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Protists as bioindicators in activated sludge: Identification, ecology and future needs☆

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Abstract

When the activated sludge process was developed, operators and scientists soon recognized protists as valuable indicators. However, only when Curds et al. (1968) showed with a few photographs the need of ciliates for a clear plant effluent, sewage protistology began to bloom but was limited by the need of species identification. Still, this is a major problem although several good guides are available. Thus, molecular kits should be developed for identification. Protists are indicators in two stages of wastewater treatment, viz., in the activated sludge and in the environmental water receiving the plant effluent. Continuous control of the protist and bacterial communities can prevent biological sludge foaming and bulking and may greatly save money for sludge oxygenation because several protist species are excellent indicators for the amount of oxygen present. The investigation of the effluent-receiving rivers gives a solid indication about the long term function of sewage works.

The literature on protist bioindication in activated sludge is widely distributed. Thus, I compiled the data in a simple Table, showing which communities and species indicate good, mediocre, or poor plant performance. Further, many details on indication are provided, such as sludge loading and nitrifying conditions. Such specific features should be improved by appropriate statistics and more reliable identification of species. Then, protistologists have a fair chance to become important in wastewater works.

Activated sludge is a unique habitat for particular species, often poorly or even undescribed. As an example, I present two new species. The first is a minute (~30 μm) *Metacystis* that makes an up to 300 μm-sized mucous envelope mimicking a sludge floc. The second is a *Phialina* that is unique in having the contractile vacuole slightly posterior to mid-body. Finally, I provide a list of species which have the type locality in sewage plants.

Keywords: Activated sludge; Ciliates; *Metacystis mucosa* n. sp.; *Phialina serranoi* n. sp.

Introduction

Farming needs about two thirds of the earth's freshwater and the industry takes further 23%. To produce 1 kg corn requires 1300 l water and 15,000 l are needed to produce 1 kg beef. An US-American consumes about 250 l/day, a European about 160 l, and an African only 20 l (Helmholtz-Zentrum 2011).

Most of the used water comes back as wastewater contaminated with organic and inorganic materials. Several methods have been developed to clean wastewater (Fig. 1) but all imitate the self-purification in running waters: the wastes are degraded by organisms, mainly bacteria, to minerals, water, CO_2, heat and new biomass, e.g. bacteria. This needs a lot of oxygen which must be added to the wastewater treatment plants to obtain "activated = oxygenated sludge" composed

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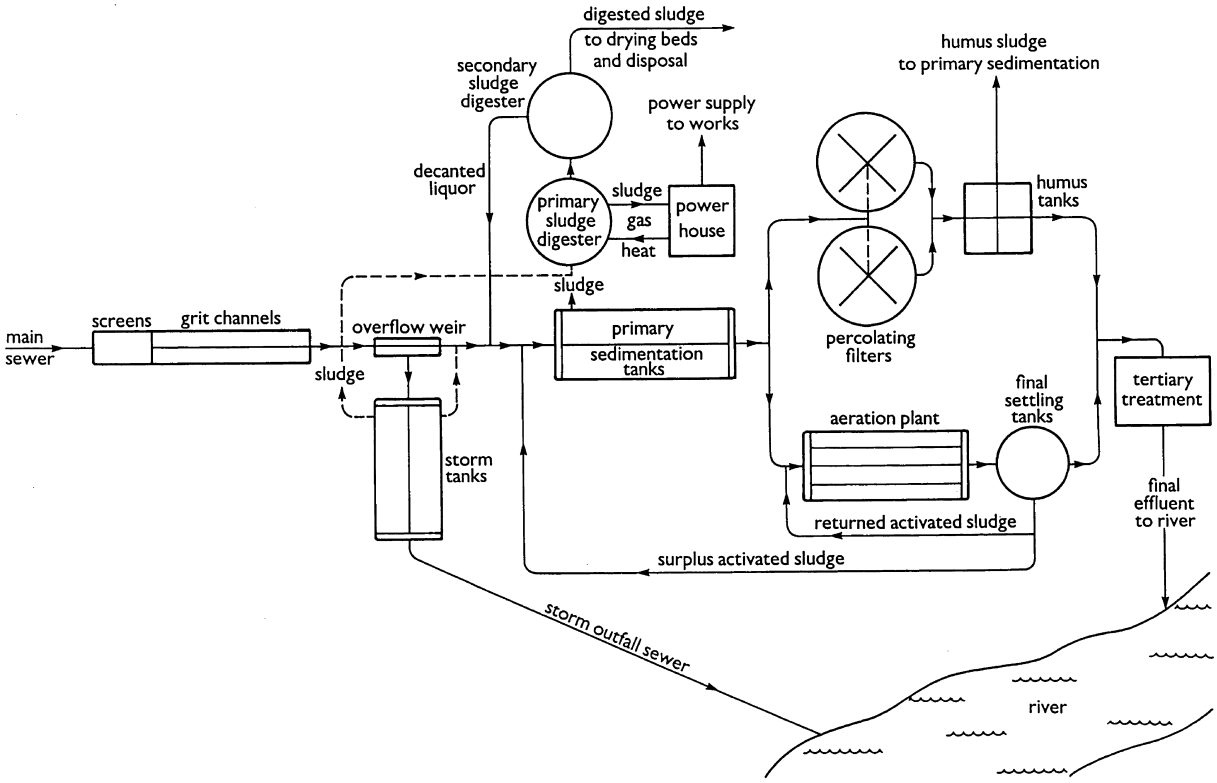

Fig. 1. Flow diagram and layout of a typical sewage-treatment work (from Curds 1992).

of about 0.5 mm-sized flocs made of inorganic and organic materials covered by a thin layer of active organisms, such as bacteria, fungi, and protists (Arregui et al. 2013; Curds 1992; Liebmann 1936, 1951, 1958; Mudrak and Kunst 1994; Pauli et al. 2001; Uhlmann 1982).

The most expensive process is the oxygenation of the activated sludge. Here, protists can help to minimize oxygenation because many of them are excellent oxygen indicators (Berger et al. 1997). The activated sludge process is frequently disturbed by "sludge foaming" and "sludge bulking", that is, part of the sludge flocs does not settle and contaminate the plant effluent, respectively, the river in which the effluent is discharged. Here, protists can serve as an early and late warning tool when the river shows signs of overloading (Berger et al. 1997; Curds 1992; Ganner et al. 2002; Liebmann 1936, 1951; Uhlmann 1982).

This review should stimulate students and group leaders to join an interesting, applied branch of protistology. It does neither review the technical basics of wastewater treatment works (for reviews see, e.g., Bayerisches Landesamt für Wasserwirtschaft 1999; Uhlmann 1982; Imhoff and Imhoff 1993; Fig. 1) nor the important role bacteria and fungi play in the activated sludge process (for reviews see, e.g., Arregui et al. 2013; Eikelboom and van Buijsen 1992; Kunst et al. 2000; Lemmer and Lind 2000). I shall concentrate on protists, i.e., the release from the taxonomic impediment, including the description of two new ciliates from an Austrian sewage plant; brief chapters on industrial wastewaters and on indices for the presentation of microscopic sludge analyses; and a detailed table on the species and communities indicating specific parameters of plant performance.

A brief history

Historically, one might recognize three principal periods in using protists as indicators in wastewater 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 Ardern and Lockett (1914) created the term "activated sludge" and researchers recognized the importance of protists in cleaning the wastewater 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 and Cockburn (1970a, b) to update bioindication in wastewater treatments plants. Their faunistic and experimental studies lay the ground for a scientific treatment of the field. They showed which ciliate

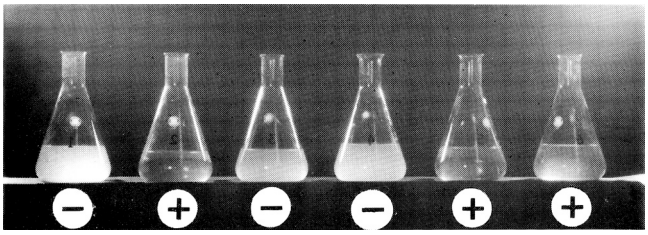

Fig. 2. Photograph of effluents issuing from laboratory-scale pilot plants operating in the presence (+) and absence (−) of ciliated protozoa (from Curds 1992).

species dominate in sewage plants and demonstrated their significance for a clear effluent (Fig. 2).

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, most now with nitrogen elimination (Bayerisches Landesamt für Wasserwirtschaft 1999; Ganner et al. 2002). 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, Fernandez-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: Bayerisches Landesamt für Wasserwirtschaft (1992, 1999), Berger and Foissner (2003), Buck and Buck (1980), Curds (1982, 1992), Eikelboom and van Buijsen (1992), Foissner and Berger (1996), Foissner et al. (1991, 1992, 1994, 1995, 1999), Ganner et al. (2002), Jenkins et al. (2004), Kinner (1984), Madoni (1991, 1994), Mudrak and Kunst (1994), Pauli et al. (2001), 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 and Horan 1991; Curds and Cockburn 1970b; Madoni 1994; Bayerisches Landesamt für Wasserwirtschaft 1992) and specific identification keys were published (Berger and Foissner 2003; Berger et al. 1997; Fernández-Galiano et al. 1996; Foissner and Berger 1996; Foissner et al. 1991, 1992, 1994, 1995, 1999, Fig. 4). 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 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 learn identification of 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 control soon stopped identification work and allocated it to technical assistants!

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.

Several books are available for the identification of microscopic wastewater organisms. For ciliates, the most comprehensive guide is that of Berger and Foissner (2003). It shows 357 common freshwater ciliates on flow charts, of course including those frequently occurring in activated sludge (Fig. 4A). This guide, which is written in English language, has 160 pages and solves not only the taxonomic impediment but informs on several other features, such as biomass, indicator value, and preferred habitat. More detailed descriptions of the morphology and ecology of the individual species and many micrographs of live and silver-impregnated specimens are contained in the monographs of Foissner et al. (1991, 1992, 1994, 1995).

