the left and right constantly; egg-shaped to leaf-shaped and partially flattened, partially with verrucous surface; posterior end lifted; capable of producing pseudopodia (not always visible); 10 - 25µm
Flagella length
Flagella equal in length
Flagella different in length
Flagella circa equal in length; capable of producing pseudopodia; strong metabolic (=changeable cell shape); amoeboid; 5- 60µm
Cercomonas Dujardin 1841
Cercomonads
Chart 11
Flagella inserting subapically in groove; usually with acronem (!) (=diminution at the end of the flagella); cell body is oval, ellipsoidal, egg-shaped or bean-shaped; relatively constant cell shape, slightly metabolic (=changeable cell shape); usually < 10µm
Free-living kinetoplastids
Chart 10
Posterior edge of the cell body is moving up and down; slightly metabolic (=changeable cell shape); < 8µm
Capable or incapable of producing pseudopodia; with or without amoeba-like movements; with or without emargination (=depression)
Relatively thick flagella, originate apically in emargination (=depression); cell body egg-shaped to ellipsoidal and flattened; rigid cell shape; 6-100µm
Euglenids
Chart 12
Second gliding flagellum sometimes under cell body and hard to see; anterior flagellum lies in a proboscis (=trunk-like protrusion of the cell); metabolic (=changeable cell shape) and some produce pseudopodia; up to 10µm
Apusomonads
Chart 13
Common genera examples:
Partly leaf-shaped cell body; without scales on the cell surface; threadlike pseudopodia may be formed
Egg-shaped cell body; with scales on the cell surface (visible using high magnification)
Leaf-shaped cell body, oval or pointed at the posterior end; without scales on the cell surface, complex life-cycle with thick cyst walls; anterior flagellum typically waving
Egg-shaped to spherical and flattened cell body; without scales on the cell surface
Pseudopodia posteriorly along the gliding flagellum; amoeboid movements; without emargination (=depression)
Incapable of producing pseudopodia; with emargination (=depression; at the flagellar insertion along the edge ("nose")); anteriorly depressed, laterally compressed; anterior flagellum thin, often difficult to see
Gyromitus Skuja 1939
Thaumatomonads
Thaumatomastix Lauterborn 1899
Thaumatomonads
Thaumatomonas De Saedeleer 1931
Thaumatomonads
Protaspis Skuja 1939
Cryomonads
Bodomorpha Hollande 1942
Glissomonads
Ancyromonas Kent 1880
Ancyromonads
Common species examples:
Common species examples:
G. disomatus Skuja 1939
T. setifera Lauterborn 1899
T. lauterborni De Saedeleer 1931
P. verrucosa Larsen & Patterson 1990
B. reniformis Zhukov 1978
B. minima (Hollande 1942) Hollande 1952
Ancyromonas Kent 1880

Chart 3.

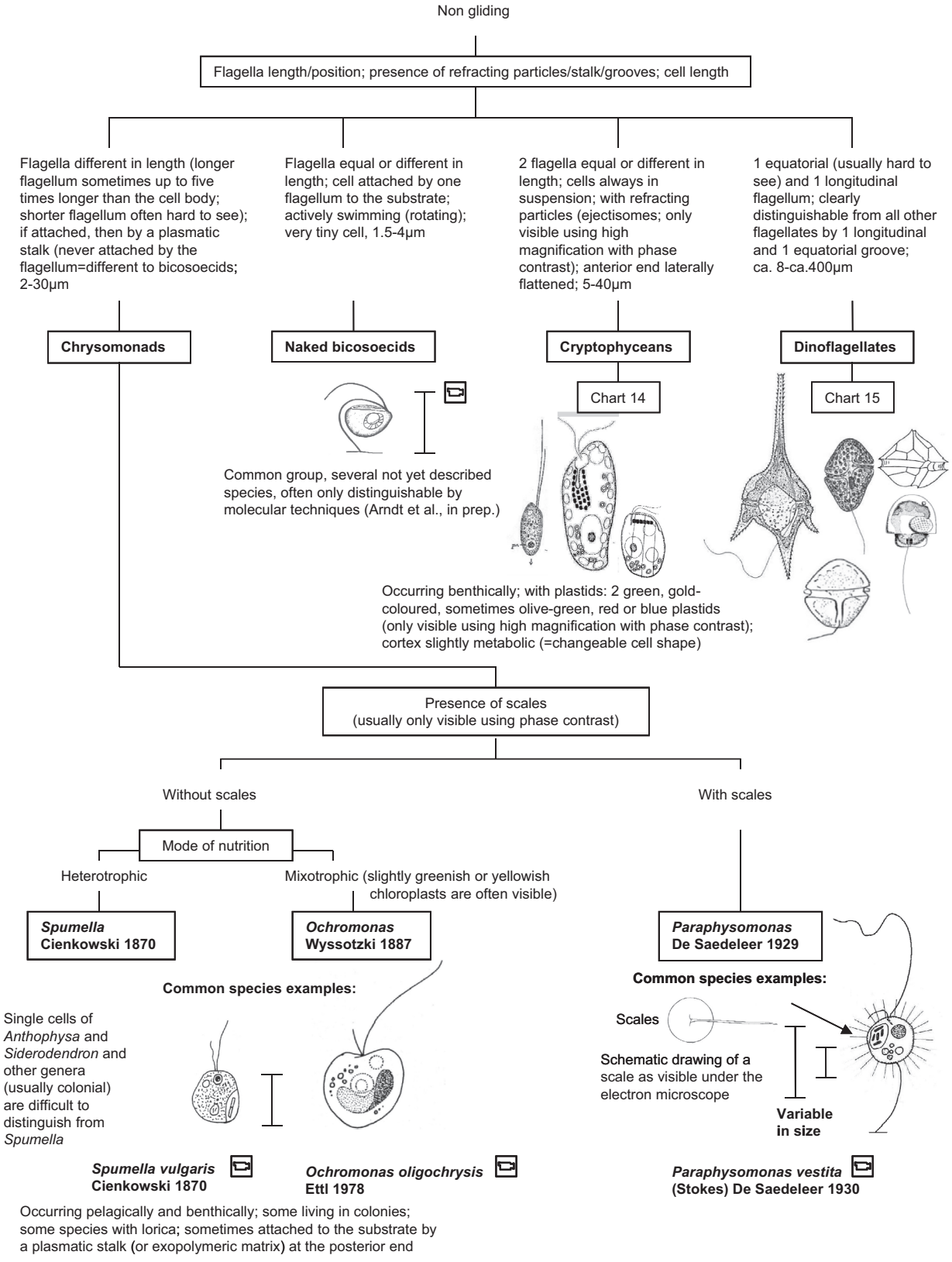

Non gliding
Flagella length/position; presence of refracting particles/stalk/grooves; cell length
Flagella different in length (longer flagellum sometimes up to five times longer than the cell body; shorter flagellum often hard to see); if attached, then by a plasmatic stalk (never attached by the flagellum=different to bicosoecids; 2-30µm
Flagella equal or different in length; cell attached by one flagellum to the substrate; actively swimming (rotating); very tiny cell, 1.5-4µm
2 flagella equal or different in length; cells always in suspension; with refracting particles (ejectisomes; only visible using high magnification with phase contrast); anterior end laterally flattened; 5-40µm
1 equatorial (usually hard to see) and 1 longitudinal flagellum; clearly distinguishable from all other flagellates by 1 longitudinal and 1 equatorial groove; ca. 8-ca.400µm
Chrysomonads
Naked bicosoecids
Cryptophyceans
Dinoflagellates
Chart 14
Chart 15
Common group, several not yet described species, often only distinguishable by molecular techniques (Arndt et al., in prep.)
Occurring benthically; with plastids: 2 green, gold-coloured, sometimes olive-green, red or blue plastids (only visible using high magnification with phase contrast); cortex slightly metabolic (=changeable cell shape)
Presence of scales (usually only visible using phase contrast)
Without scales
With scales
Mode of nutrition
Heterotrophic
Mixotrophic (slightly greenish or yellowish chloroplasts are often visible)
Spumella Cienkowski 1870
Ochromonas Wyssotzki 1887
Paraphysomonas De Saedeleer 1929
Common species examples:
Common species examples:
Single cells of Anthophysa and Siderodendron and other genera (usually colonial) are difficult to distinguish from Spumella
Scales
Schematic drawing of a scale as visible under the electron microscope
Variable in size
Spumella vulgaris Cienkowski 1870
Ochromonas oligochrysis Ettl 1978
Paraphysomonas vestita (Stokes) De Saedeleer 1930
Occurring pelagically and benthically; some living in colonies; some species with lorica; sometimes attached to the substrate by a plasmatic stalk (or exopolymeric matrix) at the posterior end

Chart 4.

