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Heterotrophic euglenoid flagellates from the Sierra Nevada Natural Park area (Southern Spain)

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Abstract – In this study we report on 22 species of colourless euglenoid flagellates that were found in the Sierra Nevada Natural Park region (southern Spain), many of them rarely and infrequently reported from nature. Twelve taxa represent first records for Spain. Two species were of uncertain taxonomic affiliation. Illustrations, taxonomic information and biogeographical data are given for species belonging to the genera *Astasia* (4 species), *Anisonema* (2), *Distigma* (4), *Entosiphon/Entosiphonomonas* (1), *Heteronema* (1), *Hyalophacus* (1), *Jenningsia* (1), *Metanema* (1), *Trachelomonas* (1), *Peranema/Pseudoperanema* (1), *Petalomonas* (3) and *Rhabdomonas* (2).

colourless / Euglenophyta / Euglenozoa / heterotrophic / Spain

Résumé — Flagellés euglénoïdes hétérotophes du Parc Naturel de la Sierra Nevada (Sud de l'Espagne). Dans ce travail**,** nous présentons 22 espèces de flagellés euglénoïdes incolores trouvées dans la région du Parc Naturel de la Sierra Nevada (Sud de l'Espagne), la plupart d'entre elles étant rarement trouvées dans la nature. Douze taxons sont mentionnés pour la première fois en Espagne. Deux espèces ont une position taxinomique incertaine. Les illustrations ainsi que les informations taxonomiques et biogéographiques sont données pour les espèces appartenant aux genres *Astasia* (4 espèces), *Anisonema* (2), *Distigma* (4), *Entosiphon/Entosiphonomonas* (1), *Heteronema* (1), *Hyalophacus* (1), *Jenningsia* (1), *Metanema* (1), *Trachelomonas* (1), *Peranema/Pseudoperanema* (1), *Petalomonas* (3) et *Rhabdomonas* (2). (Traduit par la Rédaction.)

Espagne / Euglenophyta / Euglenozoa / hétérotrophe / incolore

INTRODUCTION

The flora of colourless, heterotrophic euglenoid flagellates is poorly known in many countries and very little research has been undertaken on this group. This information gap can be attributed to a range of facts, including the lack of taxonomic expertise of this morphologically complicated and diverse group,

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deficient identification keys, the present unsatisfactory systematic framework of euglenoid flagellates, and methodological problems associated with observations. Considering the latter, Angeler (2000) and Wolowski & Walne (1997) have pointed out the need for studying the same living sample at regular intervals to detect the later appearance of heterotrophic flagellates, including euglenoids, which are apparently absent during initial observations (see also Farmer & Triemer, 1988 ; Lee & Patterson, 2000). Presumably present in a kind of resting stage when the sample is taken, these flagellates often appear after a substantial time lag when organic matter starts to decompose (sometimes several weeks). Unfortunately, the mechanisms of this process are not well understood, reinforcing the need for further research.

This study addresses this need by describing the heterotrophic euglenoids of the Iberian Peninsula. We report on 22 species, many of them infrequently found in nature. All taxa have been detected in samples from a relatively small area in the Sierra Nevada Natural Park region (Granada, southern Spain). This finding is interesting in as much as the taxa were collected in ephemeral water bodies associated with the agricultural irrigation system of the region, indicating either interesting life-history characteristics and dispersal dynamics. So far, 18 taxa of colourless euglenoids have been cited from Spain which were summarized in the phycological list by Alvarez-Cobelas (1984). Recently, Angeler (2000) rediscovered *Distigma elegans* Christen and *Khawkinea fritschii* (Pringsheim et Hovasse) Pringsheim ex Angeler, which have been cited only once before from nature. Based on these recent findings, an interesting flora of colourless euglenoids awaits to be discovered in the Iberian Peninsula.

MATERIALS AND METHODS

Study site

The Sierra Nevada Natural Park (Fig. 1), which has an area of 171,646 ha and encompasses the Sierra Nevada National Park (86,208 ha), is composed of siliceous metamorphites in the central area with the periphery containing dolomites. The region is famous for its high biodiversity, with *ca* 60 endemic plant species and 170 invertebrate taxa.

Sampling

The material for this study was collected on 8.vi.1998 and 11.x.1998 at seven sites in and around the Sierra Nevada Natural Park, as indicated in Tab. 1 and Fig. 1. Site 1 was a puddle along an irrigation channel in the municipality of Cogollos de Guadix with a high abundance of *Spirogyra* sp. Site 2 was the edge of the water reservoir of Cogollos de Guadix, where wind-driven floating organic matter accumulated. This reservoir is partly surrounded by a pine forest and does not support aquatic plants. Site 3 was an open water area of the same reservoir without floating decaying matter. Site 4 was the open water area of the water reservoir of the municipality of Albuñán, which does not support aquatic plants. Site 5 was a puddle near an irrigation channel in Albuñán. Site 6 was a concrete pool with high abundance of *Botryococcus* sp. Finally, site 7 was a small pond without vegetation.

Samples from the water column were collected in 1.5 L bottles, then transferred to and immediately observed in the laboratory, after which they were

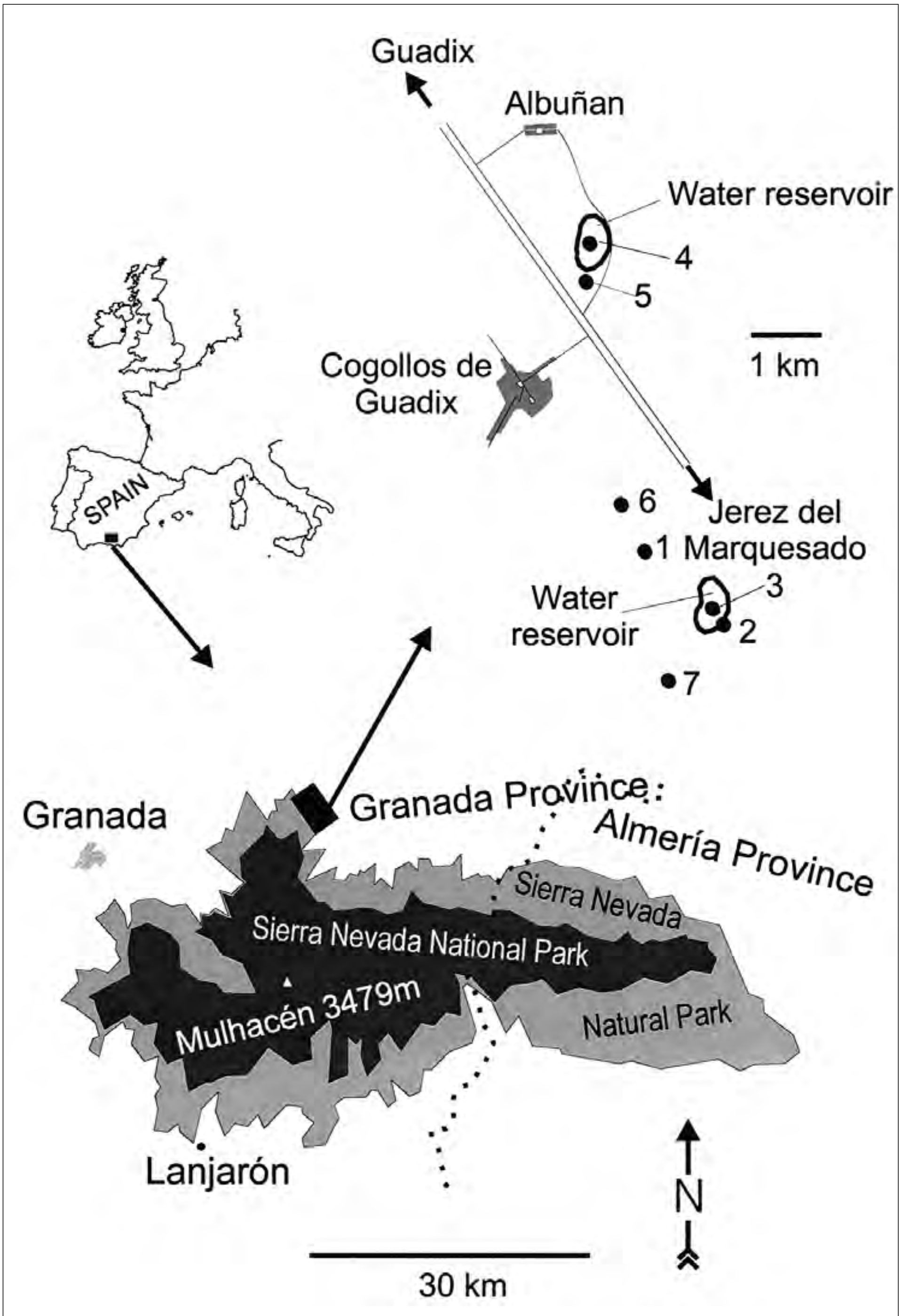


Fig. 1. Study site.