The ciliate guide by Serrano et al. (2008) has a conventional key to common genera in activated sludge and shows 61 species each by a rather crude line drawing and a single micrograph. Unfortunately, the figures lack labels and any information on similar species which might cause misidentifications, is lacking. The real values of this booklet are the ecological data, i.e., under which plant conditions the species have been found. It is written in English language.

"Das mikroskopische Bild bei der biologischen Abwasserreinigung" (The microscopic picture of biological wastewater purification) has been published by the Bayerische Landesamt für Wasserwirtschaft (1999). Very likely, this is the most comprehensive guide to the investigation of activated sludge and plant performance. It presents many good figures of about 100 species ranging from bacteria to small metazoans and other microscopic structures, such as starch grains and resting cysts of protists. Further, it is the sole book that provides help if, e.g., the sludge is bulking or foaming, the substrate concentration is not appropriate, and indicators of anaerobity are present.

The colour guide to wastewater organisms by Berk and Gunderson (1993) is a nice booklet showing a variety of organisms ranging from bacteria to eggs of parasitic worms. All are not identified to species level and thus this guide is hardly useable for an estimation of sewage plant performance.

An electronic guide is in preparation by Curds, Roberts, Salvado and Warren. It appears promising, showing 175 ciliate species and their ecology, similar as in Berger and Foissner

Table 1. Comparison of a high-rate wastewater treatment plant (ARA1) with conventional activated sludge plants (from Aescht and Foissner 1992).

Parameter	ARA1			Conventional plants		
	Min	Max	Average	Min	Max	Average
Temperature (°C)	–	–	30	5	25	–
pH	6.5	7.7	7	4	9	7
Oxygen content (mg/l)	0.4	4.0	2.2	1	2	–
Oxygen saturation (%)	5	50	26	–	–	–
Space loading (kg biochemical oxygen demand$_5$/m^3 d)	–	–	21	0.25	1.5	1
Sludge age (d)	–	–	1	13	35	–
BOD influent (mg/l, biochemical oxygen demand)	–	–	21,000	–	–	300
BOD effluent (mg/l)	–	–	3000	–	–	20
COD influent (mg/l, chemical oxygen demand)	–	–	38,000	500	600	550
COD effluent (mg/l)	–	–	3400	–	–	100
COD: BOD	–	–	1.9	–	–	<1.7
COD digestion (%)	–	–	90	–	–	90
Sludge loading (kg BOD$_5$/kg suspended solids d)	0.7	1.0	0.8	0.05	0.3	–
Sludge volume (ml/l)	–	–	>600	300	600	–
Sludge index (ml/g suspended solids)	–	–	>200	80	120	–
Floc size (μm)	–	–	<30	150	500	–
Suspended solids (%)	–	–	3	–	–	<1
Anorganic substance (%)	45	56	50	15	65	40
Organic substance (%)	44	55	50	35	85	60
Prokaryotic biomass (mg/dry mass/l)	11,000	18,800	15,000	–	–	–
Eukaryotic biomass (mg/dry mass/l)	140	1470	590	–	–	–
Dominant bacteria		Filamentous			Flocculating	
Dominant protozoa		Flagellates			Ciliates	
Amoebae (number × 1000/ml)	0	770	340	0	19	–
Flagellates (number × 1000/ml)	0	5400	2000	0	17,000	2000
Globules (number × 1000/ml)	385	6000	1000			–
Ciliates (number × 1000/ml)	10	157	54	1	50	–
Rotifers (number/ml)	0	200	29	0	2300	–
Nematodes(number/ml)	0	0	0	–	–	1

(2003). Further information see: http://www.nhm.ac.uk/research-curation/projects/wastewater-ciliate/

Molecular identification

A molecular identification kit for the common sludge ciliates would minimize the problems discussed in the forgoing chapter. Unfortunately, such a kit is not available although the 18S rRNA sequence is available for rather many sludge ciliates while community analyses are still in their infancy (Eisenmann et al. 2001; Guggiari and Peck 2008; Marsh et al. 1998; Matsunaga et al. 2014).

Four tonnes of protozoa/day in a highly loaded sewage plant

The sewage of industrial wastewaters has been poorly studied. Often most protist species have not been identified to species level or some misidentified at all, e.g. in the study of Santos dos Araújo et al. (2014).

Aescht and Foissner (1992) investigated the organism community of a heavily loaded (21 kg BOD$_5$/m^3 d) plant with pharmaceutical wastes. The organic substance on average amounted to 16 g dry mass/l. The organism community consisted of 96% bacterial and 4% protozoan biomass (Table 1). Related to suspended solids the protozoan biomass constituted 2%; four tonnes protozoa (wet mass) are produced in 1400 m^3 wastewater daily. Very likely, this is the highest production value reported for heterotrophic protists. During the 1-year-study, about two flagellate species, two amoeba, eight ciliate, one nematod, and one rotifer species were recognized. Among the ciliates, one undescribed species was discovered, *Parastrongylidium oswaldi*.

Sludge indices

To have a single, simple numerical value for sewage plant performance is the dream of government administrators. A

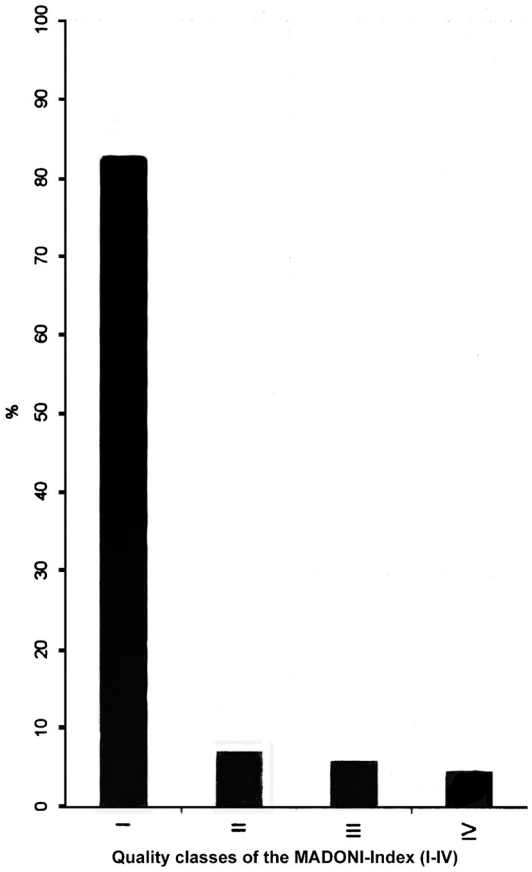

Fig. 3. Madoni-indices of 85 sewage plants from 38 wastewater works in Salzburg. I–IV: quality classes. From Ganner et al. (2002).

well known example is the saprobic index for evaluating the quality of running waters (Sládeček 1973).

Five indices/methods have been developed for the performance of activated sludge plants. Most widely applied is the sludge biotic index (SBI, Fig. 3) by Madoni (1994), while the indices of Curds and Cockburn (1970b), Morishita (1970), Al-Shahwani and Horan (1991), and the Bayerische Landesamt (1992) have been rarely and mainly locally used.

Both, the saprobic index and the SBI got heavy critic because it seems impossible to "reduce" the organism communities to a single, numerical value. However, most users recommend such indices when they are interpreted cautiously (Arévalo et al. 2009; Arregui et al. 2013; Drzewicki and Kulikowska 2011; Ganner et al. 2002 (Fig. 3); Liebmann 1951; Pedrazzani 2014; Sládeček 1973; Toman 2002).

Sewage plant performance by bioindication

Table 2 is a more complete version of a Table first put together by Arregui et al. (2013). It shows, inter alia, 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 days before and after the investigation. I think progress is possible, 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 (Jenkins et al. 2004; Kunst et al. 2000; Lemmer and Lind 2000). In contrast to conventional wastewater systems, Pérez-Uz et al. (2010) observed a high number of small heterotrophic flagellates (mainly bodonids) and small amoebae in enhanced-nitrogen removal wastewater treatment systems. Likewise, Canals et al. (2013) observed that ciliate diversity was much lower in wastewater with high ammonium concentration than in conventional systems. Further, systems which include anoxic or anaerobic periods must be evaluated with care (Dubber and Gray 2011b).

Protists are also important indicators in the environmental water bodies that receive the plant effluent. If the plant performance was good the past 2–3 weeks, then the water quality should be not or only slightly changed (Liebmann 1951, 1958).

Activated sludge, an almost untouched habitat for protist taxonomists

There is a wide belief that mostly common, usually polysaprobic protists occur in activated sludge. Indeed, several of the most common sludge ciliates belong to this domain. But there are also many less common species which are often poorly known because they are infrequent and of low abundance in their natural habitats. Some may get high abundances in activated sludge and thus can then be studied in detail, for instance, *Chilodonatella minuta*, *Prodiscophrya collini*, and *Enchelyomorpha vermicularis* redescribed by Becares and Foissner (1994), Aescht and Foissner (1992), and Foissner and Foissner (1995).

