

Ultrastructural Studies on a Model Tintinnid – *Schmidingerella meunieri* (Kofoid and Campbell, 1929) Agatha and Strüder-Kypke, 2012 (Ciliophora). I. Somatic Kinetids with Unique Ultrastructure

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Abstract. Molecular phylogenies of Oligotrichea currently do not contain all genera and families and display topologies which are often incongruent with morphological findings. In ciliates, the somatic kinetids are rather conserved, i.e., their ultrastructures, particularly the fibrillar associates, often characterise the main groups, except for the choreotrichids. Four different kinetid types are found in protargol-stained choreotrichids and used for reconstructing the taxon's evolution (the “Kinetid Transformation Hypothesis”). Proof for this hypothesis requires transmission electron microscopic studies, which are very rare in the choreotrichids and oligotrichids. Such an approach provides insights into the ultrastructural variability of somatic kinetids in spirotrichs and may also detect apomorphies characterising certain choreotrichid families. In the model tintinnid *Schmidingerella meunieri*, the ultrastructure of the three kinetid types in the somatic ciliature is studied in cryofixed cells. The data support the “Kinetid Transformation Hypothesis” regarding tintinnids with a ventral kinety. This first detailed study on kinetids in tintinnids and choreotrichids in general reveals totally new kinetid types in ciliates: beyond the three common associates, they are characterised by two or three conspicuous microtubular ribbons extending on the kinetids' left sides. These extraordinary ribbons form together with the overlapping postciliary ribbons a unique network in the cortex of the anterior cell portion. The evolutionary constraints which might have fostered the development of such structures are discussed for the Oligotrichea, the choreotrichids, and tintinnids as their first occurrence is currently uncertain. Additionally, the kinetids in tintinnids, aloriccate choreotrichids, oligotrichids, hypotrichs, and euplotids are compared.

Key words: basal bodies, fibrillar associates, phylogeny, somatic infraciliature, tintinnids, ultrastructure

Dedication: This paper is dedicated to the memory of our wonderful colleague Denis Lynn, who introduced the ultrastructure of the somatic kinetids in the ciliate systematics.

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INTRODUCTION

Lynn (1981) established the “Structural Conservatism Hypothesis” postulating that “the conservation of structures through time is inversely related to the level of biological organisation”. In ciliates and eukaryotes in general, (alpha) tubulin and microtubules are too strongly affected by selection to be useful for inferring relationships (Kilburn and Winey 2008, Chaaban and Brouhard 2017, Rajter and Vďačný 2018). Likewise, only two variants of basal bodies had been found in the more than 150 ciliates species ultrastructurally studied (Lynn 1981). However, the fibrillar associates of the basal bodies were assumed to characterise particular ciliate classes in the classification of Small and Lynn (1985). Actually, most ciliate groups distinguished by different kinetid types in their systematics also emerged as monophyletic groupings in genetic phylogenies. The less conserved number of basal bodies in a kinetid might merely be characteristic for families, and the even more variable numbers of kinetids in the somatic cortex and especially of oral organelles characterise probably only genera and species (Lynn 2017).

With increasing knowledge about cell morphology and ultrastructure, the taxa affiliated with the spirotrichs distinctly changed over time. In gene trees, they comprise six main groups: the phacodiniids, licnophorids, euplotids, hypotrichs [including halteriids; Lynn and Kolisko (2017)], oligotrichids, and choreotrichids.

The molecular phylogenies on oligotrichids and choreotrichids not only reveal several non-monophyletic genera and families but also insufficiently resolved groups and astonishing relationships which are not reflected by the overall morphology (Santoferrara *et al.* 2017).

Various mainly ultrastructural features with potentially phylogenetic significance had been proposed for Oligotrichea (Agatha and Strüder-Kypke 2007). Considering the particular importance of the somatic kinetids, Agatha and Strüder-Kypke (2014) suggested the here called “Kinetid Transformation Hypothesis” to explain the extraordinary diversity of kinetid structures in the choreotrichids and even in a single species. The different character states of the kinetid had been implemented into cladistic analyses for providing rationales (synapomorphies) for the topology of gene trees. The hypothesis is, however, based only on data from supposedly homologous somatic kineties in pro-targol-stained euplotids, hypotrichs, oligotrichids, and

choreotrichids. A dikinetid (pair of kinetosomes) with a cilium only at the anterior basal body is assumed to represent the plesiomorphic state as found in euplotids, hypotrichs, and oligotrichids. It possibly transformed several times independently into a monokinetid via three transition stages (both basal bodies ciliated; loss of the anterior cilium; loss of the anterior basal body). To put the hypothesis on a sound basis, ultrastructural data are indispensable.

The somatic kinetids of ciliates might have three associated structures: a transverse microtubular ribbon extending obliquely leftwards, a kinetodesmal fibril extending obliquely rightwards, and a postciliary microtubular ribbon extending obliquely posteriorly. The different equipment with associated structures in the two dikinetidal basal bodies enables the reconstruction of the kinetid evolution on the ultrastructural level: the “Kinetid Transformation Hypothesis” predicts the occurrence of the same fibrillar associates in a posterior dikinetidal basal body and a monokinetid.

While transmission electron microscopic data on somatic dikinetids are available for several euplotids (Ruffolo 1976, Görtz 1982, Morelli *et al.* 1996, Lynn 2008, Modeo *et al.* 2013) and hypotrichs (Grimes and Adler 1976, Görtz 1982, Lynn 2008), only scarce information on the fibrillar associates of monokinetids in aloriccate choreotrichids and tintinnids had been published (Hedin 1976, Laval-Peuto and Brownlee 1986, Grim 1987); the two types of tintinnid dikinetids (with two cilia and with a cilium only at the posterior basal body) and the oligotrichid dikinetids have not been studied in this respect.

The present ultrastructural study on *Schmidingerella meunieri* (Kofoid and Campbell, 1929) Agatha and Strüder-Kypke, 2012 provides the first data not only on somatic dikinetids in tintinnid ciliates, but also for choreotrichids and Oligotrichea in general, enabling a comparison with those of the euplotids and hypotrichs. Additionally, the more abundant monokinetids were investigated to collate them with the species’ dikinetids as well as with the sparse previously published data on monokinetids in choreotrichids.

MATERIALS AND METHODS

Cultivation

Monoclonal cultures of the tintinnid originally designated as *Favella taraikaensis* and identified here as *Schmidingerella meunieri*, had been established on specimens sampled in the Northeast

Pacific, i.e., at the coast of Washington State. They were provided by Kelley Bright and Suzanne L. Strom from the Shannon Point Marine Centre, Western Washington University, USA. The specimens obtained in July 2011 and June 2016 were further cultivated in the laboratory in Salzburg with artificial sea water (salinity 33‰) plus a f/2 trace metal solution (Guillard 1975) at a 12 h:12 h light-dark cycle and a temperature of 18 °C. The dinoflagellate *Heterocapsa triquetra* and the haptophyte *Isochrysis galbana* were used as food, but the cultures also contained a huge diversity of further flagellates of unknown identity (SA own observ.). Exclusively based on lorica features, the tintinnid has provisionally been identified by SA as *Schmidingerella meunieri*.

Live observations

From the various specimens investigated, around 20 cells were measured at up to 1,250 × magnification under a compound microscope equipped with a high-power oil immersion objective, bright-field and interference contrast optics (Leitz Diaplan, Leitz Wetzlar GmbH, Germany), and a digital camera (Leica DFC420; Leica Microsystems, Vienna).

Cryopreservation

Cells were transferred into sample holders with a depth of 200 µm and a diameter of 1.2 mm with a small amount of culture medium. Cryofixation was performed by means of a Leica EM PACT high pressure freezer (Leica Mikrosysteme GmbH, Vienna, Austria) within milliseconds at about 2,000 bar. Eppendorf tubes with the sample holders containing the fixed specimens were filled with a precooled mixture made of 2% osmium tetroxide and 0.05% uranyl acetate in acetone and placed in a Leica EM AFS (Vienna, Austria) for freeze substitution. The AFS was programmed as follows: 60 h at -80 °C, followed by a temperature increase at a rate of 10 °C per hour for 5 h to -30 °C, 4 h at -30 °C, and another temperature increase at a rate of 2.5 °C per hour for 20 h to 20 °C, and finally 10 h at 20 °C. After freeze-substitution, the samples were rinsed three or four times in anhydrous acetone followed by three times washing for 10 min each in propylene oxide before embedding in Agar Low Viscosity Resin (ALVR; Agar scientific, Stansted, UK). The embedding was done with a propylene oxide:ALVR ratio of 3:1, 1:1, and finally 1:3. Next, the samples were placed for 4 d in a desiccator for polymerization and finally for 24 h in an oven at a temperature of about 60 °C.