Tab. 1. Temporal occurrence and relative abundance of the taxa found in this study. The first date in the respective columns indicates initial observations, while the second date reflects the second observation of the same sample after the given time lag. Taxa are arranged in alphabetical order. 0, absence of taxon; 1, rare occurrence (less than 10 individuals per observed sample volume); 2, moderately present (10-30 individuals); 3, frequently present (> 30 individuals). Nomenclature follows the ICZN only.

	Site 1		*Site 2*				*Site 3*		*Site 4*		*Site 5*		*Site 6*		*Site 7*	
Taxon	*8.6.*	*28.6.*	*8.6.*	*28.6*	*11.10.*	*31.11.*	*8.6.*	*28.6.*	*8.6.*	*28.6*	*8.6.*	*28.6.*	*8.6.*	*28.6.*	*8.6.*	*28.6.*
Anisonema acinus	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anisonema ovale	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Astasia comma	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Astasia curvata	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Astasia pygmaea	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Astasia torta	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Distigma curvatum	2	3	1	3	0	0	0	0	0	0	0	0	0	0	0	0
Distigma elegans	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0
Distigma gracilis	1	2	0	1	0	0	0	0	0	0	0	2	0	0	0	0
Distigma proteus	0	0	1	3	0	0	0	0	0	0	1	2	0	0	0	0
Distigma sennii	1	3	1	3	0	0	0	0	0	0	0	1	0	0	0	0
Entosiphon sulcatum	0	1	0	0	0	1	0	0	0	1	0	1	0	0	0	1
Heteronema acus	2	2	1	2	0	0	0	0	0	0	0	1	0	0	0	1
Hyalophacus caecus	2	2	1	2	0	0	0	1	0	1	0	1	0	0	0	0
Jenningsia fusiforme	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Metanema sp.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Peranema trichophorum	1	2	1	2	0	0	0	0	0	1	1	2	0	0	0	0
Petalomonas alata	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
Petalomonas mediocanellata	1	3	1	3	1	3	0	0	0	0	0	2	0	0	0	0
Petalomonas steinii	0	0	2	2	0	0	0	0	0	0	0	1	0	0	0	0
Rhabdomonas costata	1	2	0	0	1	2	0	0	0	0	0	1	0	0	0	0
Rhabdomonas incurva	1	3	1	3	0	2	0	0	0	0	0	2	0	0	0	0
Trachelomonas sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

stored at room temperature. The material was re-examined after an arbitrarily chosen time interval (on 28.vi.1998 and 31.xi.1998, respectively) to assess community dynamics and the later appearance of additional taxa. Living as well as lugol-fixed material was examined to determine species characteristics. Occasionally, the viscosity of the medium was increased with methylcellulose to test for euglenoid movement, which can cease when cells become adapted to environmental conditions (Angeler, 1997). Cells have been documented by drawings. Dimensions are given as length x width, and unless otherwise stated refer to cells not undergoing euglenoid movement. Identifications were made using the floristic or taxonomic works of Pringsheim (1936, 1942), Huber-Pestalozzi (1955), Asaul (1975), Popova & Safonova (1976), Starmach (1983), Larsen (1987), Angeler (1997), Wolowski & Walne (1997), Lee *et al.* (1999) and Lee & Patterson (2000), as well as by comparison with original descriptions and available culture material. The same sources served for biogeographical data; exceptions are referenced.

Taxa are assigned to orders following the taxonomic framework of Farmer (1988). New records for Spain are marked with an asterisk.

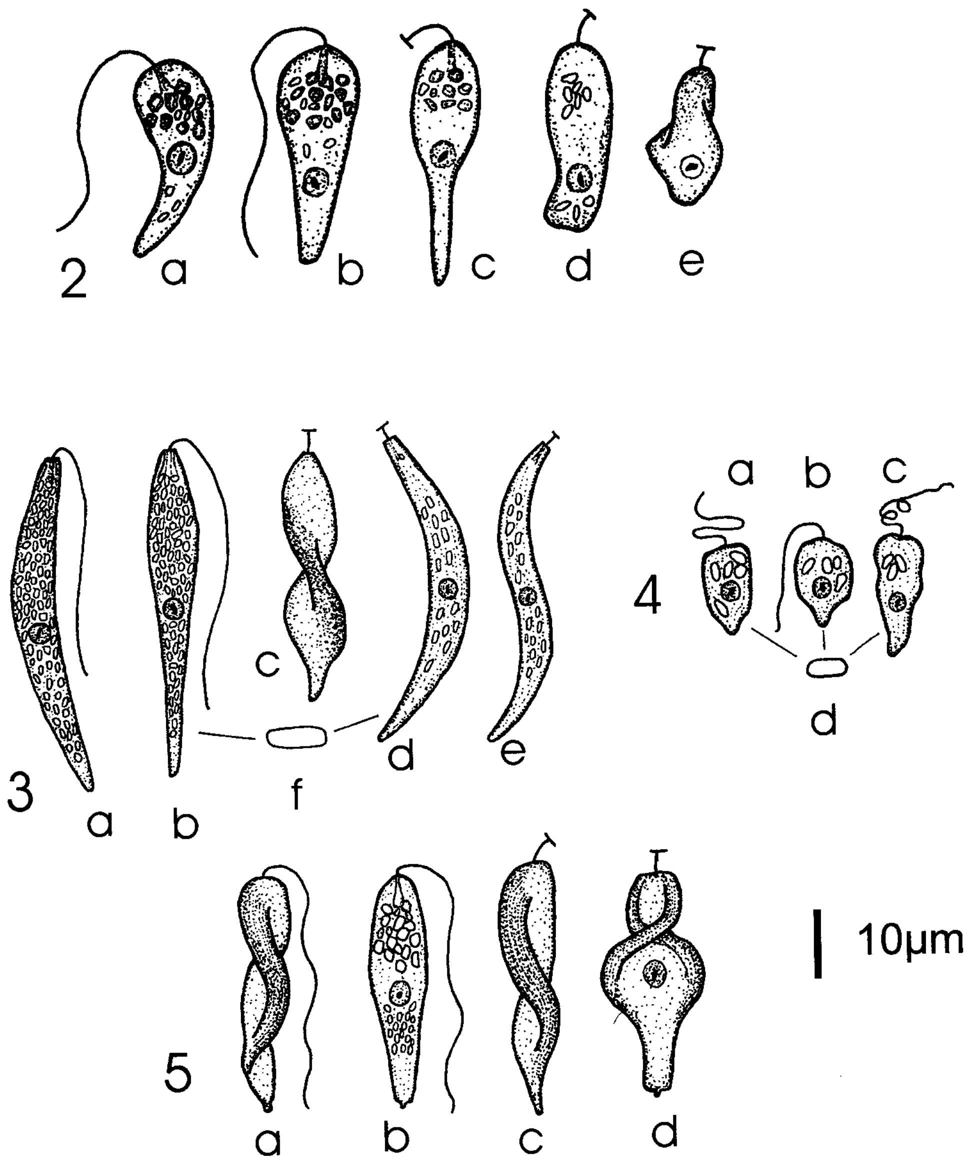
Because the organisms treated in this study have been described by both botanists and zoologists, in accordance with, respectively, the International *Code of Botanical Nomenclature* (ICBN) or the International *Code of Zoological Nomenclature* (ICZN), nomenclatural problems have arisen. These codes are independent of each other, and as such ambiregnal taxa (those taxa regarded by different workers as plants or animals) may be described with more than one name, or with a name that is acceptable under one code but not the other. In order to be formally correct, and to provide unambiguous names, we have adopted an ambiregnal nomenclature and provided names valid under both codes. For further discussion on ambiregnal nomenclature the reader is referred to Patterson (1986) and Larsen & Patterson (1990).

