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Redescription of *Chilodonatella minuta* DRAGESCO 1966 (Protozoa, Ciliophora)

E. BECARES & W. FOISSNER

Abstract: *Chilodonatella minuta*, a small cyrtophorid ciliate rather superficially described by DRAGESCO (1966), is redescribed from an activated sludge plant treating pharmaceutical wastes in León, Spain. Its morphology and infraciliature were studied in live and protargol impregnated cells. The genus and species are clearly defined by the somatic ciliature which is organized, as in other cyrtophorids, in two kinety fields which are, however, indistinctly separate at the left anterior portion of the cell because the preoral and circumoral kineties are displaced into a deep buccal cavity unique to the whole group. Thus, the somatic ciliary rows form seemingly uninterrupted archs. Improved diagnoses are provided for the genus and species and the systematic position of the genus is discussed.

1 Introduction

Industrial wastewater treatment systems allow the growth of interesting ciliate species and provide valuable data on the ecology of ciliates in general (AESCHT & FOISSNER 1992; BECARES 1991, 1994; LUNA-PABELLO et al. 1992). In this paper we shall redescribe *Chilodonatella minuta* DRAGESCO, a peculiar species which has never been found since its description, although it is common in a pharmaceutical wastewater treatment plant in León (Spain).

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2 Material and Methods

The material was collected from a two-stage activated sludge pilot plant (A+B system) treating wastewater from a pharmaceutical company in León (Spain). The system is composed of two activated sludge processes in series, viz. a highly loaded (2 kg BOD/kg MLSS) reactor (A) followed by a low loaded (0.2 kg BOD/kg MLSS) reactor (B). *Chilodonatella minuta* was very abundant (up to 70 millions cells/l) in reactor A, which had a sludge age of two days and a hydraulic retention time of 12 hours. See BECARES (1994) and BECARES & GARCIA-OLIVARES (1994) for further data on the pilot plant and wastewater.

The specimens were taken directly from the reactor. Sludge samples were fixed in 5% glutaraldehyde and stained with protargol following procedure B described in FOISSNER (1991), except that bleaching was done at a higher sodium hypochlorite concentration (4ml NaClO + 100 ml distilled water). All other procedures followed methods described in FOISSNER (1991).

3 Results

3.1 Redescription of *Chilodonatella minuta* DRAGESCO 1966

Neotype material

Three slides of protargol impregnated cells, Wilbert modification, have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz (LI), Austria. Accession numbers 94/5, 94/6, 94/7.

Redescription (Fig. 1-20, Tab. 1)

In vivo 20-50 x 15-30 µm. Acontractile but flexible. Shape rather constant, slightly reniform, very similar to that of *Chilodonella uncinata* but more slender and with anterior left end less distinctly pointed (Fig. 1, 11), in some specimens even rounded (Fig. 6). Posterior end often with small

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Table 1. Morphometric characteristics from *Chilodonatella minuta* [1)]

Character	$\bar{x}$	M	SD	$SD_{\bar{x}}$	CV	Min	Max	n
Body, length (protargol impregnation)	32	31	3.9	0.8	12.3	26	49	26
Body, length (glutaraldehyde fixed)	29	29	4.2	0.8	14.3	23	40	26
Body, width (protargol impregnation)	17	16.5	2.4	0.5	14.3	12	26	26
Body, width (glutaraldehyde fixed)	17	17	2.0	0.4	11.6	15	22	26
Distance anterior somatic end to distal curve of preoral kinety	4	4	0.5	0.1	11	4	5	26
Distance anterior somatic end to proximal vertex of preoral kinety	13	13	1.2	0.2	9.4	11	15	26
Distance anterior somatic end to posterior vertex of buccal opening	9	9.7	0.8	0.1	8.3	8	11	26
Distance anterior somatic end to anterior excretory pore	13	13	1.2	0.2	9.3	11	15	25
Distance anterior somatic end to posterior excretory pore	26	26	3.0	0.6	12	20	32	23
Distance anterior somatic end to macronucleus	13	13	1.2	0.2	9.2	12	17	25
Distance anterior somatic end to innermost kinety of left field	14	14	1.1	0.2	8.1	12	16	26
Distance anterior somatic end to innermost kinety of right field	5	5	0.8	0.2	18.3	2	6	26
Maximal postoral distance between kinety fields	8	8	1.7	0.3	20	5.5	12	26
Macronucleus, number	1	1	0	0	0	1	1	26
Macronucleus, length	14	14	2.9	0.6	22	7	18	25
Macronucleus, width	8	7	1.2	0.2	16.4	6	10	25
Micronucleus, number	1	1	0	0	0	1	1	13
Micronucleus, length	3	3	–	–	–	2	3	13
Micronucleus, width	3	3	–	–	–	2	3	13
Excretory pores, number	2	2	0	0	0	2	2	26
Right field kineties, number	5	5	0	0	0	5	5	26
Left field kineties, number	7	7	–	–	–	6	7	26
Frontal kineties, number	4	4	0	0	0	4	4	26
Preoral and circumoral kineties, number	3	3	0	0	0	3	3	26
Brush kinety, length	4	4	–	–	–	4	5	24
Brush cilia, number	10	10	1.4	0.3	15	8	12	17