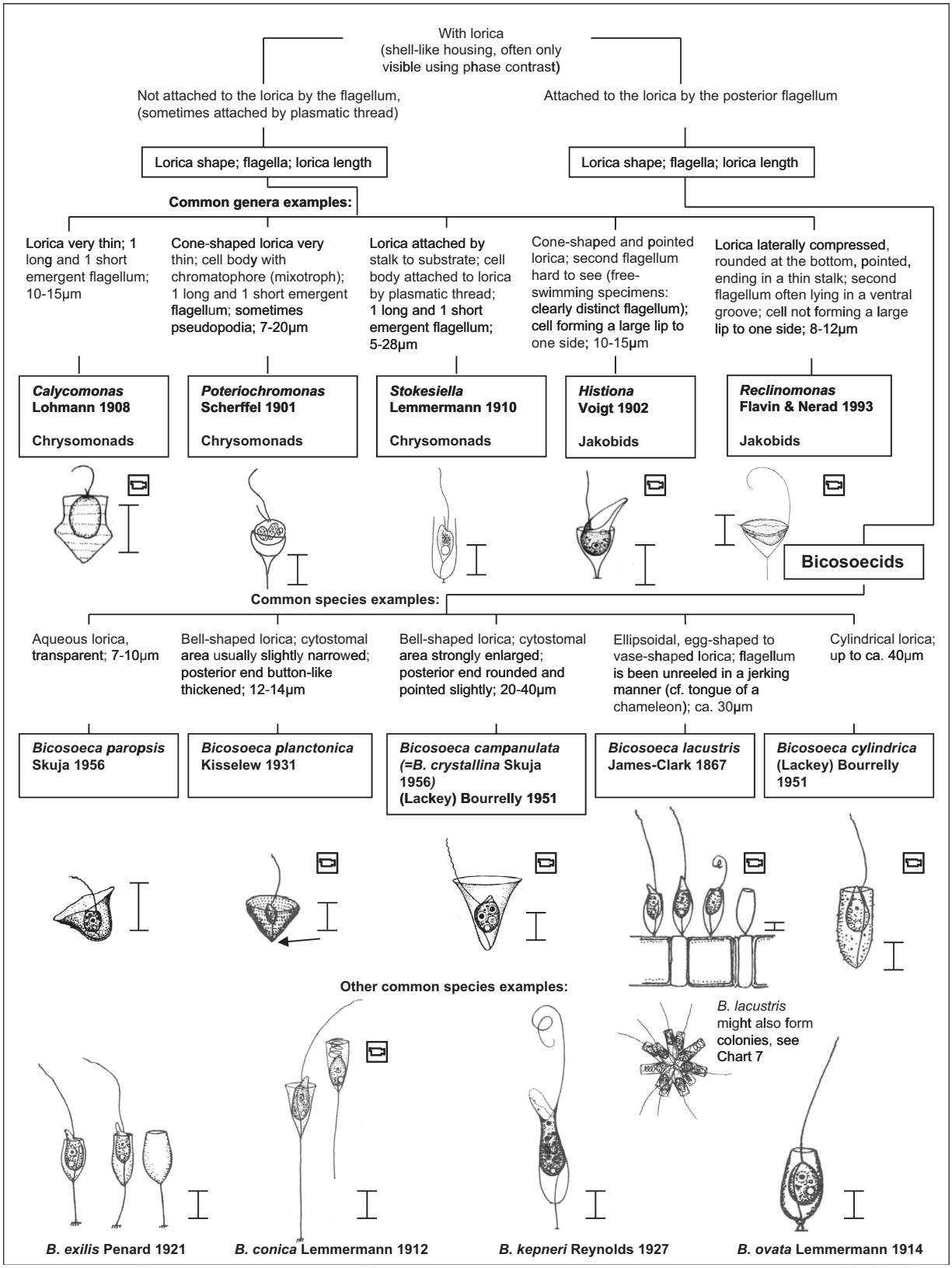

With lorica
(shell-like housing, often only visible using phase contrast)
Not attached to the lorica by the flagellum, (sometimes attached by plasmatic thread)
Attached to the lorica by the posterior flagellum
Lorica shape; flagella; lorica length
Lorica shape; flagella; lorica length
Common genera examples:
Lorica very thin; 1 long and 1 short emergent flagellum; 10-15µm
Cone-shaped lorica very thin; cell body with chromatophore (mixotroph); 1 long and 1 short emergent flagellum; sometimes pseudopodia; 7-20µm
Lorica attached by stalk to substrate; cell body attached to lorica by plasmatic thread; 1 long and 1 short emergent flagellum; 5-28µm
Cone-shaped and pointed lorica; second flagellum hard to see (free-swimming specimens: clearly distinct flagellum); cell forming a large lip to one side; 10-15µm
Lorica laterally compressed, rounded at the bottom, pointed, ending in a thin stalk; second flagellum often lying in a ventral groove; cell not forming a large lip to one side; 8-12µm
Calycomonas Lohmann 1908
Chrysomonads
Poteriochromonas Scherffel 1901
Chrysomonads
Stokesiella Lemmermann 1910
Chrysomonads
Histiona Voigt 1902
Jakobids
Reclinomonas Flavin & Nerad 1993
Jakobids
Bicosoecids
Common species examples:
Aqueous lorica, transparent; 7-10µm
Bell-shaped lorica; cytostomal area usually slightly narrowed; posterior end button-like thickened; 12-14µm
Bell-shaped lorica; cytostomal area strongly enlarged; posterior end rounded and pointed slightly; 20-40µm
Ellipsoidal, egg-shaped to vase-shaped lorica; flagellum is been unreeled in a jerking manner (cf. tongue of a chameleon); ca. 30µm
Cylindrical lorica; up to ca. 40µm
Bicosoeca paropsis Skuja 1956
Bicosoeca planctonica Kisselew 1931
Bicosoeca campanulata (=B. crystallina Skuja 1956) (Lackey) Bourrelly 1951
Bicosoeca lacustris James-Clark 1867
Bicosoeca cylindrica (Lackey) Bourrelly 1951
Other common species examples:
B. lacustris might also form colonies, see Chart 7
B. exilis Penard 1921
B. conica Lemmermann 1912
B. kepneri Reynolds 1927
B. ovata Lemmermann 1914

Chart 5.

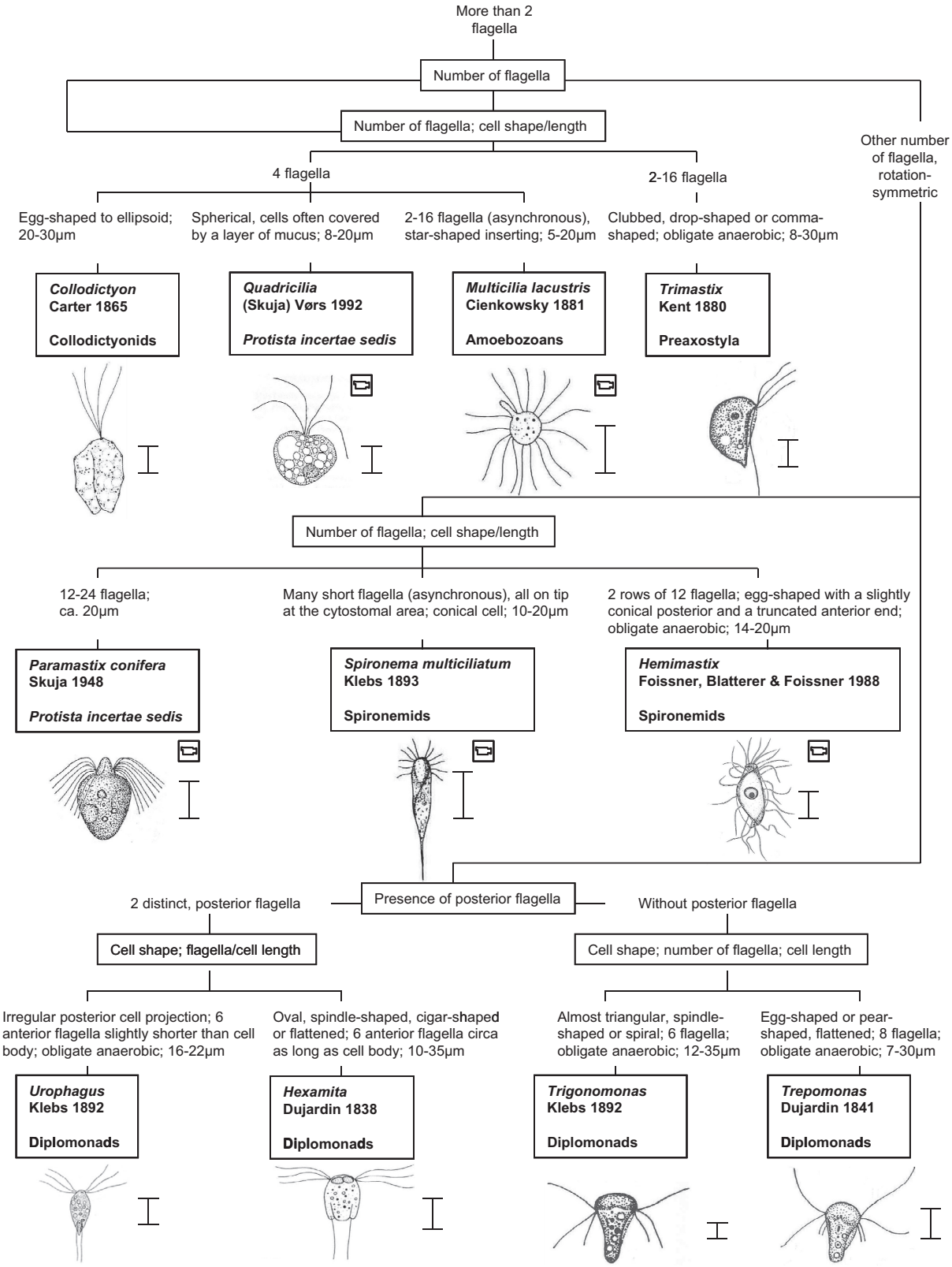

More than 2 flagella
Number of flagella
Number of flagella; cell shape/length
Other number of flagella, rotation-symmetric
4 flagella
2-16 flagella
Egg-shaped to ellipsoid; 20-30µm
Spherical, cells often covered by a layer of mucus; 8-20µm
2-16 flagella (asynchronous), star-shaped inserting; 5-20µm
Clubbed, drop-shaped or comma-shaped; obligate anaerobic; 8-30µm
Collodictyon Carter 1865 Collodictyonids
Quadricilia (Skuja) Vørs 1992 Protista incertae sedis
Multicilia lacustris Cienkowsky 1881 Amoebozoans
Trimastix Kent 1880 Preaxostyla
Number of flagella; cell shape/length
12-24 flagella; ca. 20µm
Many short flagella (asynchronous), all on tip at the cytostomal area; conical cell; 10-20µm
2 rows of 12 flagella; egg-shaped with a slightly conical posterior and a truncated anterior end; obligate anaerobic; 14-20µm
Paramastix conifera Skuja 1948 Protista incertae sedis
Spironema multiciliatum Klebs 1893 Spironemids
Hemimastix Foissner, Blatterer & Foissner 1988 Spironemids
Presence of posterior flagella
2 distinct, posterior flagella
Without posterior flagella
Cell shape; flagella/cell length
Cell shape; number of flagella; cell length
Irregular posterior cell projection; 6 anterior flagella slightly shorter than cell body; obligate anaerobic; 16-22µm
Oval, spindle-shaped, cigar-shaped or flattened; 6 anterior flagella circa as long as cell body; 10-35µm
Almost triangular, spindle-shaped or spiral; 6 flagella; obligate anaerobic; 12-35µm
Egg-shaped or pear-shaped, flattened; 8 flagella; obligate anaerobic; 7-30µm
Urophagus Klebs 1892 Diplomonads
Hexamita Dujardin 1838 Diplomonads
Trigonomonas Klebs 1892 Diplomonads
Trepomonas Dujardin 1841 Diplomonads

Chart 6.