RESULTS AND DISCUSSION

General remarks

Initial observations indicated that shallow sites, such as puddles (sites 1, 5) and the border zone of the water reservoir in Cogollos (site 2), had the highest diversity of colourless euglenoids, with from 2 to 12 species present (Tab. 1), whereas none were present in open water areas of deeper sites and ponds (sites 3, 4, 6, 7). The number of taxa generally increased by the second set of observations. Once again the samples taken from the puddles and the reservoir border had the highest diversity (7-16 taxa). In samples from several open water sites, colourless euglenoids were observed during the second examination but not during the initial observations (Tab. 1). From the seven sampling sites studied, only one (the concrete pool; site 6) lacked colourless species throughout the study.

These patterns suggest some interesting phenomena regarding the biogeographical distribution of these flagellates. Their lower diversity during initial observations and higher numbers at subsequent observations may explain their rare citations from nature. Many infrequently and rarely reported taxa emerged on the second sampling, suggesting that the decomposition of organic material in the sampling vessels may have favoured the growth of heterotrophic organisms (Angeler, 2000). Unfortunately, we did not have the opportunity to resample the original sites to compare the natural succession with that which occurred *in vitro*.



Figs 2-5. Fig. 2 *Astasia comma*. Fig. 3 *Astasia curvata*; a-e, shapes while swimming; f, view in cross section. Fig. 4 *Astasia pygmaea*; a-c, cell shapes; d, cross section. Fig. 5 *Astasia torta*; a-d, frequently observed cell shapes.

In any case, many of our study sites had dried out by the second sampling date (M.J. García, pers. comm.). While meaningful comparisons are impossible at this stage, this study does suggest that the full range of euglenoid diversity can only be assessed by laboratory as well as field observations.

Descriptions

Euglenales

***Astasia* Dujardin 1841 [ICZN + ICBN]**

**A. comma* Pringsheim 1942 (Fig. 2)

Cells club-shaped, curved, comma-like with pronounced euglenoid movement ('metaboly'), rotating while swimming; anterior end either rounded or truncated, depending on the degree of euglenoid movement. Flagellum length approx. equal to cell length. Nucleus located centrally or in posterior half of cell, easily visible, moving throughout cell during metaboly. Pellicle striation very delicate, not clearly visible under light microscopy. Paramylon grains subspherical to rod-like, usually accumulating at anterior pole. Dimensions: 16-36 × 7-11 mm, depending on degree of distortions of cell.

The specimens were in good agreement with Pringsheim's (1942) description. Taxonomic boundaries in many small *Astasia* species are unclear due to their morphological similarity. *Astasia comma* is similar to *A. clava* Pringsheim, *A. parvula* Skuja, *A. parva* Pringsheim, *A. cylindrica* Pringsheim and *A. thiophila* Huber-Pestalozzi. *Astasia clava* and *A. parvula* have longer flagella than *A. comma* (ca. 1.5 × body length), and *A. parvula* can also be distinguished by its smaller size (10-12 × 3.5-4.5 mm). The remaining species are more difficult to delineate due to overlapping cell lengths and similar lengths of flagella. We distinguish *A. comma* from *A. parva* by the formation of persistent button-like swellings at the posterior end in the latter, as well as its straight cell shape. According to Pringsheim (1942), *A. comma* can be distinguished from *A. cylindrica* by its more cylindrical cell shape. *Astasia thiophila* is more drop-like in form and is less curved.

Distribution: Asia and Europe.

**A. curvata* Klebs 1893 (Fig. 3)

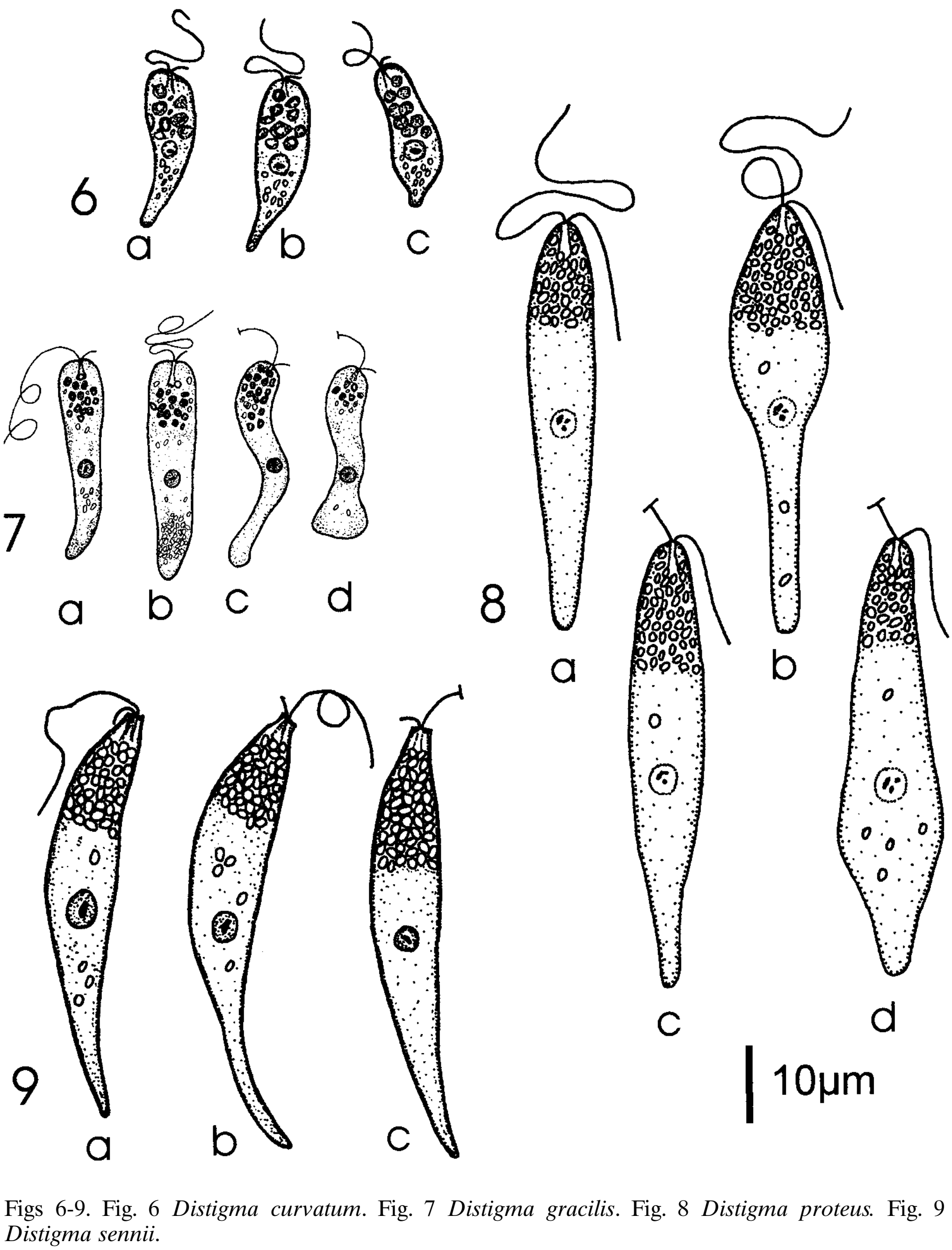
Cells elongated, flattened, at times curved and distorted; anterior end narrowed, truncated, not extended funnel-like; canal opening apical; posterior end slightly narrowed, terminating in a tip. Euglenoid movement predominantly by twisting. Flagellum length approx. 2/3 of cell length. Nucleus located centrally. Pellicle surface smooth. Paramylon granules small, subspherical to elongated. Dimensions: 40-45 × 5-6 mm.

Huber-Pestalozzi (1955) and Wolowski (1991) remarked that *A. curvata* can easily be confused with *Astasia tortuosa* (Stokes) Popova [= *Menoidium tortuosum* (Stokes) Senn]. Recently, Angeler (1999b) re-evaluated the morphological relationships between *A. harrisii* Pringsheim, *A. tortuosa* and *A. curvata,* concluding that the latter two species can be distinguished based on the size and form of their anterior pole (*A. tortuosa* with and *A. curvata* without a funnel-like extension).

Distribution: According to Wolowski (1991) this species occurs commonly in Europe, Asia and America.

