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Light microscopical observations of the sigmoid species of the genus *Cryptomonas* Ehrenberg (Cryptophyceae) and their eventual pyrenoids

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Abstract: Several species of the genus *Cryptomonas* EHRENB. are characterized by the sigmoid (S-shaped) form of their cells under natural conditions. Such species were sampled and studied by the author for decades from the plankton of freshwater lakes, dam reservoirs, fishponds, and peat pools, from among littoral vegetation, as well as in small water bodies overgrown with vegetation. After application of optical microscopes on live or chemically treated specimens, they were measured and depicted. Four species known from literature are demonstrated to be easily determinable, namely *Cryptomonas curvata*, *C. reflexa*, *C. marssonii*, *C. gracilis.* Two new varieties are described. *Cryptomonas borealis* is shown as a transient species between straight and sigmoid cells. A special attention is given to the occurrence of pyrenoids and Maupas corpuscles within the cells. The new forma of *C. cylindrica* is pictured and described, with the antapex of the cells deformed differently from sigmoid species.

Key words: *Cryptomonas*, ecology, light microscopy, pyrenoids, sigmoid cells, taxonomy

Introduction

The DNA sequences have made the sigmoid shape of cryptomonad cells doubtful as generic diacritic feature (Hoef-Emden & Melkonian 2003). Consequently, the generic name *Campylomonas* D.R.A. HILL was not adopted in the present study for the s-shaped species of the genus *Cryptomonas* EHRENB. The "cryptomorph / campylomorph pairs" described by the above mentioned authors from cultures, can be found also in natural populations but the relationship of one to the other is obvious in most cases. The interspecific differences, e.g. between *C. curvata* and *C. ovata*, is not obliterated in most cases. The validity of sigmoid cell-shape as a diacritical feature is therefore recognized on the species and variety level in the present study.

Cryptomonas curvata EHRENB., as emended by PENARD, was demonstrated to be a well-defined species that is easily recognizable. Slight differences between *C. curvata* and *C. rostratiformis* SKUJA were found but another feature (six pyrenoids) reveals that they most probably belong to the same species.

Two different varieties were demonstrated within *Cryptomonas reflexa* (MARSSON) SKUJA: the typical *C. reflexa* var. *reflexa* and the form with shorter and more thick cells, namely, *C. reflexa* var. *anas* (JAVORNICKÝ) comb. nova. The same situation was discovered in *Cryptomonas marssonii* SKUJA, i. e., typical variety *C. marssonii* var. *marssonii.* beside the short and relatively thick *C. marssonii* var. *brachys* var. nova. Both shorter forms were detected several times in shallow water bodies containing hydrogen sulphide. Probably they may be considered to be ecological varieties.

Cryptomonas cf. *borealis* SKUJA was identified from three localities. The shelled pyrenoids are scarcely detected in live sigmoid cryptomonads by optical microscopy. When the cells are still treated by iodine, they appear (typical number of six pyrenoids in *C. curvata*) but they are frequently masked by numerous structured grains of starch. However, when the cells are preserved with osmium acid and stained by azocarmine, no protein containing bodies are found within these pyrenoids. In comparison, the pyrenoids of *Plagioselmis* display the clear proteinic nuclei after application of azocarmine.

The sigmoid curvature visible in side projection is not the only deviation from symmetry of cryptophycean cells. It is demonstrated on *C. cylindrica* EHRENB. forma, the deformed antapex of which appeares in dorsiventral position.

Materials and methods

The plankton biocoenoses were sampled from the surface using plain bottles or by a Friedinger sampler from various depth strata. The material from the littoral vegetation of water bodies or from shallow pools and drains was collected together along with a periphytic coating, sometimes by wringing the immersed plants.

The plankton was concentrated by means of a low-revolution centrifuge in pointed-bottom test tubes. The periphytic biocoenosis required usually no concentration. The living, as well as preserved material, was studied under light microscopes (Carl Zeiss, Jena or Meopta, Prague). For preservation, use was made of Lugol's solution (KI+I) which also helped to make some cell organelles, in particular, starch grains or pyrenoids, more evident. Azocarmine was applied to stain the protein bodies: nuclei, nucleoli, or eventual central bodies of the pyrenoids.

The exact sources of respective samples are given in the Tables together with the cryptomonad names and their dimensions.

Results

Cryptomonas curvata EHRENBERG (Figs 1-5, 8-9)

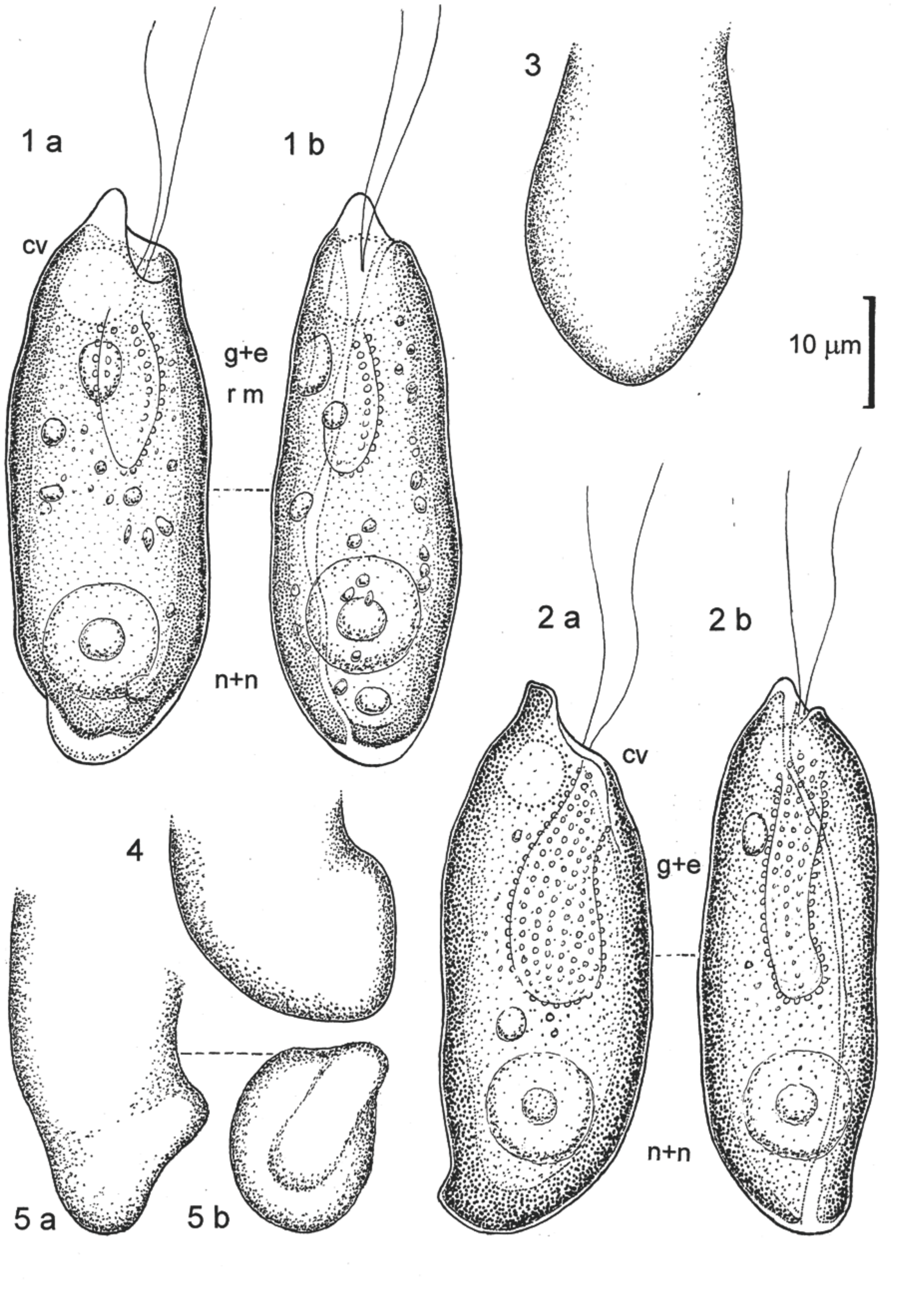
Basionym: *Cryptomonas curvata* EHRENBERG, 1838, Infusionsthierchen vollkomm. Organismen, Berlin u. Leipzig: 40-41, Tab. II: XVI; PENARD, 1922, Proc. Acad. nat. Sci. Phil. 73: 105-168.

Iconotypus: EHRENBERG, 1838, l. c., Tab. II: XVI 1 - 2.

