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Redescription of *Platyophrya sphagni* (PENARD 1922) FOISSNER 1993 (Protozoa, Ciliophora)

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A b s t r a c t : *Platyophrya sphagni*, a medium-sized colpodid ciliate with symbiotic green algae, was found in a *Sphagnum* pond, i.e. a biotope very similar to that where PENARD (1922) discovered the type population. The morphology and infraciliature of *P. sphagni* were studied in live and silver impregnated cells. The morphologic and morphometric data largely agree with those of *P. chlorelligera* KAWAKAMI 1989, supporting synonymy as suggested by FOISSNER (1993). *Platyophrya dubia* FOISSNER 1980 is also rather similar to *P. sphagni* but is smaller (30 vs. 60 µm) and thus has distinctly fewer ciliary rows (13-16 vs. 19-26) and adoral organelles (4-5 vs. 5-8). *Platyophrya sphagni* very likely prefers, but is not restricted to *Sphagnum* habitats.

1 Introduction

In 1989 KAWAKAMI described a new species of *Platyophrya* KAHL 1926, *P. chlorelligera*, from a small freshwater pond at Kurose town, a suburb of Hiroshima, Japan. Later, FOISSNER (1993) synonymized *P. chlorelligera* with *Glaucoma sphagni* PENARD 1922, a *Sphagnum* pond species, because of distinct similarities in size, shape and flexibility of body as well as in the location of the oral apparatus and the possession of symbiotic green algae (zoochlorellae). However, the synonymization could be criticized considering the different biotopes and biogeographic regions the species were found. We were thus pleased to scrutinize the synonymy on a population found in an acid moorland pond near Constance, a biotope very similar and near to that where PENARD discovered *P. sphagni*.

2 Material and Methods

Platyophrya sphagni was found in varying numbers (up to 50 cells/ml) during the years 1995 and 1996 in a small pond (about 5 x 3 m, max. depth 1 m) near Hegne, a suburb of Constance, Germany (E9°10', N47°40'), about 7 km NW of the town. The pond, which was covered by water-lilies (*Nymphaea* sp.), is located at the grassy margin of a 100 m broad *Sphagnum* stripe with dwarfed pines (*Pinus* sp.). The water was brownish and had pH 5.5-6.0. The bottom was covered with a thick layer of mud, the upper zone of which contained a rich and diverse community of bacteria, algae and protozoans, including *P. sphagni*.

The specimens were taken directly from the mud. Attempts to establish pure cultures failed, but *P. sphagni* could be maintained for weeks in the sampling jar. Specimens were studied in vivo using a high-power oil immersion objective and interference contrast. The ciliary pattern (infraciliature) and other cytological details were revealed by various silver impregnation techniques, preferable silver carbonate, all described in FOISSNER (1991). Generally, *P. sphagni* was difficult to stain with all methods tried because of the symbiotic algae, which usually stained rather heavily and thus made observation of the ciliate rather troublesome. Furthermore most specimens were within slimy bacterial masses. Fortunately, they could be disentangled from the flocs by shaking the samples several times within half an hour.

Counts and measurements on silvered specimens were performed at a magnification of X 1000. In vivo measurements were conducted at a magnification of X 100-1000. Although these provide only rough estimates it is worth giving such data as specimens may shrink in preparations or contract during fixation. Standard deviation and coefficient of variation were calculated according to statistics textbooks. Illustrations are based on free-hand sketches and micrographs. All figures are orientated with the anterior end of the organism directed to the top of page.

3 Results

3.1 Redescription of *Platyophrya sphagni* (PENARD 1922) FOISSNER 1993

1922 *Glaucoma sphagni* PENARD, Études Infusoires: 126.

1977 *Platyophrya viridis* (GELEI 1954) – GROLIÈRE, Annls Stn limnol. Besse **10**: 280.

1989 *Platyophrya chlorelligera* KAWAKAMI, VIII. Int. Cong. Protozool., Program and Abstracts: 120.

1991 *Platyophrya chlorelligera* KAWAKAMI 1989 – KAWAKAMI, Europ. J. Protistol., **26**: 245.

1993 *Platyophrya sphagni* (PENARD 1922) nov. comb. – FOISSNER, Colpodea: 574.

Material

Six neotype slides with protargol- and silver nitrate (CHATTON-LWOFF technique and KLEIN-FOISSNER method)-impregnated specimens have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens are marked by a black ink circle on the cover glass. Accession numbers: 1996/53-58.