[1)] All data based, if not otherwise stated, on randomly selected, protargol-impregnated and mounted specimens from activated sludge. Measurements in µm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of specimens investigated, SD – standard deviation, $SD_{\bar{x}}$ – standard deviation of the mean, $\bar{x}$ – arithmetic mean.

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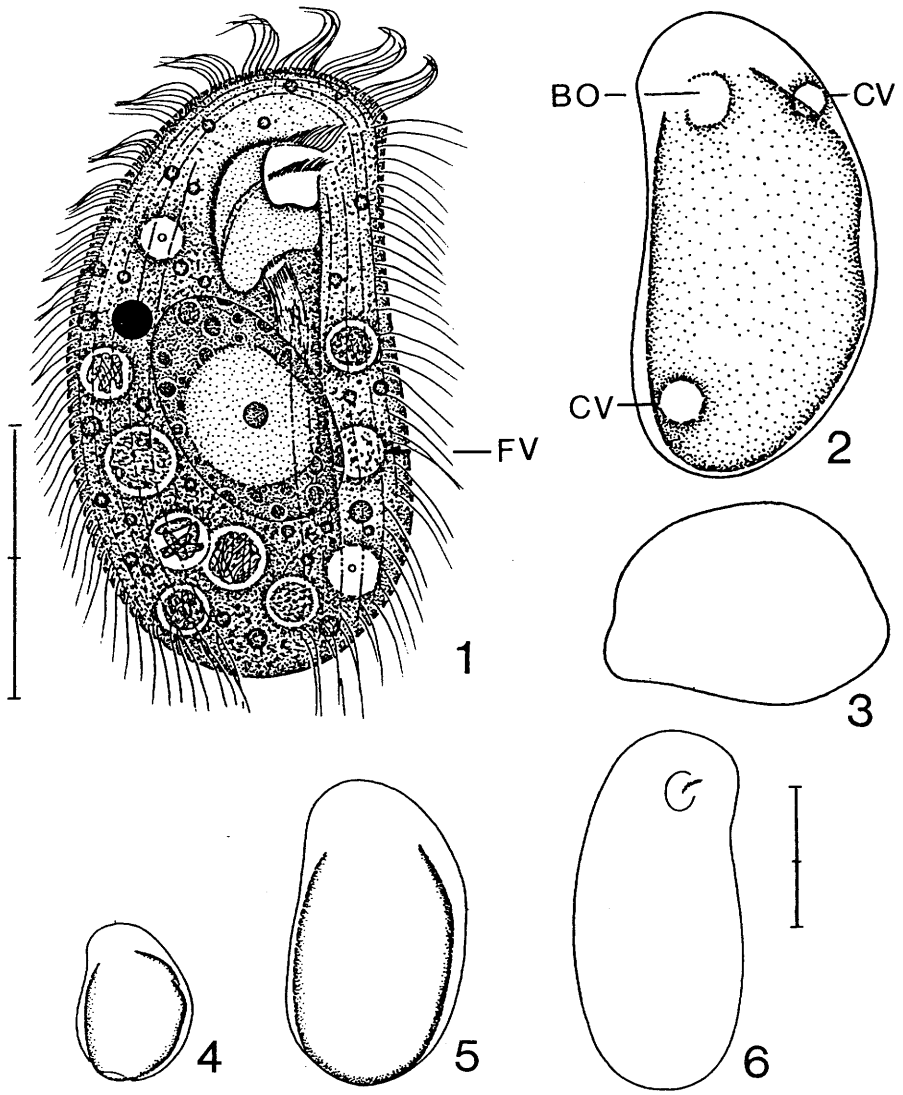

Fig. **1-6.** *Chilodonatella minuta* from life and video records. **1:** Ventral view of typical specimen. **2:** Dorsal view. **3:** Transverse view at level of buccal cavity. **4-6:** Dorsal and ventral views showing variability of size and shape; drawn to scale. BO – buccal opening, CV – contractile vacuoles, FV – food vacuole. Scale bar division: 10 µm.