**A. pygmaea* Skuja 1939 (Fig. 4)

Cells with considerable morphological variation, maize grain-shaped to irregularly curved cones, somewhat flattened. Anterior end rounded, reservoir opening slightly subapical. Euglenoid movement low during swimming, with



Figs 6-9. Fig. 6 *Distigma curvatum*. Fig. 7 *Distigma gracilis*. Fig. 8 *Distigma proteus.* Fig. 9 *Distigma sennii.*

violent distortions upon irritation. Flagellum length approx. cell length or slightly longer. Nucleus located centrally. Cell surface appearing smooth. Paramylon granules rod-like. Dimensions: 10-15 x 6-9 mm.

Astasia pygmaea is morphologically similar to *A. kathemerios* Skuja and *A. parvula*. According to Skuja (1948), *A. pygmaea* is half the size of *A. kathemerios* and differs from both species in its more pear-shaped form and bigger paramylon grains.

Distribution: Europe and Asia.

**A. torta* Pringsheim 1942 (Fig. 5)

Cell forms variable, predominately club-shaped, broadest part in anterior cell half, narrowing towards posterior pole, flattened; anterior end truncated, posterior end with characteristic stump-like extension. Cells twisting strongly, although not more than 180º. Flagellum length approx. equal to cell length. Periplast striation faint (not shown in Fig. 5). Paramylon granules small, rod-like, usually concentrated in anterior cell pole. Dimensions: 28-36 × 7-8 mm.

Astasia torta is similar to *A. curvata* and *A. harrisii* Pringsheim, but is smaller and has a longer flagellum.

Distribution: Europe.

***Distigma* Ehrenberg 1838 emend. Pringsheim 1942 [ICZN + ICBN]**

**D. curvatum* Pringsheim 1936 (Fig. 6)

Cell morphology highly variable, comma- to beet- like, curvate; anterior end rounded, during euglenoid movement appearing truncated. Metaboly usually very pronounced, sometimes ceasing when cells adapted to environmental conditions. Two unequal heterodynamic flagella, long flagellum approx. equal to cell length, short flagellum stump-like. Pellicle appearing smooth under light microscopy. Paramylon granules polymorphic, subspherical, accumulating in canalar region. Dimensions 22-29 × 7-11 mm.

The specimens observed during this study are in the size range that Pringsheim (1936) considered typical for *D. curvatum* f. *major* Pringsheim. However, Starmach (1983) and Menezes (1994) found that cell lengths overlap between *D. curvatum* fo. *major* and *D. curvatum* fo. *minor*, indicating that discrimination at the infraspecific level may not be justified. *Distigma curvatum* is morphologically similar to *Astasia comma*, but *A. comma* lacks a second emergent flagellum.

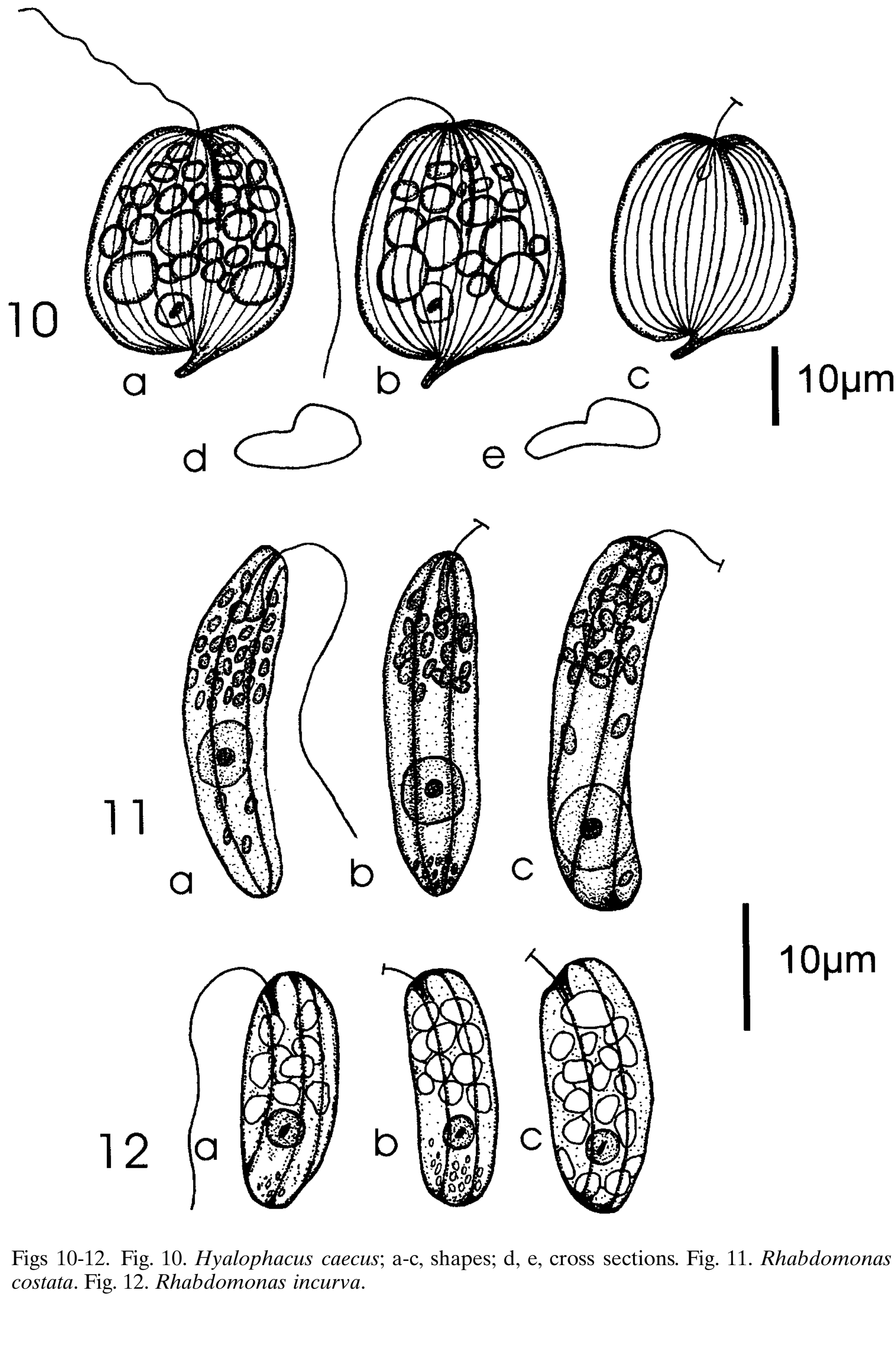
Distribution: Presumably world-wide.

**D. gracilis* Pringsheim 1942 (Fig. 7)

Cells usually slender, carrot-like, at times undulating, gradually narrowed towards posterior end, frequently curved and cylindrical; anterior pole rounded and broadest part of cell. Euglenoid movement pronounced. Long flagellum approx. equal to cell length, short flagellum stump-like. Pellicle striation hardly visible under light microscopy (not shown in Fig. 7). Paramylon granules polymorphic, larger grains accumulating in anterior pole, smaller-sized granules scattered in posterior end. Dimensions: 26-32 × 6-8 mm.

Distigma gracilis is similar to *D. curvatum*, although the latter species is shorter. *Distigma elegans* and *D. sennii* have a truncated anterior part of the cell whereas *D. gracilis* has a rounded anterior end. *Distigma gracilis* can be easily distinguished from other *Distigma* species by its undulated and carrot-like form.

Distribution: Europe.



Figs 10-12. Fig. 10. *Hyalophacus caecus*; a-c, shapes; d, e, cross sections. Fig. 11. *Rhabdomonas costata*. Fig. 12. *Rhabdomonas incurva*.

D. proteus Ehrenberg 1838 emend. Pringsheim 1942 (Fig. 8)

[Syn. *Astasia proteus* Stein pro parte, *Astasia tenax* (O. Müller) Bütschli, *Distigma pseudoproteus* Pringsheim]

Cells morphologically variable, depending on euglenoid movement, usually cylindrical to club-shaped, with a gradual transition between anterior and posterior pole; canal opening apical; with trembling swimming movement; Euglenoid movement in form of longitudinal movements of cytoplasmic dilatations. Long flagellum approx. 2/3 of cell length, second flagellum short (1/3 of cell length). Nucleus located centrally, shifting throughout the cytoplasm during euglenoid movement; with few (2-3) endosomal structures within the nucleoplasm. Pellicle striation not visible under light microscopy. Paramylon granules abundant, subspherical, stored in anterior of cell. Dimensions: 50-100 × 9-19 mm.

