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Observation of live specimens of *Pseudotontonia cornuta* (Ciliophora: Oligotrichida) reveals new distinctive characters

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A large planktonic ciliate, *Pseudotontonia cornuta*, was observed during a cruise in the North Sea, Denmark, in summer 2001. Live cells as well as fixed and protargol-stained specimens were studied. The species possessed the characteristic tail of Tontoniidae, somatic ciliature classifying it as a *Pseudotontonia*, and cell proportions and oral ciliature corresponding to *P. cornuta*. Observation of live cells, however, revealed distinctive features as chloroplast-containing tentacles emerging just below the apical membranelles and an S-shaped proximal rim of the left margin of the oral cavity. These characters are eye-catching in live specimens, but have passed unnoticed till now because all previous studies on *P. cornuta* have been made on fixed samples.

INTRODUCTION

Ciliates of the family Tontoniidae Agatha, 2004 are characteristic due to a long, contractile tail (Agatha, 2004). Recently the genus *Tontonia*, described by Fauré-Fremiet (1914), was split into four genera based on the somatic ciliature that has for long been considered an important character for the taxonomy of other oligotrichid ciliates (Agatha, 2004). Observations of live cells are needed to see the tail of the Tontonids in its extended state and, in addition, the cells need to be undisturbed, i.e. contained in a bottle with seawater and not simply in a drop of water on a slide. When fixed, cells often loose the tail or the tail contracts into a knob or 'bud' at the cell's posterior part. All species of Tontoniidae have been found in marine environments, usually in relatively low cell concentrations in coastal waters. The specimens of *Pseudotontonia cornuta* (Leegaard, 1915) Agatha, 2004 studied in this paper were collected offshore in the North Sea in the same geographical region in which Leegaard (1915) originally found the species.

MATERIALS AND METHODS

Samples were collected during a cruise (HE152) with the RV 'Heincke' (of The Alfred Wegener Institute, Bremerhaven, Germany) on the North Sea in August 2001. The majority of the samples on which this publication is based were taken at the position 56°11.32′N 06°37.60′E. Water was collected with a Niskin water sampler and fixed immediately either in Lugol's solution (final concentration: 2%) or in glutaraldehyde (GA) of a final concentration of 2%. Depth, temperature, salinity, and fluorescence were recorded with a CTD (Hydrobios). Samples were taken at various depths to recognize the vertical distribution of ciliate cells.

Cells were viewed and photographed using a Zeiss Axiovert (live cells and fixed cells) or an Olympus AX50 (fixed cells) microscope, both equipped with epifluorescent illumination and UV filter sets. Live cells were observed and photographed on-board the RV 'Heincke'. For viewing of nuclei, cells were 4′,6-diamidino-2-phenylindole (DAPI) stained as follows: Lugol's-fixed ciliates were concentrated by gravity and picked out individually with a drawn-out Pasteur pipette. The cells were then transferred to a bottle containing 1 ml of the original seawater/Lugol's solution. Two grains of sodium thiosulphate ($Na_2SO_3 \cdot 5H_2O$) were added to bleach the cells and 0.2 ml of DAPI stock solution (1 μg/ml) was added to stain the cell's nuclei. Protargol staining was done on approximately 40 GA-fixed cells that had been concentrated and isolated as described above and then stained following a standard protocol (Montagnes & Lynn, 1987).

A simple grazing experiment was performed to determine potential uptake of bacteria-sized particles. Fluorescent polystyrene microbeads (FluoSpheres®, 1 μm in diameter, Molecular Probes, USA) were added at a final concentration of 10^7 beads ml^{-1} to 600 ml culture tissue bottles. The bottles were then incubated without agitation at temperatures and irradiances corresponding to conditions at the four different depths where samples were taken. After 8 h, GA was added to the bottles and cells were then viewed with epifluorescence microscopy. Below we use standard taxonomic terminology and abbreviations suggested by Agatha (2004).