Redescription: (Figs. 1-37, Table 1)

Extended specimens in vivo about 60 x 28 µm, size, especially length little altered by preparation procedures, specimens impregnated with protargol or the dry silver nitrate method shrunken 10% on average (Tab. 1). Shape highly variable, depending on condition of cell, basically reniform to bursiform, anterior end more narrowly rounded than posterior, flattened laterally up to 2:1, transverse view thus ellipsoid

(Figs. 1, 2, 26). Specimens swimming for some time, for instance when samples were shaken repeatedly, usually become more slender[1], distinctly fusiform and slightly sigmoidal (Figs. 3, 4). Distinctly contractile and highly metabolic, fully contracted specimens about 30 µm long and bursiform (Fig. 5). Metaboly very conspicuous, but usually recognizable only under coverslip pressure and especially when burrowing for food in bacterial flocs (Figs. 6-8, 23, 24, 27). Such specimens lack a definite shape and behave like a naked amoeba.

Nuclear apparatus in centre of cell (Tab. 1), macronucleus globular, in vivo 9-13 µm across and studded with very small granules (bacteria ?), nucleoli small and pale; micronucleus lenticular, in vivo about 4 x 2.5 µm, in small indentation of macronucleus (Figs. 1, 28). Contractile vacuole distinctly subterminal near ventral side, excretory pore on right side beneath kineties 7 and 8, which are shortened, i.e. abut to pore (Figs. 1, 31, 33). Cortex very flexible, about 1 µm

Table 1: Morphometric characteristics from *Platyophrya sphagni*[1)]

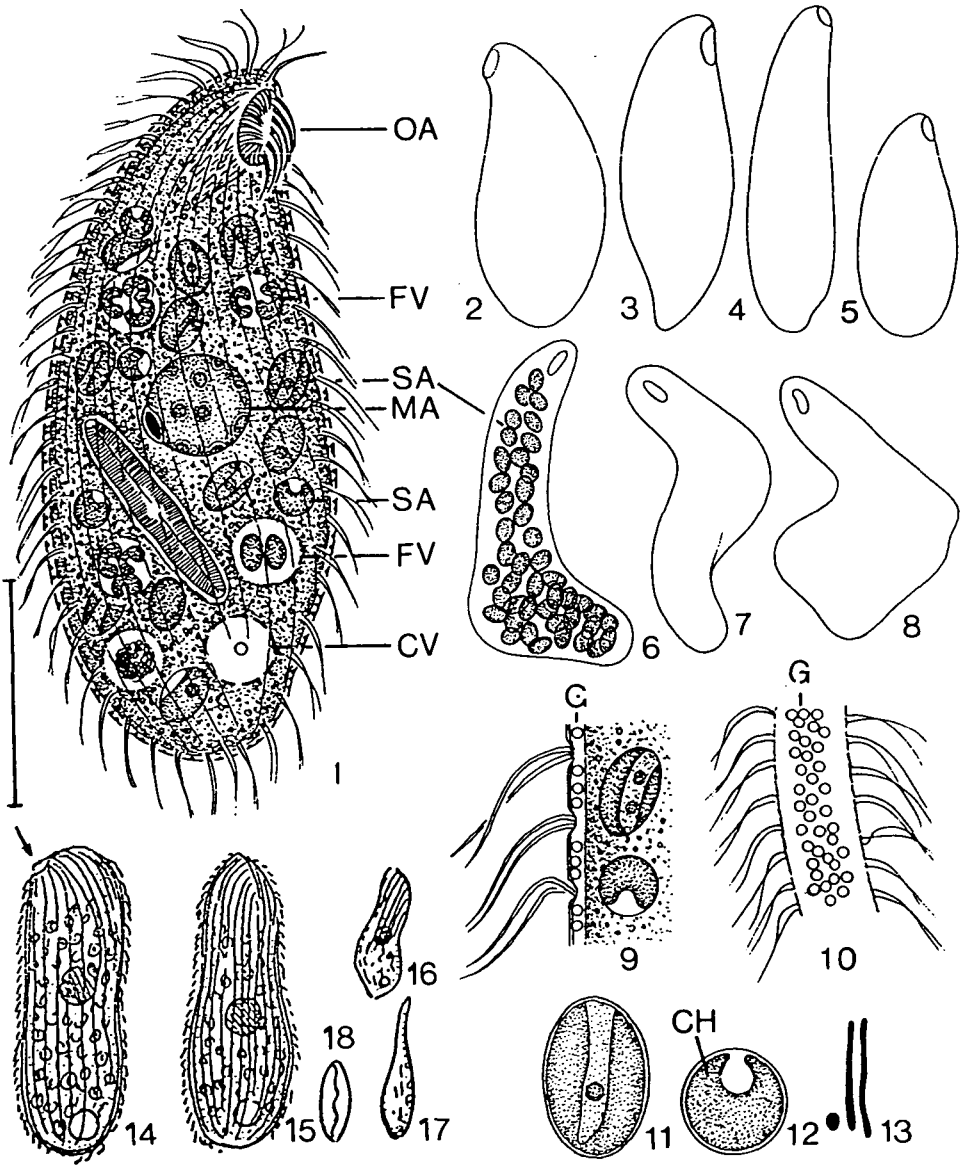
Character	Method[3)]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	IV	59.6	59	7.1	2.2	11.8	50	75	10
Body, width	IV	27.9	28	2.0	0.6	7.3	25	30	10
Body, length	CL	61.4	62	7.0	1.5	11.4	48	73	23
Body, width	CL	19.3	19	1.9	0.4	10.1	16	25	23
Body, length	DS	55.7	55	7.3	1.9	13.2	43	67	15
Body, length	P	55.4	55	6.4	1.3	11.6	42	70	23
Body, width	P	19.1	19	3.0	0.6	15.8	15	28	23
Anterior end to proximal end of oral apparatus	P	7.1	7	1.0	0.2	14.1	6	9	23
Anterior end to macronucleus	P	25.0	25	3.1	0.7	12.4	20	31	23
Macronucleus, length	P	7.7	8	0.8	0.2	9.7	7	9	23
Macronucleus, width	P	7.4	7	0.8	0.2	10.8	6	9	23
Somatic kineties, number	SC	22.3	22	1.8	0.5	8.2	20	26	15
Dikinetids in a right kinety, number	SC	42.8	42	6.8	1.8	16.0	31	52	15
Dikinetids in a left kinety, number[2)]	SC	20.5	22	5.1	2.1	24.6	12	26	6
Adoral organelles, number	SC	6.1	6	0.5	0.1	7.6	5	7	15
Paroral dikinetids, number	SC	28.1	28	1.3	0.3	4.6	25	30	15
Symbiotic algae, length	IV	5.8	6	0.4	0.1	6.1	5	6	10
Symbiotic algae, width	IV	3.8	4	0.2	0.1	6.3	3	4	10
Symbiotic algae, number	IV, SC	78.4	76	24.4	4.9	31.1	47	133	25