Ultramicrotomy and electron microscopy

The polymerized samples were ultrathin-sectioned (70 nm) with an ultramicrotome (Ultracut S, Reichert AG, Vienna, Austria). Observations were conducted by a Zeiss EM 910 transmission electron microscope (Karl Zeiss AG, Oberkochen, Germany), and images were taken with the Sharp:Eye digital camera system (Tröndle), using the computer program Image SP Viewer. More than 3,000 images were inspected, and measurements were usually performed on several subsequent sections of the same structure to ascertain the maximum dimensions. All data concerning lorica measurements come from scanning electron micrographs with a total number of about 150 loricae investigated.

The terms right and left refer to the orientation of the structures as viewed from outside the cell, and the terminology of the structures follows Lynn (1981). The orientation of the micrographs is parallel to the longitudinal axis of the cell if not otherwise mentioned.

RESULTS

General morphology

The campanulate lorica is 103–250 µm long (\bar{x} = 153 µm; n = 81) and has a subapical ring-shaped bulge and an apical ring with a diameter of 50–90 µm (\bar{x} = 72 µm; n = 111; Fig. 1A). The length:width ratio of the lorica is 1.5–3.2:1 (\bar{x} = 2.1:1). Its posterior end is acute, but has only in 27% of specimens investigated a straight process 9–38 µm long (\bar{x} = 21 µm; n = 81), which lacks ribs and a channel. The lorica wall is 1–2 µm thick and monolaminar with alveoli up to 3 µm across and minute pores. Rarely, irregular windows occur in the apical lorica portion. The outer surface of the wall has more or less distinct reticulate ridges. The paralarica is of the *Coxliella*-type, i.e., has a spiralled structure and a broadly rounded posterior end; a posterior process is absent.

In extended state, the cell proper is about 70–170 µm long and attached by a 35–85 µm long and about 5 µm wide contractile peduncle to the bottom of the lorica (Fig. 1A). The cytoplasm is colourless and contains two ellipsoidal macronucleus nodules and food vacuoles with algae. A contractile vacuole was not observed. The cytopye is near the base of the peduncle. Pin-shaped and mobile tentaculoids about 25–40 µm long and with a 1–2 µm wide distal portion insert in the outer portions of the intermembranellar ridges; they contain granules, probably extrusomes. The striae are indistinct (about 1 µm wide) and rarely seen. The cilia of the ciliary fields are about 3 µm long, except for the about 15 µm long and highly mobile anteriormost cilia of the right and left fields. The ventral kinety is separated by an unciliated stripe and a very shallow furrow from the right ciliary field (Fig. 1B). The cilia are about 6 µm long in its monokinetic anterior portion, while somewhat longer (about 8–9 µm) in its dikinetid posterior portion. The dorsal kinety extends from the zone of adoral membranelles to the base of the peduncle and has associated cilia 8–15 µm long.

The adoral zone of membranelles (described in detail in a forthcoming paper) forms a closed circle on the peristomial rim and remains perpendicular to the main cell axis in contracted specimens (Fig. 1B, C). The about 17 three-rowed collar membranelles are about 50 µm long and frayed distally; four of them are elongated, extending into the eccentric and about 30 µm deep buccal cavity, which also contains one buccal membranelle. The intermembranellar ridges are indistinct and con-

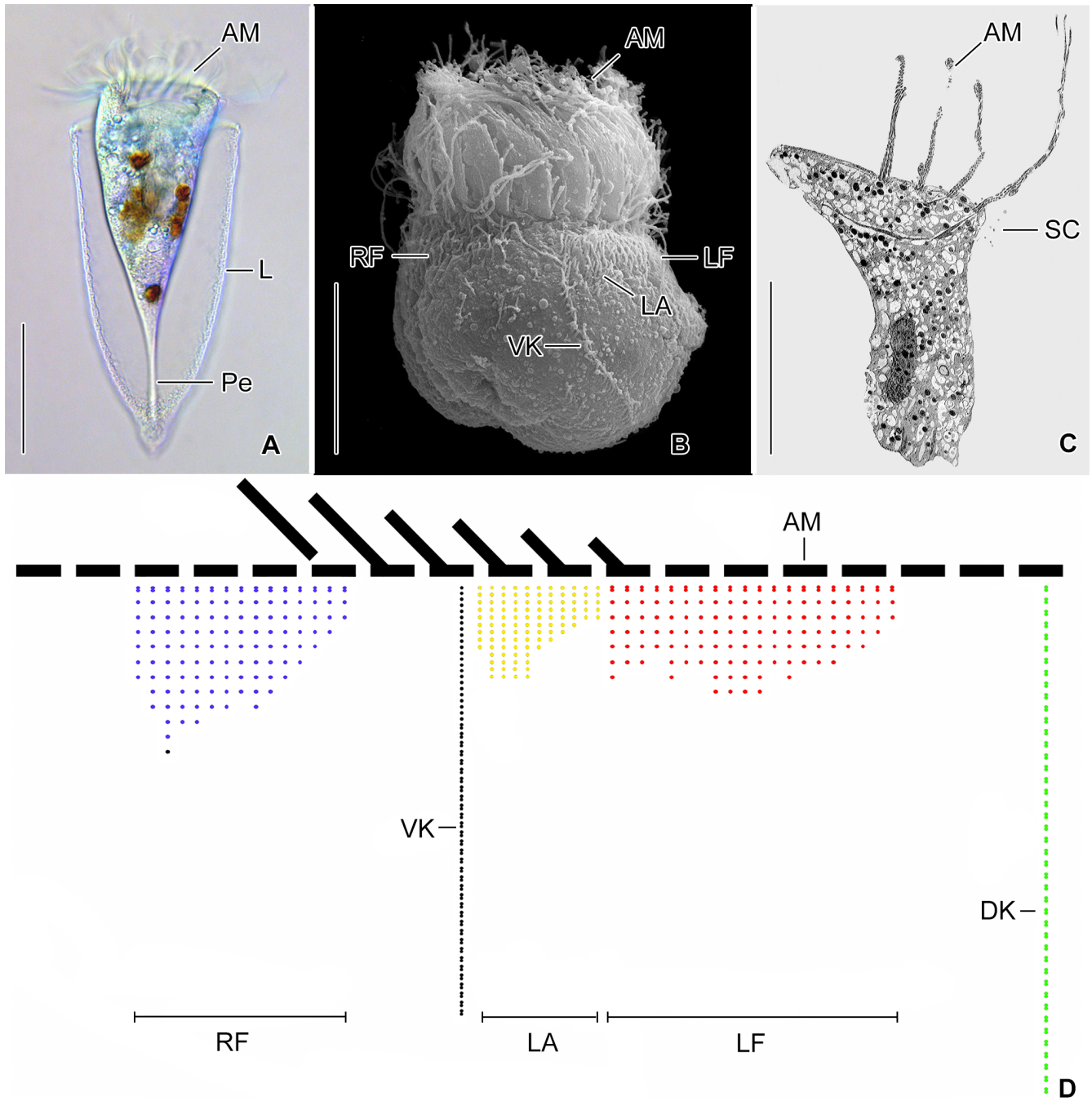


Fig. 1. *Schmidingerella meunieri* from the Northeast Pacific *in vivo* (A), in the scanning electron microscope (B), and in the transmission electron microscope (C) and a kinetal map of a congener after protargol staining (D). (A) The living cell is attached by its peduncle to the bottom of the lorica. (B) Contracted, naked specimen. (C) Longitudinal ultrathin section. (D) Scheme of ciliary pattern in *Schmidingerella arcuata* (modified from Agatha and Strüder-Kypke 2012). AM, adoral membranelles; DK, dorsal kinety; L, lorica; LA, lateral ciliary field; LF, left ciliary field; Pe, peduncle; RF, right ciliary field; SC, somatic cilia; VK, ventral kinety. Scale bars: 50 μm (A, C), 20 μm (B).

tain numerous granules, probably mitochondria and/or extrusomes. The peristomial field performs pumping movements. Conjugation and resting cysts occurred in the cultures.