SYSTEMATICS

Order OLIGOTRICHIDA Bütschli, 1889
Family TONTONIIDAE Agatha, 2004
Genus *Pseudotontonia* Agatha, 2004
Pseudotontonia cornuta (Leegaard, 1915) Agatha, 2004
(Figures 1–3)

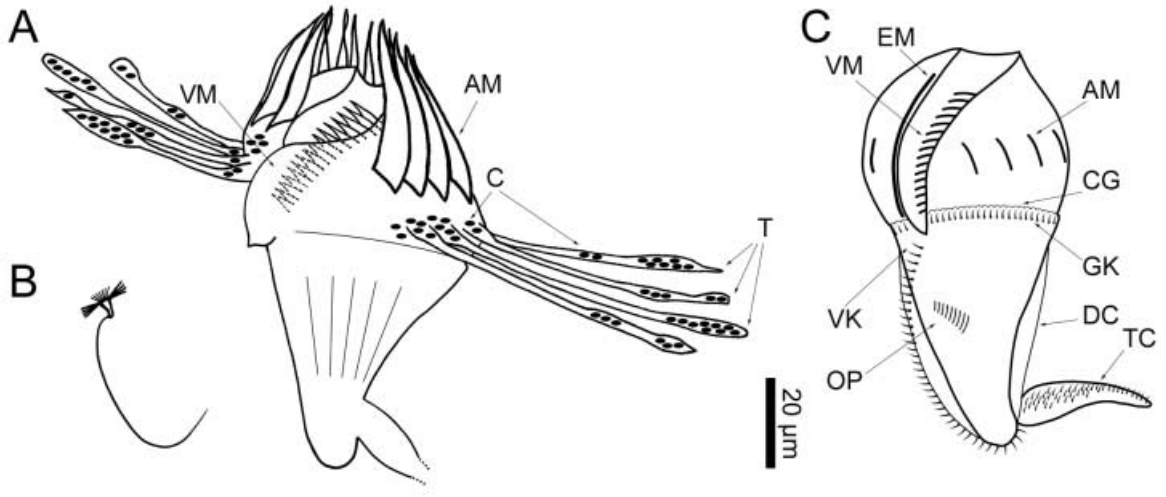

Figure 1. *Pseudotontonia cornuta*. (A) Schematic drawing of live cell; (B) schematic presentation of live cell with tentacles extended; (C) oblique ventral view of protargol-stained neotype specimen (tentacles not visible). AM, anterior membranelles; C, chloroplasts; CG, circumferential girdle; DC, distended cell surface; EM, endoral membrane; GK, girdle kinety; OP, oral primordium; T, tentacles; VK, ventral kinety; VM, ventral membranelles; TC, tail cilia. Scale bar applies to part A and C only.