Very likely, there are many undescribed protists in activated sludge plants. Some may belong to the "rare biosphere" (Weisse 2014) and may develop to recognizable numbers in activated sludge. The following ciliates have their type locality in wastewater plants: *Colpoda ecaudata* (Liebmann, 1936; Foissner, 1993) (a new colpodid from the sewer system in the town of Leipzig, Germany); *Epistylis epibioticum* Banina, 1983; *E. longicaudatum* Banina, 1983; *E. poleneci saprobicum* Banina, 1983 (three new peritrichs from sewage plants in Russia); *Gastronauta aloisi* Oberschmidleitner and Aescht, 1996 (a new cyrtophorid from a plant with mixed domestic and industrial wastewater near to the town of Asten, Upper Austria. Also found by Silva and Silva-Neto (2001) in a sludge plant in Rio de Janeiro, Brazil but misidentified as *G. membranaceus*; see their Figs. 46, 47); *Deviata brasiliensis* Siqueira-Castro, Paiva and Silva-Neto, 2009 (a new hypotrich from a sewage plant near

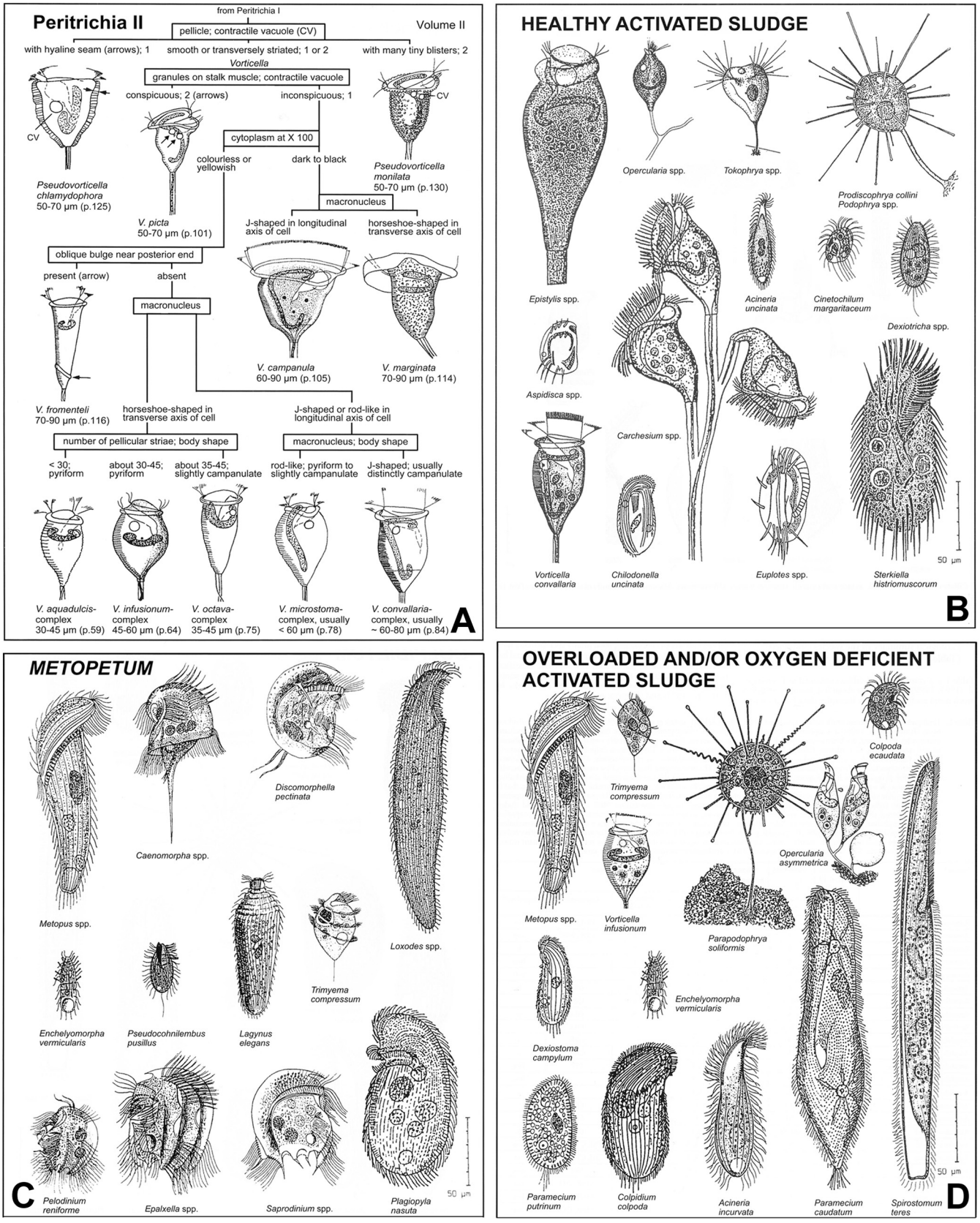

Fig. 4. (**A**) shows a flow chart from the ciliate guide by Berger and Foissner (2003). (**B**–**D**) show characteristic ciliate communities (from Berger and Foissner 2003).

Table 2. Microfaunal species and communities as indicators of sludge plant performance in conventional systems.

Organisms	Performance	Remarks	Literature[a]
Species (usually when subdominant at least)			
Acineria uncinata (Fig. 4B)	Mediocre	Instability of sludge (due to high abundance of free bacteria and insufficient cleaning; tolerates low oxygen concentration). Present with high densities and occurrence in nutrient removal systems	19
Aspidisca cicada (Fig. 4B)	Good	Stable plant conditions. Good removal of BOD_5 and COD, low oxygen and nitrate	1, 19 20, 25
Chilodonella uncinata (Fig. 4B)	Good	Good effluent quality, low oxygen concent	19, 25
Coleps hirtus	Good	Effective nitrification with good removal of ammoniacal-N in effluent	1
Enchelyomorpha vermicularis (Fig. 4C)	Poor	Microaerobic; overloading; hydraulic problems	2, 3
Euplotes aediculatus (Fig. 4B)	Mediocre	This species can be used for evaluating the toxicity of waters polluted by nickel	19
Euplotes mutabilis	–	Highly tolerant for heavy metals	22
Euplotes patella (Fig. 4B)	Mediocre	When abundant and in connection with many rotifers indicative for an increasing sludge volume index; otherwise underload	2, 6
Holophrya discolor	Mediocre	Microaerobic; intermitent and very low oxygenation; high N-reduction	4, 20
Litonotus lamella	Poor	Deficient sludge setting	19
Litonotus obtusus	Poor	Poor sludge setting	16
Metopus spp. (Fig. 4C, D)	Poor	Anaerobic conditions; overloading; hydraulic problems	1, 2, 5, 9
Opercularia articulata	–	Highly tolerant for NaCl	21
Opercularia coarctata (Fig. 4B)	Poor	Low effluent quality and toxicity (associated with high BOD_5 in the effluent)	19
Plagiocampa rouxi	Mediocre	Microaerobic; intermitent and very low oxygenation; high N-reduction	4, 20
Spirostomum teres (Fig. 4D)	Mediocre	Microaerobic; intermitent and very low oxygenation; high N-reduction. Toxicity by heavy metals, pesticides, or phenols	4, 19
Stylonychia mytilus	–	Removes Pb^{2+} from sludge	22
Tetrahymena pyriformis – complex	Poor	Related to short sludge retention time and colonization processes. Good indicator of polysaprobic to isosaprobic conditions	19
Trimyema compressum (Fig. 4C)	Poor	Microaerobic; overloading; hydraulic problems	2, 3
Trithigmostoma cucullulus	Good	Sensitive to ammonia and phosphate	20
Trochilia minuta	Good	Indicative of good nitrifying conditions; underload	19
Uronema nigricans	Poor	Low effluent quality	19
Vorticella campanula (Fig. 4A)	Good	High effluent quality; underload	2, 10, 25
Vorticella convallaria (Fig. 4B)	Mediocre	Lack of nitrification	1
Vorticella convallaria and *Arcella hemisphaerica*	Good	High sludge retention time; underload	2, 16, 17
Vorticella microstoma/infusionum (Fig. 4D) and *Opercularia* sp. (Fig. 4B)	Poor	Low clearing efficiency, especially when connected with high flagellate abundance; anaerobic; high sludge load and sludge volume index; low oxygen concentration	7, 8, 9, 17, 25
Vorticella striata – complex	Poor	Poor effluent quality and insufficient cleaning (positive relation with high volumetric load, mass load and high effluent BOD_5).	1, 19
Zoothamnium procerius	Good	Good depuration efficiency	19

Table 2. (*Continued*)

Organisms	Performance	Remarks	Literature[a]
Communities (when dominant or subdominant)			
Small flagellates	Poor	Low effluent quality; oxygen depletion; overloading; sludge maturation period; onset of nitrification	8, 18, 19
Small naked amoebae	Poor	Very high load; not easily degradable material; sludge maturation. Associated to anaerobic conditions and processes of high load poor yields in CAS. Small and large naked amoebae are compatible with good nitrification when WWTP are designed for biological nutrient removal. >50 μm are present in low organic loading rates and moderate and higher SRT, common in nutrients removal WWTP	8, 19
Small flagellates, naked amoebae, swarmers of peritrich ciliates; many dispersed bacteria	Poor	Unstable sludge; sludge maturation; toxic influences	2, 9
Testate amoeba, e.g., *Arcella*, *Euglypha*	Good	Underloading, high sludge retention time; usually found in N-removal plants. Good performance related to low mass load, sufficient oxygenation and good nitrification	8, 19, 23
Testate amoebae; crawling ciliates; attached peritrich ciliates with width peristome; nematods; rotifers	Good	Healthy, low-loaded, sufficiently aerated and well-flocculated sludge with high effluent quality	12
Glaucoma, Dexiostoma campylum (Fig. 4D), *Vorticella microstoma* and peritrich swarmers, flagellates and naked amoebae	Poor	Insufficient oxygenation; many dispersed bacteria; poor effluent quality	2, 9
Vorticella infusionum (Fig. 4D); *Opercularia coarctata* (Fig. 4B); *Acineria uncinata* (Fig. 4B); Small flagellates	Poor	High-loaded with insufficient oxygen; shock-load; high ammonia; many dispersed bacteria	12
Spirostomum minus, Euplotes affinis, Opercularia coarctata	–	Indicative of 80–85% nitrogen removal and of 90–95% of carbon compounds; do not tolerate NH_4^+ concentrations above 14 mg/l	24
Heterotrich ciliates (Fig. 4D) and many flagellates	Poor	Poor operation of RBC system	5
Epistylis (Fig. 4B), large naked amoebae, rotifers	Good	When in last stage of RBC system	13
Green algae on plant wall	Good	Underload since a long time	9
Small swimming ciliates	Mediocre	Too short sewage retention time; insufficient oxygenation. Positive relationship with effluent BOD_5 and negative correlation with sludge age	8, 19
Large swimming ciliates (Fig. 4B, D)	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	Good	Transient phenomena, such as recent sludge extraction, discontinuous load. Good management and performance of plant. Positively related to volumetric load and negatively to effluent BOD_5	1, 8, 19