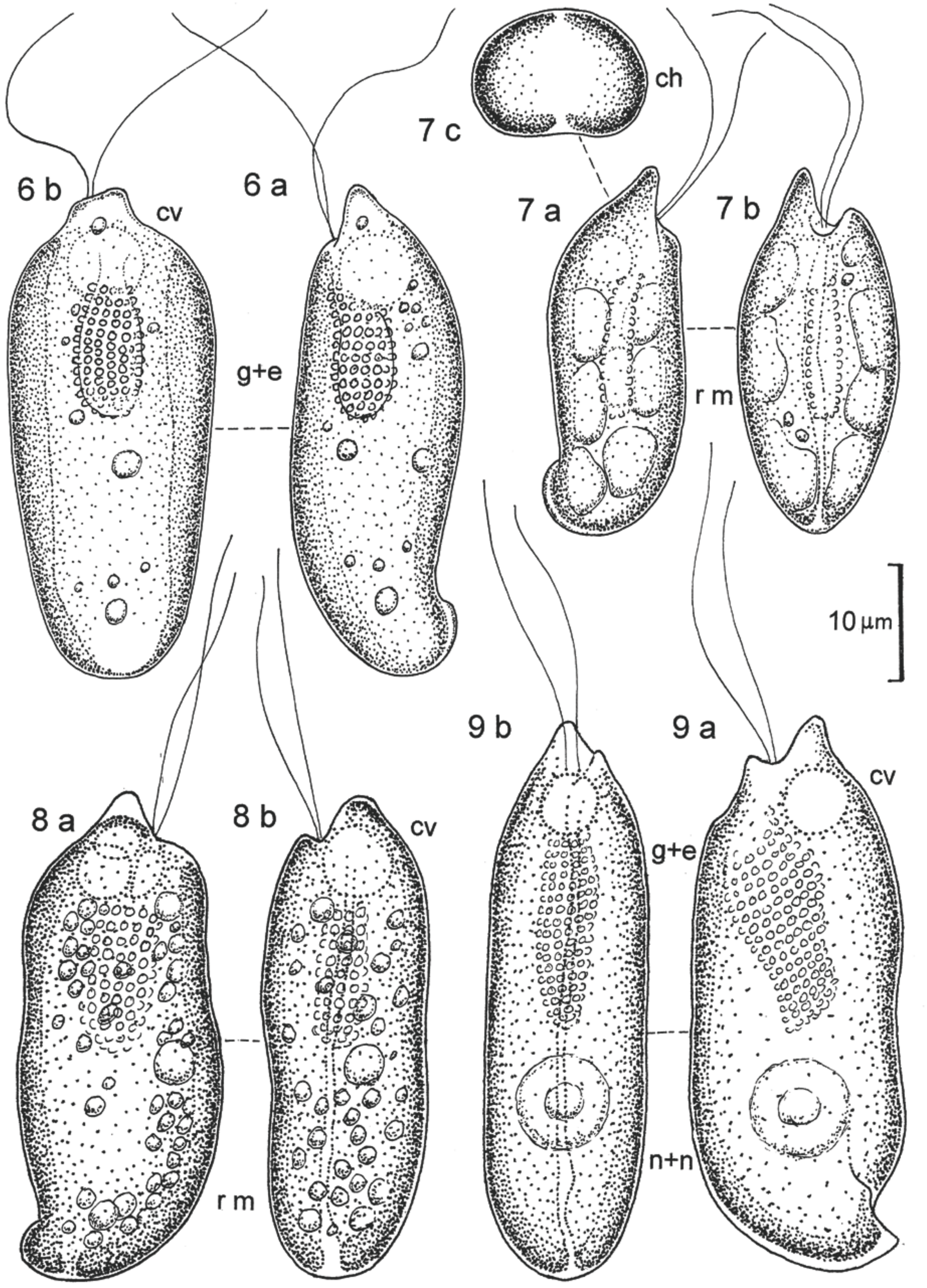
Synonyms: *Cryptomonas ovata* EHRENB. sensu STEIN pro parte, 1878, Organ. Infusionsthiere 3, 1, Leipzig, 1-154, Tab. XIX: 27 ; *Cryptomonas rostrata* TROITZKAJA, 1922, Bot. Mater. Inst. sporov. Rast. 1, 8; *Cryptomonas rostrata* SKUJA, 1948, (non *Cr. rostrata* TROITZ.), Symb. Bot. Upsal. 9: 359 – 361,Tab. 37, 40-40a; *Cryptomonas rostratiformis* SKUJA in HUBER-PESTALOZZI, 1950, Phytopl. Süsswass. Syst. Biol. 3. In THIEMANN (ed.), Binnengewässer 16, 3, Stuttgart.: 55-56; *Campylomonas rostratiformis* (SKUJA) KUGRENS & CLAY, in WEHR & SHEATH, 2003, Freshw. Alg. North Amer., Ecol. Classif.: 745-746.

The cell length varied between 32 – 61 μm in my materials (Tab. 1). The elongated cells have a conspicuous apical finger-shaped rostrum, sometimes with a cut off peak (Fig. 2a), and pseudopharynx with a funnel-shaped mouth. The wide, laterally flattened pseudopharynx is inlaid with numerous ejectosomes and reaches down to half the cell-length. The antapex is bluntly pointed, curved to the cell dorsum, and sometimes keel-shaped (Figs 4-5). The posterior cell end exceptionally may be bug-shaped but still with an enlarged dorsal part (Fig. 3). The cells are frequently deformed with a slightly undulated surface and displaying a screw-like distortion. Therefore the cell orientation is hard to define precisely. Most cells are somewhat laterally flattened and contain two lateral chloroplasts, sometimes joined with a narrow dorsal isthmus. Maupas corpuscles are not present.

In some specimens, six shelled pyrenoids were visible in live cells (Figs 7, 47, 48) or in cells treated by KI+I (Fig. 49). Six pyrenoids ought to by typical for the species (Hoef-Emden & Melkonian 2003). Due to preservation by iodine, the numerous small pyrenoids or perhaps structured starch grains were detected in some cells (Fig. 50)**.** This coincides with the electron microscopic findings of "two chloroplasts, each with numerous pyrenoids" (see Kugrens & Clay 2003, for *Cryptomonas rostratiformis*).



Figs 1-5; 8-9: *Cryptomonas curvata* EHRENB. 1a, 2a – lateral views; 1b, 2b – ventral views; 3-5 – contour of a keel-shaped cell's posterior, 3-5a – lateral view, 5b – antapical view.



Figs 6-7: *Cryptomonas curvata* EHRENB. – specimens resembling *Cryptomonas rostratiformis* SKUJA. 6a, 7a – lateral views; 6b – dorsal view; 7b – ventral view; 7c – equatorial cross section of the cell; 8a, 9a – lateral views; 8b, 9b – ventral views.

Skuja (1948) described a new species under the homonymic name *Cryptomonas rostrata*. The cell dimensions of his specimens (length 48 – 60 μm), as well as the excellent sketches Skuja drew, show the cryptomonad belonging very closely to *C. curvata* EHRENB. Skuja admitted: "Vielleicht ist mit ihr die Form, welche Stein (1878) als...*C. ovata (incl. C. curvata E.)* betrachtet, identisch." Ettl (1980) makes a difference between *C. curvata* and *C. rostratiformis*. Nevertheless, Ettl´s drawings and micrographs demonstrate that both species are hardly to be distinguished one from another.

The only specimens similar to *C. rostratiformis* I have observed and sampled from the same site (Figs 6-7), revealed two features comparable with Skuja´s depictions and description. The contour lines of their cells were conspicuously smooth and the curvature of the antapex was geometrically accurate, resembling the adornment on the helmet of a hussar. Moreover, their cells were flattened dorsiventrally and not laterally as is often the case with *C. curvata*. They were 32 – 43 μm long (Tab. 1). In spite of this, six pyrenoids visible in one of the specimens (Fig. 7), which is one of the specific features of *C. curvata,* show that the above mentioned differences are not enough important to justify the existence of a different species.

Among the population of typical sigmoid specimens of *C. curvata,* one can observe some straight, not curved specimens. In all other respects they are identical with sigmoid cells and could be considered to be cryptomorphs of *C. curvata* (Hoef-Emden & Melkonian 2003). These authors, however, found only two pyrenoids in the cryptomorphs.

In spite of some nomenclatural confusion, the species *Cryptomonas curvata* EHRENB. emended by PENARD is well defined and may be easily identified. Therefore Fott (1971) proposed to accept *C. curvata* for typus of the genus *Cryptomonas* in contrast to Butcher´s (1967) proposition to select the drawing by Stein (1878) of *C. ovata* EHRENB. as the type of the genus. The typification of the genus by *C. curvata* has been repeated by Hoef-Emden and Melkonian (2003).

Tab. 1: *Cryptomonas curvata* EHRENBERG

Collection site	Dimensions of cells, μm		
	long	thick (d/v)	wide (s/s)
South Bohemia, Řežabinec fish-pond, plankton (Figs 1, 4-5)	52	19	16
S. Bohemia, Smyslov fish-pond by Blatná, plankton	50.5	--	16
(Figs 2-3)	50.5	21.5	--
Central Bohemia, Červený fish-pond by Mšec, outlet, in sapropel (Figs 8-9)	42	15	--
North Bohemia, Bělá pod Bezdězem, marsh pool with *Utricularia,* in sapropel, H_2S	48	18	16
Central Bohemia, dam reservoir Slapy on Vltava River,	46	18	--
surface plankton	61	26	--
Southeast Bohemia, Želivka River, dam reservoir Sedlice, plankton (Fig. 8), resembling *Cr. rostratiformis*	43	15	18
The same locality, experimental nylon column in water, plankton (Fig. 7), resembling *Cr. rostratiformis*	32	12	14

All the above localities are in the Czech Republic

Cryptomonas reflexa (MARSSON) SKUJA var. ***reflexa*** (Figs 10-14)

Basionym and iconotypus: *Cryptomonas reflexa* SKUJA, 1939, Acta Horti Botanici Univ. Latviensis 7: p. 93-94, pl. 5, fig. 9.

Synonyms: *Cryptomonas erosa* EHRENB. var. *reflexa* MARSSON, 1904, Mitteil. königl. Prüfunganst. Wasserversorg. Abwässerbeseit., Berlin 4: p. 140; *Cryptomonas reflexa* SKUJA, 1939, loco citato; *Campylomonas reflexa* (SKUJA) D. R. A. HILL, 1991, Phycologia 30 (2): p. 175.

The *C. reflexa* accompanies frequently *C. curvata* in phytoplankton of freshwater-bodies. *C. reflexa* is smaller, the cells were 22 – 30 μm long (average 26. 9 μm) in my materials (Tab. 2). Their shape was sigmoid (S) with the blunt apical rostrum curved ventrally and the rounded or bluntly pointed antapex curved towards the cell dorsum. The cells were slightly laterally flattened and contained two lateral brown chloroplasts sometimes joined with dorsal isthmus. Two Maupas corpuscles were most often present, rarely absent in some cells (Fig. 12). This species may easily be distinguished from *C. curvata* not only by the presence of Maupas corpuscles absent in *C. curvata*. Except for the dimensions, the cell form of *C. reflexa* is regular, i. e., not deformed or spirally distorted. *C. reflexa* may be found in open pelagial, in littoral among the higher plants, or in shallow, overgrown pools.