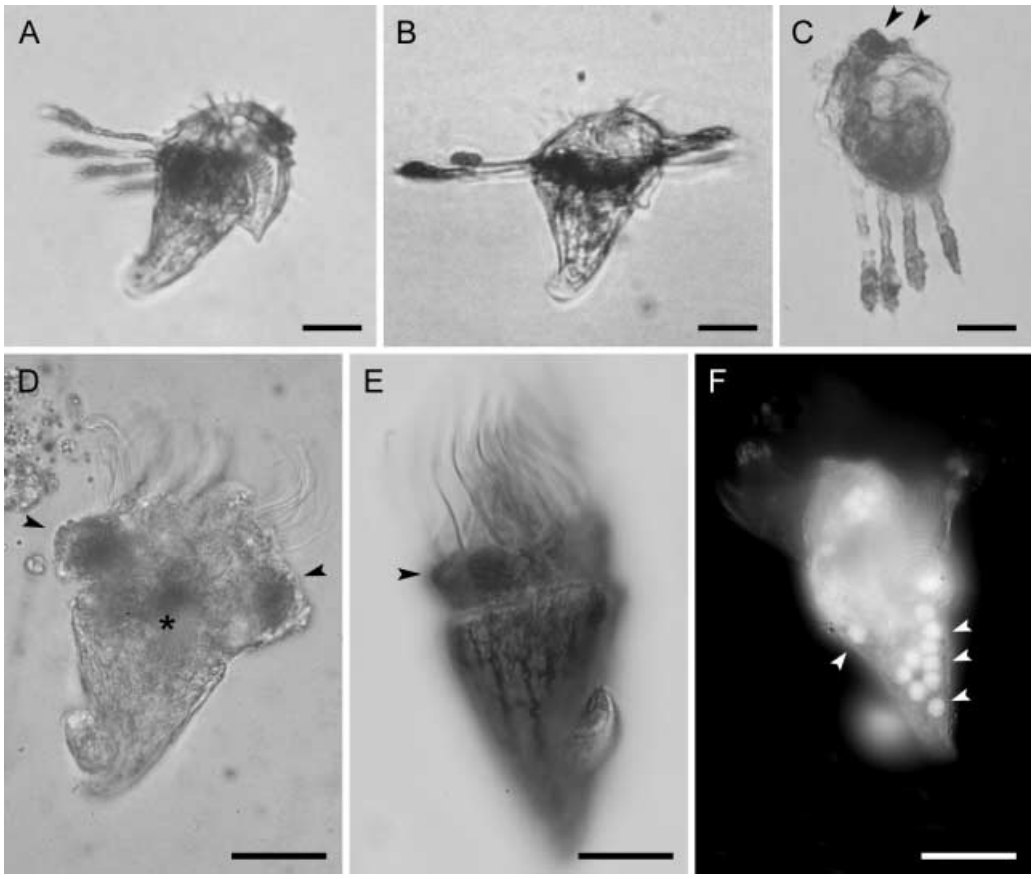

Figure 2. *Pseudotontonia cornuta*. (A–C) Live cell, same specimen; (A) lateral/anterior view showing four (right) tentacles and oral cavity with ventral membranelles. Tail not visible; (B) oblique ventral view showing tentacles (of which some are pointing towards the observer). Tail not visible; (C) apical view showing four right tentacles extended (bottom) and four left tentacles retracted (arrowheads); (D) glutaraldehyde-fixed cell. Combined transmitted and epifluorescent illumination. Dorso-lateral view. Tentacles are visible as lateral knobs (arrowheads) with red autofluorescence (in this greyscale image visible as darker taint). Fluorescence (blue) of ingested microspheres is visible as greyish area in centre of cell (asterisk); (E) Lugol's-fixed cell. Oblique dorsal view. Contracted tail and tentacles (arrowhead) visible. Striation of cell body presumably due to pattern of cortical platelets; (F) Lugol's-fixed, bleached and DAPI-stained cell showing macronuclei (arrowheads). Note that several macronuclei are outside the focal plane and, thereby, not visible on the photograph. Scale bars: 20 μm.

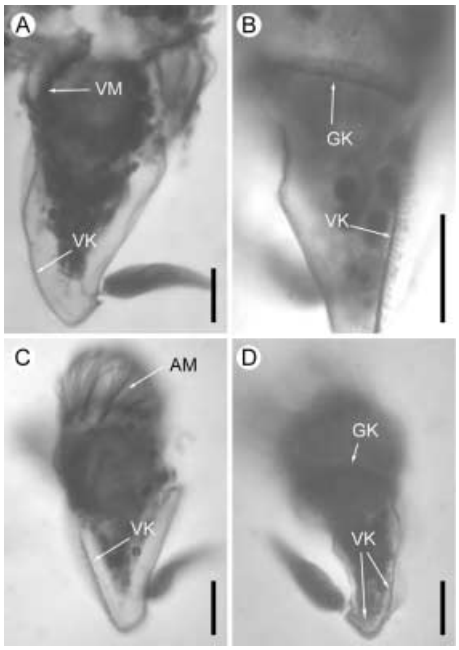

Figure 3. *Pseudotontonia cornuta*, protargol-stained. (A) Whole cell depicting ventral membranelles and ventral kinety; (B) central part of cell with girdle and ventral kineties; (C) whole cell with intact apical membranelles and ventral kinety; (D) whole cell illustrating row of ventral kinetids continuing around cell antapex. Abbreviations as in Figure 1. Scale bars: 20 μm.

Improved diagnosis

Conical *Pseudotontonia* with slightly curved posterior end, 65–146 μm long and 30–71 μm wide. Twelve to 19 anterior membranelles (AM), 11–16 ventral membranelles. Eight approximately 60 μm long, retractable tentacles emerge from anterior part of cell below AM (four on each side of oral cavity). Tentacles and anterior part of cell contain chloroplasts; 13–30 macronuclei.