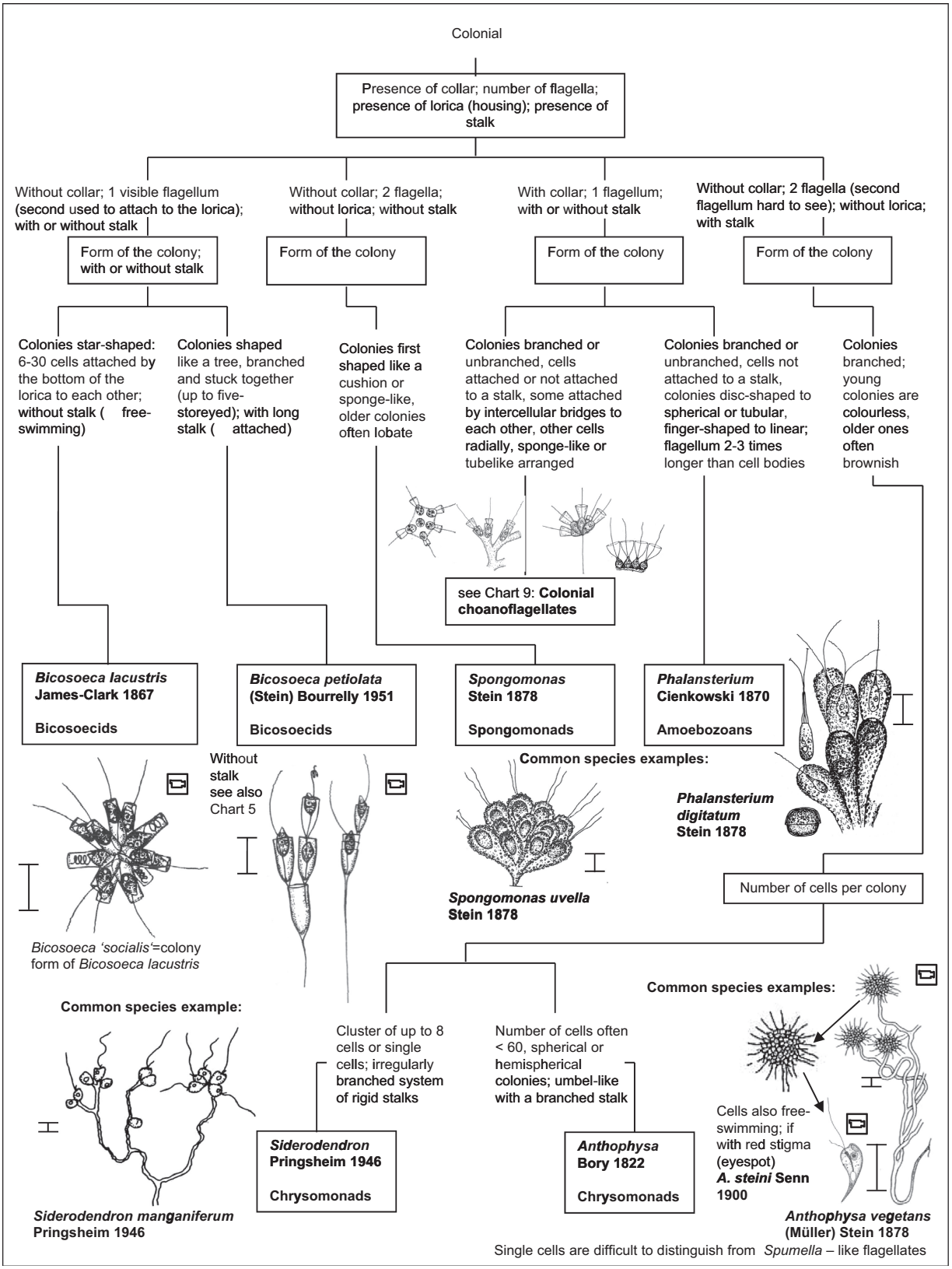

Colonial
Presence of collar; number of flagella; presence of lorica (housing); presence of stalk
Without collar; 1 visible flagellum (second used to attach to the lorica); with or without stalk
Without collar; 2 flagella; without lorica; without stalk
With collar; 1 flagellum; with or without stalk
Without collar; 2 flagella (second flagellum hard to see); without lorica; with stalk
Form of the colony; with or without stalk
Form of the colony
Form of the colony
Form of the colony
Colonies star-shaped: 6-30 cells attached by the bottom of the lorica to each other; without stalk (free-swimming)
Colonies shaped like a tree, branched and stuck together (up to five-storeyed); with long stalk (attached)
Colonies first shaped like a cushion or sponge-like, older colonies often lobate
Colonies branched or unbranched, cells attached or not attached to a stalk, some attached by intercellular bridges to each other, other cells radially, sponge-like or tubelike arranged
Colonies branched or unbranched, cells not attached to a stalk, colonies disc-shaped to spherical or tubular, finger-shaped to linear; flagellum 2-3 times longer than cell bodies
Colonies branched; young colonies are colourless, older ones often brownish
see Chart 9: Colonial choanoflagellates
Bicosoeca lacustris James-Clark 1867
Bicosoecids
Bicosoeca petiolata (Stein) Bourrelly 1951
Bicosoecids
Spongomonas Stein 1878
Spongomonads
Phalansterium Cienkowski 1870
Amoebozoans
Without stalk see also Chart 5
Common species examples:
Phalansterium digitatum Stein 1878
Bicosoeca 'socialis'=colony form of Bicosoeca lacustris
Spongomonas uvella Stein 1878
Number of cells per colony
Common species example:
Common species examples:
Cluster of up to 8 cells or single cells; irregularly branched system of rigid stalks
Number of cells often < 60, spherical or hemispherical colonies; umbel-like with a branched stalk
Cells also free-swimming; if with red stigma (eyespot) A. steini Senn 1900
Siderodendron Pringsheim 1946
Chrysomonads
Anthophysa Bory 1822
Chrysomonads
Siderodendron manganiferum Pringsheim 1946
Anthophysa vegetans (Müller) Stein 1878
Single cells are difficult to distinguish from Spumella – like flagellates

Chart 7.