[1)] Measurements in µm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of specimens investigated, SD – standard deviation, SE – standard deviation of mean, $\bar{x}$ – arithmetic mean.

[2)] Including those of postoral pseudomembrane.

[3)] CL – CHATTON-LWOFF'S silver nitrate technique, DS – FOISSNER's dry silver nitrate technique, IV – in vivo, P – protargol (based on FOISSNER's technique), SC – silver carbonate. See FOISSNER (1991) for a detailed description of all methods mentioned.

[1] This could explain the difference in width of live and prepared specimens (Tab. 1), which were isolated from the mucuous bacterial masses by shaking the samples 4-5 times for about 30 s within a period of one hour.



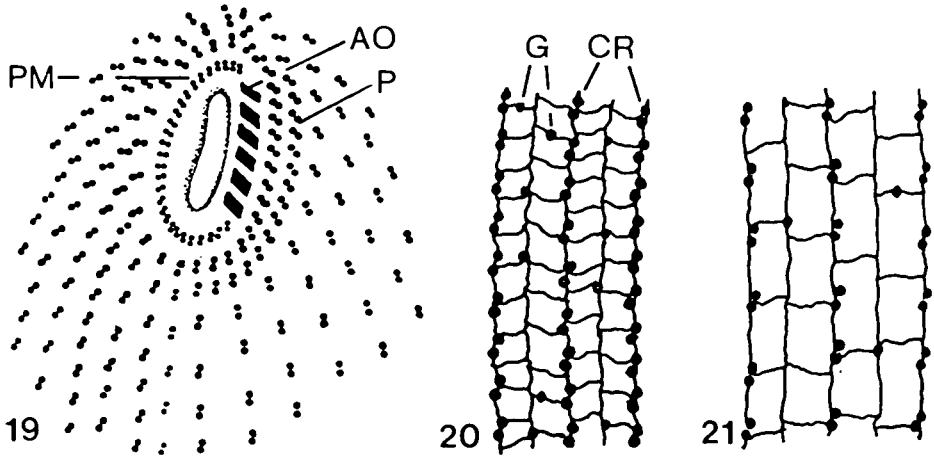
Figs. 1-18: *Platyophrya sphagni* from life (1-12, 14-18) and after silver carbonate impregnation (13); Figs. 1-13 originals, Figs. 14-18 from PENARD (1922). **1:** Right lateral view of gliding, typical specimen. **2, 3:** Shape variants of swimming specimens. **4, 5:** Extended and contracted specimen. **6-8:** Specimens rooting in slimy bacterial masses, redrawn from micrographs. **9, 10:** Optical section and surface view of cortex. **11, 12:** Longitudinal and transverse view of symbiotic algae. **13:** Resting (0.7 µm) and discharged (up to 10 µm) extrusomes. **14-17:** Gliding and resting specimens; arrow marks oral aperture. **18:** Surface view of oral apparatus. CH – chloroplast, CV – contractile vacuole, FV – food vacuoles, G – cortical granules (extrusomes), MA – macronucleus, OA – oral apparatus, SA – symbiotic algae. Bar: 20 µm.