Neotype material

Neotype as a slide with protargol-stained cells deposited at the Biological Reference Collections, Institut de Ciències del Mar, 08003 Barcelona, Spain (reference: ICMCS 8/2005).

Locality

Offshore waters, the North Sea, Denmark (56°11.32′N 06°37.60′E). Salinity, 33–34 psu; temperature, 14–17°C.

Description

Live cells conical with posterior end slightly curved, approximately 100 μm in length. Dimensions of fixed cells are listed in Table 1. Cell shape relatively well preserved in fixed material (Figures 1–3), but tail only fully extended (up to 0.5 mm or more) in live cells when undisturbed (Figure 1B). Tail in fixed cells contracted to small appendage (Figures 2D,E & 3) or absent. Polygonal cortical platelets covering the cell body at times visible in GA-fixed specimens. In live and in Lugol's fixed material, a longitudinally striated appearance of the cell surfaces may be caused by these rows of cortical platelets (Figures 1A & 2E). Tentacles and anterior part of cell with dark brownish granules (Figures 1A & 2A–C), exhibiting red autofluorescence when viewed in epifluorescence microscopy (Figure 2D). Like the tail, the tentacles are contractile and quickly retract upon physical disturbance. Contraction of tentacles lasts for approximately 0.5–2 s and is thereby slower than the contraction of tail. Re-extension of tentacles lasts several seconds. Tentacles usually lost or disintegrated in fixed cells.

Table 1. *Morphological characteristics of fixed* Pseudotontonia cornuta *cells.*

	Mean	Range	SD	N
Lugol's				
Cell length	84 μm	75–95 μm	—	4
Cell width	40 μm	38–40 μm	—	4
Glutaraldehyde				
Cell length	100 μm	71–146 μm	16 μm	25
Cell width	55 μm	45–71 μm	6 μm	25
Protargol				
Cell length	93	80–105 μm	7 μm	21
Cell width	50	40–70 μm	6 μm	21
Anterior membranelles	16	12–19	2	19
Ventral membranelles	14	11–16	2	10
Number of macronuclei	18	13–26	5	6

N, numbers of cells measured; SD, standard deviation.

Girdle kinety (GK) encircles widest part of cell, except from a small gap below the oral cavity (Figures 1C & 3B). Circumferential girdle positioned anterior to girdle kinety. Ventral kinety (VK) consisting of one straight row of ciliated kinetids extends from below oral cavity to cell antapex (Figures 1C & 3), ending at base of tail. All visible kinetids with each one cilium (1.5 and 2 μm long, GK and VK, respectively). Tail possesses row of ciliated kinetids seemingly continuing along the full length of tail, but arranged in disorganized pattern on contracted tails. Macronuclei localized irregularly throughout the cell with a tendency of most macronuclei in posterior part (Figure 2F).

Left margin of the asymmetric oral cavity with S-shaped proximal edge, extending slightly beyond cell surface (Figures 1A & 2A,B). Right margin of oral cavity with endoral membrane (Figure 1C).

Ecology

Pseudotontonia cornuta was found in the water column between the surface and the depth of 25 m with highest abundances (200 cells l^{-1}) in the fluorescence maximum at 20 m. The red autofluorescence indicates presence of chloroplasts. It was, however, not ascertained whether chloroplasts were contained individually or as part of algal cells. Similarly, it was not investigated whether these chloroplasts or algal cells constituted a permanent symbiosis or merely were retained for a limited time. In addition to possessing chloroplasts, *P. cornuta* is also an active filter feeder: 1-μm fluorescent microspheres were ingested in large numbers (Figure 2D), and 85% of cells (N=136) contained masses of ingested microspheres after 8 h of incubation. Whether particles of this size are suitable food items for *P. cornuta*, or rather coincidently follow the

feeding current, is unknown. It must be concluded, however, that the species is a functional mixotroph capable of both photosynthesis and particle ingestion.