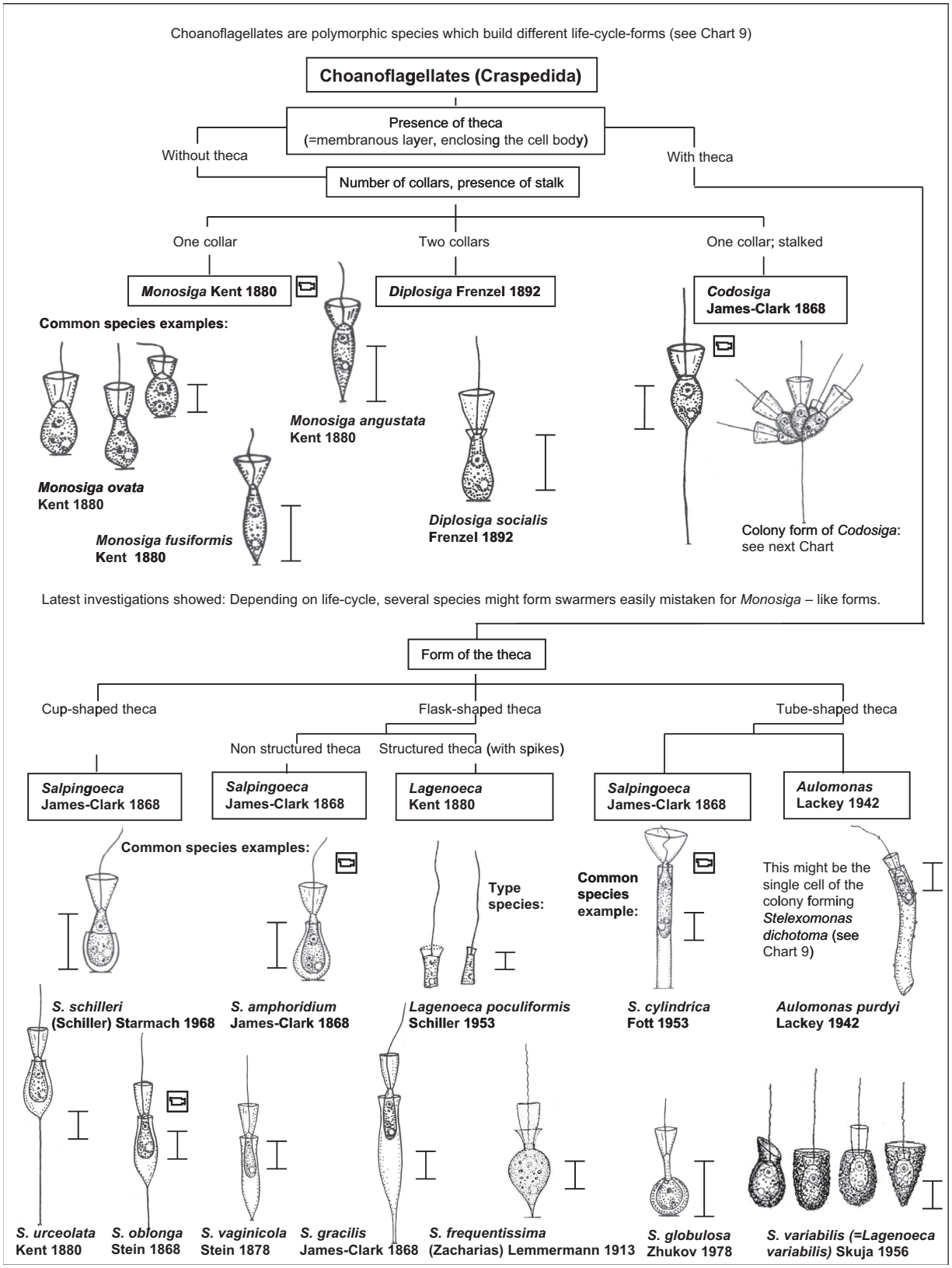

Choanoflagellates are polymorphic species which build different life-cycle-forms (see Chart 9)
Choanoflagellates (Craspedida)
Presence of theca
(=membranous layer, enclosing the cell body)
Without theca
With theca
Number of collars, presence of stalk
One collar
Two collars
One collar; stalked
Monosiga Kent 1880
Diplosiga Frenzel 1892
Codosiga James-Clark 1868
Common species examples:
Monosiga ovata Kent 1880
Monosiga fusiformis Kent 1880
Monosiga angustata Kent 1880
Diplosiga socialis Frenzel 1892
Colony form of Codosiga: see next Chart
Latest investigations showed: Depending on life-cycle, several species might form swarmers easily mistaken for Monosiga – like forms.
Form of the theca
Cup-shaped theca
Flask-shaped theca
Tube-shaped theca
Non structured theca
Structured theca (with spikes)
Salpingoeca James-Clark 1868
Salpingoeca James-Clark 1868
Lagenoeca Kent 1880
Salpingoeca James-Clark 1868
Aulomonas Lackey 1942
Common species examples:
Type species:
Common species example:
This might be the single cell of the colony forming Stelexomonas dichotoma (see Chart 9)
S. schilleri (Schiller) Starmach 1968
S. amphoridium James-Clark 1868
Lagenoeca poculiformis Schiller 1953
S. cylindrica Fott 1953
Aulomonas purdyi Lackey 1942
S. urceolata Kent 1880
S. oblonga Stein 1868
S. vaginicola Stein 1878
S. gracilis James-Clark 1868
S. frequentissima (Zacharias) Lemmermann 1913
S. globulosa Zhukov 1978
S. variabilis (=Lagenoeca variabilis) Skuja 1956

Chart 8.

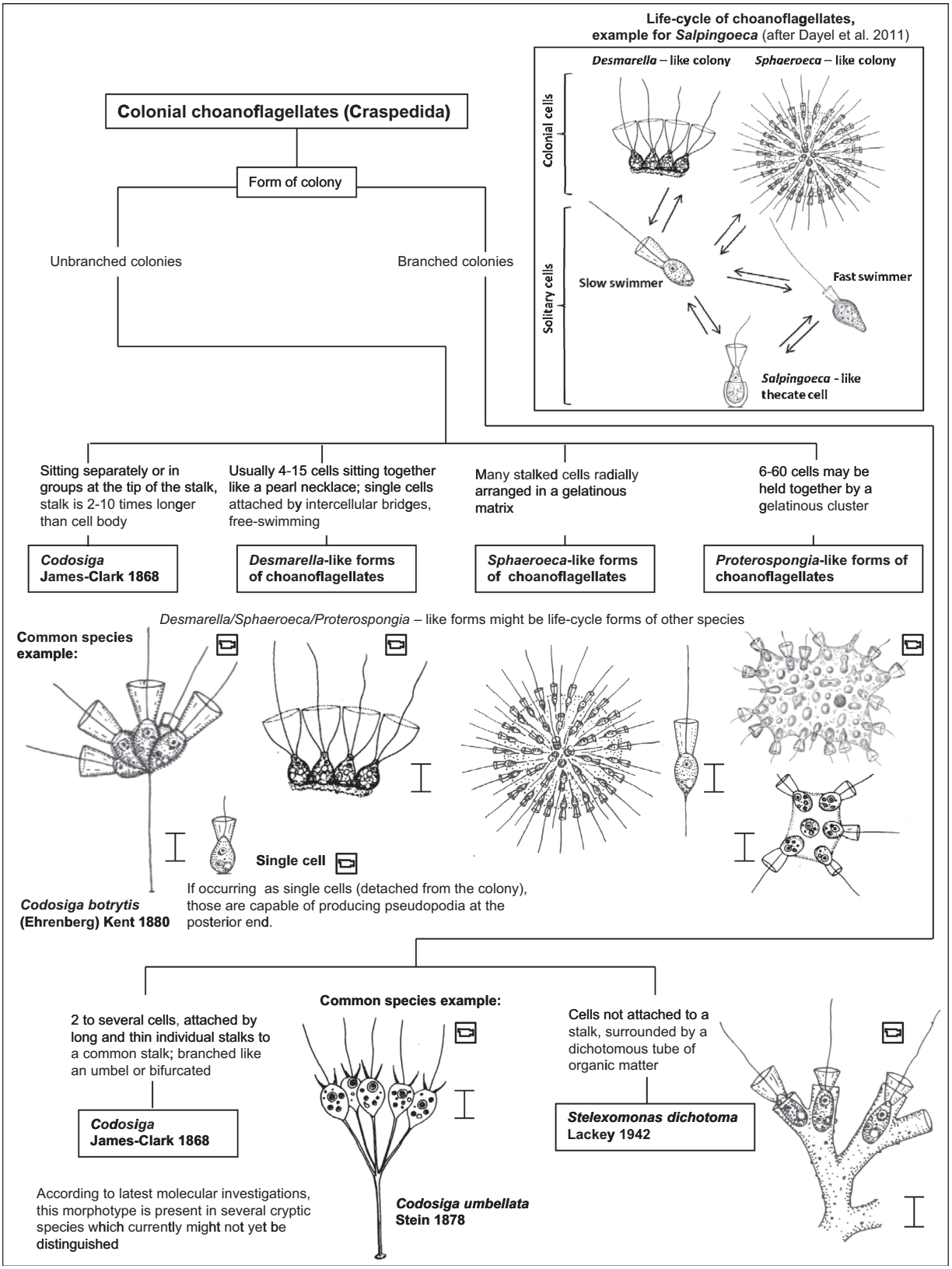

Life-cycle of choanoflagellates, example for Salpingoeca (after Dayel et al. 2011)
Desmarella – like colony
Sphaeroeca – like colony
Colonial cells
Solitary cells
Slow swimmer
Fast swimmer
Salpingoeca - like thecate cell
Colonial choanoflagellates (Craspedida)
Form of colony
Unbranched colonies
Branched colonies
Sitting separately or in groups at the tip of the stalk, stalk is 2-10 times longer than cell body
Usually 4-15 cells sitting together like a pearl necklace; single cells attached by intercellular bridges, free-swimming
Many stalked cells radially arranged in a gelatinous matrix
6-60 cells may be held together by a gelatinous cluster
Codosiga James-Clark 1868
Desmarella-like forms of choanoflagellates
Sphaeroeca-like forms of choanoflagellates
Proterospongia-like forms of choanoflagellates
Desmarella/Sphaeroeca/Proterospongia – like forms might be life-cycle forms of other species
Common species example:
Single cell
Codosiga botrytis (Ehrenberg) Kent 1880
If occurring as single cells (detached from the colony), those are capable of producing pseudopodia at the posterior end.
Common species example:
2 to several cells, attached by long and thin individual stalks to a common stalk; branched like an umbel or bifurcated
Codosiga James-Clark 1868
Cells not attached to a stalk, surrounded by a dichotomous tube of organic matter
Stelexomonas dichotoma Lackey 1942
According to latest molecular investigations, this morphotype is present in several cryptic species which currently might not yet be distinguished
Codosiga umbellata Stein 1878

Chart 9.