Recently, Angeler (1999a) described the variety *D. proteus* var. *longicauda* Angeler, which differs from var. *proteus* in its more pronounced differentiation into a head-like anterior pole and slender posterior tail, the latter also being longer in var. *longicauda*. *Distigma proteus*, with a rounded anterior part of the cell, can easily be distinguished from species with a truncated anterior part of the cell (e.g., *D. sennii, D. elegans*). The second flagellum of *D. tremens* Christen is nearly as long as the first flagellum and shows strong trembling movements.

Distribution: cosmopolitan.

**D. sennii* Pringsheim 1942 (Fig. 9)

Cells club-shaped, broadest part in first third of anterior pole, gradually tapering towards posterior end of cell, rotating while swimming; anterior end of cell truncated, funnel-like, often bent; posterior end forming a rounded tip. Euglenoid movement weak in comparison with other *Distigma* species, under strong irritation undergoing metaboly by flattening with simultaneous distortions and cytoplasmic dilatations. Long flagellum length between half and two thirds of cell length, short flagellum stump-like but actively beating. Nucleus located approx. centrally, with a single endosome in nucleoplasm. Pellicle striation hardly visible under light microscopy (not shown in Fig. 9). Paramylon granules small, located in the anterior half of cell. Dimensions: 45-68 × 10-14 mm.

Though differing in many diagnostic aspects from *D. proteus* (Angeler, 1997, 1999a; Angeler *et al*., 1999), *D. sennii* is often confused with that species, due largely to a historical unawareness of the specific differences. Accordingly, Senn's (1900) drawing, which represents *D. sennii*, but is identified as *D. proteus*, has been copied throughout the 20th century, resulting in misleading floras and ambiguous identifications. To avoid further misidentification we recommend consideration of the papers of Angeler cited above. *Distigma sennii* is morphologically very similar to *D.* elegans, but both species can be easily distinguished by the degree of euglenoid movement. While *D. sennii* is nearly rigid, *D. elegans* undergoes pronounced euglenoid movement when swimming. This difference has been related to characteristics of the pellicle (Angeler *et al*., 1999)

Distribution: unclear because of taxonomic confusion with *D. proteus*.

***Hyalophacus* Pringsheim 1936 [ICZN + ICBN]**

**H. caecus* Christen 1959 (Fig. 10)

[Syn. *Phacus pleuronectes* (O.F. Müller) Dujardin var. *hyalinus* Klebs]

Cells similar to *Phacus pleuronectes* (O.F. Müller) Dujardin; anterior end broadly rounded; posterior end forming typical small spine directed backwards; ventral side of cell flattened or concave; dorsal side of cell with a median furrow extending from anterior end of cell to approx. middle of cell. No stigma present.

Single emerging flagellum almost as long as cell. Nucleus subspherical, located in posterior part of cell. Periplast striation clearly visible under light microscopy. Paramylon granules polymorphic; small subspherical grains especially abundant in anterior 2/3 of cell, up to 6 bigger grains (*ca* 6 mm diameter) located among smaller grains; cells frequently without paramylon. Dimensions: 34-36 × 22-24 mm.

There are currently two species of *Hyalophacus*. *Hyalophacus caecus* can be distinguished from *H. ocellatus* Pringsheim by the absence of the stigma. A further distinguishing feature may be the fine papillar appearance of the pellicle of *H. ocellatus* (Pringsheim, 1936; Wolowski, 1991), but the reliability of this feature cannot be assessed at the moment.

Distribution: *Hyalophacus* without stigma have been reported only from Europe, while *Hyalophacus* with stigma is found throughout the northern hemisphere (Wolowski & Walne, 1997).

Rhabdomonadales

***Rhabdomonas* Fresenius 1858 [ICZN + ICBN]**

**R. costata* (Korshikov) Pringsheim 1942 (Fig. 11)

[Syn. *Menoidium costatum* Korshikov, *M. longum* Pringsheim, *M. semilunaris* Wermel, *M. semilunaris* var. *regularis* Wermel]

Cells rigid, elongated, curved; anterior end truncate; posterior end rounded. Nucleus located almost always in posterior part of cell, with a single endosome visible. Flagellum length approx. equal to cell length or slightly longer. Periplast forming pronounced longitudinal keels. Paramylon granules polymorphic, with bigger grains in anterior part of cell, smaller grains in posterior part of cell. Dimensions: 25-28 × 8-9 mm

Rhabdomonas costata is clearly distinguishable from other *Rhabdomonas* species by its "banana" shape and longitudinal keels.

Distribution: Freshwaters in America, Asia and Europe.

R. incurva Fresenius 1858 (Fig. 12)

[Syn. *Menoidium incurvum* (Fresenius) Klebs, *Astasia proteus* Stein *pro parte*]

Cells rigid, bean-shaped; anterior and posterior ends rounded. Flagellum length approx. equal to cell length. Nucleus located in posterior half of cell. Periplast with pronounced longitudinal keels. Dimensions: 14-17 × 6-8 mm.

Pringsheim (1942) distinguished between a *R. incurva* type with dimensions of 13-15 × 5-7 mm and *R. incurva* var. *major* with dimensions of 16-18 × 6-7.5 mm. Size ranges of our material overlap so that infraspecific discrimination does not seem to be justified (see also discussion in Huber-Pestalozzi 1955).

Distribution: Freshwaters in America, Asia, Australia and Europe. This species may possibly have a cosmopolitan distribution.