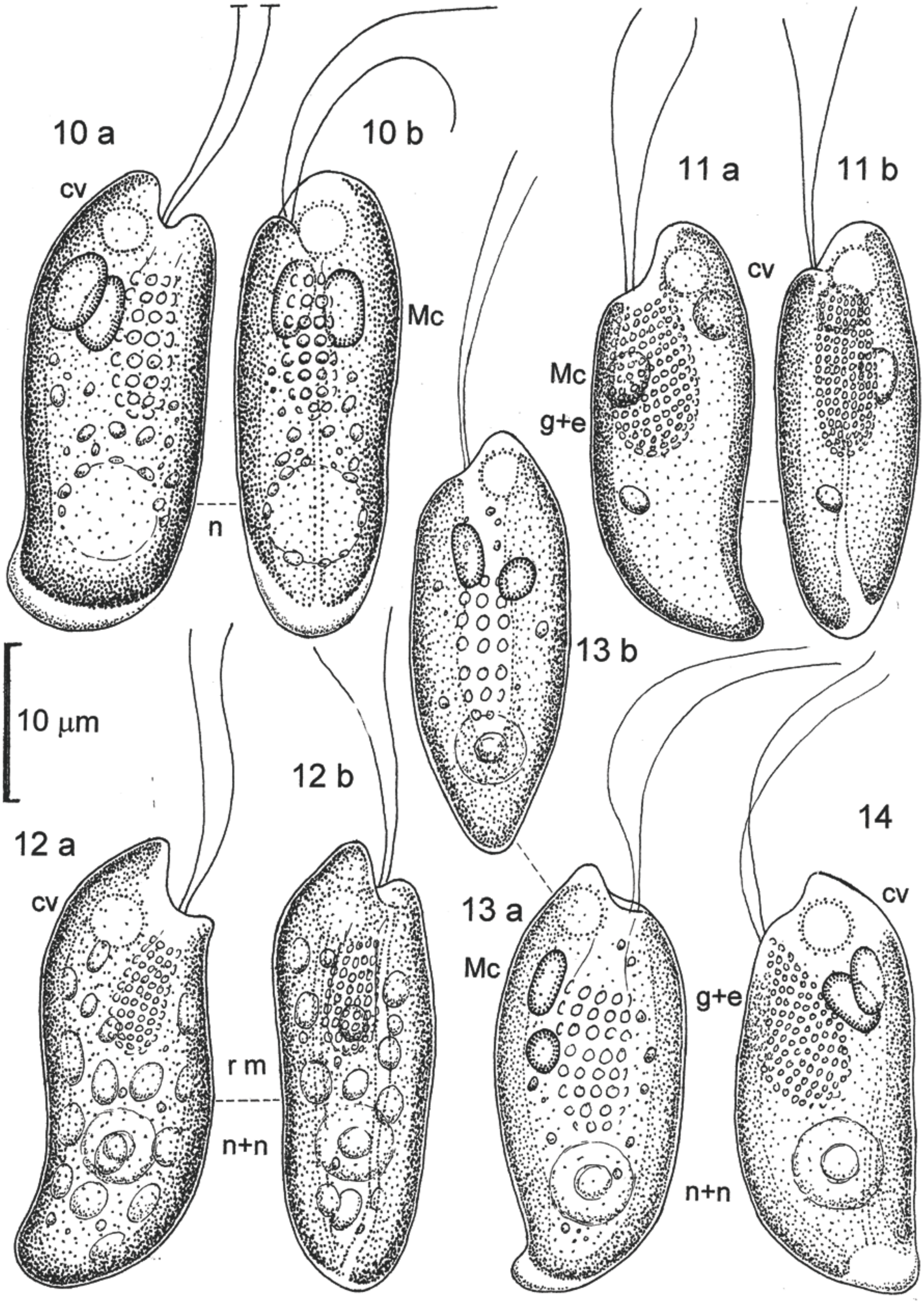
Two pyrenoids are mostly inconspicuous in live cells under a light microscope. They are sometimes conspicuous after preserving phytoplankton with iodine or dyeing by azocarmine (Fig. 53). Also some specimens of *C. reflexa* may be found practically without any sigmoid distortion, so probable the cryptomorph and campylomorph stages could be recognized.

Tab. 2: *Cryptomonas reflexa* (MARSSON) SKUJA var. ***reflexa***

Collection site	Dimensions of cells, μm			Shares		
	long	thick (d - v)	wide (s – s)	lo./th.	lo./wi.	th./wi.
A	26	9.8	---	2.65		
	30	10.5	---	2.85		
B	27	10.8	9.5	2.50	2.84	1.14
C	30	11.4	10.4	2.63	2.88	1.10
D	27	9.6	8.7	2.81	3.10	1.10
E	21.6	9.8	7.3	2.20	2.96	1.33
F	25	10	---	2.50		
	27	11.6	---	2.33		
	25	---	10.2		2.45	
G	30	13.7	12	2.19	2.50	1,14
Range	21.6 – 30	9.6 – 13.7	7.3 – 12	2.19 – 2.85	2.45 – 3.10	1.10 –1.33
Average	26.86	10.80	9.68	2.52	2.79	1.16

Collection sites A – E : Czech Republic

A – South Bohemia, drain in peat bog Červená blata by Třeboň, brown water, pH 3.2
B – Northeast Bohemia, Velká Jizerská louka, inundation pool of the river Jizera, pH 4.5
C – South Bohemia, fish pond Řežabinec by Ražice, plankton
D – Central Bohemia, fish pond Labeška, park of Průhonice Castle
E – Central Bohemia, dam reservoir Slapy on Vltava River, plankton
F – Central California, Clear Lake by Woodland, plankton
G – Northeast Germany, Hiddensee Island, small village pond



Figs 10-14: *Cryptomonas reflexa* (MARSSON) SKUJA var. *reflexa.* 10a, 11a, 12a, 13a, 14 – lateral views; 10b, 11b – ventral views; 12b – oblique view; 13b – dorsal view.

Cryptomonas reflexa var. ***anas*** (JAVORNICKÝ) JAVORNICKÝ, **comb. nova** (Figs 15-22)

Basionym: *Cryptomonas anas* JAVORNICKÝ, 1957, Universitas Carolina, Biol. 3, 3: 251.
Iconotypus: JAVORNICKÝ, 1957, l. c., Fig. 1, a-c (copied in the present study, Fig. 15, a-c).
Synonym: *Cryptomonas anas* JAVORNICKÝ, l. c.; JAVORNICKÝ, 1978, Cryptoph., in HINDÁK (ed.): Sladkovodné riasy (Freshwater algae), p. 453, Fig. 149: 1, a-c.

Beside the typical specimens of *C. reflexa* there are special shortened forms sampled from shallow pools with decaying leaves and water containing humic acids and hydrogen sulphide (H_2S). This form was described as *Cryptomonas anas* by Javornický (1957). The short and relatively wide cells of this species are similar to those of a typical variety *C. reflexa* var. *reflexa.* They are sigmoid, but at the same time bag-shaped, resembling the body of a duck (*Anas* in Latin). They were 12.5 – 21 μm (average 18.2 μm) long (Tab. 3). The average length was 26.9 μm for *C. reflexa* var. *reflexa.* The thickness (dorsiventral) was practically the same in both varieties so that the ratio of length to thickness was 2.52 for the typical variety and 1.86 for *C. reflexa* var. *anas.*

The cells of *C. reflexa* var. *anas* are either laterally slightly flattened or they were not flattened at all. The characteristic view of the species is that from the side. Some specimens are in this perspective typically sigmoid (Figs 15a, 16, 18a, 22a); some are more bag-shaped with only a slight curvature of the cell dorsum (Figs. 17, 19, 21a). The dorsiventral projection of the cell axis is more or less straight (Figs 15b, 18b, 20b, 22b).

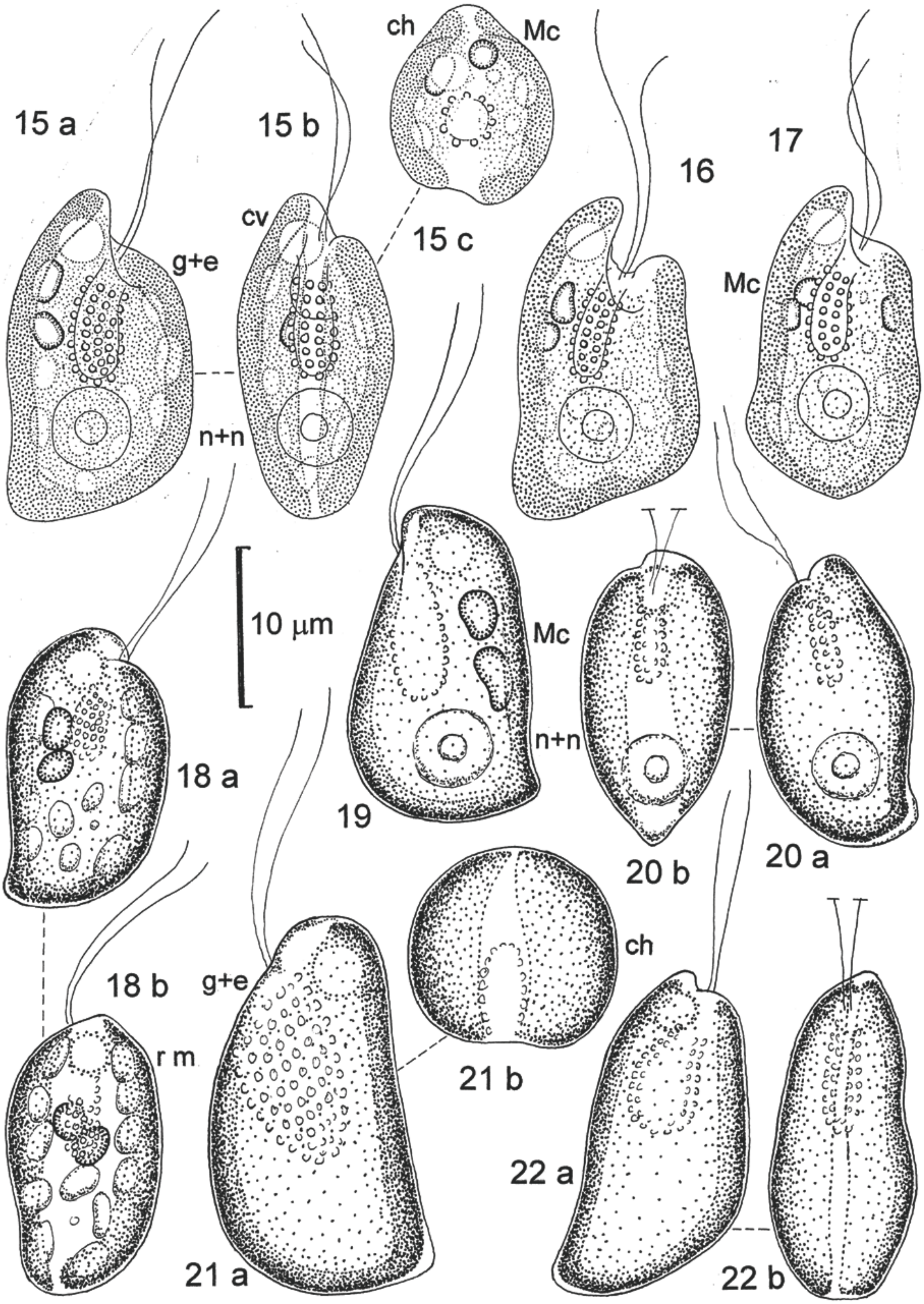
A pair of two Maupas corpuscles, exceptionally three of them (Fig. 17), frequently of irregular shape, were observed in a majority of specimens.

The original Latin diagnosis of *C. anas* is repeated here as the journal Universitas Carolina is not widely known and appeared for a limited time.