Table 2. (*Continued*)

Organisms	Performance	Remarks	Literature[a]
Ciliates	Good	When abundance is 10^6/l or more	8
Ciliates	–	Abundance <10^4/l (poor), 10^4–10^6/l (mediocre), >10^6 (good)	11, 17
Metopetum (Fig. 4C)	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
Vorticella microstoma/infusionum (Fig. 4D) and *V. campanula*	Good	Well-setting sludge	10
Cyrtophorids, hypotrichs, scuticociliates, pleurostomatids (Fig. 4B)	Good	Good operation of RBC system (Rotation Biological Contactor)	5
Opercularia, Uronema, nematods	Poor	Indicate overloading when in last stage of RBC system	13
Carnivorous ciliates, e.g., *Litonotus lamella, Amphileptus*	Poor	Poor-setting sludge	10
Aspidisca cicada (Fig. 4B), *Chilodonella* spp. (Fig. 4B), *Vorticella striata*	–	High sludge retention time	10
Epistylis plicatilis and *Vorticella striata*	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 = Pérez-Uz et al. (1998), 4 = Ganner et al. (2002), 5 = Martín-Cereceda et al. (2001), 6 = Cingolani et al. (1991), 7 = Gori et al. (1991), 8 = Madoni (1994), 9 = Bayerisches Landesamt für Wasserwirtschaft (1992, 1999), 10 = Lee et al. (2004), 11 = De Marco et al. (1991), 12 = Drzewicki and Kulikowska (2011), 13 = Berri and Casaschi (1991), 14 = Hu et al. (2013), 15 = Bedogni et al. (1991), 16 = Zhou et al. (2006), 17 = Toman (2002), 18 = De, Cybis and Horan (1997), 19 = Arregui, Liébana, Calvo, Pérez-Uz, Salvado and Serrano (2013), 20 = Dubber and Gray (2011a), 21 = Salvadó et al. (2001), 22 = Rehman et al. (2006), 23 = Chen et al. (2004), 24 = Luna-Pabello et al. (1996), 25 = Čech et al. (1994).

to the town of Rio de Janeiro, Brazil); *Euplotes muscicola lahorensis* Chaudhry and Shakoori, 2012 (a new subspecies from industrial wastewater in Pakistan); *Metacystis galiani* Arregui et al., 2010 (a new prostome from the wastewater of a health resort in Valencia, Spain); *Metacystis mucosa* (see description below); *Parastrongylidium estevesi* Paiva and Silva-Neto, 2005 (a new hypotrich from a sewage plant in Brazil); *Parastrongylidium oswaldi* Aescht and Foissner, 1992 (a hypotrich discovered in a heavily loaded pharmaceutical sewage plant in Tyrol, Austria); *Parentocirrus brasiliensis* Paiva and Silva-Neto, 2004 (a new hypotrich from a sewage plant in Rio de Janeiro, Brazil); *Phialina serranoi* (see description below); *Propyxidium elongatum* Shizheng and Zhengxue, 1995 (a new peritrich from paper mill sludge in China); *Telotrochidium matiense* (Martín-Cereceda et al., 1996) Martin-Cereceda et al., 2007 (a new, stalkless peritrich from a sewage plant in Madrid, Spain); *Vorticella aerotenci* Banina, 1983; *V. geispicae* Banina, 1983; *V. hyalina* Banina, 1983; *V. peterhoffi* (four new *Vorticella* species from sludge plants in Russia); and *Trochiliopsis australis* Foissner et al., 1988 (a new microthoracid from a sludge plant in southern Australia).

Future needs

Wastewater technology has reached a high standard. When biologists want not disappear from the wastewater industry, they should improve (i) the identification of the organisms and redescribe or describe insufficiently known or new species, (ii) the knowledge on the ecology of the individual indicator species, (iii) the knowledge on plant technology and statistics, (iv) data interpretation by incorporating plant type, physico-chemical parameters and the bacterial community, (v) the molecular identification of sludge protists by inventing methods that can be handled by technicians.

Description of new species

Both species are from an oxygenated, well working wastewater plant in Austria (see type locality and Ganner et al. 2002). They were discovered during wastewater courses in 1993 and 1999. One species is outstanding because it makes a voluminous mucous coat thus appearing like a sludge floc. For ciliate preparation methods and terminology, see Foissner (2014a) and Lynn (2008).

***Metacystis mucosa* nov. spec.** (Figs 5A–M, 6A–L, 7A–P; Table 3)

Diagnosis: Lives in a mucous coat up to 300 μm across. Size in vivo about 40 × 17 μm, contractile up to half of body length. Extended specimens lageniform or bursiform, contracted cells barrel-shaped or ovate. With a conspicuous posterior vacuole. Macronucleus in posterior half, globular. Contractile vacuole in or near mid-body. Lorica lageniform, made of a thin, colourless membrane. Cortex and silverline pattern as typical for genus. On average 31 longitudinal ciliary rows forming eight transverse ciliary rings; caudal cilium about as long as body. On average nine circumoral dikinetids and five perioral ciliary rings composed of monokinetids.

Type locality: Activated sludge plant for the town of Seekirchen, Salzburg county, Austria, 47°53′8.4″N 13°7′14.26″E.

Etymology: The Latin adjective *mucosa* refers to the voluminous mucous coat.

Type material: The holotype slide (reg. no. 832/2015) and three paratype slides (reg. no. 833/2015–835/2015) with protargol-impregnated specimens, three paratype slides (reg. no. 836/2015–838/2015) with Chatton–Lwoff silver nitrate-impregnated cells, and four slides (reg. no. 839/2015–842/2015) with Klein–Foissner silver nitrate-impregnated specimens have been deposited in the Biology Centre of the Upper Austrian Museum in Linz (LI). The holotype and other relevant specimens have been marked with black ink circles on the coverslip.

Description: *Metacystis mucosa* has an ordinary variability with all important features having variation coefficients ≤ than 15%.

Body size difficult to study because contractile by up to 50%. Several measurements suggest (Table 3): size of extended specimens in vivo 35–50 × 15–20 μm, usually about 40 × 17 μm; slightly smaller (mean: 34 × 12.8 μm, respectively, 36 × 14 μm) after Chatton–Lwoff silver nitrate impregnation when 5% preparation shrinkage are added; strongly shrunken in protargol preparations (mean 27 × 10 μm). Body shape also highly variable because very different in extended and contracted state. When extended elongate bursiform or lageniform with a length: with ratio of 2.0–3:1 and a slender more or less curved neck and a

Table 3. Morphometric data on *Metacystis mucosa*.

Characteristics[a]	Method	Mean	M	SD	SE	CV	Min	Max	*n*
Body, length	IV	39.3	37.0	5.5	2.0	14.1	35.0	50.0	8
	CHLE	34.0	33.0	4.5	1.0	13.4	27.0	44.0	21
	CHLC	21.2	21.0	4.3	0.9	20.2	17.0	34.0	21
	PE	26.5	27.0	2.9	0.6	11.0	22.0	31.0	21
	PC	14.6	14.0	1.8	0.4	12.5	12.0	18.0	21
Body, width	IV	16.8	16.0	2.1	0.7	12.3	15.0	19.0	8
	CHLE	12.8	13.0	1.6	0.3	12.4	10.0	16.0	21
	CHLC	16.5	16.0	2.9	0.6	17.3	13.0	24.0	21
	PE	10.2	10.0	0.8	0.2	8.0	9.0	12.0	21
	PC	11.5	11.0	1.5	0.3	13.1	9.0	15.0	21
Body length: width, ratio	IV	2.4	2.4	0.4	0.1	15.5	1.8	3.0	8
	CHLE	2.7	2.6	0.3	0.1	10.0	2.3	3.3	21
	CHLC	1.3	1.3	0.1	0.1	8.0	1.1	1.4	21
	PE	2.6	2.6	0.2	0.1	7.6	2.3	3.0	21
	PC	1.3	1.3	0.1	0.1	7.5	1.0	1.4	21
Anterior body end to macronucleus	P	9.3	9.0	2.5	0.5	26.3	5.0	14.0	21
Oral bulge, height	P	2.1	2.0	–	–	–	2.0	3.0	20
Anterior body end to last ciliary ring	P	18.6	19.0	2.2	0.5	11.9	15.0	22.0	20
Macronucleus, length	P	6.2	6.0	0.6	0.1	10.0	5.0	7.0	21
Macronucleus, width	P	6.1	6.0	0.6	0.1	9.8	5.0	7.0	21
Micronucleus, length	P	1.6	2.0	–	–	–	1.0	2.0	8
Micronucleus, width	P	1.3	1.0	–	–	–	1.2	2.0	8
Circumoral dikinetids, number	P	9.0	9.0	0.6	0.2	6.4	8.0	10.0	7
Perioral ciliary rings, number	P	5.0	5.0	0.0	0.0	0.0	5.0	5.0	8
Somatic ciliary rings, number	P	8.0	8.0	0.3	0.1	4.1	7.0	9.0	20
	CHL	8.1	8.0	0.6	0.1	7.9	7.0	9.0	22
Longitudinal ciliary rows, number	P	31.3	31.0	1.8	0.5	5.7	28.0	34.0	13
	CHL	31.2	32.0	2.0	0.5	6.3	26.0	33.0	13

[a]Data based on environmental, partially selected specimens. Measurements in μm. CV, coefficient of variation in %; CHL, Chatton–Lwoff silver nitrate impregnation; CHLC, more or less contracted, silver nitrate-impregnated specimens; IV, in vivo; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, number of cells investigated; P, protargol impregnation; PC, more or less contracted, protargol-impregnated specimens; PE, more or less extended, protargol-impregnated specimens; SD, standard deviation; SE, standard error of arithmetic mean.