Although the somatic ciliature had not morphometrically been analysed in our specimens, it was obvious that the general pattern matches that of the genus *Schmidingerella* Agatha and Strüder-Kypke, 2012 in comprising (i) a left and a right ciliary field composed of monokinetidal kineties with usually one anterior dikinetid (Fig. 1D), (ii) a ventral kinety separated by a distinct gap from the right field and consisting of a monokinetidal anterior portion and a dikinetidal posterior portion with cilia only at each posterior basal body (Fig. 1B), (iii) a monokinetidal lateral ciliary field (Fig. 1D), and (iv) a dorsal kinety composed of dikinetids each having associated a cilium only with the posterior basal body (Figs 2E, 5); a posterior kinety is absent. All kineties start about at the same level, i.e., a few micrometres posteriorly to the collar membranelles.

Basal bodies

The basal bodies (kinetosomes) of mono- and dikinetids invariably consist of nine microtubular triplets forming a cylinder about 216 nm across and 480 nm long. The individual basal body is delimited proximally by a cartwheel (Figs 3E, 4D, 6A) and distally by a plate directly underneath the axosome, probably the axosomal plate; but, the terminal plate as continuation of the epiplasm was not recognisable or could not be distinguished from the axosomal plate. Two, rarely three (observed once) electron-dense core granules about 50 nm across occur in the lumen of the basal body; yet, they are seen only in half of the longitudinal sections because of their scattered distribution (Figs 2B, 3C, 4C, 5, 6D, 7). The axosome is globular, about 120 nm across, and electron-dense. It almost fills the lumen of the basal body (Figs 2D, 3B, 4A–D, 5, 6A, E, F, 7). Electron-dense material forms a cuff covering the proximal third of the basal body including the cartwheel. Neither parasomal sacs nor alveoli were found near the basal bodies. Densely arranged longitudinal microtubules form a cortical layer (Figs 3B, 5, 6A, B, E, F, 7). The cell membrane is about 9 nm thick. The entire cell including the cilia is covered by a perilemma (Figs 4D, 6A, E, F).

Dikinetids

Dikinetids occur in the dorsal kinety (Figs 2–4), in the posterior portion of the ventral kinety (Fig. 1D), and at the anterior ends of the kineties in the right and left

ciliary fields (Fig. 6D, E) and share an identical ultrastructure. Because of their higher numbers in the dorsal kinety, the following data come mainly from studies on this specialised ciliary row.

The dikinetids are clockwise inclined to the kinety axis with angles of 20°–30° (Figs 2A–C, 3A–D). The basal bodies are perpendicular to the cell surface and parallel to each other. Occasionally, the posterior basal body seems to insert about 70 nm more distally than the anterior one, i.e., when the posterior basal body already displays its axosome in optimal cross sections, the anterior basal body shows its lumen (Fig. 2D). The distance between the two basal bodies is about 90 nm and they are connected by a desmose (electron-dense connection; Figs 2A, B, 3B, 5, 6D). The electron-dense cuffs covering the proximal third of the basal bodies are probably the origin of several fibrillar associates (Figs 2A, B, 3B–E, 5, 6D, E, 7).

Besides the three common fibrillar associates (kinetodesmal fibril, postciliary microtubular ribbon, and transverse microtubular ribbon), the dikinetids comprise three extraordinary – as yet unknown – microtubular ribbons (Fig. 8A). All microtubules are 25 nm across as typical. The kinetodesmal fibril originates in the proximal portion of the posterior basal body between triplets 6 and 8 (Figs 2A–C, 3B, C, 5, 8A); the exact position could not be determined. The fibril is probably elongate conical with a broad base and extends parallel to the kinety axis anteriorly and obliquely towards the cell surface for an undetermined distance until it converges with the cortical microtubules; it is not periodically striated but has a fibrous structure as recognisable in cross/oblique sections (Fig. 2D).

A divergent postciliary ribbon originates near the proximal portion of the posterior basal body at triplet 9, apparently performs a sharp curvature above the electron-dense cuff, and extends on the right side of the kinety posteriorly, terminating near the second following kinetid. Accordingly, the postciliary ribbons of each two kinetids overlap. The individual ribbon consists of a layer with probably four or five microtubules (exact number not determinable), approximately 2,800 nm long (Figs 2B, C, 3B, C, 5, 6D, 8A). The anterior basal body lacks a postciliary ribbon.

A tangential transverse ribbon originates near the proximal portion of the anterior basal body probably near triplets 4 and 5. Its four or five microtubules extend parallel to the anterior basal body towards the cell surface; whether they converge with the cortical layer of longitudinal microtubules is uncertain (Figs 2B–F, 5,