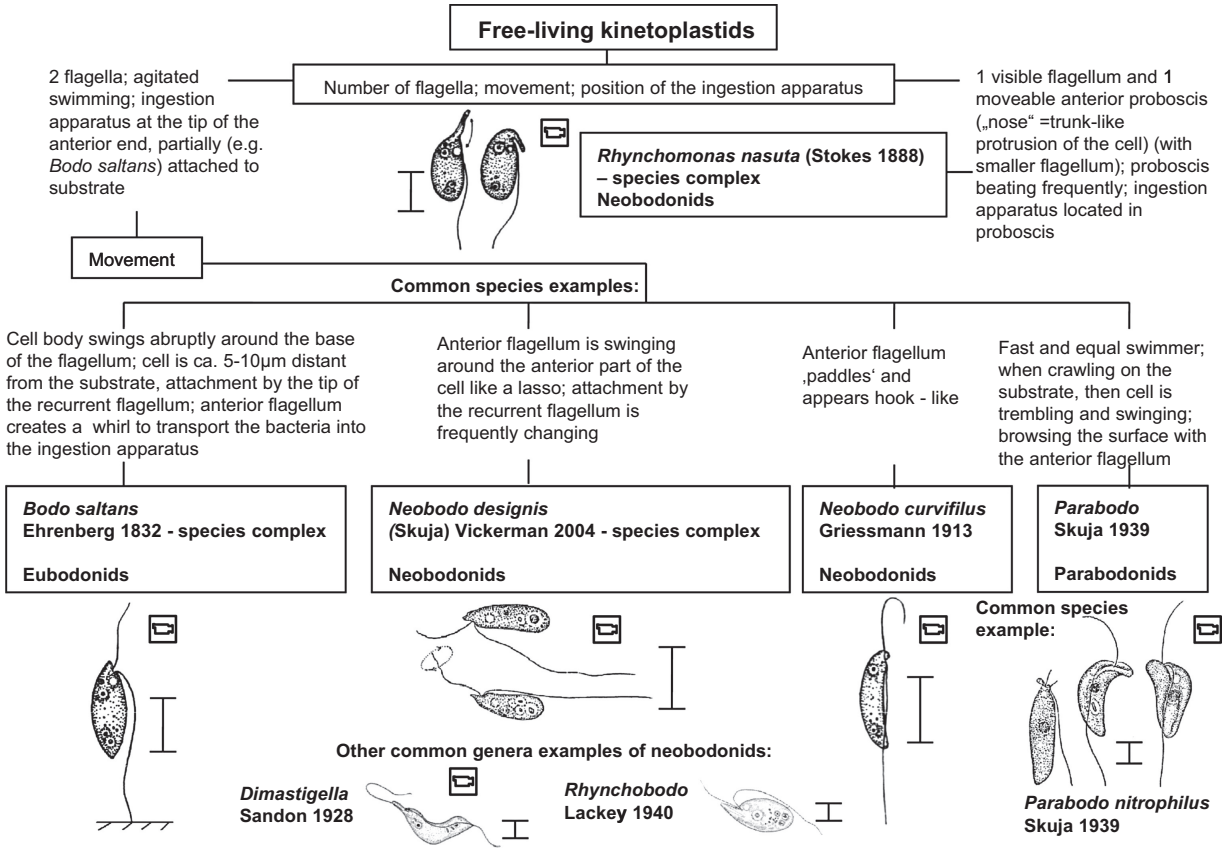


Chart 10.

(e.g. Pawlowski et al. 2011, Stoeck et al. 2010, and Domonell et al., unpubl. observ.). However, their application still bears a lot of problems concerning the following points: 1) many specific primers are necessary to cover the whole HF diversity due to the wide molecular diversity of HF (some groups will be overlooked), 2) active and inactive forms are difficult to differentiate, 3) databases are incomplete (Will and Rubinoff 2004) and contain high numbers of errors (Prosdocimi et al. 2013). Quantitative morphotype studies will provide helpful information to increase reliability of databases and offer important knowledge on the ecology and evolution of HF groups significant in ecosystems (Pawlowski et al. 2012). We would like to increase the resolution of these quantitative morphotype studies by this taxonomic guide.

How to Use the Guide

The present guide is intended to help also unexperienced researchers to identify HF morphotypes, although it is recommended to have a general knowledge of HF (recommended literature: e.g. Hausmann et al. 2003; Leadbeater and Green 2000; Patterson and Larsen 1991). The systematic position of flagellate groups considered in the guide is given in Figure 1 following the recent revision by Adl et al. (2012). Following the idea of Foissner and Berger (1996) with regard to ciliates, we designed a polytomous guide. The first Chart contains a general overview of the most important characters of the main groups of protists. The user is led to a “fast-track” regarding the main groups of HF (Charts 1–15) by schematic drawings with arrows pointing to the important characters. If identification is already sufficient the user is led to the special Chart of the organism. If not, he should proceed to Chart 2. The user is led from obvious characters (e.g. colonial or single cells, number of flagella) to more detailed descriptions. Features of organisms which are used to discriminate between different taxa are set within boxes while the discriminator for the subsequent path of identification is shown without boxes. Figures are added to the descriptions of characters to accelerate and increase the chance of identification. All scale bars in the guide indicate

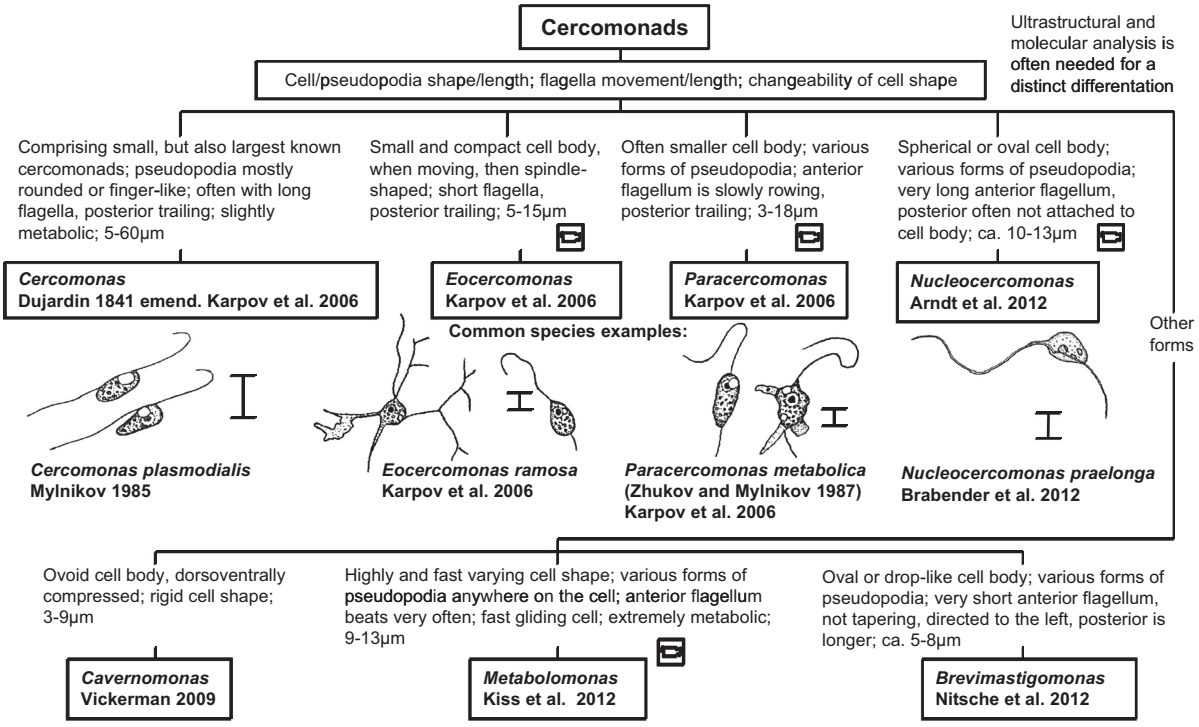


Chart 11.

10 µm. The guide leads only to the species or genus morphotype. It is widely accepted that many protistan morphotypes contain a few up to hundreds of genotypes (typical examples are the common species *Neobodo designis* (Scheckenbach et al. 2006) or *Codosiga botrytis* (Stoupin et al. 2012)), see above. If video sequences (Supplementary Material) are available for the organisms, they are marked by a video icon (▭). Some additional literature is given on the Charts (Cavalier-Smith and Chao 2010; Bass et al. 2009; Brabender et al. 2012; Karpov et al. 2006). Due to the fact that major forms of heterotrophic flagellates are ubiquitous in marine and freshwater environments, this guide may also be useful in parts for an orientation regarding flagellate groups in brackish water systems.

Methods

The guide is based on an analysis using the classical living-droplet method by phase contrast/DIC light microscopy (e.g. Arndt and Mathes 1991; Gasol 1993; Massana and Güde 1991). This method is reliable to detect morphological and behavioural characteristics of even very small HF regarding their main taxonomic groups – in contrast to hidden characteristics within samples treated by fixatives. Pelagic flagellates (undiluted samples) and benthic flagellates (diluted by a factor of 5- >20 with filtered water) can be studied in droplets of 5-20 µl on a prepared microscope slide or an Utermöhl chamber (Utermöhl 1958; HydroBios GmbH, Kiel, Germany; Fig. 2). The HF composition has to be analysed within one hour after sampling by means of a phase contrast microscope equipped with high resolution video-recording which is helpful for further and later identification. 20x, 40x objectives are helpful for rough morphological observations and quantitative counts. Individual samples can be analysed within a few minutes (particularly critical for pelagic samples). The use of a 63x long distance objective or water immersion objectives (63x and 100x; with a long working distance) are recommended for morphotype identifications. Phase contrast equipment is mostly indispensible to analyse position and movement of flagella, the presence of collar, lorica, or stalk of some flagellates. Differential interference contrast (DIC) equipment might additionally help to identify cell structures such as paramylon, ingestion apparatus, vacuoles or extrusomes.

A realistic compromise should be a combination of counts by the living-droplet method with counts of chemically fixed samples and analysis by epifluorescence microscopy. The following fixatives are commonly in use: 2% glutaraldehyde (e.g. Caron 1983; Choi and Stoecker 1989), 2% formaldehyde (e.g. Porter and Feig 1980; Sherr et al. 1989), buffered formaldehyde (e.g. Børsheim and Bratbak 1987; Sherr and Sherr 1983) and 0.5% acidic Lugol' solution (10 g I_2, 20 g KI, 10 g sodium acetate in 140 ml aq. dest.) plus 3% formaldehyde (e.g. Sherr et al. 1989).