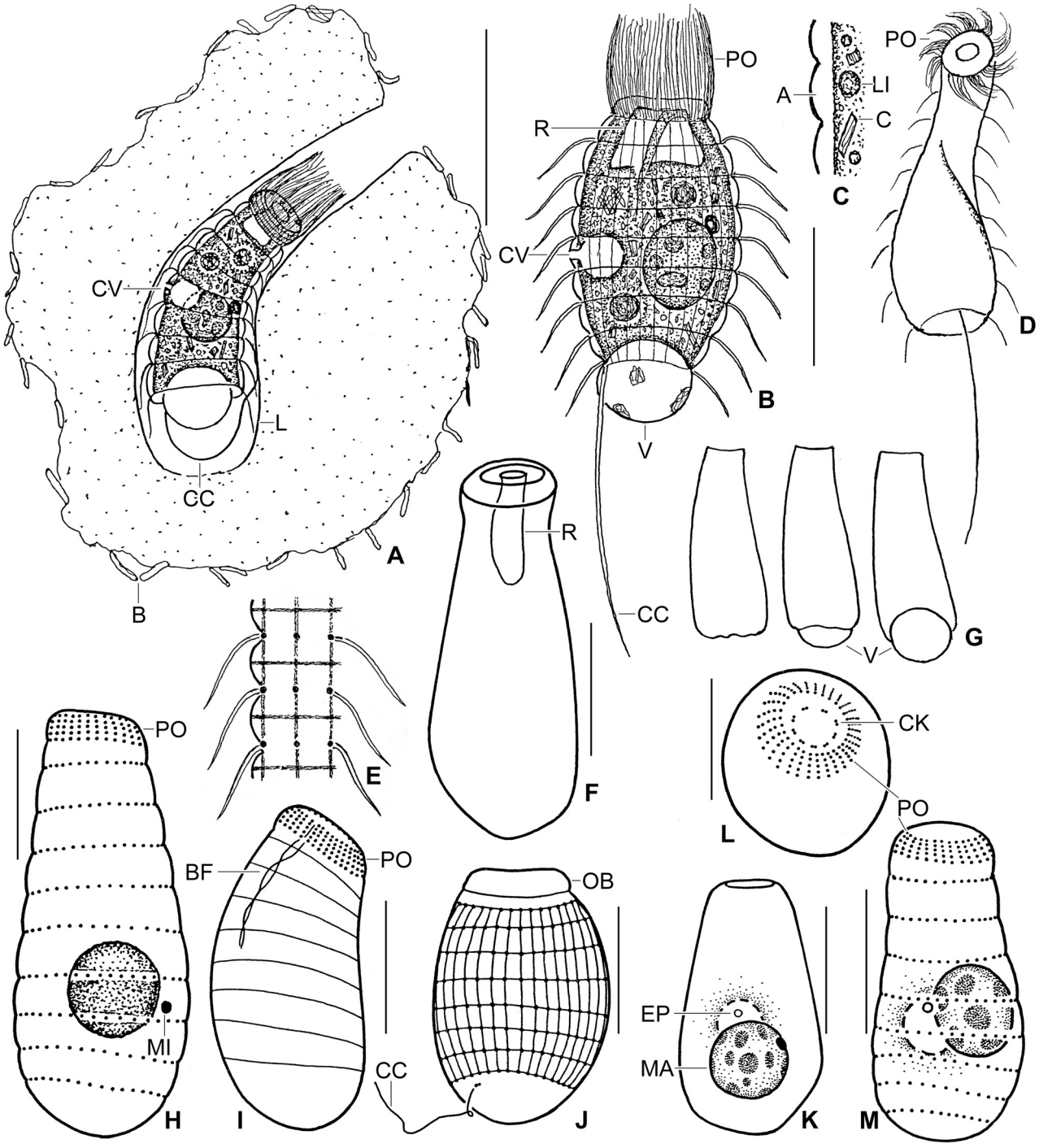

Fig. 5. A–M. *Metacystis mucosa* from life (A–E, G) and after protargol impregnation (F, H–M). **A:** An extended, 40 μm long specimen within its mucous coat the opening of which is lined by the membranous lorica. **B:** A freely motile, contracted specimen, length 23 μm, showing the main organelles, especially the contractile vacuole and the long caudal cilium. **C:** Cortex. **D:** An extended specimen, showing the perioral ciliary waves. **E:** Cortex structure; cilia in mid of figure not shown. **F:** An extended specimen, showing the receptaculum. **G:** Formation of the posterior vacuole. **H:** Basal body pattern of a slightly contracted specimen. **I:** brush fibres? **J:** Cortex structure and caudal cilium. **K:** Excretory pore and macronucleus. **L:** Slightly oblique anterior polar view, showing the oral basal body (ciliary) pattern. **M:** Holotype specimen, length 22 μm. A, alveolus; B, bacteria; BF, brush fibres? C, cytoplasmic crystal; CC, caudal cilium; CK, circumoral kinety; CV, contractile vacuole; EP, excretory pore; L, lorica; LI, lipid droplet; MA, macronucleus; MI, micronucleus; OB, oral bulge; PO, perioral ciliature; R, receptaculum; V, posterior vacuole. Scale bars 10 μm (B, F, H–M) and 30 μm (A).

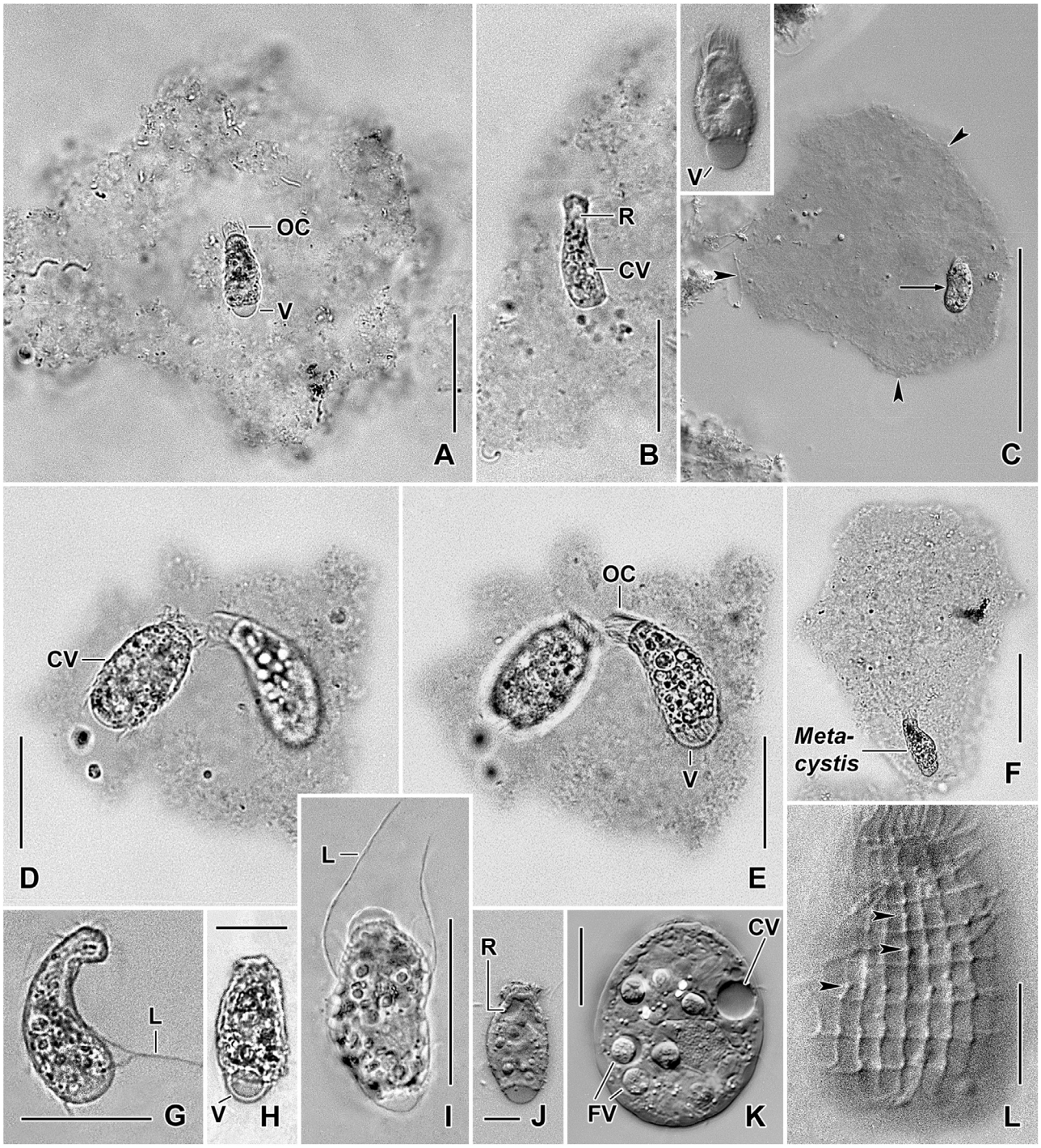

Fig. 6. A–L. *Metacystis mucosa* from life, bright field (A–H) and interference contrast (C, inset; I–L) micrographs. **A:** A slightly contracted specimen in a large slime floc. Note the characteristic posterior vacuole. **B:** An extended, swirling specimen, showing the receptaculum and the contractile vacuole slightly posterior to mid-body. **C:** A contracted specimen (arrow) within a homogenous mucous coat (arrowheads). The inset shows a cell with a conspicuous posterior vacuole. **D, E:** A contracted and a moderately contracted specimen forming the mucous coat. **F:** An extending specimen in a large slime floc. **G:** An extended, feeding specimen with lorica destroyed by the preparation. **H:** A moderately contracted specimen. **I:** A slightly pressed specimen leaving the lorica through the posterior end. The lorica is very thin and colourless. **J:** A specimen showing the receptaculum. **K:** A strongly pressed specimen, showing compact food vacuoles and the contractile vacuole near mid-body. **L:** Surface view, showing the cortex pattern and the transverse basal body (ciliary) rings (arrowheads). CV, contractile vacuole; FV, food vacuoles; L, lorica; OC, oral cilia; R, receptaculum; V, posterior vacuole. Scale bars 10 μm (J–L), 15 μm (H), 30 μm (D, E, G, I), 40 μm (B), 50 μm (A, F), and 100 μm (C).