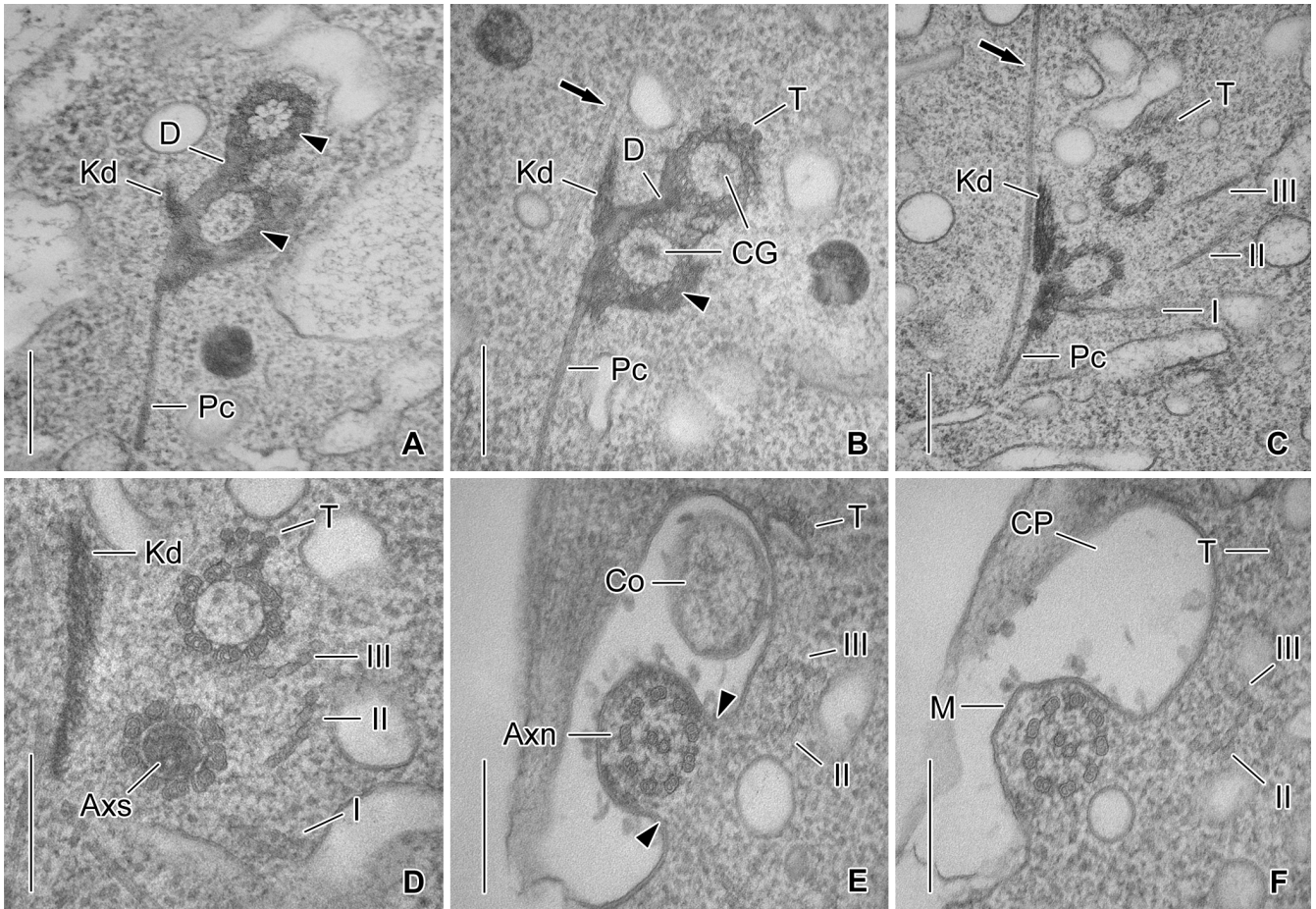


Fig. 2. Cross sections of dorsal dikinetids at different levels (from proximal to distal) in *Schmidingerella meunieri* in the transmission electron microscope. (A) The two basal bodies are sectioned at different levels, indicating a more proximal position of the anterior one. Arrowheads mark the electron-dense cuffs. (B) The dikinetid shows the electron-dense cuff (arrowhead) around the posterior basal body and the postciliary ribbon extending parallel to that of the more anterior dikinetid (arrow). (C) Slightly oblique section showing the three extraordinary microtubular ribbons extending perpendicularly or obliquely to the kinety axis leftwards: ribbons I and II originate near the posterior basal body, ribbon III commences near the anterior one. The arrow marks the long postciliary ribbon from the previous dikinetid. (D) The extraordinary ribbons I, II, and III are shown in cross sections. Note the fibrillar structure of the kinetodesmal fibril. (E) The dikinetid is located in a ciliary pit. While the anterior basal body terminates with a condylocilium, the posterior cilium displays a typical axoneme and is attached to the pit's left wall by a cytoplasmic connection (arrowheads). (F) The short condylocilium at the anterior basal body is not visible any longer. The ribbons II and III and the transverse ribbon terminate close to the cell cortex. I–III, extraordinary microtubular ribbons I–III; Axn, axoneme; Axs, axosome; CG, core granule; Co, condylocilium; CP, ciliary pit; D, desmose; Kd, kinetodesmal fibril; M, cell membrane; Pc, postciliary ribbon; T, transverse ribbon. Scale bars: 250 nm.

8A). On the posterior basal body, a transverse ribbon is lacking.

Three extraordinary – as yet unknown – fibrillar associates, the microtubular ribbons I, II, and III, arise from the electron-dense cuff at the cartwheel level on the left side of the dikinetid (Figs 2C, D, 3A–D, 5, 8A). Ribbon I (connective ribbon) originates near triplet 1 of the posterior basal body and probably slightly distally of ribbon II. It consists of about six microtubules approximately 2,400 nm long arranged in two layers

and extends perpendicularly to the kinety axis and the postciliary ribbon leftwards, probably after performing a sharp curvature above the level of the electron-dense cuff (exact course not determinable; Figs 2C, D, 3B–D, 5). In the anteriormost dikinetids of the right and left ciliary fields, ribbon I extends leftwards, terminating near triplet 5 of the posterior basal body of the adjacent dikinetid (Figs 6D, 7). Likewise, the dikinetids of the ventral kinety are connected with those of the dorsal kinety in the posterior portion of cell proper where the

kineties extend almost parallel at a distance of about 1,500 nm. Ribbons I originating in the posterior dikinetidal portion of the ventral kinety terminate near triplets 5 of the posterior basal bodies of the dorsal dikinetids (Fig. 3E); ribbons I of the dorsal kinety seem to have no contact with other kinetids (Fig. 3A).

Ribbon II (posterior oblique ribbon) originates near triplet 2 of the posterior basal body and slightly proximally of the connective ribbon (Figs 5, 7, 8A). It comprises five microtubules about 2,500 nm long and extends with its proximal portion almost parallel to the basal body, while its distal portion extends obliquely anteriorly, converging with the cortical microtubules; thus, ribbon II forms an angle of about 45° with ribbon I in surface views (Figs 2C–F, 3A–D, 5, 8A).

Ribbon III (anterior oblique ribbon) originates proximally near triplet 2 of the anterior basal body and comprises four to six microtubules about 2,200 nm long arranged in one, rarely two layers (Figs 5, 8A). It extends like ribbon II obliquely towards the cell surface, i.e., it accompanies the proximal portion of the basal body before it curves obliquely anteriorly, converging with ribbon II about 1,500 nm apart from the basal body; finally, it extends parallel to the cortical microtubules (Figs 2C–F, 3A–D, 5).

The dikinetids of the dorsal kinety and the posterior portion of the ventral kinety have a cilium associated only with the posterior basal bodies, while the anterior basal bodies have associated a condylocilium (Figs 2E, 3B, 4A, D, 5). The almost globular condylocilia of the dorsal kinety are about 280 nm across and enclosed by perilemma. They contain a granular cytoplasm, nine microtubular triplets, and an axosome; no central microtubules were observed (Fig. 4A–D). The dikinetids are located in pits about 400–600 nm deep (rough value). Interestingly, the cilia associated with the posterior basal bodies have broadened proximal portions which are connected with the left walls of the ciliary pits (Figs 2E, F, 3B, 5).

The dikinetids at the anterior ends of the kineties in the right and left fields have two elongated cilia [soies; Fauré-Fremiet (1924)] with typical axonemes (Fig. 7). Again, the posterior cilia are connected with the left walls of the ciliary pits; in one of the few sections available, such a cytoplasmic connection was also observed in the anterior cilium. Additional dikinetids which occasionally occur in the left field (Fig. 6E) very likely result from the intrakinetal proliferation of basal bodies.

There is probably a slight variability concerning the orientation and curvature of the microtubular asso-

ciates in the dikinetids of the various kineties, e.g., in the course of microtubular ribbons III (cp. Fig. 2D with 2E).

Monokinetids

Monokinetids constitute the lateral ciliary field, the anterior portion of the ventral kinety, and the main portions of the right and left ciliary fields (Fig. 1D). They seem to be always ciliated and match in their fibrillar associates (kinetodesmal fibril, postciliary ribbon, connective ribbon, and posterior oblique ribbon) the posterior dikinetidal basal bodies. Likewise, they are located in ciliary pits and their cilia are proximally connected with the left walls of the pits (Fig. 6A). Accordingly, transverse ribbons and ribbons III are lacking (Figs 6B–F, 7, 8B).

The monokinetids are 1,100–1,500 nm apart in a kinety and about 2,200 nm apart from those of the adjacent ciliary rows in the left ciliary field; however, distances vary within and between ciliary fields (Figs 6B–F, 7).