Tab. 3: ***Cryptomonas reflexa*** (MARSSON) SKUJA var. ***anas*** (JAVORN.) JAVORNICKÝ

Collection site	Dimensions of cells, μm			Shares		
	long	thick (d - v)	wide (s – s)	lo./th.	lo./wi.	th./wi.
A	20	11.3	10.3	1.77	1.94	1.12
	20	10.5	9.8	1.90	2.04	1.07
	21	12.2	10.5	1.72	2.00	1.16
	19	11.4	10.6	1.67	1.79	1.07
	17.5	8.6	7.8	2.03	2.24	1.10
B	21	9.7	8.5	2.16	2.47	1.14
C	14.6	---	7.1	---	2.06	---
	12.3	7	---	1.76	---	---
Range	12.3 – 21	7 – 12.2	7.1 – 10.6	1.67 – 2.16	1.79 – 2.47	1.07–1.16
Average	18.17	10.1	9.23	1.86	2.08	1.11

Collection sites
A – small forest pond, pH of water 6.6 – 6.8, 2 mg/l H_2S, vicinity of the town Čelákovice, Central Bohemia, Czech Republic
B – Czech Republic, South Bohemia, fish pond Řežabinec by Ražice, plankton
C – Central California, Clear Lake by Woodland, plankton



Figs 15-22: *Cryptomonas reflexa* (MARSSON) SKUJA var. *anas* (JAVORN.) JAVORN. 15a, 16, 17, 18a, 19, 20a, 21a, 22a – lateral views; 15b, 18b, 20b, 22b – ventral views; 15c, 21b – apical views.

Latin diagnosis

Cryptomonas reflexa var. ***anas***

Forma cellulae rara: pars anterior oblique cuneata in rostrum longum, ad partem ventralem curvatum, progreditur; cellula a latere visa latissima sub media parte est et ad partem dorsalem in processum caudalem obtusum progreditur; a ventre visa paulo compressa est, minime super mediam partem, maxime in parte posteriore; corpus saepe paulo spiraliter tortum est. Faux ad ventrem curvata, trichocystidis parvis obtecta, ad medium cellulae attingit, sub rostro apicali in sulcum perbrevem influit. Hic flagella, longitudine distincta (13 μm, 16 μm) inserta sunt. Chromatophora bina lateraliter disposita sunt, colore fusco-olivaceao. Bina sed terna corpuscula Maupasii parva, lucem refringentia, forma irregulare, apud faucem disposita sunt. Amylum in forma parvorum granulorum ovalium dispersum est. Vacuolum pulsans in rostro apicali. Nucleus nucleolatus in parte posteriore cellulae.

Dimensiones: long. 17.5 – 23.5 μm, lat. 11.5 – 14 μm, crass. 10-11 μm.
Holotypus (iconotypus): Figurae 1, a-c in Javornický, 1957, Universitas Carolina, Biol. 3, 3: 251.

Cryptomonas marssonii SKUJA var. ***marssonii*** (Figs 23-28)

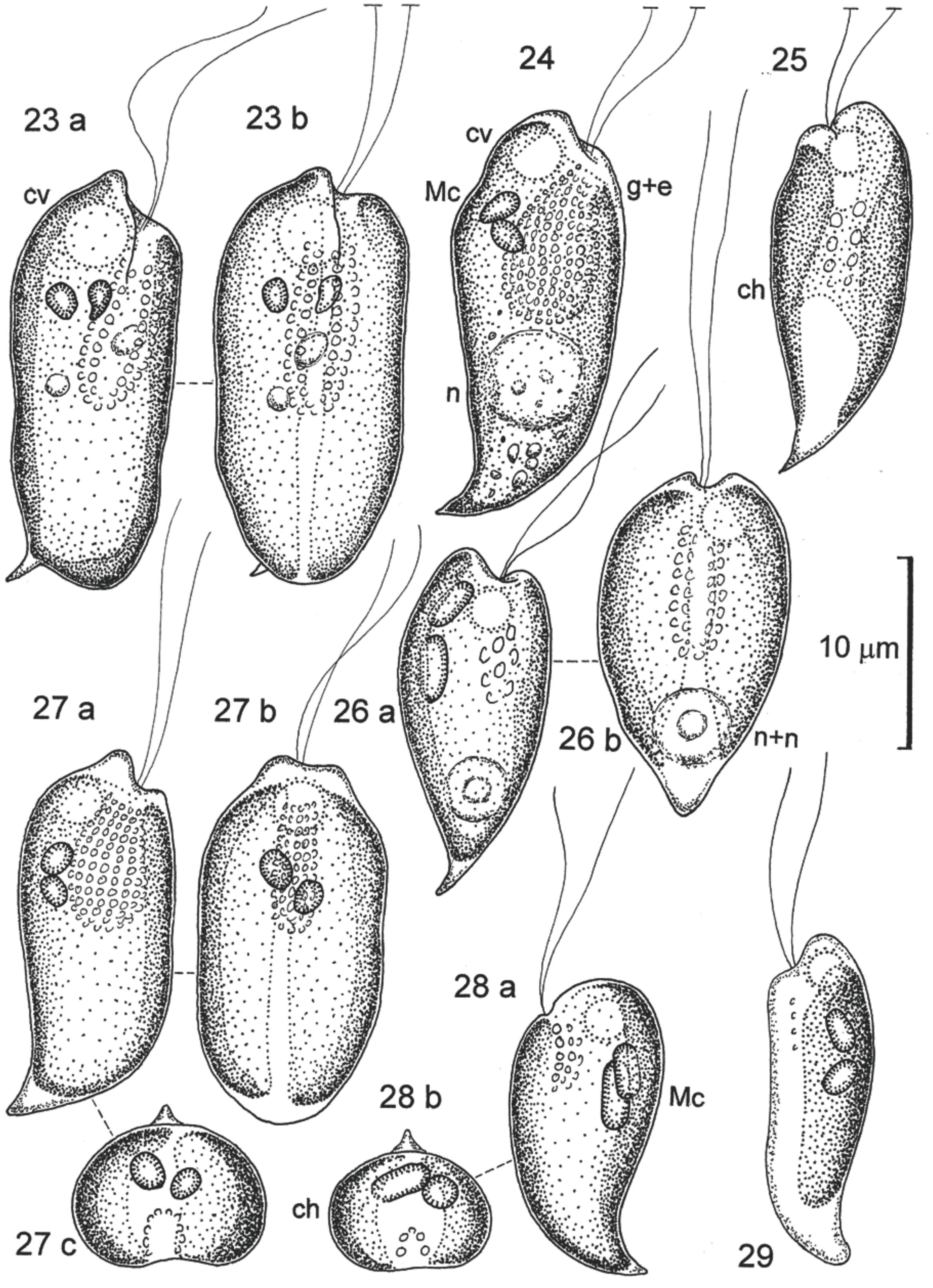
Basionym: *Cryptomonas marssonii* SKUJA, 1948, Symbol. botan. upsal. 9, 3: 357.
Ikonotypus: SKUJA, l. c., Fig. XXXVII: 41.
Synonyms: *Campylomonas reflexa* (SKUJA) D. R. A. HILL, 1991, Phycologia 30 (2): p. 175;
Campylomonas marssonii (SKUJA) KUGRENS & CLAY, in Wehr & Sheath, 2003, Freshw. Alg. North Amer., Ecol. Classif.: 746.

Another member of the frequent phytoplankton assemblage in fresh waters is *Cryptomonas marssonii* SKUJA. Some authors doubt this species to be a separate one but it is easy to see that it really is. It is smaller than *C. reflexa* even though the dimensions of both species partially overlap: the cells of *C. marssonii* in my materials were 13.5 – 24 μm (average 19 μm) long (Tab. 4). The main difference, however, is the shape of cell antapex. That of *C. marssonii* is sharply pointed particularly from the side projection. The short hyaline tail may be evident (Figs 23, 25, 26, 27a, c, 28ab). Two Maupas corpuscles are almost always present; they are relatively small, and sometimes of irregular shape. Pyrenoids were not observed in live material. The lack of pyrenoids was demonstrated also by electron microscopy (Kugrens & Clay 2003). On the contrary, pyrenoids were observed in the cells of the *C. marssonii* when dyed by azocarmine (Fig. 54), or treated by iodine (Figs 42 – 44).

Tab. 4: *Cryptomonas marssonii* SKUJA var. ***marssonii***

Collection site	Dimensions of cells, μm			Shares		
	long	thick (d - v)	wide (s – s)	lo./th.	lo./wi.	th./wi.
A	19	5.7	6.2	3.33	3.06	0.92
	19	7.5	11.4	2.53	1.67	0.66
	21	9	---	2.33	---	---
	21.5	8	9	2.69	2.39	0.89
B	15	6.6	7.7	2.27	1.95	0.86
C	21	8.6	---	2.44	---	---
	24	10.8	---	2.22	---	---
D	17	7	---	2.43	---	---
	13.5	---	7.5	---	1.80	---
Range	13.5 – 24	5.7 – 10.8	6.2 – 11.4	2.22 – 3.33	1.67 – 3.06	0.66 – 0.92
Average	19.00	7.90	8.36	2.53	2.17	0.83

A - Czech Republic, South Bohemia, fish pond Řežabinec by Ražice, plankton
B - Czech Republic, South Bohemia, forest fish pond Žabinec by Třeboň, plankton
C - Czech Republic, Central Bohemia, ponds close to Slapy reservoir on the Vltava River, plankton
D – Central California, Clear Lake by Woodland, plankton



Figs 23-29: *Cryptomonas marssonii* SKUJA var. *marssonii.* 23a, 24, 25, 26a, 27a, 28a – lateral views; 23b, 26b, 27b – ventral views; 27c, 28b – apical views. 29 – *Cryptomonas gracilis* SKUJA – lateral view.

There is one more diacritic character: while the cells of *C. reflexa* are slightly laterally flattened or not flattened at all, those of *C. marssonii* are slightly flattened dorsiventrally. The position of a cell may be easily oriented because of the dorsal curvature of the posterior tail.

Cryptomonas marssonii var. ***brachys*** JAVORNICKÝ, **var. nova** (Figs 30-37)

Similarly to *C. reflexa*, there exist the "small water-bodies pendant" of *C. marssonii*. The cells of this form are relatively wider so that the whole cell has the form of a reversed drop of water. They are sigmoid and possess more or less sharp, dorsally curved antapices.
1–2 Maupas corpuscles are deposited near the dorsal, sometimes also near the ventral inner wall of the cell. The cells are dorsiventrally flattened as are those of the typical form. The average cell length of *C. marssonii* var. *brachys* was 16.6 µm in my materials. While the ratio of length to thickness was 2.53 µm on average for *C. marssonii* var. *marssonii*, it was 1.95 µm for the variety brachys (Tab. 5).