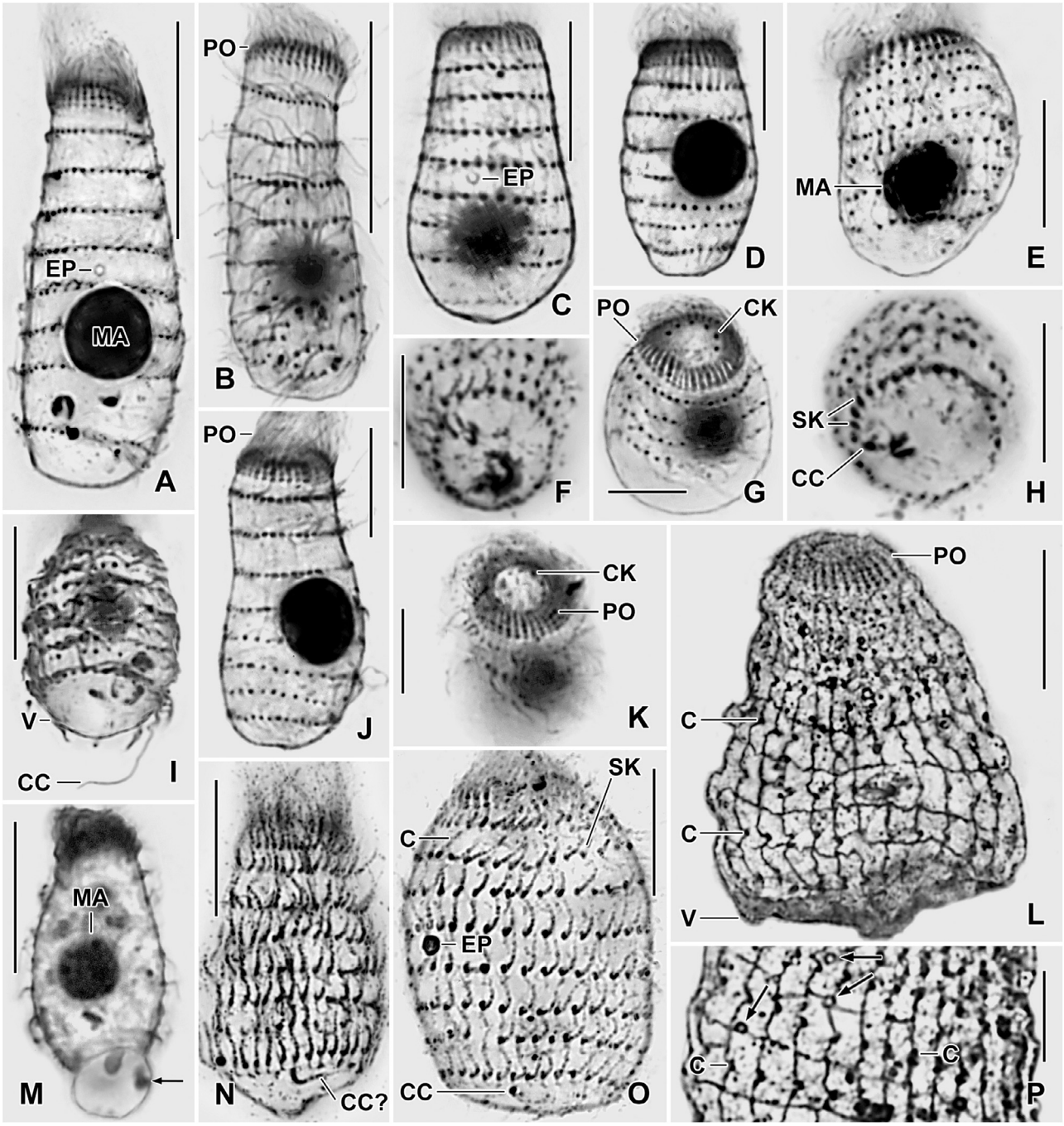

Fig. 7. A–P. *Metacystis mucosa*, basal body (~ciliary) pattern after protargol impregnation (A–K, M) and after Chatton–Lwoff (N, O) and Klein–Foissner (L, P) silver nitrate impregnation. **A, B, J:** More or less extended specimens, showing the ciliary pattern on average composed of 31 meridional ciliary rows forming 8 transverse ciliary rings. The pore of the contractile vacuole is in or slightly posterior to mid-body while the macronucleus is in the posterior third of the cell. **C–E, I:** Moderately (C) and strongly (D, E, I) contracted specimens are bursiform (C), barrel-shaped (D), ellipsoid (E), or ovate (I). Note the long caudal cilium in (I). **F, H:** Posterior polar views, showing the circular shape of the ciliary rings and the basal body of the caudal cilium. **G, K:** Oblique anterior polar views, showing the dikinetids of the circumoral kinety and the monokinetids of the five perioral ciliary rings. **L, P:** Silverline pattern with docked extrusomes marked by arrows in (P). **M:** A contracted specimen with a large posterior vacuole having attached some argyrophilic material (arrow). **N, O:** Cilia or kinetodesmal fibres impregnate with the Chatton–Lwoff silver nitrate method. Note the basal body of the caudal cilium and the excretory pore between the fourth and fifth ciliary ring (O). C, cilia (basal bodies); CC, caudal cilium; CK, circumoral kinety (dikinetids); EP, excretory pore of contractile vacuole; MA, macronucleus; PO, perioral ciliature; SK, somatic kineties; V, posterior vacuole. Scale bars 5 μm (G, K, P), 10 μm (C–F, H–J, L, M, O), and 15 μm (A, B, N).

broad posterior quarter (Figs 5A, D, F, H, 6B, G, 7A, B, J; Table 3); unflattened (Fig. 7F, H). More or less contracted specimens bursiform (Figs 5K, F, 6A, E, 7C), barrel-shaped (Figs 5B, J, 6D, 7D, O), ellipsoid (Figs 5I, J, 6C, H) or ovate (Figs 5K, 7I, M, N); about 23 × 17 μm in size (Table 3). Macronucleus usually in posterior half or third of cell, globular to broadly ellipsoid, about 7 μm across in vivo; with distinct nucleoli about 2 μm in size. Micronucleus near or attached to macronucleus, broadly ellipsoid, about 2.5 × 1.5 μm (Figs 5A, H, M, 7A, D, E, J, M; Table 3). Contractile vacuole usually between fourth and fifth somatic transverse ciliary ring, i.e., in or near mid-body, about 3 μm across in vivo, excretory pore about 1 μm in diameter (Figs 5A, B, K, M, 6B, D, K, 7A, C, O). Posterior vacuole not contractile, one third usually within cell, two thirds projecting (Figs 5A, B, 6A, C inset, E, J, 7A, E, I, N). Very likely bursts occasionally because it can disappear and is rebuilt (Fig. 5G). Vacuole contents hyaline, frequently some fluffy material attached to inner side of vacuole membrane (Figs 5B, 7M). Cortex very flexible, of typical *Metacystis* structure, i.e., with series of longitudinal and transverse ridges, forming oblong, rectangular, comparatively large alveoli with cilia inserted in mid (Figs 5B, C, E, J, 6L); alveolar pattern recognizable in vivo (Fig. 6L) and, occasionally, also in protargol-impregnated specimens (Figs 5J, 7B). Silverline pattern very similar to cortex pattern, docked extrusomes appear as minute rings in the transverse ridges (Fig. 7L, P); extrusomes in vivo not recognized possibly because only 1 μm long and of very similar refractivity as cortex. Cytoplasm colourless, usually crammed with food vacuoles, crystals, and granules (Figs 5A–C, 6A, D, E, G, I–K). Food vacuoles 3–4 μm across in vivo, very likely contain compacted bacteria. Crystals scattered throughout body, 0.2–3 μm in size, sometimes in yellowish vacuoles about 2 μm across.

Lives in a highly flexible lorica difficult to recognize because very thin, colourless, and very near to the cell (Figs 5A, 6A–C, F, G, I). Lorica possibly open at both ends, shape as described for the extended body, extends through the mucous coat, does not stain with alcian blue. Coat up to 300 μm in size, stains with alcian blue indicating acid mucopolysaccharides, very hyaline often becoming recognizable mainly due to attached bacteria (Figs 5A, 6A–C, F). Most cells leave coat when transported to the microscope slide but soon begin to secrete a new coat (Fig. 6D, E).