DISCUSSION

Due to the characteristic tail it is beyond doubt that the species studied here is a member of the family Tontoniidae, and the somatic ciliature unambiguously places it in the genus *Pseudotontonia* (Agatha, 2004). Somatic ciliature seemed to be of monokinetid nature. It cannot be excluded, however, that non-ciliated basal bodies of dikinetids were present but not recognized due to insufficient staining. Tentacles have not before been seen in Oligotrichida, just as chloroplasts so far have not been reported in *Pseudotontonia* (Agatha, 2004) although oligotrichid ciliates are commonly found to contain chloroplasts (Stoecker et al., 1987). Except for tentacles and chloroplasts, all characters suggest that the species studied here is *P. cornuta*. Data on morphology of *P. cornuta* have been reported twice (*Laboea cornuta* in Leegaard, 1915; *Tontonia cornuta* in Suzuki & Song, 2001), but in both cases only fixed specimens were studied. We found signs of tentacles in only two specimens among 195 fixed cells investigated, and in these two cases the tentacles were just faintly recognizable as knobs or small projections (Figure 2D,E). In the 21 intact protargol-stained cells investigated, no signs of tentacles were apparent. On the contrary, more than 100 live cells were observed at low magnification and they all had distinctive tentacles. It is therefore probable that tentacles have been overlooked in previous studies based solely on fixed material. Detection of chloroplasts may, likewise, not be feasible when cells are fixed and stained, just as the S-shaped edge of the left margin of oral cavity is evident only in live cells.

Cell proportions of our cell material concur with the original description of *P. cornuta* in which one cell was depicted as having a belt-like dilation at the widest part of the cell (figure 8A,B in Leegaard, 1915), and cell content was noted to be granular. The depicted belt has similarity with the retracted tentacles as they appear in Figure 2E, and the granular cell content might have been due to contained chloroplasts. Cells reported by Suzuki & Song (2001) were smaller than those of the original description (Leegaard, 1915) as well as the cell material referred to in the present paper. However, all three reported size-ranges are wide and do overlap. In the present redescription the cell size-range is, therefore, extended to cover all three reported ranges. The first data on somatic ciliature of *P. cornuta* (Suzuki & Song, 2001) agree with the observations in the present paper: the somatic ciliature is identical and numbers of membranelles and macronuclei are comparable. In addition these authors depicted a drawing in which a cell had characteristic fibres at the point where we have found tentacles to be inserted (figure 1B in Suzuki & Song, 2001). These fibres may represent tentacles in their contracted state.

Many planktonic, oligotrichid ciliates have been described solely from fixed material. When working with fixed material, staining techniques like protargol are invaluable tools for elucidating oral and somatic ciliatures, which are recognized as key characters for the taxonomy of Oligotrichida. However, one must bear in mind that other important features may be overlooked in fixed specimens and that it may be necessary also to observe live cells to achieve a full understanding of the morphology of a species. Future studies on live material of the other present member of the genus *Pseudotontonia*, *P. simplicidens* (Lynn & Gilron, 1993) Agatha, 2004, are needed in order to determine whether this species possesses tentacles. If this proves not to be the case, the genus may need re-evaluation.

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REFERENCES

Agatha, S., 2004. Evolution of ciliary patterns in the Oligotrichida (Ciliophora, Spirotricha) and its taxonomic implications. *Zoology*, **107**, 153–168.

Fauré-Fremiet, E., 1914. Deux infusoires planctoniques *Tontonia appendiculariformis* (n. gen., n. sp.) et *Climacostomum diedrum* (n. sp.). *Archiv für Protistenkunde*, **34**, 95–107.

Leegaard, C., 1915. Untersuchungen über einige Planktonciliaten des Meeres. *Nytt Magasin for Naturvidenskapene*, **53**, 1–37.

Montagnes, D.J.S. & Lynn, D.H., 1987. A quantitative protargol stain (QPS) for ciliates: method description and test of its quantitative nature. *Marine Microbial Food Webs*, **2**, 83–93.

Stoecker, D.K., Michaels, A.E. & Davis, L.H., 1987. Large proportion of marine planktonic ciliates found to contain functional chloroplasts. *Nature, London*, **326**, 790–792.

Suzuki, T. & Song, W., 2001. A redescription of *Tontonia cornuta* (Leegaard, 1915) comb. nov., a planktonic oligotrichous ciliate (Ciliophora: Oligotrichia) from the northern Pacific Ocean. *Hydrobiologia*, **457**, 119–123.

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