Tab. 5: ***Cryptomonas marssonii*** SKUJA var. ***brachys*** JAVORNICKÝ

Collection site	Dimensions of cells, µm				Shares	
	long	thick (d - v)	wide (s – s)	lo./th.	lo./wi.	th./wi.
A	17	7.9	13	2.15	1.31	0.61
	17	---	10.4	---	1.63	---
	15.5	9.4	---	1.65	---	---
	14.5	8.1	9.2	1.79	1.58	0.88
	15.2	7.7	9.8	1.97	1.55	0.79
	17	8.4	10.7	2.02	1.59	0.78
	15.2	7.8	---	1.95	---	---
B	20	10.5	12	1.90	1.67	0.87
	16.5	7.9	8.5	2.09	1.94	0.93
C	18	9.8	12.7	1.84	1.42	0.77
D	16.5	7.6	9.7	2.17	1.70	0.78
Range	14.5 – 20	7.6 – 10.5	8.5 – 13	1.65 – 2.17	1.31 – 1.94	0.61– 0.93
Average	16.58	8.51	10.67	1.95	1.60	0.80

A – Czech Republic, Central Bohemia, Prague – Ďáblice, small forest pond
B - Czech Republic, South Bohemia, fish pond Smyslov by Blatná, plankton
C - Czech Republic, West Bohemia, small forest ponds "Kvapilova jezírka" near Plzeň, plankton
D – Central California, Clear Lake by Woodland, Konocti Bay, plankton

Latin diagnosis

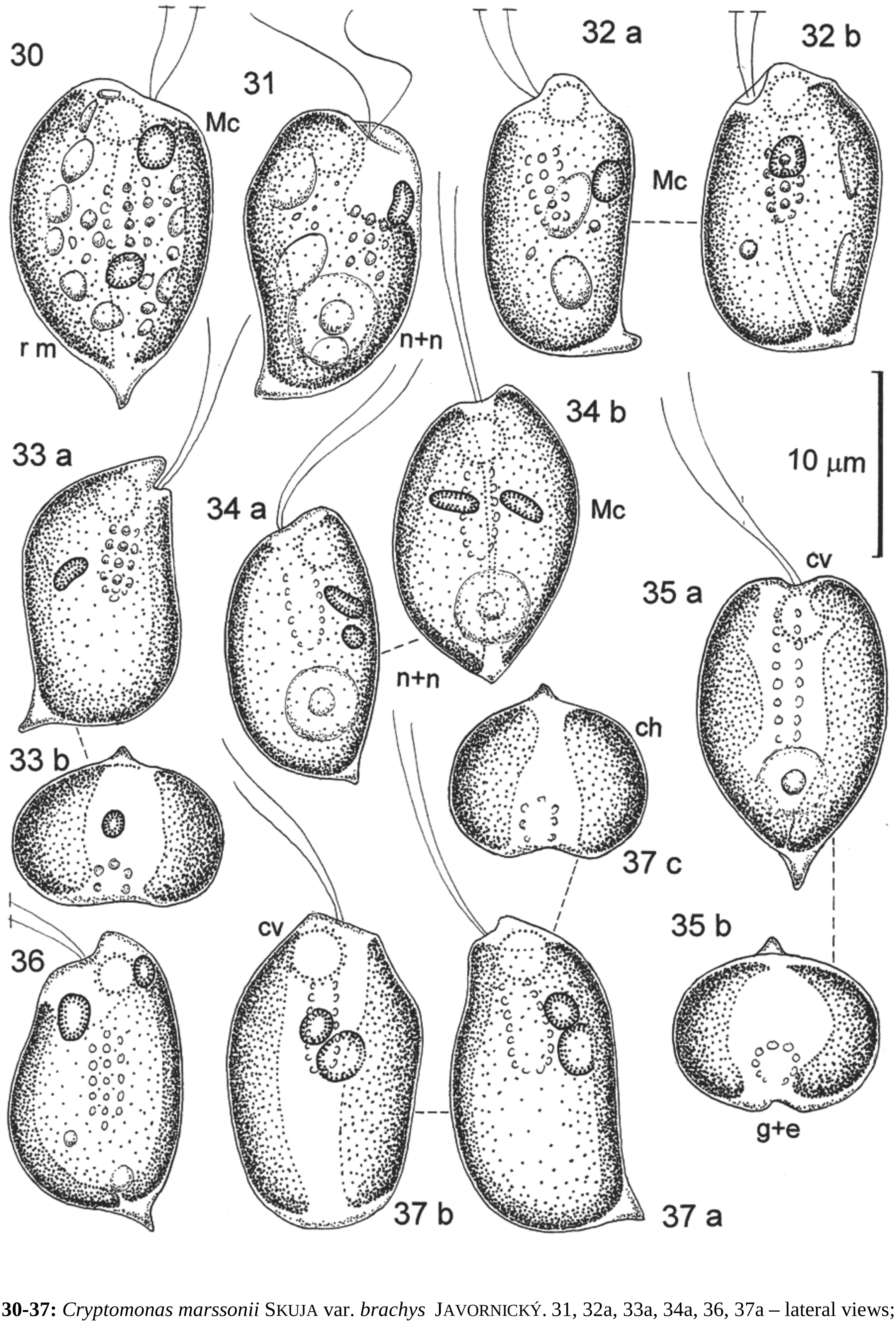
Cryptomonas marssonii var. ***brachys***

Cellulae relativiter breves obtusaeque, forma guttae reversae. Apex oblique cuneatus, antapex in rostrum breve sed acutum, ad partem dorsalem curvatum, angustatus. Cellulae e latere visu sigmoideae, dorsiventraliter paulo compressae. Pseudopharynx trichocystis paucis obtecus ad medium cellulae longitudinis attingit. Chloroplasta fusco-olivacea bina, lateraliter disposita. Vacuola pulsans in parte anteriore cellulae, nucleus nucleolatus in parte posteriore cellulae. Flagella bina sub rostro inconspicuo apicale inserta sunt. Corpuscula Maupasii (lucem refringentia), raro singularia, saepe bina, forma irregulare, postice faucis, raro in parte ventrale disposita sunt.

Cellulae circiter 16.6 µm longae. Relatio lengitudinis ad crassitudinem circiter 2.53 in *C. marssonii* var. *marssonii* et 1.95 in varietate *brachys* (Tab. 6).

Holotypus (iconotypus): Figurae nostrae 37 a-c.

These two varieties of *C. reflexa* and *C. marssonii* may be supposed to be "ecospecies" in the conception by Klaveness 1985. Their characteristic milieu are small ponds and puddles with decaying organic matter (leaves) and H_2S content.



Figs 30-37: *Cryptomonas marssonii* SKUJA var. *brachys* JAVORNICKÝ. 31, 32a, 33a, 34a, 36, 37a – lateral views; 30, 34b, 35a – ventral views; 32b – oblique view; 37b – dorsal view; 33b, 35b, 37c – apical views.

Cryptomonas gracilis SKUJA (Fig. 29)

Basionym: *Cryptomonas gracilis* SKUJA, 1948, Symbol. botan. Upsal. 9, 3: 358.
Ikonotypus: SKUJA, l. c., Fig. XXXVIII: 3.
Synonym: *Campylomonas reflexa* (SKUJA) D.R.A. HILL, 1991, Phycologia 30 (2): p. 175.

Hill (1991) observed the wide variability of the cultivated flagellates which is, of course, a well known phenomenon in cultures. Skuja (1948) defined and illustrated *C. gracilis* exactly and recognized the species also in his book published in 1956. The species was rare in my samples but it was clearly distinguishable by the slender form of the cells reminding a small fish. The cells were 17 – 19 μm long, 5.2 – 5.5 μm thick, containing the single olive green bilobed dorsal chloroplast and two Maupas corpuscles. The specimens were sampled from eutrophic fishponds named Řežabinec and Žabinec in South Bohemia in Czech Republic.

Cryptomonas cf. ***borealis*** SKUJA (Figs 38-41)

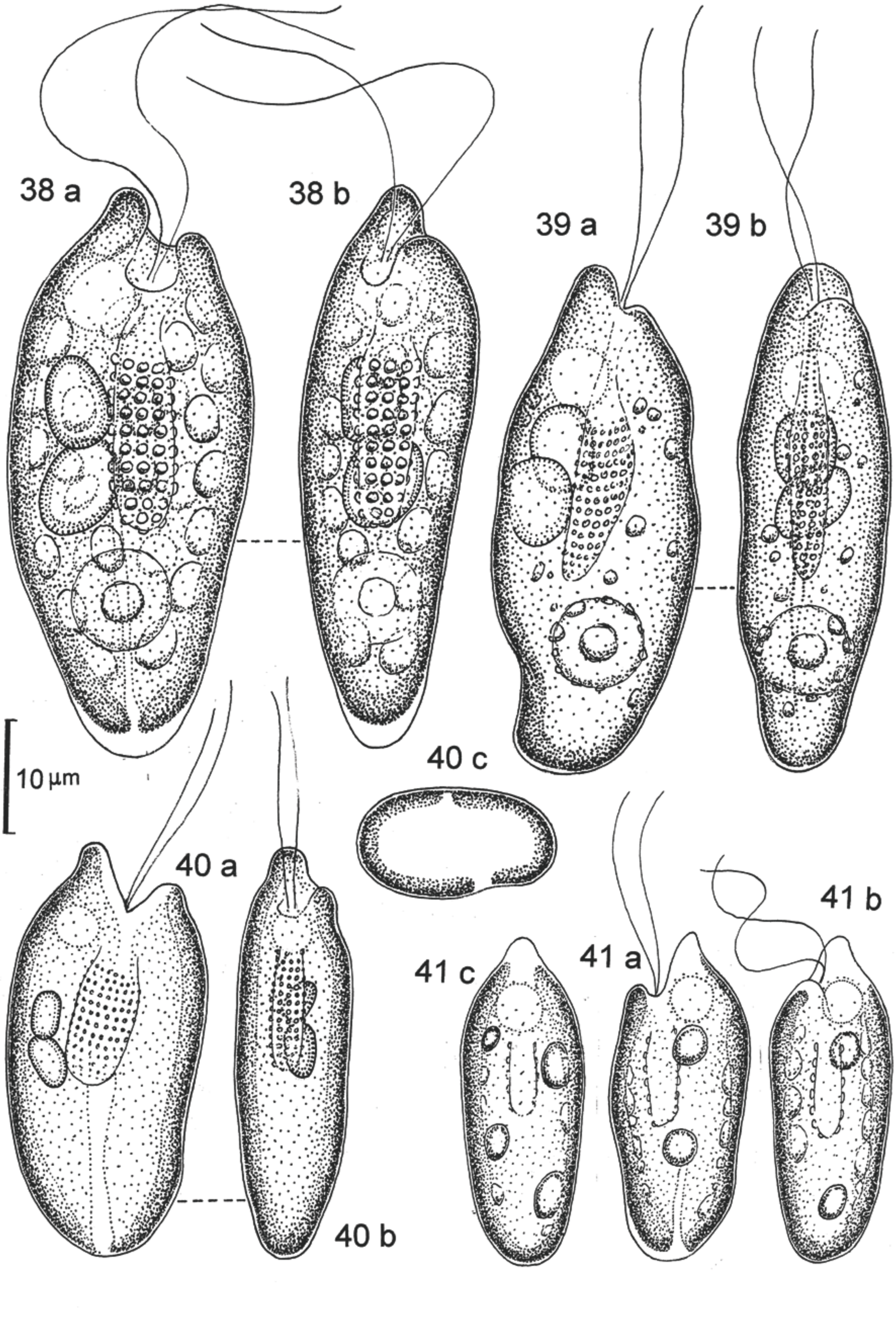
Cryptomonas borealis as described and pictured by Skuja (1956) has the cells only rarely of a slightly sigmoid shape; some of his specimens possess straight or even slightly C-shaped longitudinal axes. Nevertheless, the cells display the apparent apical rostrum and funnel shaped vestibule similar to the *Cryptomonas curvata.* Skuja gave the length of the laterally flattened cells as 20-40-50 μm. 1 – 2, rarely 3 light-breaking corpuscles (Maupas corpuscles) should be present; shelled pyrenoids were not observed.