Somatic cilia only 5–6 μm long in vivo, form an average of 31 very narrowly and equidistantly spaced, longitudinal rows with cilia ordered so strictly that seven to nine, usually eight transverse somatic and five perioral ciliary rings originate. Caudal cilium about as long as body, inserts between cell and posterior vacuole, turned around posterior body region in loricate cells, easily overlooked (Figs 5A, B, D, J, 7H, L, N, O; Table 3).

Oral opening in centre of anterior end, broadly ellipsoid and about half as width as body, guides into a large, conical receptaculum with a membranous structure moving to and fro (Figs 5A, B, F, 6B, J). Oral opening surrounded by an average of nine circumoral dikinetids (Figs 5L, 7G, K; Table 3) with about 3 μm long cilia forming conical "oral flaps" as in prostomatid ciliates (Foissner et al. 1999). Five, rarely only four very narrowly spaced, monokinetal, perioral ciliary rings with 8–10 μm long cilia making nice metachronal waves when swirling bacteria into the oral opening (Figs 5A, B, D, L, M, 6A, E, 7B, J, G, K, L; Table 3).

Occurrence and ecology: As yet found only at type locality. *Metacystis* occurs in limnetic and marine habitats, and Arregui et al. (2010) discovered a new species attached to activated sluge flocs in a Spanish wastewater plant. About half of the species possibly lack a lorica while the loricate species are attached to a firm substrate (Arregui et al. 2010; Kahl 1930). I suppose that the enormous mucous coat of *M. mucosa* simulates a sluge floc, i.e., making the cell independent from wastewater flocs which tend to break or become coated by filamentous bacteria.

Species comparison: According to the reviews by Kahl (1930) and Arregui et al. (2010), *M. mucosa* is similar to *M. recurva* Penard, 1922; *M. exigua* Penard, 1922; and *M. tesselata* Kahl, 1926. However, none has such a voluminous slime coat as *M. mucosa*, which is thus an important diagnostic feature.

Metacystis recurva differs from *M. mucosa* not only by the absence of a slime coat but also by body length (~50 μm vs. ~40 μm), the number of transverse ciliary rings (~15 vs. 8), and the number of longitudinal ciliary rows (20 vs. 31). *Metacystis exigua*, a species with a mucous coat, differs from *M. mucosa* by body length (13–20 μm vs. ~40 μm), the number of transverse ciliary rings (~4 vs. 8), the posterior vacuole (absent vs. present), and the mucous coat (a few micrometre vs. >100 μm thick). Freely motile cells of *M. mucosa* highly resemble *M. tesselata*. However, *M. tesselata* possibly lacks a lorica and a slime coat, is only slightly (vs. distinctly) contractile, and has about 15 (vs. 8) transverse ciliary rings formed by about 20 (vs. 31) longitudinal ciliary rows.

Metacystis galiani, which Arregui et al. (2010) discovered in an aerobic wastewater treatment plant differs from *M. mucosa* by having a conspicuous, annulated lorica and by the absence of both, a caudal cilium and a mucous coat. Further, it has two perioral ciliary girdles composed of an anterior ring of dikinetids and four posterior rings of monokinetids.

***Phialina serranoi* nov. spec.** (Figs 8A–K, 9A–I; Table 4)

Diagnosis: Size in vivo and in protargol preparations about 85 × 27 μm when extended. Elongate ellipsoid with oral bulge and head rather distinctly set off from trunk; broadly ellipsoid when fully contracted. Macronucleus and micronucleus broadly ellipsoid. Contractile vacuole far subterminal. Two types of rod-shaped extrusomes attached to oral bulge: type I about 12 μm long, type II 2.5–3 μm. On average 15 ciliary rows each with one to four dikinetids anteriorly.

Type locality: Activated sludge plant for the town of Seekirchen, Salzburg county, Austria, 47°53′8.4″N 13°7′14.26″E.

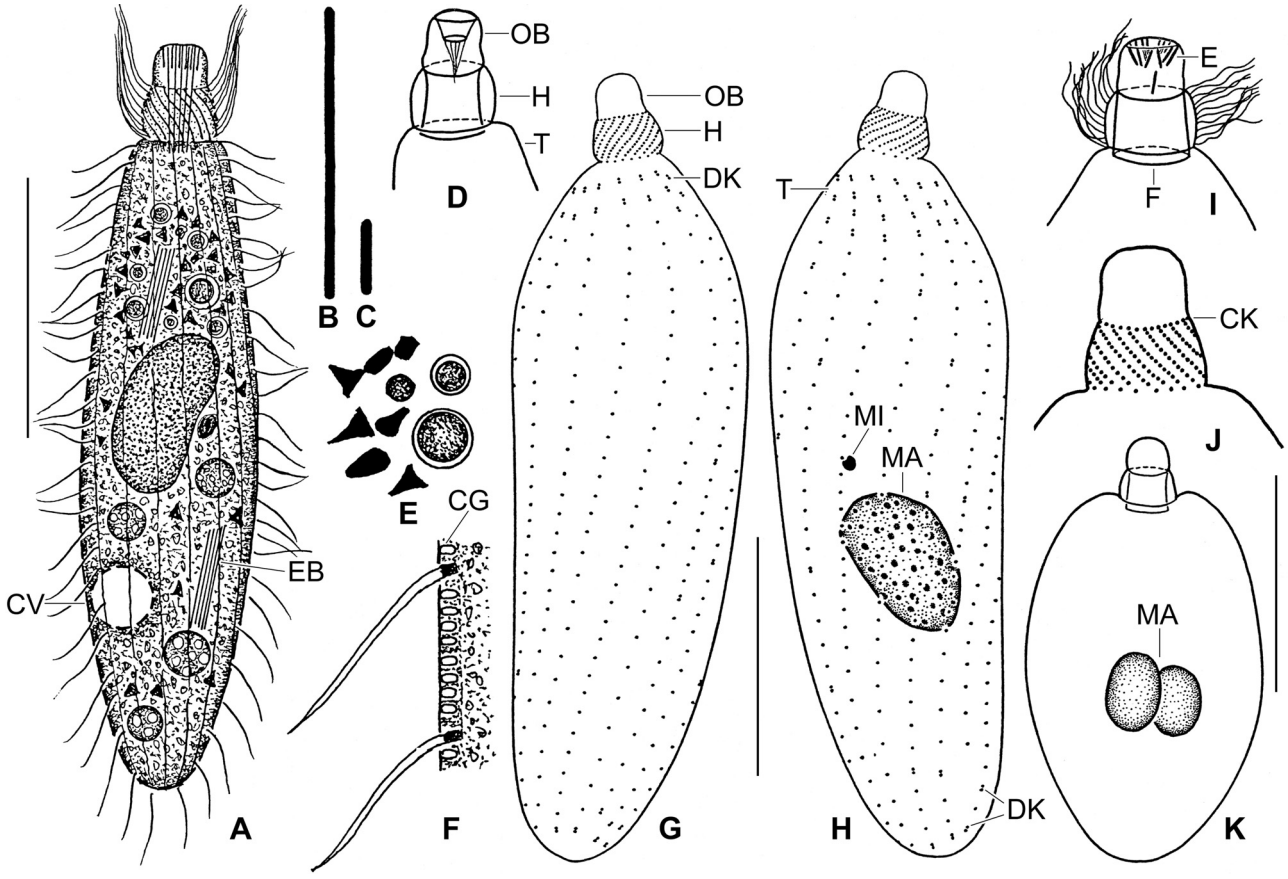

Fig. 8. A–K. *Phialina serranoi* from life (A–C, E, F) and after protargol impregnation (D, G–K). **A:** Overview of a representative specimen, showing the most important feature of this new species, viz., the far subterminally located contractile vacuole; length 85 μm. **B, C:** Type I and type II extrusomes, length 12 μm and 3 μm. **D, I, J:** Details of oral bulge and head. **E:** Cytoplasmic inclusions. The conical structures are possibly spines for the resting cyst. **F:** Optical section, showing cortical granulation. **G, H:** Basal body (ciliary) pattern of holotype specimen, length 80 μm. **K:** A contracted specimen with two macronuclear nodules, very likely a post-conjugate. CG, cortical granules; CK, circumoral kinety; CV, contractile vacuole; DK, dikinetids; E, extrusomes; EB, extrusome bundle; F, fibrous cylinder; H, head; MA, macronucleus; MI, micronucleus; OB, oral bulge; T, trunk. Scale bars 20 μm (K) and 30 μm (A, G, H).

Table 4. Morphometric data on *Phialina serranoi*.

Characteristics[a]	Mean	M	SD	SE	CV	Min	Max	*n*
Body, length	72.3	70.0	8.4	1.8	11.7	58.0	87.0	21
Body, width	23.0	23.0	2.3	0.5	9.9	18.0	27.0	21
Body length: width, ratio	3.2	3.1	0.4	0.1	12.0	2.7	4.4	21
Anterior body end to macronucleus, distance	35.7	34.0	6.3	1.4	17.7	26.0	46.0	21
Macronucleus, length	13.8	14.0	2.1	0.5	15.2	9.0	17.0	21
Macronucleus, width	9.4	10.0	1.9	0.4	20.3	5.0	12.0	21
Micronucleus, length	1.7	2.0	–	–	–	1.0	2.0	7
Micronucleus, width	1.3	1.0	–	–	–	1.0	2.0	7
Somatic ciliary rows, number	15.0	15.0	0.6	0.1	4.2	14.0	16.0	21
Monokinetids in a ciliary row, number	25.0	25.0	4.4	1.0	17.4	17.0	35.0	21
Dikinetids at begin of ciliary rows, number	2.6	3.0	0.7	0.2	25.6	2.0	4.0	21
Head, hight	4.2	4.0	0.5	0.1	12.2	3.0	5.0	21
Head, width	5.1	5.0	0.7	0.2	13.3	4.0	7.0	21

[a]Data based on environmental, protargol-impregnated, extended specimens. Measurements in μm. CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, number of individuals investigated; SD, standard deviation; SE, standard error of arithmetic mean.