thick, bright, slightly orange-coloured by mucocysts. Mucocysts arranged in loose rows between somatic kineties, globular, 0.5-0.7 µm across, very compact and thus distinctly bright, extend to up to 10 µm long rods in silver carbonate impregnated cells (Figs. 1, 9, 10, 13, 20, 21, 31-33). Cytoplasm colourless, but specimens appear bright green due to symbiotic algae. Food vacuoles 4-5 µm across, usually containing U-shaped, colourless bacteria and/or ellipsoidal, reddish bacteria, both found in the slimy bacterial masses usually inhabited by *P. sphagni*; rarely, large diatoms are ingested (Figs. 1, 25-27). Swims rather fast, but usually *P. sphagni* burrows within slimy bacterial masses showing great metaboly, as described above.

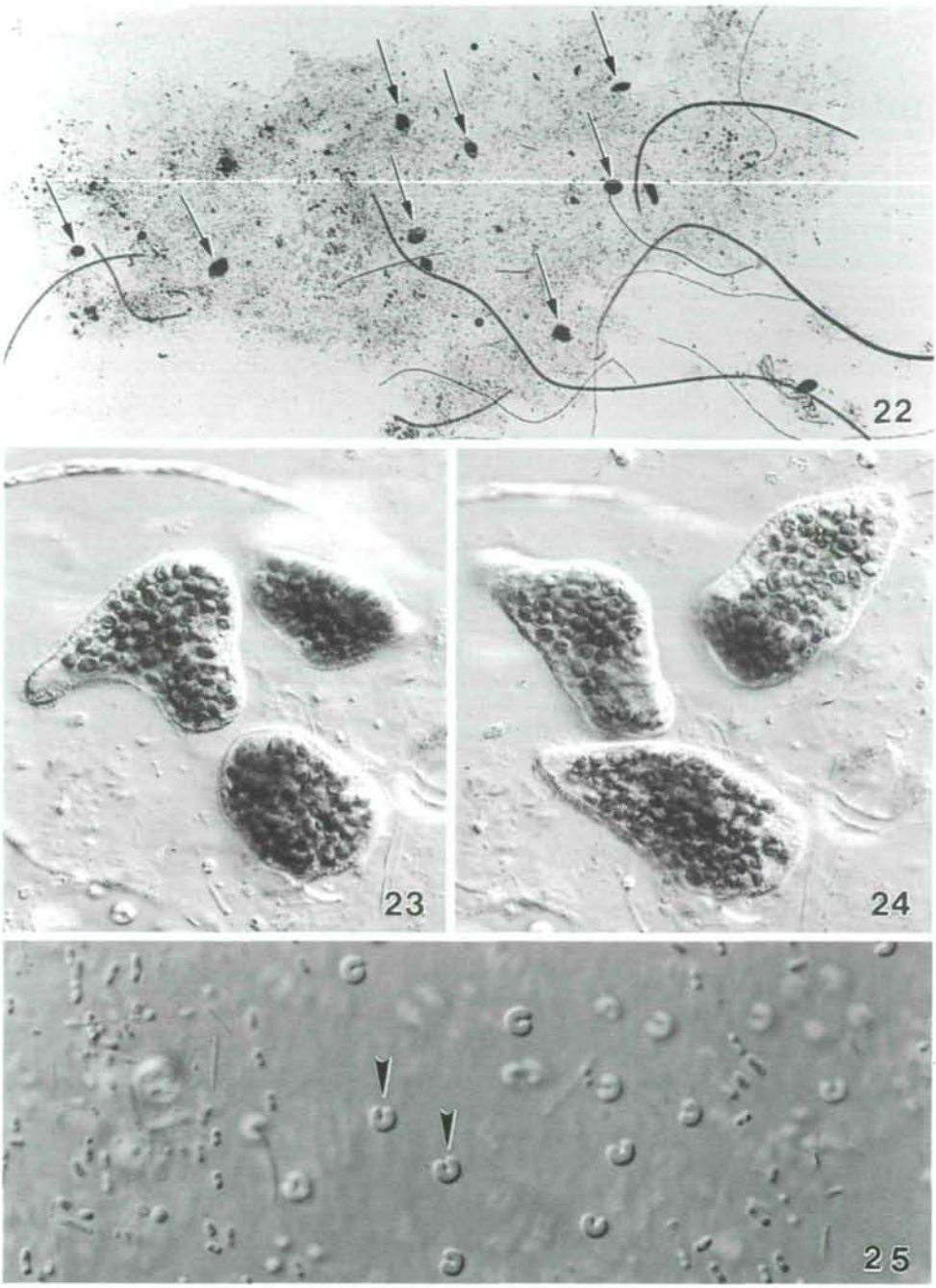
Symbiotic algae ellipsoidal and highly variable in number (Tab. 1), provide cell with conspicuous green colour. Chloroplast cylindroidal with longitudinal furrow and 1-2 globular, acentral pyrenoids (Figs. 1, 6, 9, 11, 12, 23, 24, 26-29).