The difference between two Skuja’s species, namely the *Cryptomonas platyuris* and *C. borealis* is not clear. My specimens resemble *C. borealis* by their widely opened gullet mouth but they may as well be classified in *C. platyuris*. *C. platyuris* has been introduced by Kugrens & Clay (2003) as the *Campylomonas platyuris* but without the necessary quotations. Skuja placed both species inbetween *C. curvata* and *C. erosa.*

Ettl (1965) found several populations of this species in fish-ponds in North Moravia in Czech Republic. His specimens had two more or less lateral chloroplasts. Their cells demonstrated an almost straight longitudinal axis, 3 light-breaking (Maupas) corpuscles, and particularly large pseudopharynx with numerous ejectosomes. The morphology of apex was typical, with the conspicuous rostrum and funnel-like ostium faucis. The cells were 35 – 48 μm long. Ettl, however, described from the same localities the new species *Cryptomonas skujae* Ettl. It demonstrates almost the same morphology as his specimens of *C. borealis*. Ettl gave these differences: the cells of *C. skujae* were smaller, 30 – 38 μm long (the ranges of dimensions of the two species were overlapping); a single chloroplast with two parietal lobes and the dorsal isthmus (Skuja gave 1-2 chloroplasts); some cells were slightly spirally distorted along the longitudinal axis.

The cells sampled in one of my Czech localities (Fig. 38) occupied the upper range of dimensions as given both by Skuja and Ettl for *C. borealis* (Tab. 6). They resembled *C. curvata* particularly by the morphology of the apex and mouth of the gullet but their axes were straight and they carried one pair of the robust Maupas corpuscles. The ejectosomes were relatively large and not very numerous. The olive-green chloroplasts were in dorsal and ventral positions.

My specimens from another Czech locality (Fig. 39) looked very much the same, but their chloroplasts were in lateral position. The uncertainty of the lateral or dorsiventral position of chloroplasts is apparent also in Skuja’s drawings. Moreover, the cryptomonads from this locality were slightly twisted screw-like with caudas bowed backward. The Maupas corpuscles again were easily visible, large, and almost spherical.



Figs 38-41: *Cryptomonas borealis* SKUJA. 38a, 39a, 40a, 41a – lateral views; 38b, 39b, 40b, 41b – ventral views; 40c – equatorial cross section of the cell; 41c – dorsal view.

The specimens from another Bohemian fishpond (Fig. 40) and from Teufelsee in Germany (Fig. 41) were smaller and their morphology was closer to Skuja´s type and to Ettl's findings. The cell axes were almost straight, the cells were more flattened laterally, their apices resembled an open mouth. The cells from Czech material displayed two small ovoid Maupas corpuscles, those from German lake two or four light-breaking balls spread in cytoplasm. The olive-green chloroplasts were dorsiventrally disposed.

Tab. 6: ***Cryptomonas*** cf. ***borealis*** SKUJA

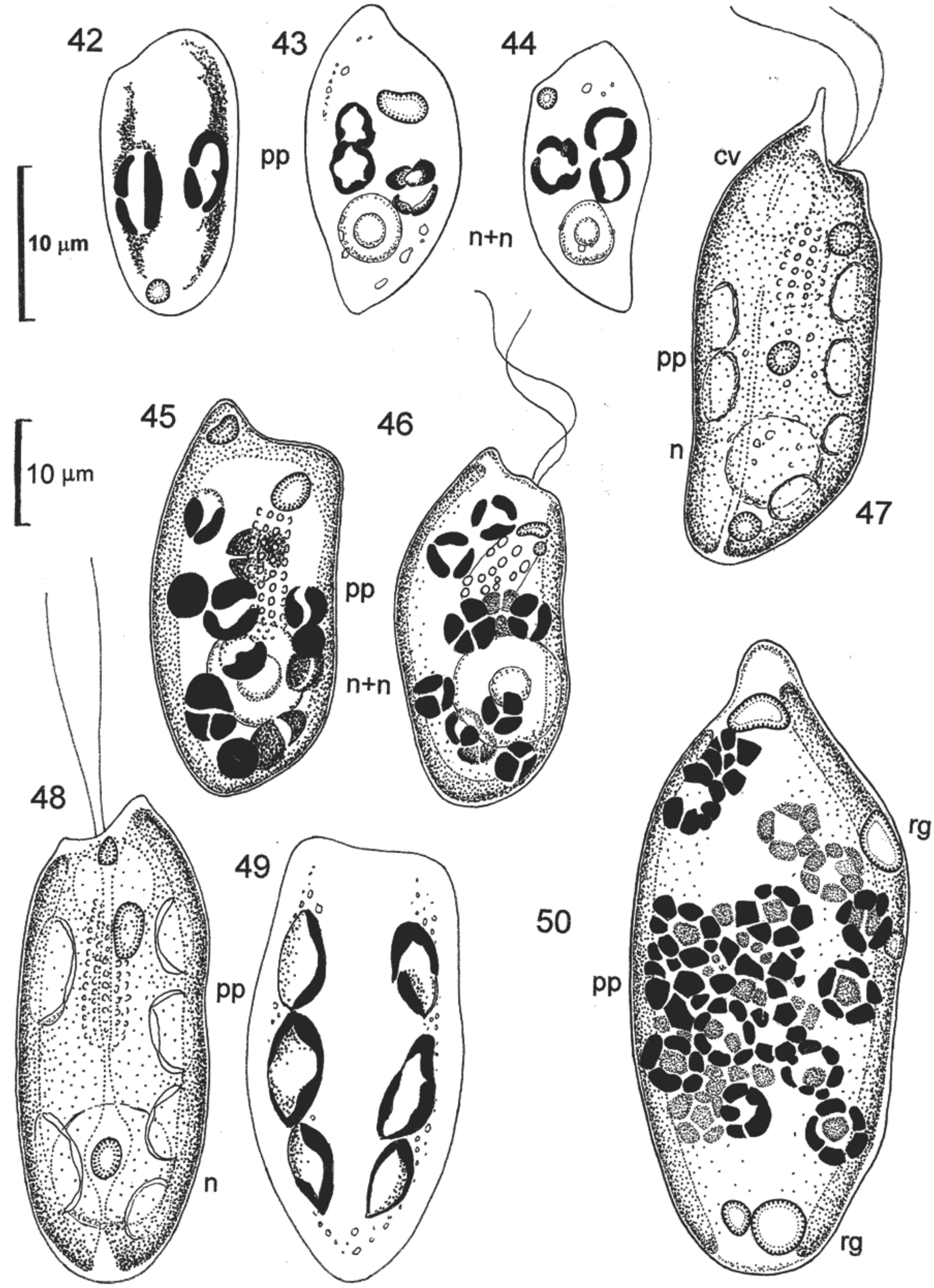
Collection site	Dimensions of cells, µm long	wide (s/s)	thick (d/v)
South Bohemia, inundated stonepit near Novosedly on Nežárka River, planton (Fig. 38)	50	14.5	21.5
South Bohemia, Řežabinec pond near Ražice, plankton, (Fig. 39)	45	---	19
South Bohemia, Žabinec pond by Třeboň, outlet, pH 6 (Fig. 40)	37	10	18
Northeast Germany, lake Teufelsee, Brandenburg, (Fig. 41)	29	11	13
Range	29 – 50	10 – 14, 5	13 – 21.5
Average	40.25	11.83	17.87

Pyrenoids in the cells of sigmoid cryptomonads as observed under a light microscope