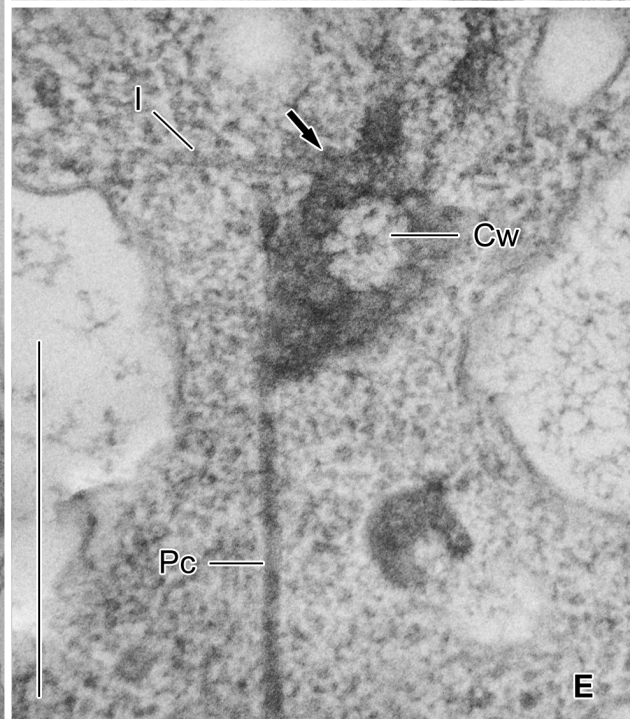
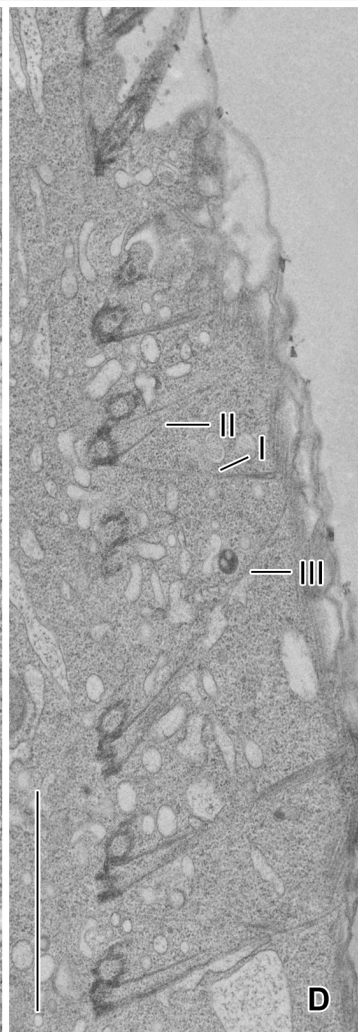
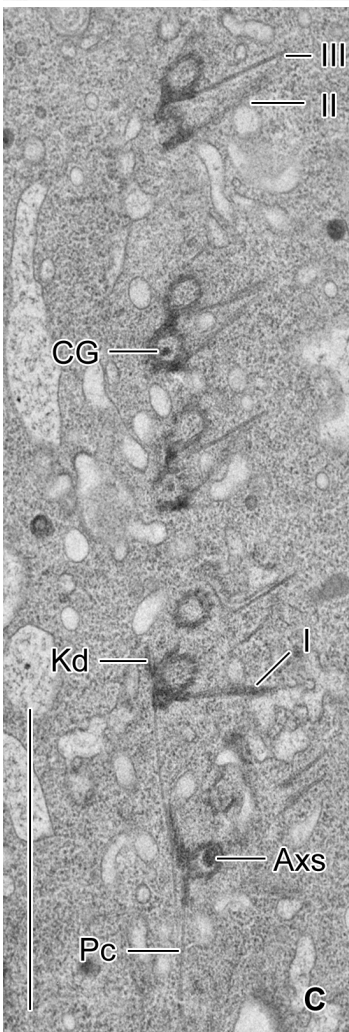
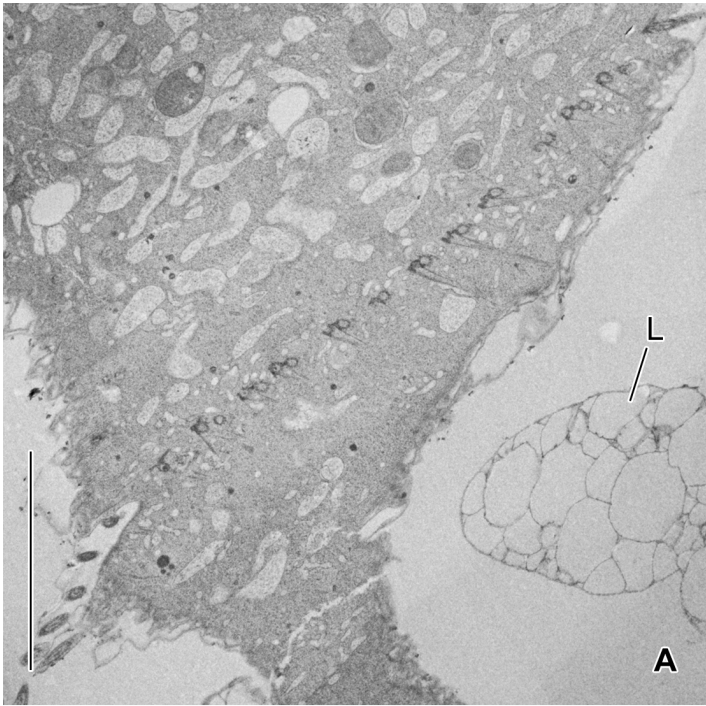
The kineties in the ciliary fields have different lengths. The last monokinetids in the kineties may have elongated postciliary ribbons extending for some micrometres, and ribbon I (connective ribbon) terminates in the cytoplasm in those monokinetids which lack a kinetid on their left sides. Further, ribbon I comprises only about four microtubules and seems to be single layered (vs. about six double layered microtubules in the other monokinetids), and ribbon II (posterior oblique ribbon) is about 1,600 nm long (vs. about 2,500 nm long).

The linkage of adjacent ciliary rows by the connective ribbons (ribbons I) causes not only an arrangement of the kinetids at the same level in a field, but also forms a dense network of fibrillar structures together with the overlapping postciliary ribbons in the ciliated anterior portion of the tintinnid (Figs 6B–F, 7).

DISCUSSION

Justification of species identification and general somatic ciliary pattern

The specimens investigated were identified as *Schmidingerella meunieri* (Kofoid and Campbell, 1929) Agatha and Strüder-Kypke, 2012 based on lorica features recognisable *in vivo* and in the scanning electron microscope. *Favella meunieri* had been established by Kofoid and Campbell (1929) for the speci-



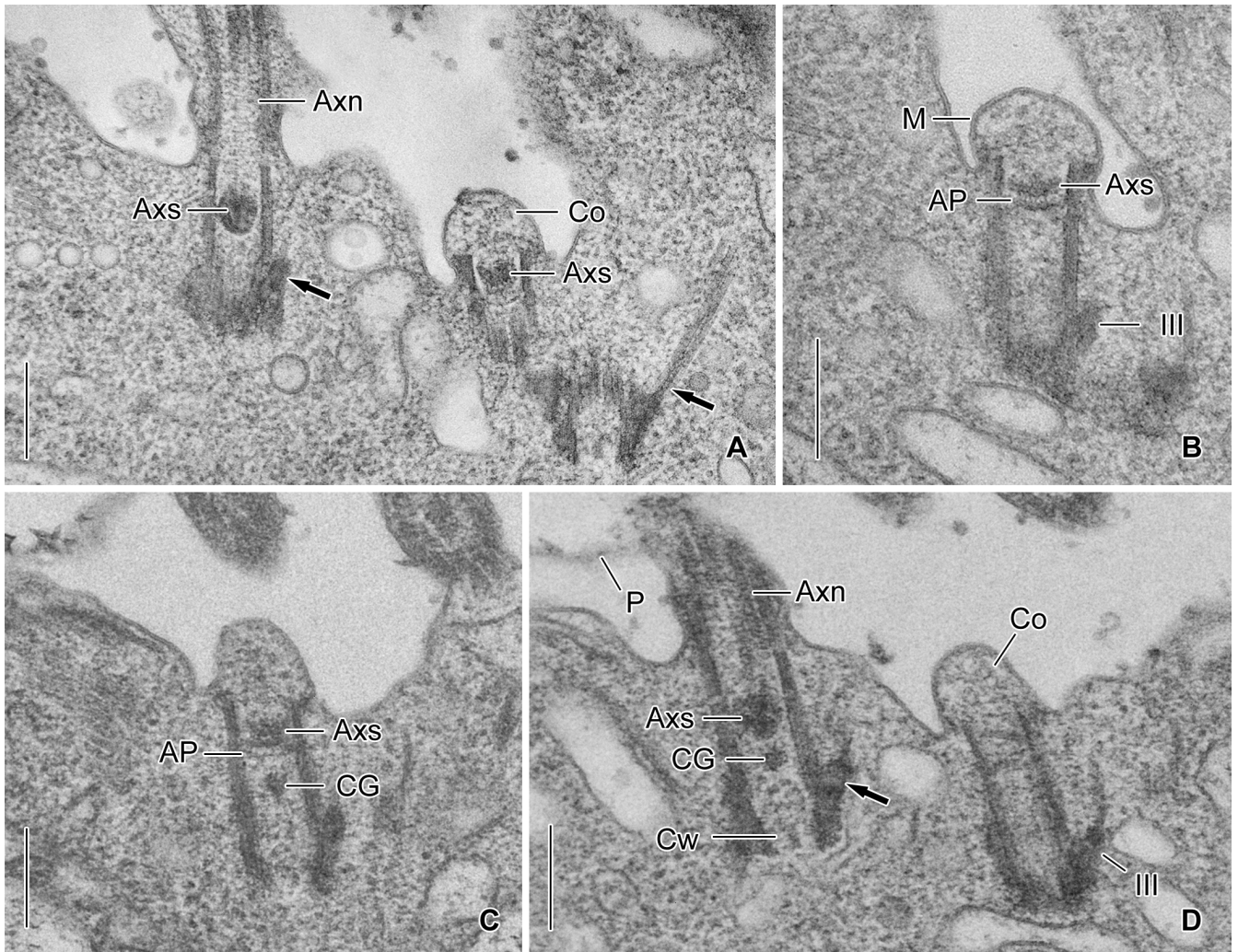


Fig. 4. Longitudinal sections of dorsal dikinetids in *Schmidingerella meunieri* in the transmission electron microscope. **(A, D)** Two dikinetids each. The posterior dikinetidal basal body (on the left) has associated a cilium, whereas the anterior basal body (on the right) displays a condylocilium. Arrows mark extraordinary ribbons I or II. **(B, C)** Longitudinal sections of condylocilia. III, extraordinary microtubular ribbon III; AP, axosomal plate; Axn, axoneme; Axs, axosome; CG, core granule; Co, condylocilium; Cw, cartwheel; M, cell membrane; P, perilemma. Scale bars: 250 nm.