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indentation in stained cells. Slightly flattened, ventral surface basically convex, but left kinety field usually rather distinctly depressed, i.e. at deeper level than oral opening (Fig. 1, 3). Dorsal hump usually distinct, slightly irregular, not or only inconspicuously projecting above ventral surface, in some specimens evenly rounded and indistinctly set off from lateral sides. Macronucleus large compared with size of cell, in middle subequatorial portion of body, usually oblique to main cell axis, ellipsoid, in small specimens sometimes globular and in posterior half of body; contains small, globular chromatin bodies surrounding hyaline area having central globule. Micronucleus spherical, usually near upper right end of macronucleus, sometimes a few µm distant from macronucleus (Fig. 1, 8, 9). Two contractile vacuoles, anterior pore between 1st and 2nd kinety of right field near level of proximal end of buccal cavity, posterior pore subterminal between 3rd and 4th inner kinety of left field. At right lateral side, near mid-body, small cylindroid structure (cytopyge?) rather distinctly stained with protargol (Fig. 8, 15). Cytoplasm colourless with many small food vacuoles containing bacteria. Movement moderately rapid, also crawling on sludge flocs like other small cyrtophorids.

Cilia in stained specimens 5-9 µm long, arranged in a left and right kinety field with 5 and 6-7 ciliary rows, respectively (Fig. 7, 14, 16). Four outer kineties of right field extend along anterior margin of cell to pointed left body end, contacting more or less distinctly outer 2-3 kineties of left field, thereby producing the genus-specific kinety arches, even prominent in live specimens (Fig. 9, 11, 14, 19). Innermost kinety of right field distinctly shortened anteriorly, ends usually at level of upper margin of buccal cavity. Four innermost kineties of left field gradually shortened in pairs, i.e. commence near distal end of preoral kinety and at level of cytopharyngeal opening, respectively (Tab. 1). All kineties, except very short outermost kinety of left field, extend near posterior end of cell. Dorsal brush at anterior left end, composed of an average of 10 narrowly spaced, long cilia (Fig. 8, 12, 17).

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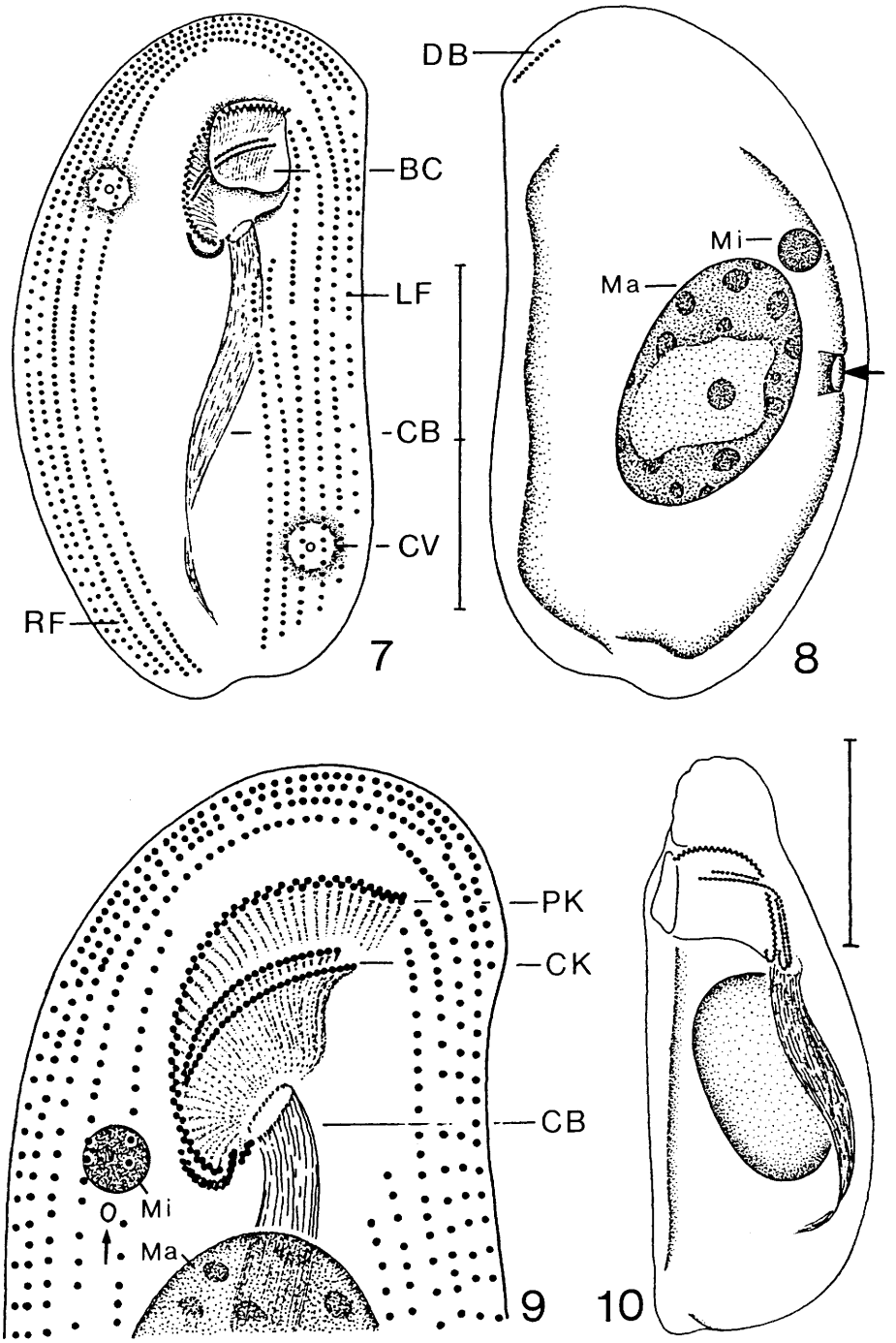