The fixed samples need to be kept at 4 °C in the dark until staining. Fluorescent dyes (e.g. DAPI (= 4′6-diamidino-2-phenylindole, Sigma Lot 47H4107, Porter and Feig 1980) are suitable for staining. After staining the sample should be filtered onto a 0.2-µm membrane filter using vacuum not exceeding 150 mm Hg. For an even distribution of the particles, another membrane (0.6 µm pore size) may be placed

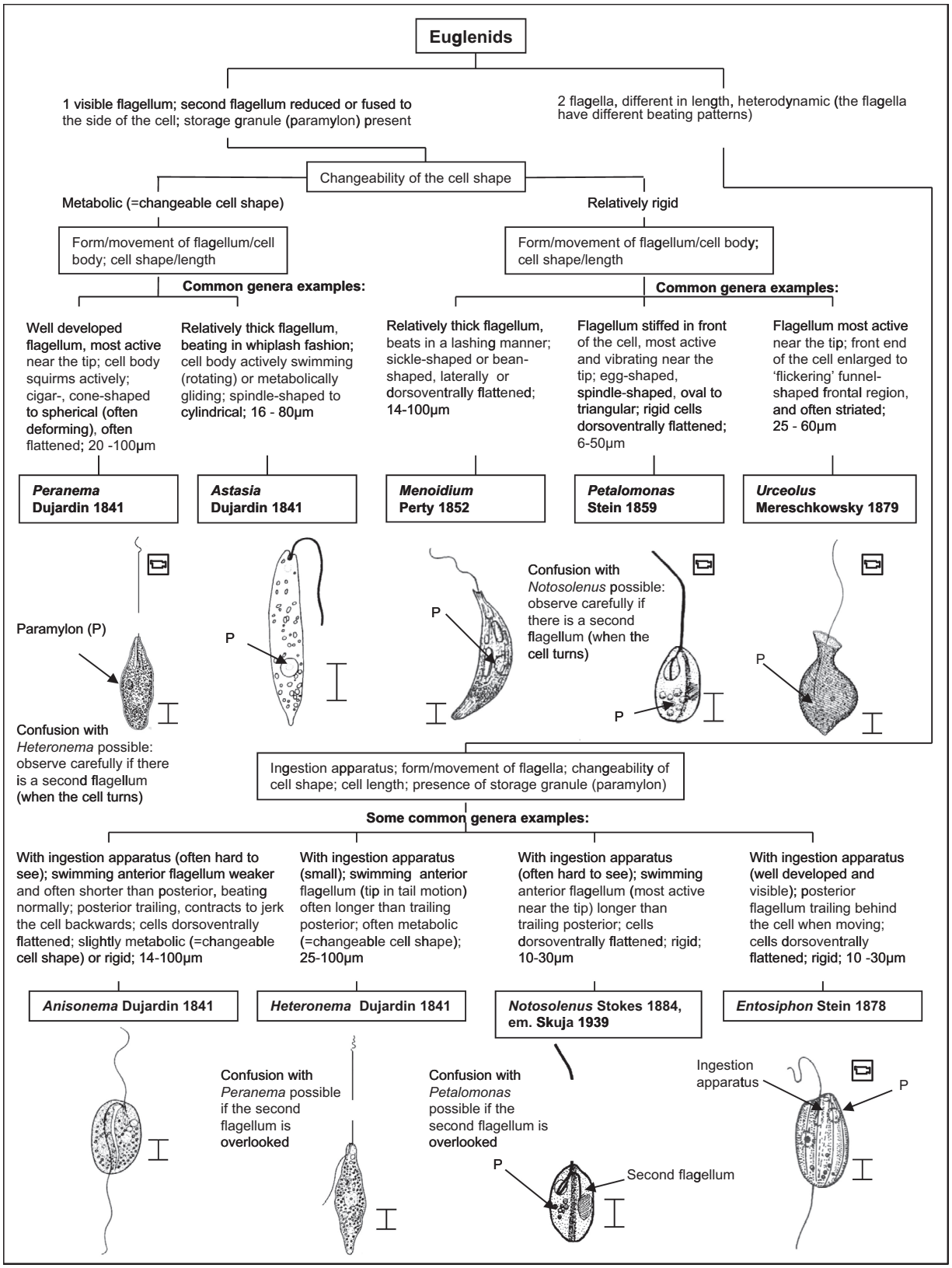

Euglenids
1 visible flagellum; second flagellum reduced or fused to the side of the cell; storage granule (paramylon) present
2 flagella, different in length, heterodynamic (the flagella have different beating patterns)
Changeability of the cell shape
Metabolic (=changeable cell shape)
Relatively rigid
Form/movement of flagellum/cell body; cell shape/length
Form/movement of flagellum/cell body; cell shape/length
Common genera examples:
Common genera examples:
Well developed flagellum, most active near the tip; cell body squirms actively; cigar-, cone-shaped to spherical (often deforming), often flattened; 20 -100µm
Relatively thick flagellum, beating in whiplash fashion; cell body actively swimming (rotating) or metabolically gliding; spindle-shaped to cylindrical; 16 - 80µm
Relatively thick flagellum, beats in a lashing manner; sickle-shaped or bean-shaped, laterally or dorsoventrally flattened; 14-100µm
Flagellum stiffed in front of the cell, most active and vibrating near the tip; egg-shaped, spindle-shaped, oval to triangular; rigid cells dorsoventrally flattened; 6-50µm
Flagellum most active near the tip; front end of the cell enlarged to 'flickering' funnel-shaped frontal region, and often striated; 25 - 60µm
Peranema Dujardin 1841
Astasia Dujardin 1841
Menoidium Perty 1852
Petalomonas Stein 1859
Urceolus Mereschkowsky 1879
Paramylon (P)
P
P
Confusion with Notosolenus possible: observe carefully if there is a second flagellum (when the cell turns)
P
P
Confusion with Heteronema possible: observe carefully if there is a second flagellum (when the cell turns)
Ingestion apparatus; form/movement of flagella; changeability of cell shape; cell length; presence of storage granule (paramylon)
Some common genera examples:
With ingestion apparatus (often hard to see); swimming anterior flagellum weaker and often shorter than posterior, beating normally; posterior trailing, contracts to jerk the cell backwards; cells dorsoventrally flattened; slightly metabolic (=changeable cell shape) or rigid; 14-100µm
With ingestion apparatus (small); swimming anterior flagellum (tip in tail motion) often longer than trailing posterior; often metabolic (=changeable cell shape); 25-100µm
With ingestion apparatus (often hard to see); swimming anterior flagellum (most active near the tip) longer than trailing posterior; cells dorsoventrally flattened; rigid; 10-30µm
With ingestion apparatus (well developed and visible); posterior flagellum trailing behind the cell when moving; cells dorsoventrally flattened; rigid; 10 -30µm
Anisonema Dujardin 1841
Heteronema Dujardin 1841
Notosolenus Stokes 1884, em. Skuja 1939
Entosiphon Stein 1878
Confusion with Peranema possible if the second flagellum is overlooked
Confusion with Petalomonas possible if the second flagellum is overlooked
P
Second flagellum
Ingestion apparatus
P

Chart 12.

Apusomonads

Position of the anterior and recurrent flagellum; changeability of the cell shape

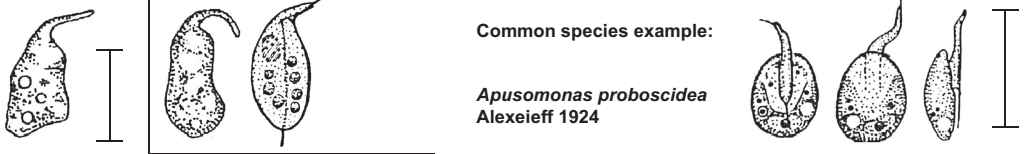
Anterior flagellum lies in a proboscis (=trunk-like protrusion of the cell); recurrent flagellum often hard to see; cells are metabolic (=changeable cell shape) and some produce pseudopodia

Anterior trailing flagellum protrudes from the proboscis (=trunk-like protrusion of the cell); recurrent flagellum lies directed posteriorly under the cell; flattened cell body is slightly metabolic (=changeable cell shape)

Apusomonas **Alexeieff 1924**

Common species example:

Apusomonas proboscidea **Alexeieff 1924**



Easily confused with *Rhynchomonas nasuta*!

Amastigomonas **De Saedeleer 1931**

Manchomonas **Cavalier-Smith 2010**

Podomonas **Cavalier-Smith 2010**

Thecamonas **Larsen & Patterson 1990**

Multimonas **Cavalier-Smith 2010**

For detailed descriptions of these genera see: **Cavalier-Smith and Chao 2010**

Chart 13.

Cryptophyceans

Flagella length; presence of dark refracting particles (=ejectisomes) (partly hard to see); presence of bright refracting storage granulum; presence of striations; cell shape; mode of movement; cell length

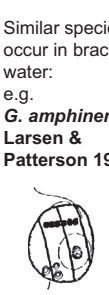
Flagella approximately equal in length; ejectisomes orientated in a row around the anterior end of the cell (only visible using high magnification with phase contrast); without storage granulum; with 3 - 6 striations; clearly dorsoventrally flattened; swimming close to the substrate; 5 -12µm

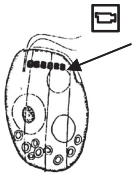
Goniomonas **(Fresenius) Stein 1878**
Synonym: *Cyathomonas* Fromentel 1874

Cryptomonads

Common species examples:

Similar species occur in brackish water: e.g. ***G. amphinema*** **Larsen & Patterson 1990:**





Goniomonas truncata **(Fresenius) Stein 1878**

Flagella approximately equal in length; ejectisomes are clustered around a emargination (=depression) at the anterior end (only visible using high magnification with phase contrast); with storage granulum; without striations; circular section; swimming fast in the water column; 15 - 30µm

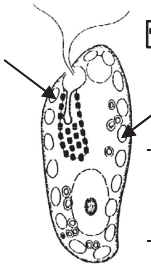
Cryptomonas **Ehrenberg 1831 emend. Hoef-Emden & Melkonian 2003**
Synonym: *Chilomonas* Ehrenberg 1831

Cryptomonads

Common species example:

Refracting ejectisomes

Many food vacuoles



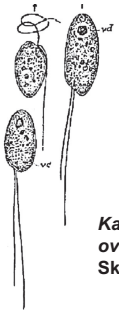
Cryptomonas paramaecium **Ehrenberg 1832 emend. Hoef-Emden & Melkonian 2003**

Flagella different in length; ejectisomes scattered within the cell body (only visible using high magnification with phase contrast); with storage granulum; without striations; circular section; swimming and jumping in the water column; 10 -18µm

Kathablepharis **Skuja 1939**

Kathablepharids

Common species example:



Movement: fast, rotating in a waving manner, shorter flagellum beats around the anterior end; only pelagically occuring.