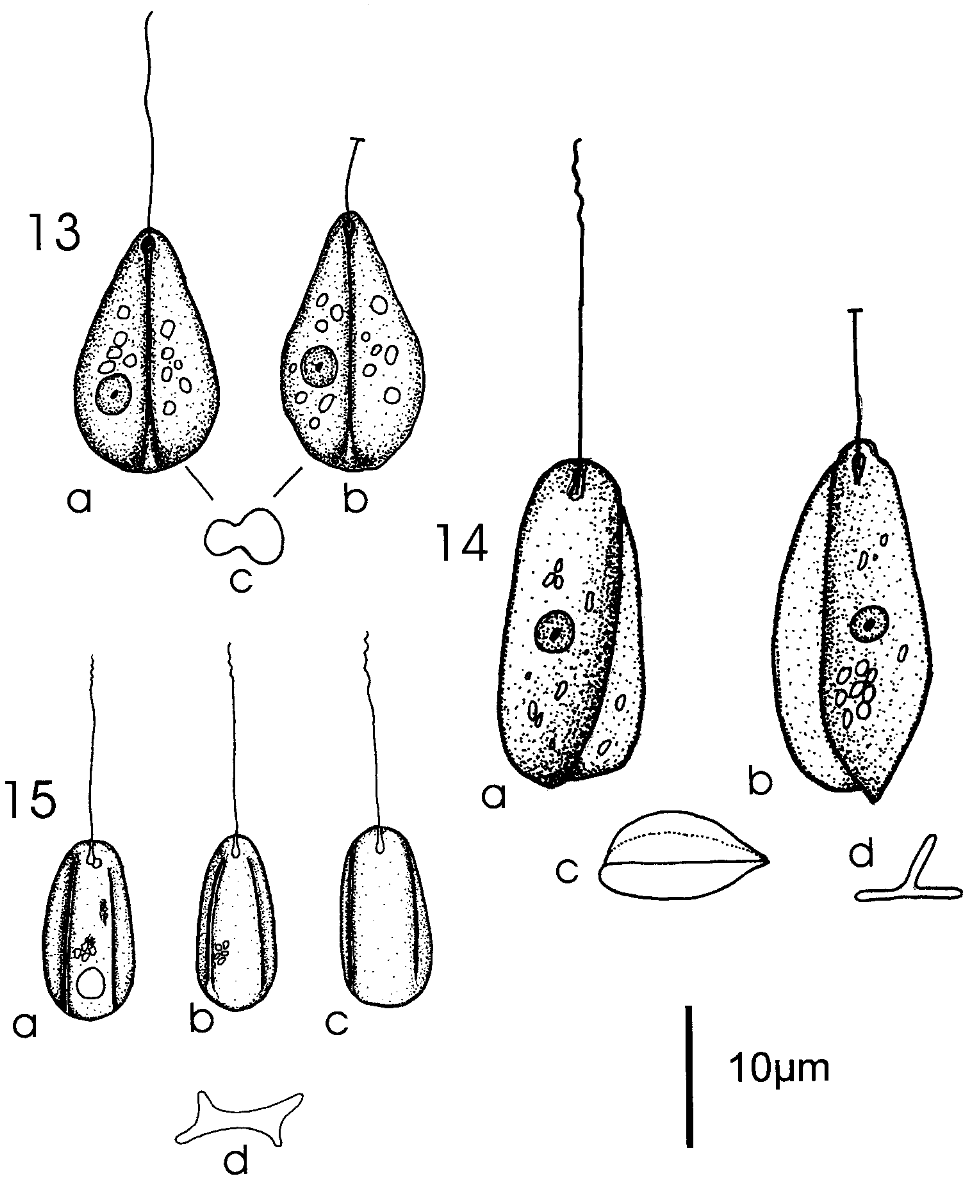
Petalomonadales

***Petalomonas* Stein 1859 [ICZN + ICBN]**

P. mediocanellata Stein 1878 (Fig. 13)

Cells flat, rigid, egg-shaped; anterior end slightly protracted and truncated; longitudinal furrows present at dorsal and ventral sides, dividing cells in two unequally thick parts. Flagellum length approx. equal to cell length. Paramylon granules subspherical, abundant. Dimensions: 21 × 14 mm.

Petalomonas mediocanellata includes four varieties [var. *disomata* (Stokes) Lemmermann, var. *pleurosigma* (Stokes) Lemmermann, var. *asymmetrica* Shi and var. *minor* Shawan et Jahn], which differ clearly in their shapes or sizes from *P. mediocanellata*. Schroeckh & Patterson (unpublished) found a tiny second



Figs 13-15. Fig. 13. *Petalomonas mediocanellata*; a, b, shapes; c, cross section. Fig. 14. *Petalomonas steinii*; a, b, shapes; c, d, sketches of keels. Fig. 15. *Petalomonas alata*; a-c, shapes; d, cross section.

flagellum in *P. mediocanellata* and *P. steinii*, so that these two species will have to be transferred to *Notosolenus*.

Distribution: Europe, Asia, America.

P. steinii Klebs 1893 (Fig. 14)

Cells ovate to egg-shaped, rigid, flat, triangular in cross section; with a pronounced median or diagonal dorsal inclined keel; anterior end narrowed, posterior end rounded. Flagellum as long as cell body, flickering at its tip only. Nucleus with single endosome located centrally, hardly visible in living cells but becoming so after fixing with lugol solution. Dimensions: 24-36 x 14-24 mm

According to Huber-Pestalozzi (1955) this species is highly polymorphic. Our species differed from drawings of other authors in the diagonal orientation of the dorsal keel in *ca* 90% of the cells observed here.

Distribution: Europe, Asia and America.

**P. alata* Stokes 1888 (Fig. 15)

Cells flat, egg-shaped; anterior and posterior ends rounded; with four longitudinal carinae, two dorso-lateral and two ventro-lateral, in cross section asymmetrical; with wide furrows between carinae. Flagellum directed forward and flickers at its tip, slightly longer than cell length. Nucleus (5 mm diam.) situated in posterior half of cell. Dimensions: 12 x 7 mm.

The specimens observed in this study are morphologically similar to what Stokes (1888) described as *P. alata*. He and subsequent authors (e.g. Shawan & Jahn, 1947; Starmach, 1983) reported the flagellum to be twice as long as the cell body, but those proportions do not agree with their figures, in which flagellar length is only slightly longer than cell length, corresponding to our observations. Our specimens further deviate from other species in *Petalomonas* by the asymmetry of the carinae.

Distribution: Europe, Asia and America.

Ploeotiales

***Entosiphon* Stein 1878 (ICZN) = *Entosiphonomonas* Larsen et Patterson 1991 (ICBN)**

Entosiphon sulcatum (Dujardin) Stein 1878 non *Entosiphon sulcatum* Skvortzov et Noda 1969 (*nom. illeg.*) = *Entosiphonomonas sulcata* (Stein) Larsen et Patterson 1991. (Fig. 16)

[Syn. *Anisonema sulcatum* Dujardin, *A. entosiphon* (Stein) Klebs, *Heteronema sulcata* Mereschkovski]

Cells rigid, ovoid, flattened; anterior end obliquely incised with an apical depression; posterior end slightly narrowed and rounded. Conical protrusive siphon prominent, as long as cell. Two unequal emergent flagella; anterior as long as cell; recurrent flagellum shorter. Pellicle with pronounced ridges. Dimensions: 26-33 × 10-16 mm.

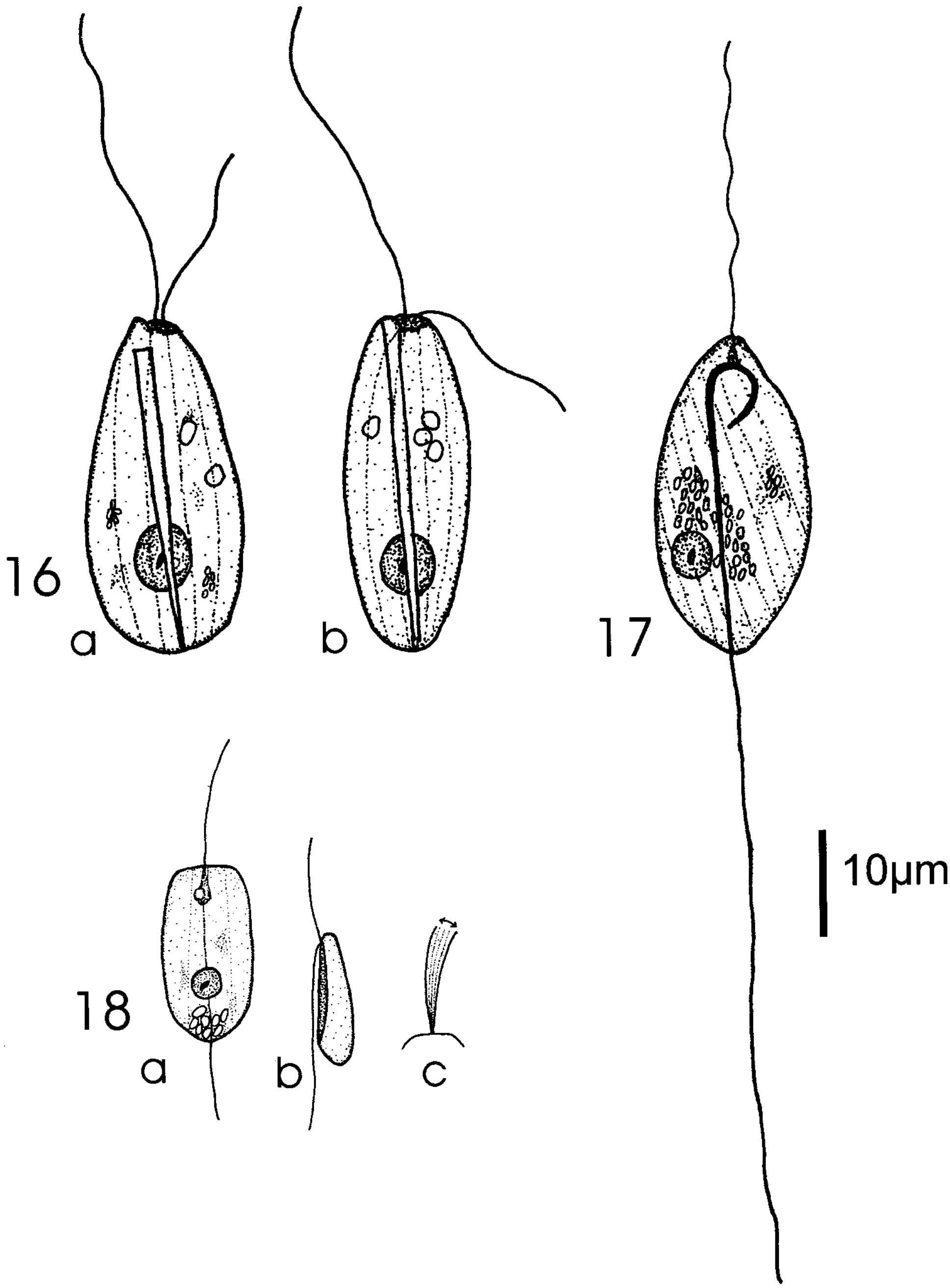
Entosiphon sulcatum/Entosiphonomonas sulcata can be distinguished from *E. sulcatum* var. *acuminatum* by its shorter siphon (about 0.7 times the cell length) and pointed posterior end.