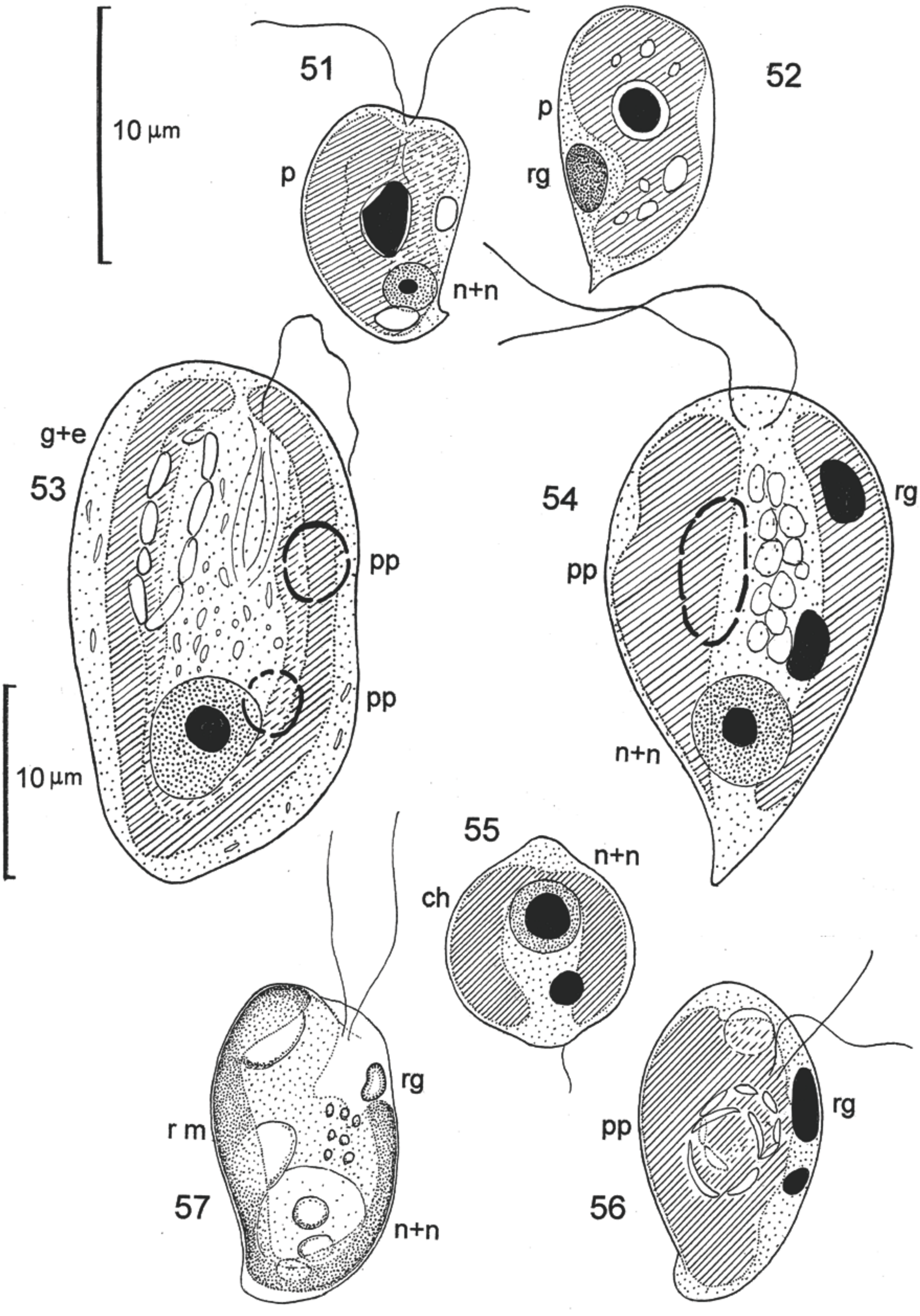
The original descriptions of the sigmoid cryptomonads based on light microscopic observations, do not reveal any pyrenoids. The electron microscopists, however, found in *Campylomonas rostratiformis* "two chloroplasts, each with numerous pyrenoids" (Kugrens & Clay 2003). The same authors ascertain that in *Campylomonas reflexa* two pyrenoids are present but for *Campylomonas marssonii* and *Campylomonas platyuris* they report "two chloroplasts without pyrenoids, which differs from *Campylomonas reflexa*".

When cryptomonads are preserved with iodine, the starch grains and structures become easily visible and dyed blue. Segmented shells of starch are sometimes recognizable. In the majority of specimens, however, they are hardly distinguishable from simple starch grains. In some specimens belonging very probably to *Cryptomonas marssonii,* I have found 2 - 3 structures resembling shelled pyrenoids (Figs 42-44). In some cells of the larger species *C. curvata*, both live or preserved with iodine, six clear shelled pyrenoids were observed (Figs. 47-49); moreover, the numerous (up to 20) artifacts similar to pyrenoids appeared (Figs 45-46, 50). They were mixed with the single starch grains of various sizes. They might be pyrenoids of some other kind or just the structured starch grains. The cells of *C. reflexa* possess two pyrenoids apparent after some kind of dyeing (Fig. 53). The pyrenoids in sigmoid species of *Cryptomonas* are different from the pyrenoids shown in the cells of *Plagioselmis* after azocarmine application (Figs 51-54). The starch shells of *Plagioselmis* are assymetric showing half of the pyrenoid being immersed into the chloroplast body, with the other half remaining outside. This type of pyrenoid is called *amphosoma* and is always clearly visible even in the live cells.

When the cells of cryptomonads had been preserved with osmium acid and then treated by azocarmine, the proteins in nucleoli and in some separate grains were stained intensively red. The rest of the nucleoplasma was stained pink. This appeared in *C. reflexa* (Fig. 53), *C. marssonii* (54), and in *C. marssonii* var. *brachys* (Fig. 55-56). The proteinic corpuscles forming the nuclei of the supposed pyrenoids were expected to be stained red as well but they were not. The centres of starch shells were found empty with respect to proteins.



Figs 42-50: Pyrenoids in the cells of different species of *Cryptomonas*: 42-46; 49-50: dyed with iodine – black areas were coloured dark blue; 47-48 – visible in live cells. 42-44 – *Cryptomonas marssonii* SKUJA; 45-50 – *Cryptomonas curvata* EHRENB. 43-48, 50 – lateral views; 42, 48, 49 – ventral views.



Figs 51-57: Pyrenoids in the cells of different species: 51-56: preserved by osmium acid and dyed by azocarmine – black areas were coloured red, dotted areas were coloured more or less intensive pink, cross hatched areas were yellow chloroplasts; 57 – live cell; 51-52 – *Plagioselmis nannoplanctica* (SKUJA) NOVARINO, LUCAS & MORRAL; 53 – *Cryptomonas reflexa* (MARSSON) SKUJA. 54 – *Cryptomonas marssonii* SKUJA; 55-57 – *Cryptomonas marssonii* SKUJA var. *brachys* JAVORN. 51-54, 56-57 – lateral views; 55 – equatorial cross section of the cell.

The difference between the pyrenoids in the cells of sigmoid species of *Cryptomonas* and those in the cells of *Plagioselmis nannoplanctica* (Figs 51-52) is obvious. The proteinic nuclei of pyrenoids in the latter genus were stained intensively red by azocarmine. The lack of such proteinic nuclei in their middle of pyrenoids in *Cryptomonas* show they are of different character. Santore (1985) observed: “Light-microscopic descriptions of pyrenoids in members of this genus *(Cryptomonas)* reflect the difficulties with which this organelle can be identified, even if the starch grains have been stained with iodine to reveal those pyrenoids which happen to be ensheathed.” Kugrens & Clay (2003) observed that the pyrenoids in cryptomonads are not traversed by thylakoids.

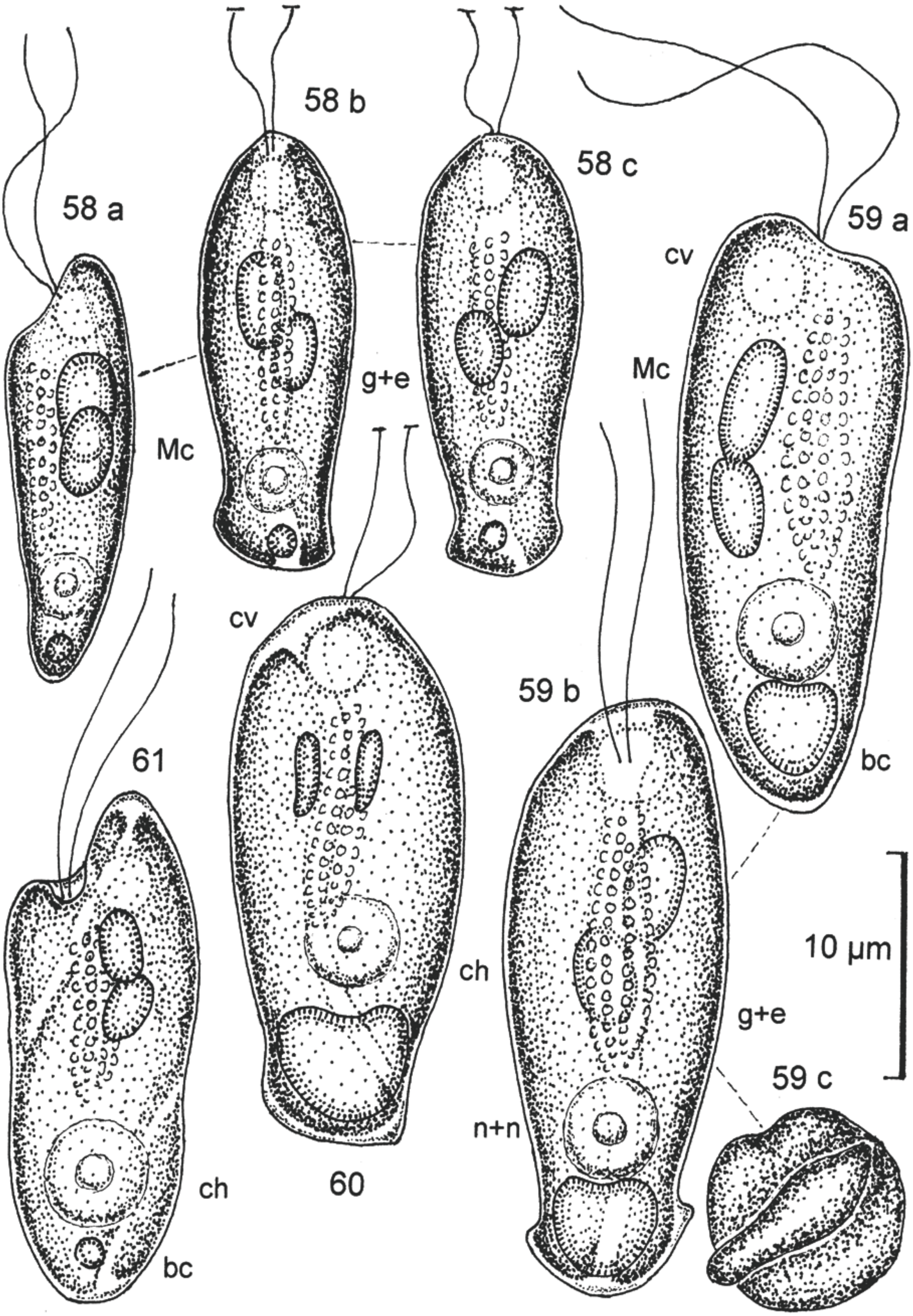
Cryptomonas cylindrica EHRENBERG forma (Figs 58-61)

The sigmoid curvature visible in side projection is not the only deviation from symmetry of cryptophycean cells. It is demonstrated here on *C. cylindrica* Ehrenb. forma, the deformed antapex of which appeares in dorsiventral position.