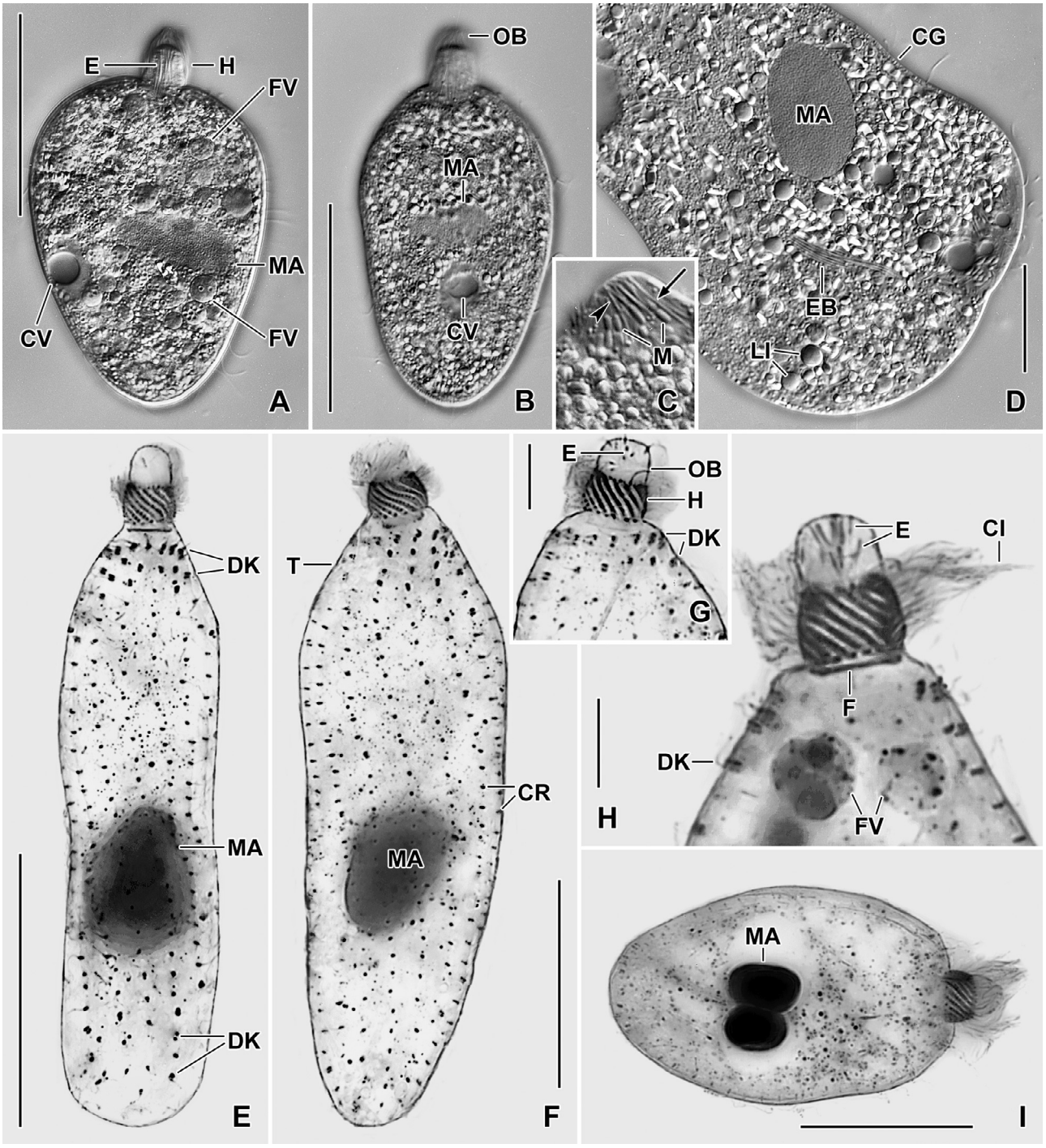

Fig. 9. A–I. *Phialina serranoi* from life (A–D) and after protargol impregnation (E–I). **A, B:** Two pressed specimens, showing the far subterminal contractile vacuole, the most important feature of this new species. **C:** Oral bulge, showing type I (arrow) and type II extrusomes (arrowhead). **D:** A squashed specimen studded with conical inclusions, very likely spines for the resting cyst. **E, F:** Basal body (ciliary) pattern of a paratype specimen and of the holotype. All ciliary rows begin with two to four dikinetids and scattered dikinetids occur throughout the rows, especially in posterior region. **G, H:** Details of anterior body region. The cilia are very narrowly spaced in the oblique head kineties and the last basal body of each kinety is slightly separate. **I:** A contracted specimen with two macronuclear nodules and thus very likely a late post-conjugate. CG, cortical granules; CI, cilia; CR, ciliary rows; CV, contractile vacuole; DK, dikinetids; E, type I (A) and type II (G, H) extrusomes; EB, type I extrusome bundle; F; fibrous cylinder; FV, food vacuoles; H, head; LI, lipid droplets; M, mitochondria; MA, macronucleus; OB, oral bulge; T, trunk. Scale bars 5 μm (G, H), 20 μm (A, B, D, F, I), and 30 μm (E).

Dedication: I dedicate this species to Prof. Dr. Aurelio Serrano Delgado, president of the Spanish Microbiology Society and of the VII ECOP-ISOP joint meeting 2015 in Seville, Spain.

Type material: The holotype slide (reg. no. 843/2015) and four paratype slides (reg. no. 843/2015–847/2015) with protargol-impregnated specimens have been deposited in the Biology Centre of the Upper Austrian Museum in Linz (LI). The holotype and other relevant specimens have been marked with black ink circles on the coverslip.

Description: *Phialina serranoi* has an ordinary variability with most variation coefficients lower than 15% (Table 4), except of macronucleus width (CV 20.3%), the number of monokinetids in a ciliary row (CV 17.4%), and the number of dikinetids at begin of the ciliary rows (CV 25.6%).

Size of extended specimens in vivo 67–100 × 20–30 μm, usually about 85 × 27 μm, as calculated from some in vivo measurements and the morphometric data in Table 4 adding 15% preparation shrinkage. Contracts and extends slowly and contracts only moderately when fixed with Stieve's solution. Extended body elongate ellipsoid with an average length: width ratio of 4:1 in vivo and of 3.2:1 in protargol preparations (Figs 8A, G, H, 9E, F; Table 4); unflattened. Most contractile in anterior third of cell; fully contracted cells broadly ellipsoid or obovate with retracted head (Figs 8K, 9A, B, I). Nuclear apparatus on average in or slightly posterior to mid-body (Figs 8A, H, 9A, B, D–F; Table 4). Macronucleus broadly ellipsoid or indistinctly reniform, on average 14 × 10 μm in protargol preparations, becomes globular (possibly contracts) under slight coverslip pressure; of 100 cells investigated, 19 have two broadly ellipsoid macronuclear nodules, very likely post-conjugates (Foissner and Xu 2007). Micronucleus attached or near macronucleus, ellipsoid, about 2 × 1 μm in protargol preparations. Contractile vacuole conspicuous because far subterminal, i.e., between mid-body and posterior end; excretory pore not impregnated with the protargol method used (Figs 8A, 9A, B). Two types of rod-shaped extrusomes attached to oral bulge and scattered in cytoplasm (Figs 8A–C, I, 9A, C, D, G, H). Type I about 12 × 0.4 μm in size, forms bundles in cytoplasm, does not impregnate with the protargol method used. Type II extrusomes only 2.5–3 μm long, likely form a ring in oral bulge, impregnate with protargol. Cortex very flexible, studded with rows of rather refractive granules about 0.8 × 0.4 μm in size (Figs 8F, 9D). Cytoplasm crammed with food vacuoles 4–7 μm across most containing a corroded starch grain (Figs 8A, 9A), anterior third dark under bright field illumination because studded with crystals and globular inclusions 2–5 μm in size; both scattered also throughout body; some cells studded with about 3 μm high, strongly refractive cones, very likely cyst spines (Figs 8A, E, 9A–D). Swims fast with head moving to and fro; creeps between sludge flocs like a small worm, showing great flexibility.

Trunk cilia about 8 μm long in vivo and in protargol preparations, arranged in an average of 15 ordinarily spaced, meridional or slightly spiral rows composed of an average of three anterior dikinetids followed by 25 monokinetids with some dikinetids irregularly interspersed. Anterior cilium of anterior dikinetids possibly lacking or reduced to an inconspicuous stump (Figs 8A, G, H, 9E–G; Table 4).

Oral bulge and head each about 5 × 5 μm in vivo, of typical *Phialina* structure, i.e., with very narrowly spaced head kineties having 10 μm long cilia and last kinetid slightly set off; a circumoral kinety difficult to recognize; and a fibrous cylinder in head (Figs 8A, D, G–J, 9A, B, E–H; Table 4).

Occurrence and ecology: As yet found only at type locality. *Phialina serranoi* became rather abundant in the one-week-old sludge sample, suggesting that it prefers microaerobic conditions.

Species comparison: Many *Phialina* species have been described since the last revision of the genus by Kahl (1930); a more recent review is not available. I checked most descriptions, e.g., Foissner (1983) and Vuxanovici (1959), but all species have the contractile vacuole in or very near to the posterior body end; in some species with acute body end, the contractile vacuole is slightly subterminal, e.g., in *P. minima* (Kahl, 1927; redescribed by Foissner et al. 2002) and in *P. vermicularis*, as redescribed by Foissner (1983). Thus, *P. serranoi* is unique in having the contractile vacuole far subterminal, i.e., between mid-body and body end.

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