Resting cysts globular, 34-38 µm across (n = 4), green by symbiotic algae, have some brownish inclusions, possibly digested zoochlorellae. Cyst wall about 0.5 µm thick, colourless, without recognizable ornamentation.

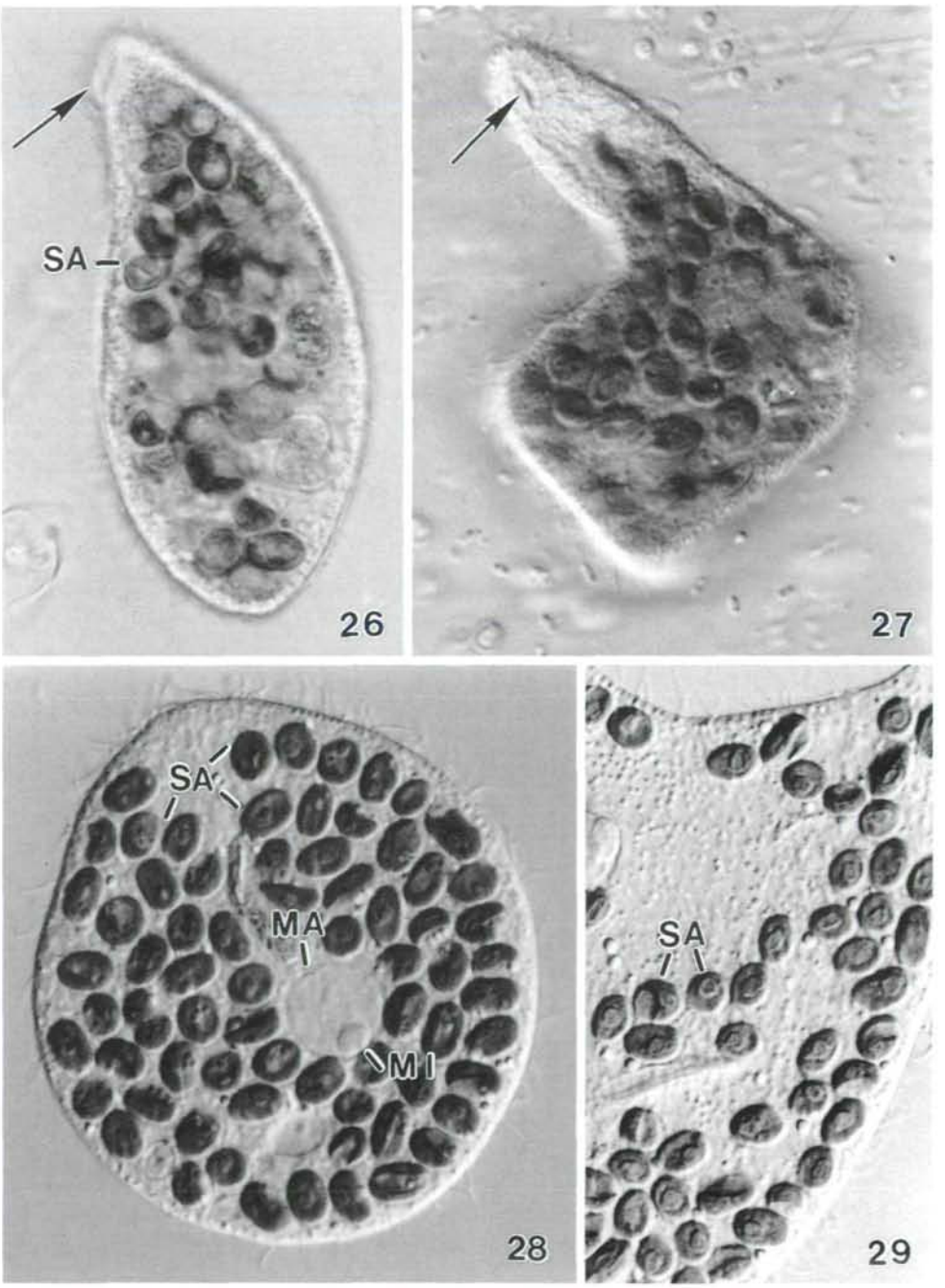
Somatic and oral infraciliature as described by KAWAKAMI (1991) and in other members of genus (FOISSNER 1993), differing mainly in morphometric details (Tab. 1). Ciliary rows slightly spirally arranged, composed of dikinetids throughout, both basal bodies of dikinetids ciliated at right side, anterior cilium lacking in most dikinetids of left side. Left side distinctly more loosely ciliated than right (Figs. 19-21, 30-33; Tab. 1). Oral opening slightly subapical, minute (about 4 x 3 µm) and thus difficult to recognize in live specimens (Figs. 26, 27).