Anisonematales

***Anisonema* Dujardin 1841 [ICZN + ICBN]**

A. acinus Dujardin 1841 (Fig. 17)

Cells rigid, oval to egg-shaped, flattened. Two flagella; anterior flagellum length approx. equal to cell length, moving in a whiplash fashion; flagellum three



Figs 16-18. Fig. 16. *Entosiphon sulcatum* = *Entosiphonomonas sulcata*. Fig. 17. *Anisonema acinus*. Fig. 18. *Anisonema ovale*; a, b, shapes; c, movement of flagellum.

times longer than cell and thicker than anterior flagellum, lying in a ventral groove. Nucleus subspherical, located in posterior part of cell, with single endosome. Periplast striation almost longitudinal, individual strips relatively broad. Dimensions: 26-31 × 12-17 mm (thickness 7-9.5 mm).

The specimens were in good agreement with previous descriptions of *A. acinus. Anisonema acinus* is similar to *A. prosgeobium* Skuja, however the latter species bears a smooth pellicle and it is smaller.

Distribution: worldwide in freshwater and marine environments.

**A. ovale* Klebs 1893 (Fig. 18)

Cells rigid, oval, with a curved appearance in cross sections; anterior end blunt; posterior end rounded. Anterior flagellum length approx. equal to cell length, moving from base to tip; trailing flagellum slightly longer than cell. Nucleus not visible in living cells. Periplast apparently smooth under light microscopy. Paramylon granules in posterior half of cell. Dimensions: 16-17 × 7 mm.

Morphologically, these specimens were in agreement with other descriptions of *A. ovale*, with the exception that the cells had a shorter flagellum. Previously reported cell length was found to be 11 to 26 µm (Huber-Pestalozzi, 1955; Shi, 1999). *Anisonema ovale* shows similarities to *A. emarginatum* Stokes, but the anterior flagellum in *A. emarginatum* is twice as long as the body.

Distribution: Europe, Asia, Africa, America.

***Jenningsia* Schaeffer 1918 [ICZN + ICBN]**

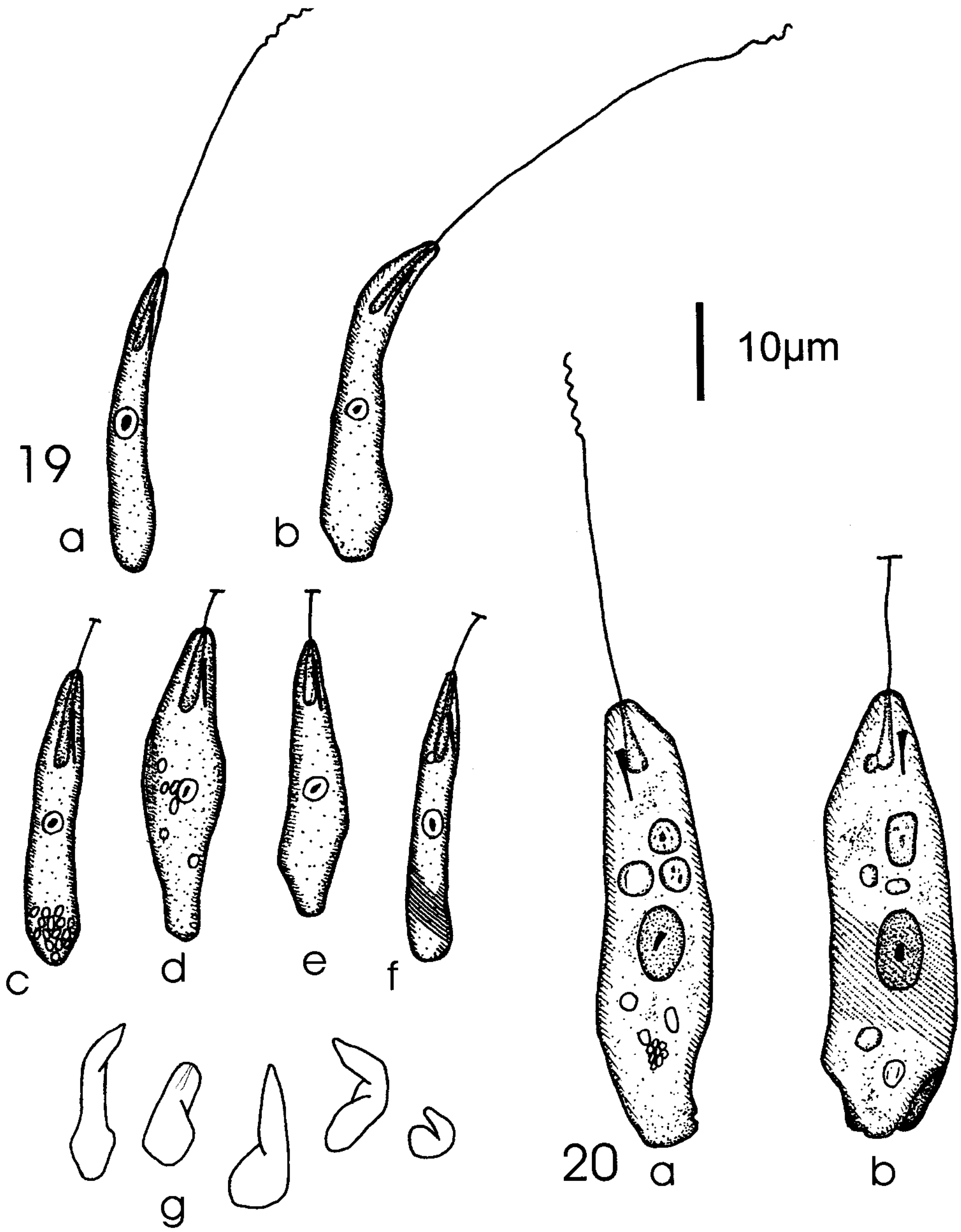
**J. fusiforme* (Larsen) Lee *et al.*, 1999 (Fig. 19)

[Syn. *Pseudoperanema fusiforme* Larsen, 1987 (ICBN); *Peranema fusiforme* (Larsen) Larsen et Patterson, 1991 (ICZN)]

Cells more or less spindle-shaped, sometimes curvate, metabolic. Ingestion organelle consisting of two parallel rods. Reservoir well developed, occupying 1/3 of the total cell length. Single emergent flagellum length approx equal to cell length, flickering at the tip; no second emergent flagellum. Nucleus subspherical, situated centrally or in posterior part of cell. Periplast striation visible under light microscopy. Dimensions: 26-38 × 7 mm.

This species has been cited thus far only from marine environments, under the name *Peranema fusiforme* (Larsen, 1987; Larsen & Patterson, 1990; Ekebom *et al.*, 1996; Patterson & Simpson, 1996; Lee *et al.*, 1999; Lee & Patterson, 2000). Our specimens conform closely to previous descriptions of this species, with the exception that our strain had a relatively pronounced reservoir. Lee *et al.* (1999) recently transferred several species of *Peranemopsis* and *Peranema* to *Jenningsia*, because the structure previously interpreted as a second emergent flagellum that is tightly adpressed to the body was shown to be a cytoskeletal element associated with the ingestion apparatus. Hence a single emergent flagellum is characteristic for *Jenningsia*, while two emergent flagella are typical for *Peranema/Pseudoperanema*. *Peranemopsis* was considered a junior synonym for *Jenningsia* (Lee *et al.*, 1999)

Distribution: marine sediments in Australia (Larsen & Patterson, 1990; Ekebom *et al.*, 1996; Patterson & Simpson, 1996; Lee *et al.*, 1999; Lee & Patterson, 2000), Fiji and Brasil (Larsen and Patterson, 1990), and Danish Wadden Sea (Larsen, 1987).



Figs 19-20. Fig. 19. *Jenningsia fusiforme*. Fig. 20. *Peranema/Pseudoperanema trichophorum*.