Tab. 7: *Cryptomonas cylindrica* EHRENBERG forma

Collection site	Dimensions of cells, µm		
	long	thick (d – v)	wide (s – s)
The locality called “Swamp”			
favoured by Adolf Pascher:	19	7, 2	---
the left peatbog near Doksy,	23	---	13
North Bohemia	22	10	---
(filled up later, does not exist any more)	21	7,7	9
	21	9	10.3
	26	10	---
	23	---	10
	24	9	---
Range	19 – 26	7.2 – 10	9 – 13
Average	22.37	8.82	10.57

Twice from the same locality, the abandoned and later destroyed peatbog called Swamp, once the favourable collecting site of Adolf Pascher and Bohuslav Fott, I sampled the identical form of *Cryptomonas.* The findings were made in the time range of nine years, so that the flagellate made there the stable part of the plankton assemblage. From the lateral view, the specimens suggested *Cryptomonas cylindrica* Ehrenb. as recognized in 1967 (Javornický 1967). This lateral projection of cells was slightly conical with wider anterior part, flat venter and convex dorsum (Figs 58a, 59a, 61). Two apparent corpuscles of Maupas were present in every cell. Two yellow-brown chloroplasts were either lateral, split by wide slots (Figs 58b-c, 59b), or of an indeterminate position, with slots winding screw likely (Figs 60, 61). The same variability of chloroplast shape was observed in *C. cylindrica* (Javornický 1967). Also the cell dimensions corresponded with *C. cylindrica*, reaching in the “Swamp” material the following averages: length 22.4 µm, thickness (dorsum – venter) 8.8 µm, width (between sides) 10.6. The cells in question were slightly dorsiventrally flattened in comparison of the cylindrical shape of the typical species. Another difference was the constant presence of the single basal refractive corpuscle of different size in every cell from”Swamp”.



Figs 58–61: *Cryptomonas cylindrica* EHRENBERG forma. 58a, 59a, 61 – lateral views; 58b, 59b – ventral views; 58c, 60 – dorsal views; 59 c – antapical view.

The reason for presenting this flagellate here is the deformation of cell posterior end, in these cryptomonads visible in dorsiventral projection. The narrowed pole formed a sort of the keel oriented from side to side, wider than the cell walls above it, proceeding into more or less sharp side processions of cauda (Figs 58b-c, 59b-c, 60). This deformation was not caused by big basal refractive corpuscles because it could be observed also in the specimens with very small corpuscles (Fig. 58).

The finding of this forma on different localities could justify the existence of the new taxon, variety or species. However, as it was sampled from the single locality, even though repeatedly after years, the author prefers to attach the organism to similar existing species, namely *C. cylindrica.* A slight widening in both directions, lateral and dorsiventral, gives Czosnowski (1948) for his species *Cryptomonas woloszynskae,* but its antapex is rounded, definitely not forming any keel or processions.

Discussion

The first drawings and description of a sigmoid cryptomonad were given by Ehrenberg (1838) under the name *Cryptomonas curvata* Ehrenb. Ehrenberg's Latin diagnosis is concise: *"C. corpore valde compresso magno, duplo longiore quam lato, 48vam lineae partem aequante, antico fine infra, postico supra emarginato seu leviter sigmoideo, viridi"* (p. 40). The pictures are very schematic but the total habitus of the cells is clear enough. Their length may be calculated respecting the given magnification of the pictures (310 times) and it turns out by 42 – 48 μm. The species was documented later by Stein (1878). His depiction is perfect; however, the flagellate was included in Stein's wide concept of the species *Cryptomonas ovata* Ehrenb. Stein describes this particular specimen: "Ein altes starres Individuum mit übergebogenem Hinterleib *(Cryptom. curvata* Ehrbg.*)*". *C. curvata* was later emended by Penard (1922). Both Stein and Penard did not find "light-breaking corpuscles" (later named Maupas corpuscles) in the cells.

The nomenclatural confusion of this species surprisingly continued. Troitzkaja (1922) described a sizable cryptomonad (long up to 65 μm) as *Cryptomonas rostrata*. Despite its documentation by means of rather poor graphics, *C. rostrata* O.V. Troitzkaja was emended by Kiselev (1931) and later recognized and properly illustrated by Czosnowski (1948). The last author's drawings and description are undoubtedly of *C. curvata* Ehrenb.

When describing his homonymous *Cryptomonas rostrata*, later renamed *C. rostratiformis* Skuja in Huber-Pestalozzi (1950), Skuja (1948) wrote: "Allerdings ist jetzt nicht mehr sicher zu entscheiden, welche von den reflexen Cryptomonaden Ehrenberg unter seiner *C. curvata* verstanden hat." Nevertheless, Skuja admitted the identity of his newly described species with the sigmoid specimen of "*C. ovata*" was given by Stein (1878). Stein indeed demonstrated in his figure XIX: 27 the cell with a smooth contour line similar to the figure by Skuja (1948, Tab. XXXVII: 39-40) and to Figs. 6a and 7a in the present study. Moreover, the comparison of Stein's figures 27 and 28 (28: "Normal Individuum von der Bauchseite") demonstrates the cell dorsiventrally compressed which is one of the features of *C. rostratiformis*. In spite of this, the distinction between *C. curvata* and *C. rostratiformis* can hardly justify the existence of two different species.

Further on Skuja (1939) promoted the variety *Cryptomonas erosa* Ehrenb. var. *reflexa* Marsson (1904) to the well-defined species *C. reflexa* (Marsson) Skuja. Later Skuja (1948) asserted that his species differs from Marsson's variety and dropped Marsson's name but kept the specific name *C. reflexa* Skuja. Consequently, he described the new species *Cryptomonas marssonii* Skuja. This nomenclatural operation is not correct; the original combinaton *C. reflexa* (Marsson) Skuja must be retained.

However, according to electron microscopic research, the genus *Cryptomonas* Ehrenb. is not homogenous but breaks up into several genera. Two of the described genera, namely

Cryptomonas Ehrenb. and *Campylomonas* D.R.A. Hill, are common in freshwater bodies. The species of both genera possess furrow and gullet (Brett *et al.* 1994). Members of both genera may or may not possess pyrenoids; if present, they are not traversed by thylakoids. There is a difference between the two types of furrow (Kugrens *et al.* 1986). Another difference appears in the flagellar apparatus. The keeled rhizostyle of *Campylomonas* is believed to cause the sigmoid shape of the cells (Hill 1991). Moreover the periplast structure of both genera is dissimilar.

If such a simple visible characteristic were joined with the ultrastructures of the cell, the light microscopic determination of *Campylomonas* would be easy. This would be a very constructive solution of the problem, but more evidence is necessary. However, Novarino (2003) writes that all later reports of a genus (referring to *Komma* Hill) "should be based on electron microscopy because the distinctive features of the genus are not visible under the light microscope. ...it is important for all later reports of cryptomonad species to be based on the same observation methods used to describe the species in the original taxonomic protologue."

Hill (1991) defined the new genus *Campylomonas* by means of an ultramicroscopic structure and established *Campylomonas reflexa* (Skuja) D.R.A. Hill as the type species of the new genus. Its proper name should be of course *C. reflexa* (Marsson) D.R.A. Hill. Hill wrote that the variability in size and shape of the cells in his single culture was wide and that his cryptomonad could be identified with *C. reflexa, C. marssonii,* and *C. gracilis.* Some more species are included in this genus according to Kugrens & Clay (2003): *Campylomonas rostratiformis, C. marssonii* and *C. platyuris,* even if the authors did not provide the transmission electron micrographs. They even declined to publish the valid nomenclatural combinations of these names. Therefore *Campylomonas* includes so far nomenclaturally only the type species, i.e. *Campylomonas reflexa* (Marsson) D.R.A. Hill (Novarino 2003).

However, Hoef-Emden & Melkonian (2003), and Hoef-Emden (2007) found no genetic information justifying existence of two different genera in question based upon the above given features. They identified several cryptomorph/campylomorph pairs within some species and synonymized *Campylomonas* with *Cryptomonas* accordingly. The coherence of genotypes lies beyond any discussion. However, the cells of cryptomonads are rather plastic which may produce different cell-shapes particularly under different conditions in cultures. Observing the populations collected from natural water bodies, we are not finding such wide variability of cells in a determinate population of cryptomonads as it occurs in culture. Therefore we may hold the sigmoid cryptomonads for different species and varieties if not for a separate genus.

According to Hoef-Emden (2007) even the electron microscopy is not enough for determining Cryptophyceae, but to know their genotype sequences is necessary. These requirements are very exact but make determining of cryptomonads impossible for majority of researchers.

Therefore it is advocated by the present study that the descriptions and the accurate depictions of natural specimens of Cryptophyceae, either drawings or photographs, identified with the explicit taxons, are still useful for the work in hydrobiology and in other branches of the field research.

Key to the identification of freshwater species of the sigmoid species of *Cryptomonas* by means of light microscope

NOTE: Cells of these species are sigmoid (S – shaped) in lateral view, i. e., the posterior cell end is always curved to the cell dorsum, the apical rostrum is frequently curved to the cell venter.

1 a. Cells (32) 40 – 60 μm long………………………………………………………….…… **2**
1 b. Maximum cell length 30 μm, one pair of Maupas corpuscles mostly present……….. **3**

2 a. Cells always sigmoid, frequently slightly deformed with somewhat undulated surface and the light screw-like distortion; they are a little laterally, rarely dorsiventrally compressed. The apical rostrum elongated, sometimes finger-shaped. Maupas corpuscles absent, six pyrenoids visible mainly after iodine preservation..................................... ***Cryptomonas curvata*** (Figs 1-9, 45-50)
2 b. Cells similar to those under 2a but having rarely sigmoid long axes, frequently straight, bearing two, exceptionally more Maupas corpuscles; pyrenoids were not observed…..……………………….. ***Cryptomonas borealis*** (Figs 38-41)

3 a. Posterior cell ends bluntly attenuated; cells slightly laterally compressed **4**
3 b. Posterior cell ends are sharply pointed, frequently terminating in the short hyaline tail; cells slightly dorsiventrally compressed **5**

4 a. Cells 22 – 30 (in average 26.9) μm long; the average ratio of cell length to cell thickness is 2.52 …..... ***Cryptomonas reflexa*** var. ***reflexa*** (Figs 10-14, 53)
4 b. Cells 12 – 20 (in average 18.2) μm long; the average ratio of cell length to cell thickness 1.86 .... ***Cryptomonas reflexa*** var. ***anas*** (Figs 15-22)

5 a. Cells 13.5 – 24 (in average 19) μm long, the average ratio of cell length to cell thickness 2.53 .... ***Cryptomonas marssonii*** var. ***marssonii*** (Figs 23-28, 42-44, 54)
5 b. Cells 15 – 20 (in average 16, 6) μm long, the average ratio of length to cell thickness 1, 95 .... ***Cryptomonas marssonii*** var. ***brachys*** (Figs 30-37, 55-57)
5 c. Cells slender, "fish" shaped, 17 – 19 μm long, 5.2 – 5.5 μm thick ……. .... ***Cryptomonas gracilis*** (Fig. 29)

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The abbreviations used in the figure plates:

bc = basal refractive corpuscle, **ch** = chloroplast, **cv** = contractile vacuole, **g+e** = gullet with ejectosomes, **Mc** = Maupas corpuscles, **n** = nucleus, **n+n** = nucleus with nucleolus, **p** = pyrenoid, **pp** = presumable pyrenoid, **rg** = refractive grain, **rm** = reserve material.