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Buccal opening in anterior quarter to third of cell, slightly left of median, almost square, appears as bright spot in live specimens, in fixed and stained cells larger than in live individuals (Fig. 1, 2, 11-13). Buccal cavity spacious, about 10 x 8 x 8 µm, extends obliquely to dorsal side, its right posterior portion elongated sac-like. Preoral kinety composed of basal bodies arranged in zigzag, commences at left anterior corner of buccal opening close to middle kineties of left ciliary field, extends as flat spiral on curved right and proximal wall of buccal cavity to cytopharyngeal opening. Two rather closely spaced circumoral kineties, possibly composed of narrowly spaced monokinetids, extend on dorsal surface of buccal cavity; commence at mid left margin of buccal opening and extend to right side of cytopharyngeal opening, approaching preoral kinety. Upper circumoral kinety always slightly shorter than lower. Rather distinct fibres lining buccal cavity and possibly also cytopharyngeal basket originate from all oral basal bodies (Fig. 1, 7, 9-20). Cilia of distal, uncovered portion of oral kineties form two distinct „membranelles" in live specimens (Fig. 1, 13). Pharyngeal opening at proximal bottom of buccal cavity left of its sac-like elongation, elliptical, oblique to main body axis. Pharyngeal basket narrow, funnel-shaped, extends near dorsal side to rear end of cell; very delicate, hardly recognisable in live specimens, also faintly stained by protargol, apparently composed of many fine fibres.

3.2 Occurrence and ecology

Chilodonatella minuta was often the dominant species in reactor (A), achieving numbers of up to 70 millions cells/l. It occurred together with *Opercularia asymmetrica* (BICZOK), *Drepanomonas revoluta* PENARD, *Acineria uncinata* TUCOLESCO and *Euplotes muscorum* DRAGESCO (checked by silver nitrate impregnation), which ingested small individuals of *C. minuta*. It is also readily fed on by a suctorian, possibly

Fig. **7-10.** *Chilodonatella minuta* after protargol impregnation. **7, 8:** Infraciliature of ventral and dorsal side. Arrow marks cylindroid structure in mid-body of right side. **9:** Ventral somatic and oral infraciliature in anterior body half at higher magnification; buccal opening omitted for sake of clarity. Arrow marks excretory pore of contractile vacuole. **10:** Lateral view. BC – buccal cavity, CB – cytopharyngeal basket, CK – circumoral kineties, CV – contractile vacuole, DB – dorsal brush, LF – left ciliary field, Ma – macronucleus, Mi – micronucleus, PK – preoral kinety, RF – right ciliary field. Scale bar division: 10 µm.