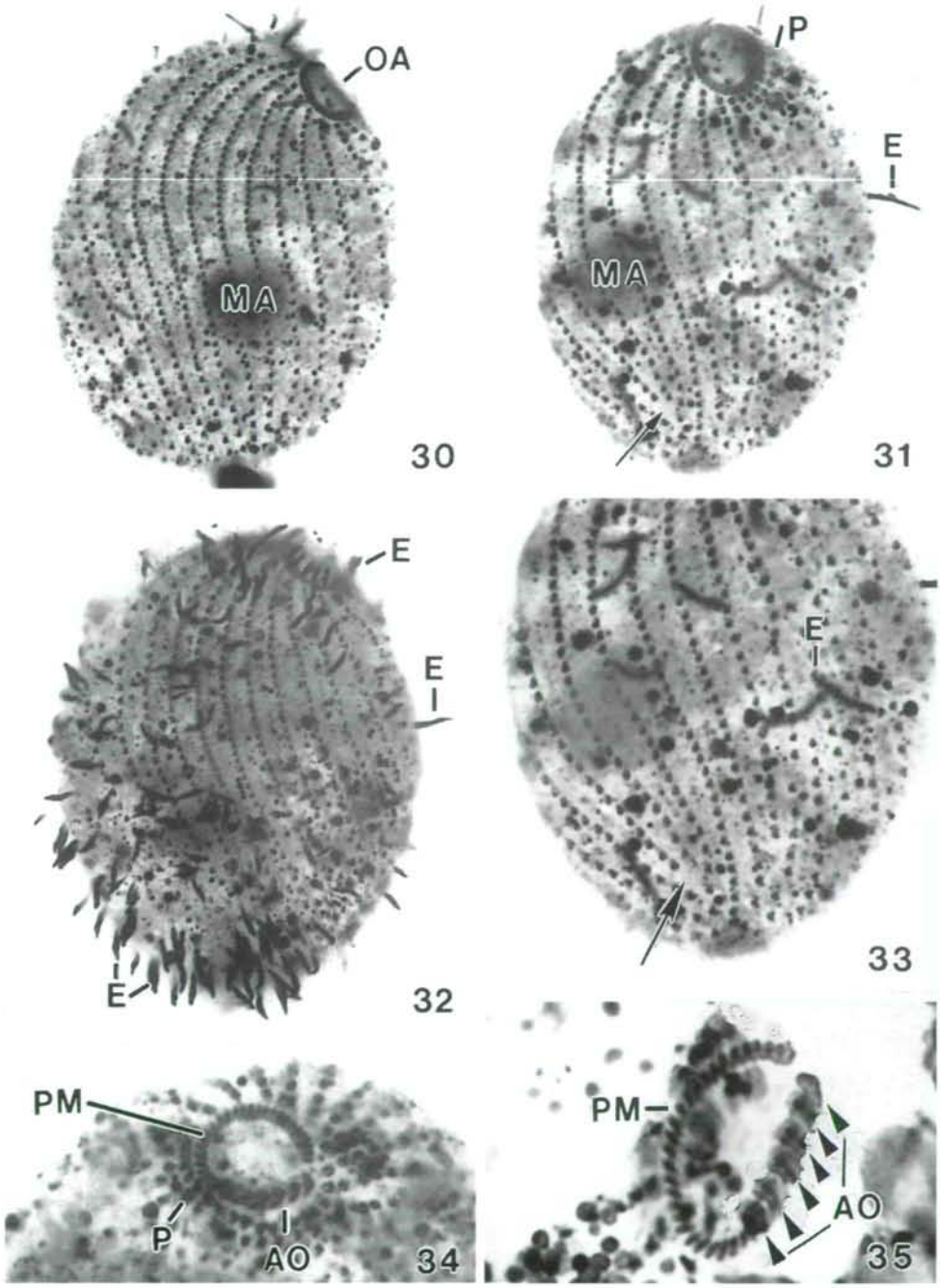
Figs. 19-21: *Platyophrya sphagni*. **19:** Oral infraciliature, redrawn and slightly schematized from silver carbonate impregnated specimens. **20, 21:** Silverline system of right and left side, drawn to scale and slightly schematized, dry silver nitrate impregnation. AO – adoral organelles, CR – ciliary rows, G – granules, possibly resting extrusomes, P – postoral pseudomembrane, PM – paroral membrane.