***Peranema* Dujardin 1841 [ICZN]) =*Pseudoperanema* Christen 1962 [ICBN]**

Peranema trichophorum (Ehrenberg) Stein 1878 = *Pseudoperanema trichophorum* (Ehrenberg) Larsen 1987 (Fig. 20)

[Syn. *Trachelius trichophorus* Ehrenberg, *Peranema trichophorum* sensu Stein *pro parte*]

Cells morphologically variable, depending on euglenoid movement; more or less cylindrical while swimming; triangular forms in cross section. Ingestion organelle visible. Anterior flagellum length approx. equal to cell length, with a flickering tip; trailing flagellum (not shown in the drawing) tightly adpressed to body, running in a tiny spiral groove with a length *ca* 3/4 of cell length. Nucleus oval, located centrally, with a single endosome. Periplast striation clearly visible. Dimensions: 45-55 x 11-16 mm.

This species has been widely reported from freshwater sites, with cells 22-81 µm long (e.g., Chen, 1950; Huber-Pestalozzi, 1955) and from marine sites with cells 35-60µm (Larsen, 1987; Larsen & Patterson, 1990, Ekebom *et al.*, 1996). While cell length is known to vary with environmental conditions [42-65 µm (Chen, 1959)], Lee & Patterson (2000) suggest that more than one species has been included under this name. *Peranema/Pseudoperanema trichophorum* is similar to *P. inflexum* Skuja and *P. pleururum* Skuja, which have been reported from freshwater habitats. *P inflexum* (30-41 µm) is shorter and *P. pleururum* (62-75µm) larger than the specimens reported here. However, based on size ranges available in the literature, Lee & Patterson (2000) argued that further study is required to establish the identity of these species.

Distribution: Cosmopolitan in freshwater and marine habitats.

***Heteronema* Dujardin 1841 [ICZN + ICBN]**

H. acus (Ehrenberg) Stein 1878 (Fig. 21)

Cells spindle-shaped; anterior and posterior poles narrowed. Ingestion organelle located in vicinity of reservoir. Euglenoid movement pronounced, occurring in form of squirming movements. Anterior flagellum length approx. equal to cell length, recurrent flagellum about 0.3 times the cell length. Pellicle striation visible under light microscopy. Dimensions: 70 × 14 mm.

Distribution: cosmopolitan.

Species of uncertain taxonomic position

Metanema sp. (Fig. 22)

Cells flat, oval; in contrast to *Anisonema* slightly metabolic; anterior end rounded or obtuse; posterior end with a slight tip; canal opening slightly subapical. Rod like structure present in canal region. Flagella of equal thickness; length of anterior flagellum approx. equal to cell length; trailing flagellum twice as long as cell. Pellicle striation visible under light microscopy. Dimensions: 26 × 13 mm.

With regard to cell morphology and metaboly, these specimens fit well within the genus *Metanema.* Within that genus, our cells showed similarities to *M. strenuum* (Skuja) Larsen, which has flagella slightly longer than the cell length and a helical to longitudinal pellicle striation. Our cells differed from *M. strenuum* in the length of the trailing flagellum and, moreover, we observed exclusively longitudinal pellicle striations. If the rod-like structure could be identified as an ingestion organelle than the species must be placed in the genus *Heteronema*.

Trachelomonas sp. (Fig. 23)

Cells elongated, with lorica surface hyaline and smooth; size and shape of lorica similar to *T. mucosa* var. *hyalina* Skvortzov; chloroplasts absent. Eyespot

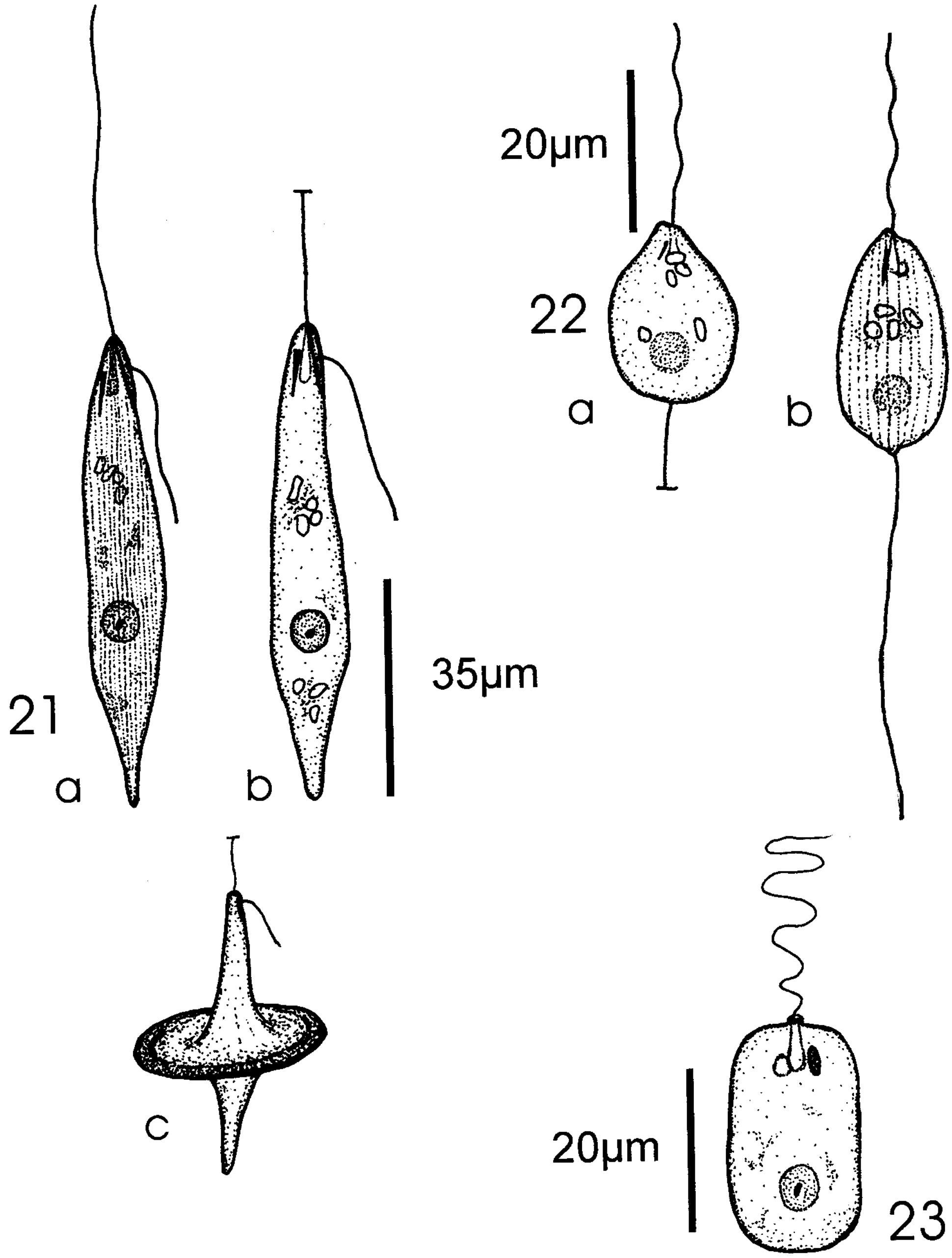


Fig. 21-23. Fig. 21. *Heteronema acus*. Fig. 22. *Metanema* sp. Fig. 23. *Trachelomonas* sp.

orange red, located near reservoir wall. Length of flagellum not determined exactly but exceeding cell length. Paramylon grains not observed. Dimensions are 26 × 17 mm.

Colourless *Trachelomonas* species are very rare in nature. Klebs (1893) reported on two species, *T. volvocina* f. *hyalina* Klebs and *T. reticulata* (= *T. obovata* var. *klebsiana* Deflandre; Huber-Pestalozzi 1955), which differ from our species in their shapes (*T. volvocina* fa. *hyalina* is roundish and *T. reticulata* is egg-shaped). In contrast to other euglenoid genera, colourless *Trachelomonas* species are currently not assigned to a different genus because data on morphology and evolutionary biology are still scarce for this genus. Three individuals of this species developed in the sample taken from Site 2 during the interval before the second observations were made.

Previous records of colourless euglenoids from the Sierra Nevada Natural Park.

Distigma elegans Christen 1959 (see Angeler, 2000)

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