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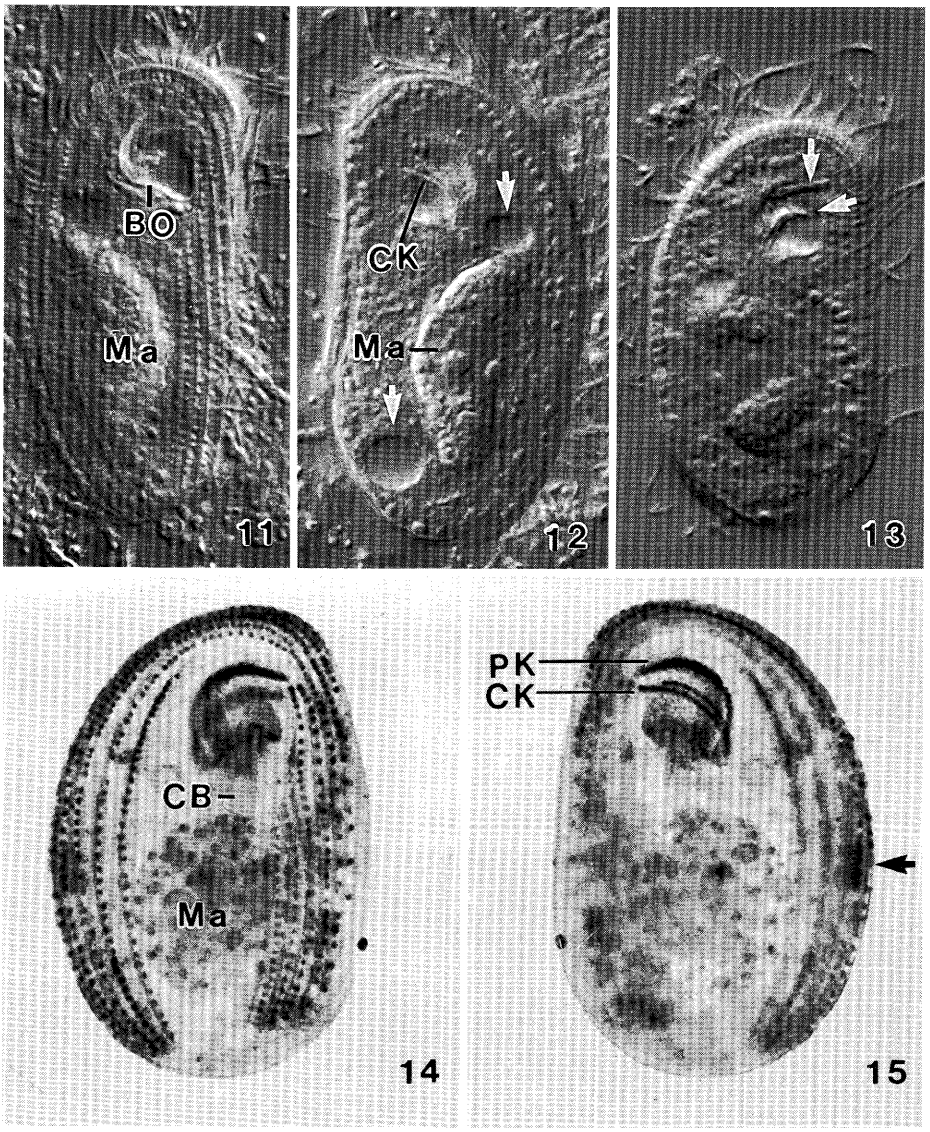

Fig. **11-15:** *Chilodonatella minuta* after glutaraldehyde fixation (11 -13) and protargol impregnation (14, 15). **11:** Ventral view focused to plane of buccal opening. **12:** Dorsal view focused to plane of circumoral kineties. **13:** Ventral view focused to distal end of oral kineties whose cilia form membranellar-like plates (arrows). **14, 15:** Infraciliature of ventral and dorsal side. Arrow marks cylindroid structure in right side of cell. BO – buccal opening, CB – cytopharyngeal basket, CK – circumoral kineties, Ma – macronucleus, PK – preoral kinety.