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Fig. 3. Cross and oblique sections of the dorsal kinety in *Schmidingerella meunieri* in the transmission electron microscope. **(A, C, D)** Overview and details. The dikinetids are clockwise inclined to the kinety axis. **(B)** Three dikinetids sectioned at different planes, i.e., a proximal section of the anterior dikinetid and a distal section of the posterior kinetid. The arrow marks ribbon I originating in the adjacent ventral kinety. **(E)** Detail of a dikinetid showing the ribbon I of the adjacent ventral kinety (arrow) which terminates near triplet 5 of the posterior basal body. I–III, extraordinary microtubular ribbons I–III; Axn, axoneme; Axs, axosome; CG, core granule; CM, cortical microtubules; Co, condylocilium; Cw, cartwheel; D, desmose; Kd, kinetodesmal fibril; L, lorica; Pc, postciliary ribbon. Scale bars: 5,000 nm (A), 1,000 nm (B), 2,000 nm (C, D), 500 nm (E).

mens misidentified as *Cyttarocylis ehrenbergii* by Meunier (1919). According to the original description, the lorica is large, cylindrical, and narrowed in its posterior portion merging into a more or less distinct, obconical, and bluntly pointed process. The smooth opening rim is formed by a short collar and has the same diameter as the main apical lorica portion. The lorica wall is rather stiff and composed of small polygonal alveoli. The three illustrations provided by Meunier (1919) show an irregular outer lorica surface. The author even described the encystment process and the resting cyst, which is orientated with its cap (papula) towards the lorica bottom and its two broad, truncate horns obliquely towards the lorica opening. The species had been transferred to the genus *Schmidingerella* by Agatha and Strüder-Kypke (2012) based on its rough lorica surface and the subapical lorica bulge.

Meunier (1919) did not provide measurements, but merely mentioned the magnification of his drawings. Our own calculations and those of Kofoed and Campbell (1929) resulted in lorica lengths of 146–188 µm and opening diameters of 56–66 µm. Thus, the specimens from the North Pacific investigated here fall into these size ranges and perfectly match the lorica shape and wall texture of *Schmidingerella meunieri*. Additionally, similar resting cysts occurred in our culture (they will be described in detail in a forthcoming paper). Scanning electron micrographs of cells that had fallen out of their loricae during the preparation procedure (Fig. 1B) and live observations revealed a somatic ciliary pattern with the same characteristics as described for *Schmidingerella arcuata*, i.e., with an unciliated stripe separating the right field from an extremely long ventral kinety (Agatha and Strüder-Kypke 2012).

The specimens used in various ecological and electrophysiological experiments by Strom *et al.* (2007) and Echevarria *et al.* (2014, 2016) reveal a rough lorica surface and a subapical lorica bulge; thus, they do not belong to the genus *Favella*, but should also be assigned to the genus *Schmidingerella*. The transcriptome of *Schmidingerella arcuata* [*Favella ehrenbergii*] MMETSP0123-20130129 (Gentekaki *et al.* 2014) is from specimens cultivated by the S. L. Strom laboratory like the specimens ultrastructurally studied here. However, the SSU rRNA sequence included in the metadata for this transcriptome (e.g., in the iMicrobe portal and some MMETSP alignments) was incorrect (L. Santoferrara pers. commun.), as it corresponds to the sequence of a real *Favella*, namely *F. ehrenbergii*, provided by Kim *et al.* (2010). The correct SSU rRNA

gene sequence actually corroborates its affiliation with the genus *Schmidingerella*; yet, conspecificity with *S. arcuata* as sequenced by Agatha and Strüder-Kypke (2012) is uncertain owing to minute differences in the nucleotide sequence (L. Santoferrara pers. commun.) and lorica morphology (present study).

Kinetids in tintinnids

The tintinnids are monophyletic in both cladistic and genetic genealogies. The few mainly freshwater taxa with two ventral organelles form an adelphotaxon to the huge number of mainly marine species having a ventral kinety. The genus *Schmidingerella* Agatha and Strüder-Kypke, 2012 belongs to the latter group and its somatic ciliature consists of dikinetids having cilia associated with both basal bodies (anteriormost kinetids in the right and left ciliary fields), dikinetids with cilia only at the posterior basal bodies (dorsal kinety and posterior portion of the ventral kinety), and ciliated monokinetids (remaining kinetids).

In *Schmidingerella meunieri*, the monokinetids match the posterior dikinetidal basal bodies in the fibrillar associates (types, insertion sites, and orientations; this study); they merely deviate by fewer constituting microtubules and somewhat shorter lengths. The data from *S. meunieri* are the first on the ultrastructure of tintinnid dikinetids and the first detailed ones on tintinnid monokinetids. These findings confirm the “Kinetid Transformation Hypothesis” established by Agatha and Strüder-Kypke (2014) at least concerning the development of monokinetids from the posterior dikinetidal basal bodies in tintinnids with a ventral kinety. A similar kinetid transformation and co-occurrence of kinetid types had been described by Eisler (1988, 1989) for the nassulid ciliate *Furgasonia blochmanni*.

In *S. meunieri*, not only all fibrillar associates of the somatic kinetids are well developed but also three additional ribbons occur; hence, the kinetid structures in this tintinnid contradict the “rule of adjacent triplets” (Raikov *et al.* 1975) or “rule of excluded sectors” (Lynn 1981), i.e., when one associated structure is well developed, the adjacent ones are reduced.

According to Hedin (1976), the somatic cilia of *Ptychocylis minor* insert in indentations of the myoneme. Microtubules originate (i) at the proximal ends of the basal bodies and extend into the myoneme pockets and (ii) at the sides of the basal bodies and extend parallel to the kinety axis anteriorly and posteriorly, overlapping those of the previous and following basal bodies. The reinvestigation of the micrographs provid-