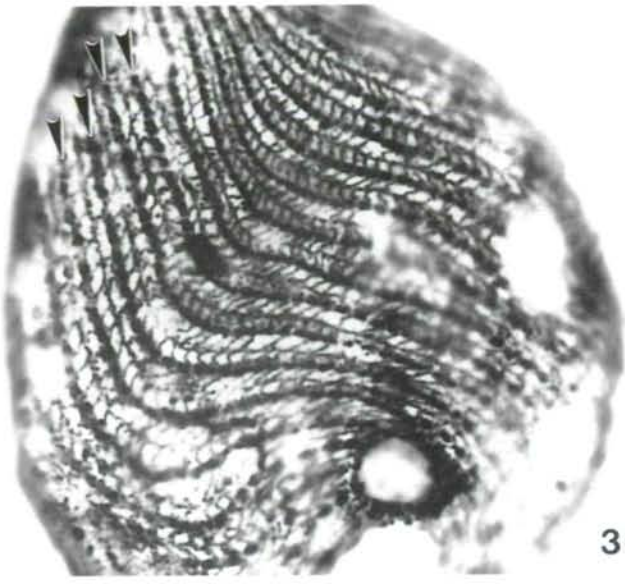
Figs. 22-25: *Platyophrya sphagni* from life. **22:** Slimy bacterial flake with many *P. sphagni* inside (arrows). **23, 24:** Three specimens rooting in a slimy bacterial flake. The micrographs, taken at an intervall of about 1 min, show impressively the amoeboid metaboly of *P. sphagni*. **25:** Bacterial flake at high magnification showing croissant-shaped bacteria, the preferred food of *P. sphagni*.



Figs. 26-29: *Platyophrya sphagni* from life. **26:** Gliding, typical specimen. Arrow marks oral aperture. **27:** *Platyophrya sphagni* usually inhabites slimy bacterial flakes exploiting them for appropriate food bacteria (cp. Fig. 25), thereby showing a conspicuous, amoeba-like metaboly. Arrow marks reniform oral aperture. **28, 29:** Squashed specimens showing nuclear apparatus and symbiotic green algae, which are distinctly ellipsoidal. FV – food vacuoles, MA – macronucleus, MI – micronucleus, SA – symbiotic green algae.



Figs. 30-35: *Platyophrya sphagni*, infraciliature after silver carbonate impregnation. **30, 31, 33:** Right lateral and ventral views. Arrows mark shortened ciliary rows at the site of the excretory pore of the contractile vacuole. **32:** Specimen with discharged extrusomes. **34, 35:** Oral infraciliature. AO – adoral organelles, E – extrusomes, MA – macronucleus, OA – oral apparatus, P – postoral pseudomembrane, PM – paroral membrane.



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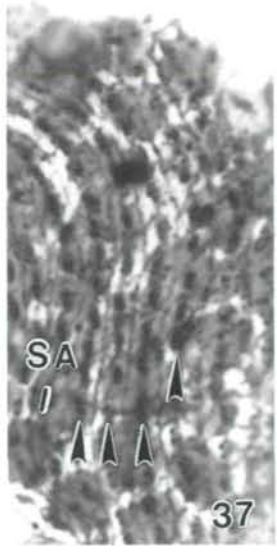

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Figs. 36, 37: *Platyophrya sphagni*, silverline system of right and left side after dry silver nitrate impregnation. Arrowheads denote ciliary rows. SA – symbiotic algae.

Paroral membrane C-shaped, cilia only 3 µm long, stick together forming distinct membrane (Figs. 1, 19, 31, 34, 35). Anteriormost adoral organelle frequently smaller than others, as in Japanese population (KAWAKAMI 1991), cilia of adoral organelles about 5 µm long and directed backwards when inactive (Figs. 1, 19, 31, 34, 35). Postoral pseudomembrane distinct, composed of 2 closely spaced rows each comprising 13-16 ciliated dikinetids (Figs. 19, 27, 31, 34).

Silverline system also as in congeners (FOISSNER 1993), i.e. reticulate with distinct median silverline between each two ciliary rows on both sides of the cell. Frequently, granules occur in silverlines, very likely indicating mucocyst sites (Figs. 20, 21, 36, 37).

3.2 Occurrence and ecology

PENARD (1922) supposed that *P. sphagni* possibly occurs only in *Sphagnum*: "Cette espèce est peut-être particulière au *Sphagnum*, dans lequel elle a été exclusivement trouvée, à la tourbière de Valavran (Switzerland)". We and possibly also GROLIÈRE (1977; see FOISSNER 1993 for discussion of synonymy) found *P. sphagni* also in acid moorland ponds, supporting PENARD's view. However, KAWAKAMI (1991) isolated her strain from normal freshwater in Japan. Thus, *P. sphagni* is obviously not re-

stricted to *Sphagnum* but possibly prefers such biotopes. KAWAKAMI (1991) could cultivate *P. sphagni* on 0.02% KNOPP solution, either with or without 0.1 µg/ml yeast extract, and on SUD's culture medium. The vegetative cells were highly phototactic, obviously due to the symbiotic algae, and crept on the bottom of the culture dishes. Resting cysts were produced in aged cultures. They excysted when fresh culture medium was added.