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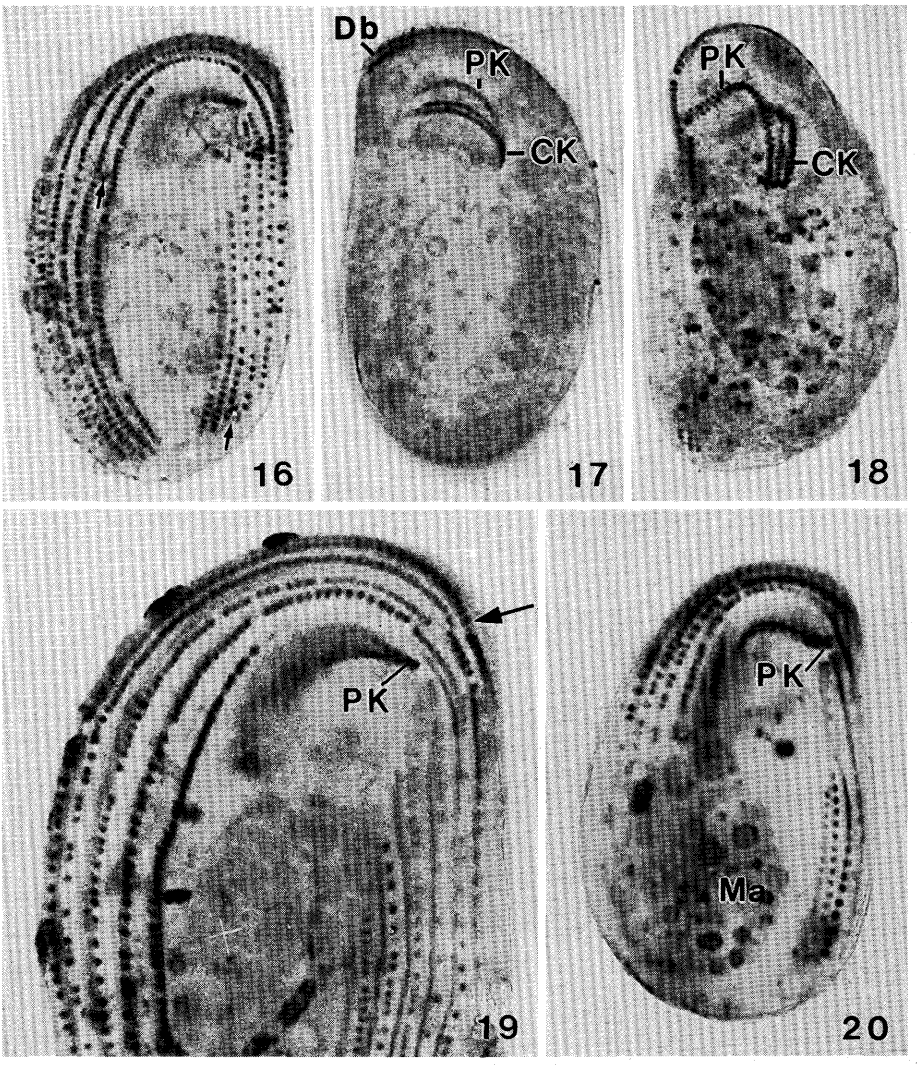

Fig. **16-20.** *Chilodonatella minuta* after protargol impregnation. **16, 17:** Somatic and oral infraciliature of ventral and dorsal side. Arrows mark excretory pores of contractile vacuole. **18:** Lateral view of oral ciliature. **19, 20:** Details of the somatic and oral infraciliature. Arrow marks inconspicuous interruption of right and left side kineties at anterior left end of cell. CK – circumoral kineties, Db – dorsal brush, Ma – macronucleus, PK – preoral kinety.

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Prodiscophrya collini (ROOT). *Chilodonatella minuta* grows well under high loading conditions (BOD up to 5000 mg/l) and tolerates various organic compounds contained in pharmaceutical wastewater (BECARES et al. 1994). It obviously has a short generation time (< 2 days) because it reached high numbers in a sludge with an average age of only two days. Very likely, *C. minuta* prefers high concentrations of free bacteria, and we were able to cultivate it on squashed wheat grains. Unfortunately, the cultures dried out and we failed to recover *C. minuta*, indicating that no cysts were formed. More detailed data on the ecology of *C. minuta* will be published later.

4 Discussion

4.1 Identification

DRAGESCO (1966) provided a rather rough description of *C. minuta* which we cite here in full to facilitate comparison with our observations.

„*Chilodonatella minuta* n. g; n. sp.: C'est dans l'eau douce, katharobe, d'un terrain inondé à la „Brague" (Alpes Maritimes) que nous avons trouvé cet extraordinaire Cilié que sa taille minuscule (L= 22 µm) et sa rareté ne nous a pas permis d'étudier autrement que sur le vivant.

Diagnose du nouveau genre: Cilié Gymnostome Cyrtophore, très voisin des *Chilodonella*. Le nouveau genre se caractérise, toutefous, par une simplification de l'infraciliature, qui entraîne une modification de la forme du corps. Le cinétome est, en effet, constitué par une écharpe de 4 longues cinéties qui entourent, de façon parfaitement symétrique, l'ouverture buccale ventrale. Ce „fer à cheval" étant parfaitement régulier, la forme même du Cilié devient un ovoide, symétrique par rapport à l'axe longitudinal du corps. Nasse, appareil nucléaire et vacuole pulsatile sont du type *Chilodonella*.