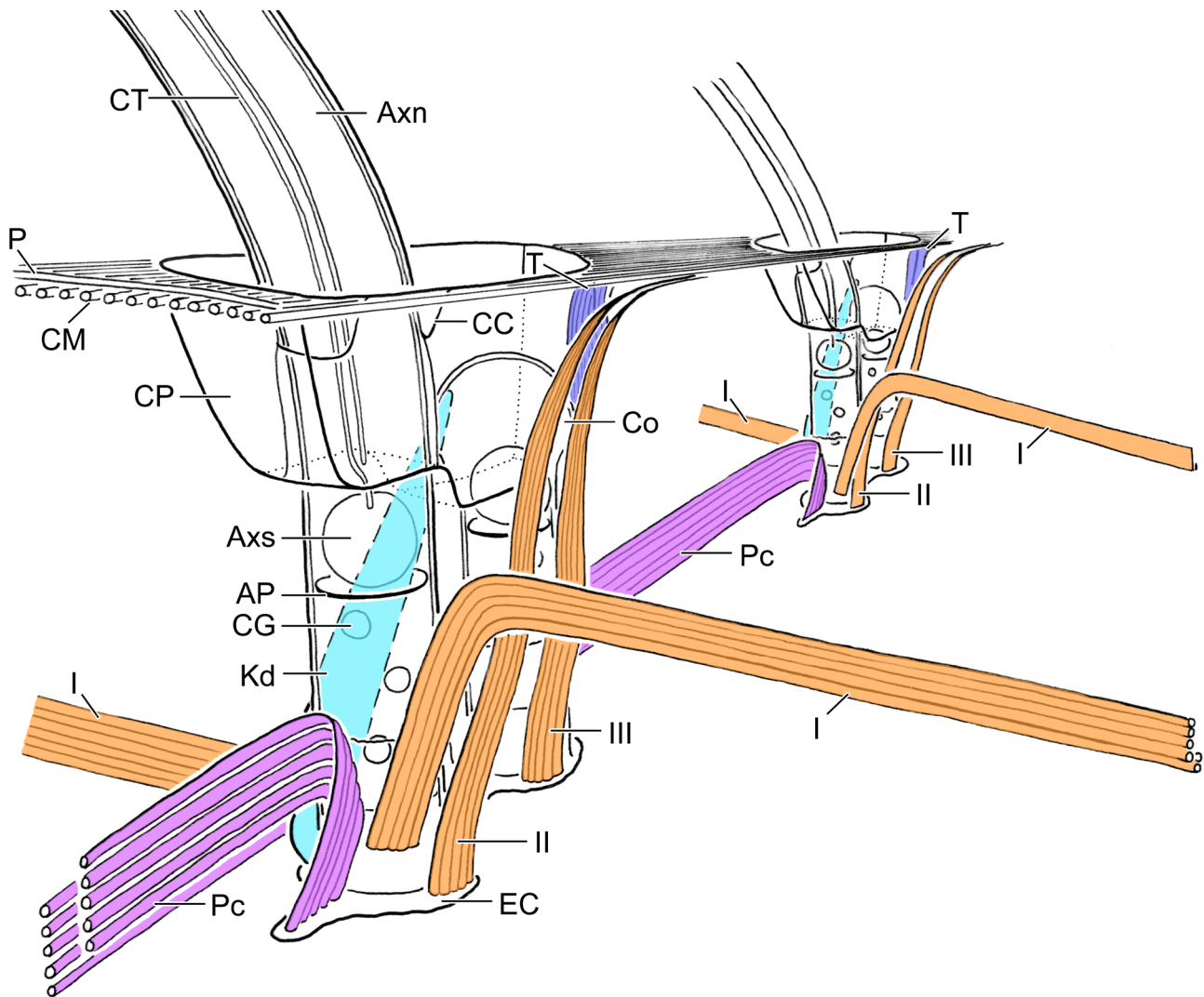
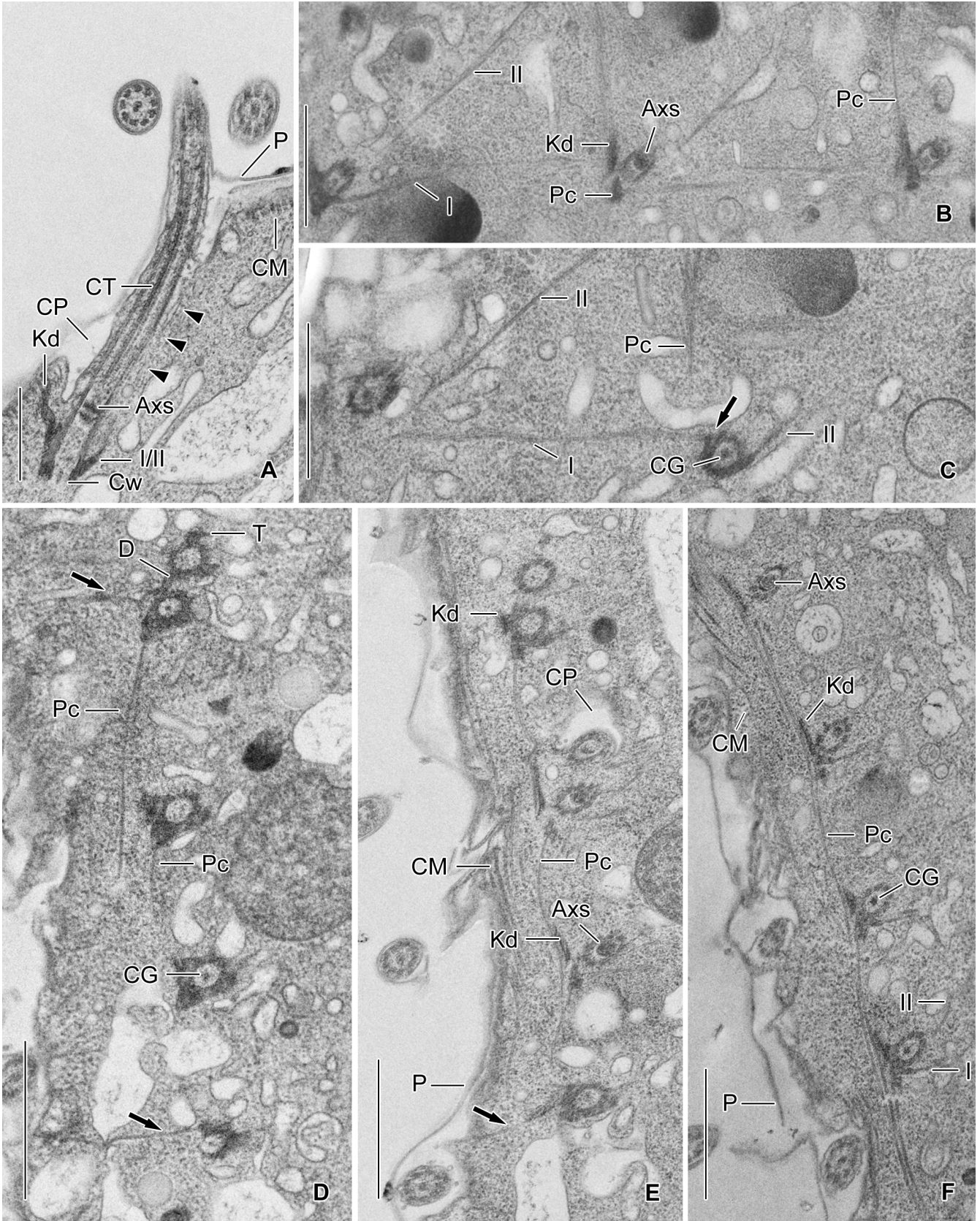


Fig. 5. Schematic drawing of two consecutive dikinetids in an obliquely orientated dorsal kinety or posterior portion of the ventral kinety in *Schmidingerella meunieri* compiling all observations from transmission electron micrographs. The 20° – 30° angles of the dikinetids are not considered. The posterior basal bodies insert somewhat more distally than the anterior ones and their cilia are connected with the left walls of the pits. The postciliary ribbons are shown in abridged form. I–III, extraordinary microtubular ribbons I–III; AP, axosomal plate; Axn, axoneme; Axs, axosome; CC, cytoplasmic connection; CG, core granule; CM, cortical microtubules; Co, condylocilium; CP, ciliary pit; CT, central tubules; EC, electron-dense cuff; Kd, kinetodesmal fibril; P, perilemma; Pc, postciliary ribbon; T, transverse ribbon.

ed by Hedin (1976) indicates that he somehow misinterpreted the sections and structures; actually, his figure 9 displays monokinetids with the same fibrillar associates as those of *S. meunieri*. Later, Laval-Peuto and Brownlee (1986), summarised their observations on several tintinnid genera (including *Ptychocyclus*). However, they described the monokinetids as equipped only with a short kinetodesmal fibril (triplets 6 and 7) and a divergent postciliary ribbon; transverse ribbons and parasomal sacs were not found. The overlapping post-

ciliary ribbons of the kinetids in a row were considered to represent postciliodesmata.

The taxon Postciliodesmatophora had been characterised by Gerassimova and Seravin (1976) to possess “postciliary microtubules that extend into the cortex to form overlapping ribbons”. Hence, Small and Lynn (1985) assigned the spirotrichs comprising the stichotrichs (= hypotrichs), choreotrichids, and heterotrichs to the Postciliodesmatophora. With improved insights into the phylogenetic relationships among



ciliates, the definition of postciliodesmata changed and thus the taxa affiliated. So, the spirotrichs now belong to the subphylum Intramacronucleata (Lynn 1996), and the term “postciliodesmata” is restricted to “stacked ribbons of overlapping postciliary microtubules, involved in extension of the body following contraction” (Lynn 2008) by means of microtubule arms (Lynn 2017). Ac-

cordingly, *S. meunieri* does not possess “true” postciliodesmata and confirms the lack of such structures in tintinnid choreotrichids (Lynn 2008).