Kathablepharis ovalis **Skuja 1948**

Chart 14.

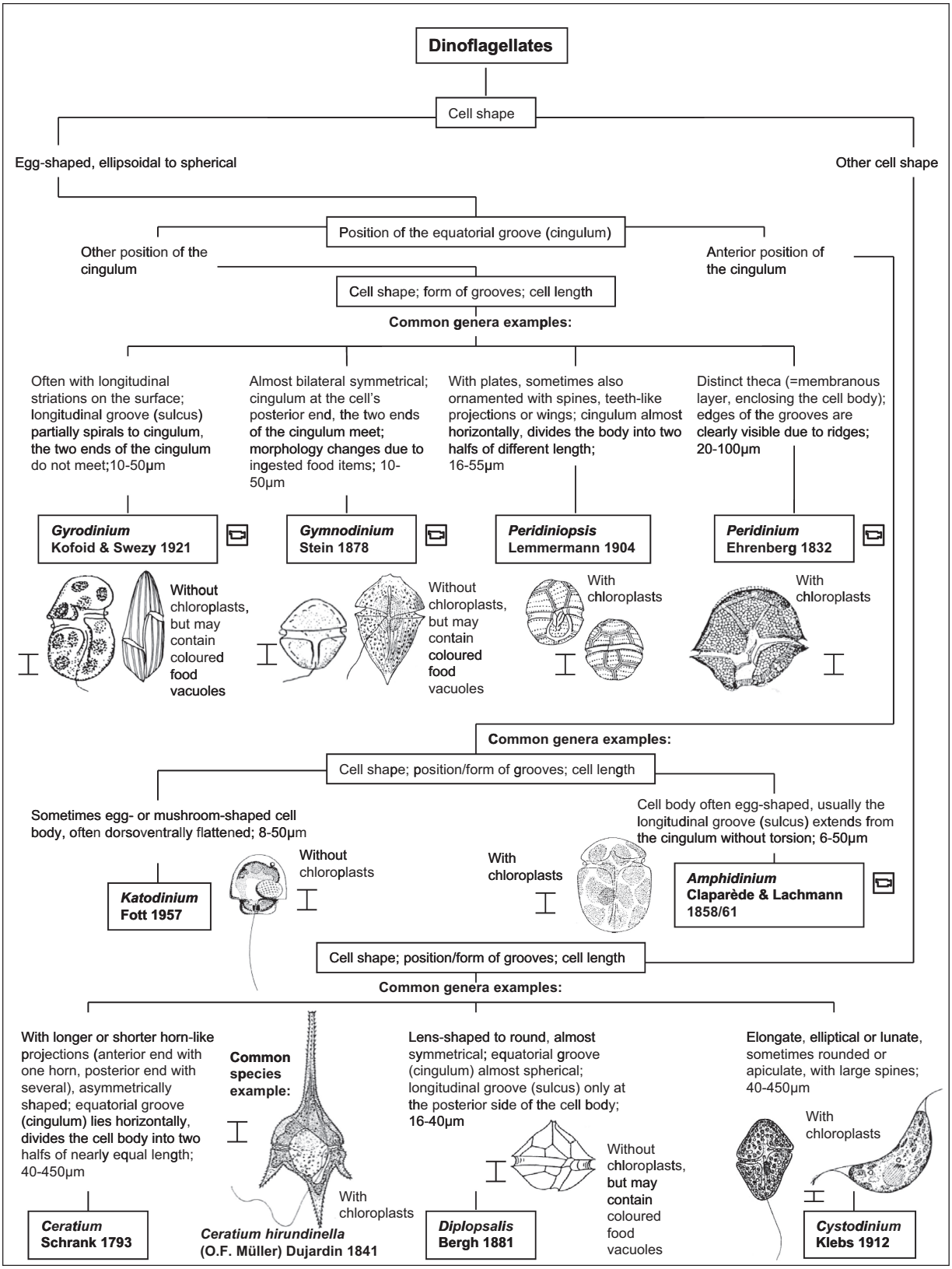

Dinoflagellates
Cell shape
Egg-shaped, ellipsoidal to spherical
Other cell shape
Position of the equatorial groove (cingulum)
Other position of the cingulum
Anterior position of the cingulum
Cell shape; form of grooves; cell length
Common genera examples:
Often with longitudinal striations on the surface; longitudinal groove (sulcus) partially spirals to cingulum, the two ends of the cingulum do not meet;10-50µm
Almost bilateral symmetrical; cingulum at the cell's posterior end, the two ends of the cingulum meet; morphology changes due to ingested food items; 10-50µm
With plates, sometimes also ornamented with spines, teeth-like projections or wings; cingulum almost horizontally, divides the body into two halfs of different length; 16-55µm
Distinct theca (=membranous layer, enclosing the cell body); edges of the grooves are clearly visible due to ridges; 20-100µm
Gyrodinium Kofoid & Swezy 1921
Gymnodinium Stein 1878
Peridiniopsis Lemmermann 1904
Peridinium Ehrenberg 1832
Without chloroplasts, but may contain coloured food vacuoles
Without chloroplasts, but may contain coloured food vacuoles
With chloroplasts
With chloroplasts
Common genera examples:
Cell shape; position/form of grooves; cell length
Sometimes egg- or mushroom-shaped cell body, often dorsoventrally flattened; 8-50µm
Cell body often egg-shaped, usually the longitudinal groove (sulcus) extends from the cingulum without torsion; 6-50µm
Without chloroplasts
With chloroplasts
Katodinium Fott 1957
Amphidinium Claparède & Lachmann 1858/61
Cell shape; position/form of grooves; cell length
Common genera examples:
With longer or shorter horn-like projections (anterior end with one horn, posterior end with several), asymmetrically shaped; equatorial groove (cingulum) lies horizontally, divides the cell body into two halfs of nearly equal length; 40-450µm
Common species example:
With chloroplasts
Ceratium hirundinella (O.F. Müller) Dujardin 1841
Lens-shaped to round, almost symmetrical; equatorial groove (cingulum) almost spherical; longitudinal groove (sulcus) only at the posterior side of the cell body; 16-40µm
Without chloroplasts, but may contain coloured food vacuoles
Elongate, elliptical or lunate, sometimes rounded or apiculate, with large spines; 40-450µm
With chloroplasts
Ceratium Schrank 1793
Diplopsalis Bergh 1881
Cystodinium Klebs 1912

Chart 15.

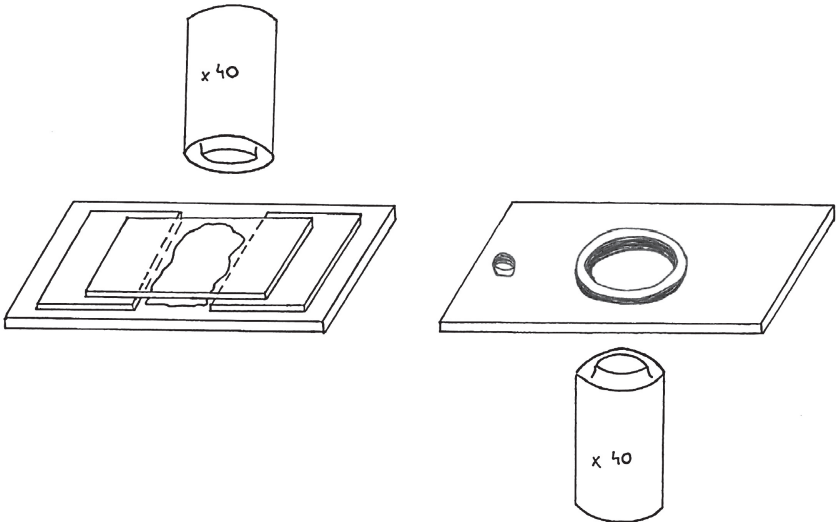


Figure 2. Schematic drawing of some helpful counting chambers. a) Simple microchamber: Two cover slips are placed on the slide (each on every end). A 10 μl droplet is placed between them and a third cover slip above the droplet and the two slips. b) Utermöhl chamber for counting living flagellates or sedimented, chemically fixed samples.

underneath the filter. The filters are generally stored deep-frozen at –20 °C until examination by an epifluorescence microscope.

It is additionally recommended to establish cultures of HF isolated from the samples (e.g. simply by adding autoclaved wheat grains etc. as a carbon source for food bacteria to parts of raw samples). This should aid in morphotype identification of bacterivorous forms and to check whether the determined morphotypes are life-cycle forms (swarmer, colony etc.) of other types, as it is often the case with e.g. choanoflagellates where extrinsic factors can induce the formation of multicellular forms (Alegado et al. 2012; Dayel et al. 2011). However, it has to be considered that all cultivation and fixation methods (and also molecular methods) can be very selective. According to our own experience, the percentage of live-counted organisms appearing in cultures ranges between 1 and 30 per cent.

Acknowledgements

Special thanks go to Frank Nitsche, Anja Scherwass, Åron Kiss, and Brigitte Gräfe who helped to improve the guide. We are indebted to Alexander P. Mylnikov for introducing us to the world of flagellate diversity in 1995. We are also thankful to the CCAC (Barbara Melkonian, http://www.ccac.uni-koeln.de/) for providing *Peridinium cinctum* (CCAC 0102) and *Amphidinium carteri* (CCAC 2266B) and to many students who tested and improved the guide. This study was supported by grants from the German Research Foundation to H.A. (HA 288/7,16, 17)

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.protis.2013.08.003.