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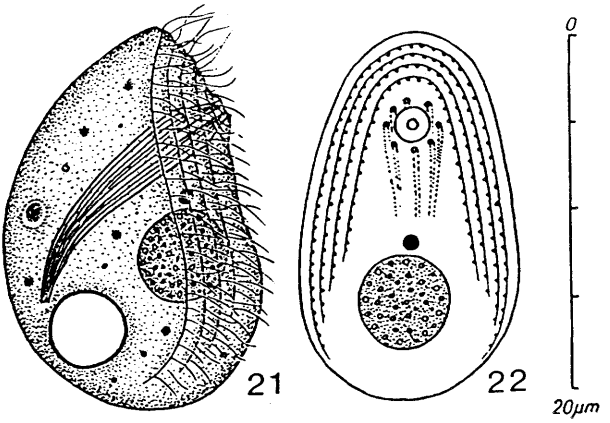

Fig. **21, 22.** *Chilodonatella minuta* (from DRAGESCO 1966). **21:** Lateral view from life. **22:** Ventral view schematized.

Espèce type *Chilodonatella minuta* n. sp. (Fig. 21, 22): La face ventrale n'est pas plane, comme chez la plupart des Chilodonelliens mais légèrement bombée. La première cinétie, c'est-à-dire celle qui entoure la face ventrale du Cilié, suivant sa plus forte dimension, est aussi la plus longue et prend naissance tout-à-fait postérieurement. Les trois cinéties suivantes sont de plus en plus courtes. Ces cinéties portent de gros cinétosomes d'où partent des cils assez longs.

La nasse, du type *Chilodonella*, est constituée par huit baguettes tubulaires assez longues. La nasse augmente de diamètre dans son premier quart pour diminuer ensuite progressivement, tout en se recourbant vers le bas. Au centre de l'ouverture de la nasse et du plateau buccal, on aperçoit une ouverture buccale se continuant par un pharynx, tubulaire, assez profond. L'appareil nucléaire est constitué par un macronucleus sphérique, à gros nucléoles, et un micronucleus adjacent. La vacuole pulsatile est postérieure et dorsale.

Contrairement à la grande majorité des Ciliés des genres voisins, *C. minuta* montre une face ventrale bombée et une face dorsale presque demisphérique, d'ou une épaisseur de même ordre que la largeur. Par tous des caractère, et surtout par son infraciliature si particulière, *Chilodonatella* reste un Cilié facile à reconnaître.

La disposition des cineties est tres aberrante et il faudra de nouvelles observations, basées sur des impregnations argentiques pur conclure quant à la realité absolue de cette simplification".

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Since our observations differ in many aspects from those of DRAGESCO we asked him for further information. In a letter he told us the „*Chilodonatella* story": „Some day in year 1946, I found a very strange and small ciliate near Cannes. I was able to observe 5 or 6 living individuals under oil immersion, when they become pressed and almost motionless under the cover glass. I was surprised by the fact of seeing kinetosomes in life, even without phase contrast. I did some drawings. I never found the species later. In 1948, I showed my drawings to FAURÉ-FREMIET who decided that such a beast cannot exist and is based on poor observations. In 1952, I showed the material to Gilbert DEROUX who agreed with FAURÉ-FREMIET. Thus, I published my observations only 20 years later, namely 1966.

I remember that, despite its small size, it was not difficult to see the strange kineties and many other details; the ciliate was very clear. It was possible to observe even the micronucleus without staining. Certainly, my specimens were in bad conditions, as I had to wait for the moment where the movement becomes very slow, i.e. the cells were near cytolysis. It is also possible that I mixed observations on two different species. The most important character is the strange arrangement of the somatic kineties, clearly recognisable in life specimens. Do not forget that these observations were done by a youngster of 26. You must consider that the data could not be as accurate as those of PENARD and others".

Our identification is based mainly on the peculiar arrangement of the somatic kineties, which DRAGESCO clearly recognized and used as main character for the genus and species. In this respect our organisms match those seen by DRAGESCO almost perfectly (Fig. 7, 9, 11, 16, 19, 22).

Concerning the second main character, the deep buccal cavity, it is reasonable to assume that it could not be recognized by DRAGESCO in his squashed and deformed specimens, which were „near cytolysis". We observed that the whole oral area can protrude above the cell surface in morbid specimens (video records) and in silver carbonate treated cells. Furthermore, the buccal cavity is rather small in some individuals. The distinct pharyngeal rods described by DRAGESCO are more difficult to explain. However, as mentioned above, he is uncertain whether he mixed different species. This is in fact possible, because there are a lot of small cyrtophorids, some not yet even described, which have a distinct cytopharyngeal basket (FOISSNER et al. 1991) and are difficult to

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distinguish in squashed condition. This interpretation is supported by the single contractile vacuole mentioned by DRAGESCO. There are indeed some small cyrtophorids, e.g. *Chlamydonella rostrata*, which have only one contractile vacuole near the posterior end (SONG WEIBO & WILBERT 1989).