We found *P. sphagni* all over the year in a small *Sphagnum* pond, where it usually inhabited bacterial flocs in the algal mud (Fig. 22). However, cells often also accumulated in dead pieces of crustaceans, where they also encysted. We could not clarify whether the accumulation was due to special food requirements or for other reasons.

4 Discussion

4.1. Identification and comparison with literature data

Our observations perfectly match those of PENARD (1922), who provided several figures (Figs. 14-18) and the following description: "Corps cylindrique, arrondi à ses deux extrémités, légèrement comprimé à sa partie antérieure, plastique et métabolique. Cils fins et courts, serrés, disposés sur des lignes longitudinales rapprochées, recourbées à la partie antérieure pour rejoindre une sorte d'arête terminale, qui elle-même se prolonge jusqu'à l'ouverture buccale; cette dernière, très petite, elliptique, et bordée d'une (double ?) membrane ondulante très petite également, s'ouvre tout près de l'extremité du corps. Cytoplasme normalement coloré en vert par des Zoochlorelles, qui le remplissent tout entier sauf à la partie antérieure, plus claire. Nouyau sphérique, central, avec micronoyau fusiforme adjacent. Vésicule contractile petite, latéro-terminale. Longueur 60 à 65 µ. – *Sphagnum*".

Thus, there can be no doubts about the identification and, in turn, the synonymy suggested because our data also perfectly match the description of *P. chlorelligera* KAWAKAMI 1991, as evident from a comparison of the main characteristics (Tab. 2). The German population has, on average, 1.5 somatic kineties more and 1 adoral organelle less than the Japanese strain – small differences indeed.

Table 2. Morphometric comparison of *Platyophrya sphagni* German population (PG), *P. sphagni* Japanese population (PJ, from KAWAKAMI 1991, named *P. chlorelligera* in that paper), and *P. dubia* (PD, from FOISSNER 1980 and new measurements from type slides).

Character[1)]	Population	Method[2)]	$\bar{x}$	SD	CV	Min	Max	n
Body, length	PG	IV	59.6	7.1	11.8	50	75	10
Body, length	PJ	IV	57.4	5.5	9.6	44	70	103
Body, length	PG	DS	55.7	7.3	13.2	43	67	15
Body, length	PD	DS	31.9	3.8	11.9	25	39	14
Body, width	PG	IV	27.9	2.0	7.3	25	30	10
Body, width	PJ	IV	23.8	3.0	12.4	18	30	103
Somatic kineties, number	PG	SC	22.3	1.8	8.2	20	26	15
Somatic kineties, number	PJ	P	21.5	1.5	7.0	19	24	50
Somatic kineties, number	PD	DS	about 13-16					
Adoral organelles, number	PG	SC	6.1	0.5	7.6	5	7	15
Adoral organelles, number	PJ	P	7.3	0.5	6.4	7	8	38
Adoral organelles, number	PD	IV, DS	about 4-5					
Symbiotic algae, number	PG	IV, SC	78.4	24.4	31.1	47	133	25
Symbiotic algae, number	PJ	IV	about 30-60					
Symbiotic algae, number	PD	IV	≤ about 20					

[1)] From stationary growth phase cells. Measurements in µm. CV – coefficient of variation in %, Max – maximum, Min – minimum, n – number of specimens investigated, SD – standard deviation, $\bar{x}$ – arithmetic mean.

[2)] DS – FOISSNER's dry silver nitrate technique, IV – in vivo, P – protargol (our values based on FOISSNER's technique), SC – silver carbonate. See FOISSNER (1991) for a detailed description of all methods mentioned.

There is only one other species in the genus which has symbiotic green algae, viz. *Platyophrya dubia* FOISSNER 1980. This species is very similar to *P. sphagni* differing only in morphometric features (Tab. 2). It is distinctly smaller and has fewer ciliary rows and adoral organelles than *P. sphagni*. These characteristics hardly overlap in *P. sphagni* and *P. dubia*, and thus both can be readily separated. However, subspecies rank would probably be more appropriate for *P. dubia*. The reinvestigation of the type slides proved that the symbiotic algae of *P. dubia* are ellipsoidal, like those of *P. sphagni*.

4.2 Improved diagnosis of *P. sphagni* (PENARD 1922) FOISSNER 1993

In vivo about 60 x 25 µm, slightly reniform or bursiform, very flexible and, under certain conditions, even highly metabolic. Macronucleus globular. Oral aperture minute and distinctly smaller than maximum body width. Cytoplasm colourless, cells, however, bright green by ellipsoidal symbiotic green algae. 19-26, usually about 22 somatic kineties and 5-8, usually 6-7 adoral organelles.

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