References

Adl SM, Simpson AGB, Lane CE, Lukes J, Bass D, Bowser SS, Brown MW, Burki F, Dunthorn M, Hampl V, Heiss A, Hoppenrath M, Lara E, Le Gall L, Lynn DH, McManus H, Mitchell EAD, Mozley-Stanridge SE, Parfrey LW, Pawlowski J, Rueckert S, Shadwick L, Schoch CL, Smirnov A, Spiegel F (2012) The revised classification of eukaryotes. J Eukaryot Microbiol **59**:429–493

Alegado RA, Brown LW, Cao S, Dermenjian RK, Zuzow R, Fairclough SR, Clardy J, King N (2012) A bacterial sulfonolipid triggers multicellular development in the closest living relatives of animals. eLIFE **1**:e00013

Arndt H, Mathes J (1991) Large heterotrophic flagellates form a significant part of protozooplankton biomass in lakes and rivers. Ophelia **33**:225–234

Arndt H, Dietrich D, Auer B, Cleven E, Gräfenhan T, Weitere M, Mylnikov A (2000) Functional Diversity of Heterotrophic Flagellates in Aquatic Ecosystems. In Leadbeater BSC, Green JC (eds) The Flagellates. Taylor & Francis, London, pp 240–268

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser **10**:257–263

Bass D, Howe AD, Mylnikov AP, Vickerman K, Chao EE, Smallbone JE, Snell J, Cabral JrC, Cavalier-Smith T (2009)

Phylogeny and classification of Cercomonadida (Protozoa, Cercozoa): *Cercomonas, Eocercomonas*, *Paracercomonas*, and *Cavernomonas* gen. nov. Protist **160**:483–521

Bird DF, Kalff J (1986) Bacterial grazing by planktonic lake algae. Science **231**:493–495

Boehme A, Risse-Buhl U, Kuesel K (2009) Protists with different feeding modes change biofilm morphology. Microb Ecol **69**:158–169

Boenigk J, Arndt H (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie van Leeuwenhoek **81**:465–480

Bonkowski M (2004) Protozoa and plant growth: the microbial loop in soil. New Phytol **162**:617–631

Børsheim KY, Bratbak G (1987) Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar Ecol Prog Ser **36**:171–175

Brabender M, Kiss AK, Domonell A, Nitsche F, Arndt (2012) Phylogenetic and morphological diversity of novel soil cercomonad species with a description of two new genera (*Nucleocercomonas* and *Metabolomonas*). Protist **163**:495–528

Caron DA (1983) Technique for enumeration of heterotrophic and phototrophic nanoplankton, using epifluorescence microscopy, and comparison with other procedures. Appl Environ Microbiol **46**:491–498

Cavalier-Smith T, Chao EE (2010) Phylogeny and evolution of Apusomonadida (Protozoa: Apusozoa): New genera and species. Protist **161**:549–576

Choi JW, Stoecker DK (1989) Effects of fixation on cell volume of marine planktonic protozoa. Appl Environ Microbiol **55**:1761–1765

Christoffersen K, Nybroe O, Jürgens K, Hansen M (1997) Measurement of bacterivory by heterotrophic nanoflagellates using immunofluorescence labelling of ingested cells. Aquat Microb Ecol **13**:127–134

Dayel MJ, Alegado RA, Fairclough SR, Levin TC, Nichols SA, McDonald K, King N (2011) Cell differentiation and morphogenesis in the colony-forming choanoflagellate *Salpingoeca rosetta*. Dev Biol **357**:73–78

Fenchel T (1987) Ecology of Protozoa. The Biology of Free-living Phagotrophic Protists. Science Tech Publishers/Springer Verlag, Madison/Wisconsin, Berlin

Fenchel T (1991) Flagellate Design and Function. In Patterson DJ, Larsen J (eds) The Biology of Free-living Heterotrophic Flagellates. Clarendon Press, Oxford, pp 7–19

Foissner W, Berger H (1996) A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshw Biol **35**:375–482

Gasol JM (1993) Benthic flagellates and ciliates in fine freshwater sediments: Calibration of a live counting procedure and estimation of their abundances. Microb Ecol **25**: 247–262

Güde H (1979) Grazing by protozoa as selection factor for activated sludge bacteria. Microb Ecol **5**:225–237

Hausmann K, Hülsmann N, Radek R (2003) Protistology. 3rd ed. Schweizerbart Science Publishers, Stuttgart

Jones RI (2000) Mixotrophy in planktonic protists: an overview. Freshw Biol **45**:219–226

Jürgens K, Matz C (2002) Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. Antonie van Leeuwenhoek **81**:413–434

Karpov SA, Bass D, Mylnikov AP, Cavalier-Smith T (2006) Molecular phylogeny of Cercomonadidae and kinetid patterns of *Cercomonas* and *Eocercomonas* gen. nov. (Cercomonadida, Cercozoa). Protist **157**:125–158

Kim E, Archibald JM (2013) Ultrastructure and molecular phylogeny of the cryptomonad *Goniomonas avonlea* sp. nov. Protist **164**:160–182

Leadbeater BSC, Green JC (eds) The Flagellates. Taylor & Francis, London,

Lee JL, Leedale G, Bradburry P (eds) An Illustrated Guide to the Protozoa. Second edition, Allen Press, Lawrence, Kansas

Massana R, Güde H (1991) Comparison between three methods for determining flagellate abundance in natural waters. Ophelia **33**:197–203

Nauwerck A (1963) Die Beziehungen zwischen Zooplankton und Phytoplankton im See Erken. Symb Bot Ups **17(5)**:1–163

Patterson DJ, Hedley S (1992) Free-living Freshwater Protozoa. A Colour Guide. Wolfe Publishing, London

Patterson DJ, Larsen J (eds) The Biology of Free-living Heterotrophic Flagellates. Clarendon Press, Oxford

Pawlowski J, Christen R, Lecroq B, Bachar D, Shahbazkia HR, Amaral-Zettler L, Guillou L (2011) Eukaryotic richness in the abyss: Insights from Pyrotag Sequencing. PLoS One **6**:e18169

Pawlowski J, Audic S, Adl S, Bass D, Belbahri L, Berney C, Bowser SS, Cepicka I, Decelle J, Dunthorn M, Fiore-Donno AM, Gile GH, Holzmann M, Jahn R, Jirku M, Keeling PJ, Kostka M, Kudryavtsev A, Lara E, Lukes J, Mann DG, Mitchell EAD, Nitsche F, Romeralo M, Saunders GW, Simpson AGB, Smirnov AV, Spouge JL, Stern RF, Stoeck T, Zimmermann J, Schindel D, de Vargas C (2012) CBOL Protist Working Group: Barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. PLoS Biol **10**:e1001419

Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. Nature Rev Microbiol **3**:537–546

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr **25**:943–948

Prosdocimi EM, Novati S, Bruno R, Bandi C, Mulatto P, Giannico R, Casiraghi M, Ferri E (2013) Errors in ribosomal sequence datasets generated using PCR-coupled 'panbacterial' pyrosequencing, and the establishment of an improved approach. Mol Cell Probes **27**:65–67

Sanders RW (1991) Mixotrophic protists in marine and freshwater ecosystems. J Eukaryot Microbiol **38**:76–81

Sanders RW, Porter KG, Bennett SJ, DeBiase AE (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers,

and cladocerans in a freshwater planctonic community. Limnol Oceanogr **34**:673–687

Scheckenbach F, Wylezich C, Mylnikov AP, Weitere M, Arndt H (2006) Molecular comparisons of freshwater and marine isolates of the same morphospecies of heterotrophic flagellates. Appl Environ Microbiol **72**: 6638–6643

Scherwass A, Fischer Y, Arndt H (2005) Detritus as a potential food source for protozoans: consumption and digestion of plant detritus by a heterotrophic flagellate (*Chilomonas paramecium*) and a ciliate (*Tetrahymena pyriformis*). Aquat Ecol **39**:439–445

Sherr EB (1988) Direct use of high molecular weight polysaccharide by heterotrophic flagellates. Nature **335**: 348–351

Sherr BF, Sherr EB (1983) Enumeration of heterotrophic microprotozoa by epifluorescence microscopy. Estuar Coast Shelf Sci **16**:1–7

Sherr EB, Sherr BF (1994) Bacterivory and herbivory: Key roles of phagotrophic protists in pelagic food webs. Microb Ecol **28**:223–235

Sherr BF, Sherr EB, Pedros-Alio C (1989) Simultaneous measurement of bacterioplankton production and protozoan bacterivory in estuarine water. Mar Ecol Prog Ser **54**: 209–219

Sonntag B, Posch T, Psenner R (2000) Comparison of three methods for determining flagellate abundance, cell size, and biovolume in cultures and natural freshwater samples. Arch Hydrobiol **149**:337–351

Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner H-W, Richards TA (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol **19**:21–31

Stoupin D, Kiss AK, Arndt H, Shatilovich AV, Gilichinsky DA, Nitsche F (2012) Cryptic diversity within the choanoflagellate morphospecies complex *Codosiga botrytis* – Phylogeny and morphology of ancient and modern isolates. Eur J Protistol **48**:263–273

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt Int Verein Limnol **9**:1–38

Wilken S, Huisman J, Naus-Wiezer, Van Donk E (2013) Mixotrophic organisms become more heterotrophic with rising temperature. Ecol Lett **16**:225–233

Will KW, Rubinoff D (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics **20**:47–55

Zhukov BF (1993) Atlas of Freshwater Heterotrophic Flagellates (Biology, Ecology, Taxonomy) [in Russian]. Russian Academy of Sciences, Institute of the Biology of Inland Waters