The third main character, the cell size, looks rather different at first glance, viz. 22 µm in DRAGESCO's specimens and 30-50 µm in our material (average in fixed specimens 30 µm; Tab. 1). DRAGESCO found few individuals, indicating that the population was small and weak. In our experience, individuals from such populations are frequently smaller than those from flourishing cultures. Thus, not too much weight should be given to such size differences.

Of course, our identification is questionable. However, we find it reasonable to identify a species with an insufficiently described taxon if it matches at least one main character. This helps to reduce poorly defined genera and species, so richly found in the protozoological literature.

4.2 Systematic position of *Chilodonatella minuta*

The arched, seemingly uninterrupted somatic kineties give *C. minuta* an unusual appearance. However, a closer examination proves that the kineties have irregularities at that site where in ordinary chilodonellids the preoral kinety resides (Figs. 7, 9, 14, 19). Thus, it is reasonable to assume that this pattern is caused simply by a dislocation of the preoral kinety into the deep buccal cavity. Other characters, like the number of preoral and circumoral kineties, the non-ciliated postoral field and the position of the contractile vacuoles also match the chilodonellid pattern very well.

In spite of these similarities, we suggest that *C. minuta* is more closely related to the Chlamydodontidae, especially to *Gastronauta*, than to the Chilodonellidae because of its delicate cytopharynx originating at the bottom of a buccal cavity, which is however less pronounced in *Gastronauta* (BLATTERER & FOISSNER 1992). Furthermore, the marginal somatic kineties of *Gastronauta* form arches like those found in *C. minuta*. In addition, the peculiar cylindroid structure at the right dorsal margin of *C. minuta* (Fig. 8, 15) has as yet been found only in *Gastronauta* (BLATTERER & FOISSNER 1992).

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The deep buccal cavity of *C. minuta* is unique within the cyrtophorids and might be considered as a family character. However, in most large groups of ciliates there are families comprising genera with very differently sized buccal cavities, e.g. in the colpodids (FOISSNER 1993), which are not separated at family level. The ecological meaning of the deep buccal cavity of *C. minuta* remains obscure since the species feeds on bacteria like many other cyrtophorids (FOISSNER et al. 1991).

4.3 Improved diagnosis of *Chilodonatella* DRAGESCO 1966

Chlamydodontidae (?) with right and left field of somatic kineties, some of which join at anterior left region forming seemingly uninterrupted arches framing non-ciliated postoral area. Oral apparatus in deep cavity containing cytopharyngeal opening and preoral and circumoral kineties.

Type species: *Chilodonatella minuta* DRAGESCO 1966.

4.4 Improved diagnosis of *Chilodonatella minuta* DRAGESCO 1966

In vivo about 20-50 x 15-30 µm, slightly reniform. 1 contractile vacuole each in anterior right and posterior left kinety field. On average, 7 kineties in left and 5 kineties in right kinety field. Dorsal brush at anterior left end, composed of an average of 10 cilia. 1 preoral kinety and 2 closely spaced circumoral kineties, all terminating at cytopharyngeal opening.

Zusammenfassung

In einer Belebtschlammanlage von León in Spanien, mit der pharmazeutische Abwässer gereinigt werden, fanden wir *Chilodonatella minuta*, ein kleines cyrtophorides Ciliat, das von DRAGESCO (1966) recht oberflächlich beschrieben wurde. Unsere Wiederbeschreibung basiert auf der Untersuchung lebender und Protargol imprägnierter Individuen. Die Gattung und die Art sind klar definiert durch die somatische Bewimperung, die so wie bei anderen Cyrtrophoriden aus zwei Wimpernfeldern besteht, die im vorderen linken Teil der Zelle aber nur undeutlich getrennt sind, weil die praeorale Wimpernreihe und die beiden circumoralen Wimpernreihen in eine tiefe Mundhöhle verlagert sind, die in der ganzen Gruppe einmalig ist. Daher bilden die

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somatischen Wimpernreihen scheinbar nicht unterbrochene Bögen. Für die Gattung und die Art werden verbesserte Diagnosen gegeben und die systematische Stellung der Gattung wird diskutiert.

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