Comparison with kinetids in aloricate choreotrichids

According to the “Kinetid Transformation Hypothesis”, the ancestral kinetid structure (dikinets having

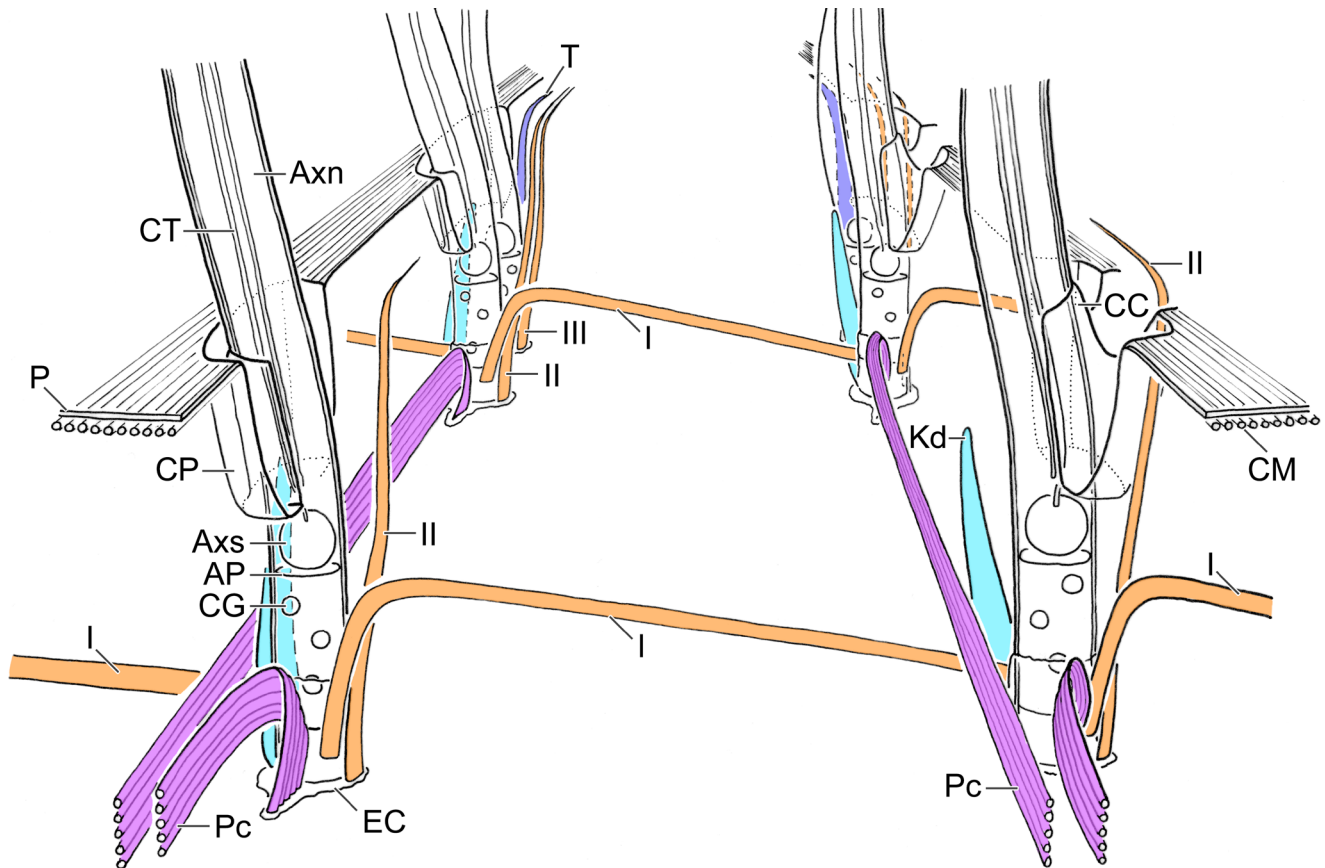


Fig. 7. Schematic drawing of the anterior portion of two adjacent kinetids in the left ciliary field of *Schmidingerella meunieri* compiling all observations from transmission electron micrographs. The 20°–30° angles of the kinetids are not considered. The kinetids are monokinetidal, except for one anterior dikinetid. The microtubules of the connecting (ribbons I) and postciliary ribbons form a network. I–III, extraordinary microtubular ribbons I–III; AP, axosomal plate; Axn, axoneme; Axs, axosome; CC, cytoplasmic connection; CG, core granule; CM, cortical microtubules; CP, ciliary pit; CT, central microtubules; EC, electron-dense cuff; Kd, kinetodesmal fibril; P, perilemma; Pc, postciliary ribbon; T, transverse ribbon.

◀

Fig. 6. Longitudinal (A) and cross (B–F) sections of kinetids in the left ciliary field of *Schmidingerella meunieri* in the transmission electron microscope. Arrows mark ribbons I of the neighbouring kinetid on the right side terminating near triplets 5 of the mono- and dikinetids. (A) The cilium of the monokinetid inserts in a pit and is connected with the left wall by a cytoplasmic bridge (arrowheads). (B) Two monokinetids (left, middle) and possibly a dikinetid (right). (C) Monokinetids in neighbouring kinetids. (D, E) Anterior kinetid portions. Occasionally, instead of a single anterior dikinetid two pairs are found, the second of which might be the result of intrakinetal proliferation. (F) Posterior kinetid portion. I–II, extraordinary microtubular ribbons I–II; Axs, axosome; CG, core granule; CM, cortical microtubules; CP, ciliary pit; CT, central tubules; Cw, cartwheel; D, desmose; Kd, kinetodesmal fibril; P, perilemma; Pc, postciliary ribbon; T, transverse ribbon. Scale bars: 500 nm (A), 1,000 nm (B–F).

associated a cilium only with each anterior basal body) changed dramatically in the choreotrichids after separation from the oligotrichid sister group. In both cladistic and phylogenetic analyses, the aloricate choreotrichids represent a paraphyletic grouping (Agatha and Strüder-Kypke 2007, 2014; Santoferrara *et al.* 2017). In contrast to the cladograms, the molecular genealogies currently do not comprise all genera and show some inconsistencies and unresolved relationships.

Only *Rimostrombidium lacustre* (Foissner, Skogstad and Pratt, 1988) Petz and Foissner, 1992 [reported as *Strobilidium velox* Fauré-Fremiet, 1924 by Grim (1987)] had ultrastructurally been investigated concerning its somatic kinetids. This species and strobilidiids in general are extraordinary in having monokinetidal somatic kineties and kinety flaps, which cover the bases of the somatic cilia extending parallel to the cell surface. The monokinetids have apparently associated only two-layered transverse ribbons. This peculiar kinetid ultrastructure of the supposedly functionless cilia in *R. lacustre* possibly represents an autapomorphy of the Strobilidiidae; yet, confirmation based on data from the other two strobilidiid genera (*Strobilidium* and *Pelagostrobilidium*) is needed.

Monokinetids in the tintinnids *Schmidingerella meunieri* (this study) and probably *Ptychocyclus minor* (Hedin 1976) differ distinctly from those of *R. lacustre* in the presence of kinetodesmal fibrils and overlapping postciliary ribbons; however, a homology of the ribbons I or II with the two-layered transverse ribbons in *R. lacustre* cannot be excluded.

Obviously, the choreotrichid ciliates are an exception of the structural conservatism of the somatic kinetids in ciliates (Lynn 1981) not only because of some taxa possessing three different kinetid types in a single cell, but mainly because of the differences between the monokinetids. Actually, the “Kinetid Transformation Hypothesis” suggests at least three independent transformations of dikinetids into monokinetids (Agatha and Strüder-Kypke 2014).

Comparison with oligotrichid, hypotrich, and euplotid kinetids

Molecular phylogenies are congruent with cladistic analyses (Agatha and Strüder-Kypke 2007, Santoferrara *et al.* 2017) in a sistergroup relationship of oligotrichids and choreotrichids and in placing the hypotrichs as adelphotaxon to this cluster. The euplotids again share the last common ancestor with the Perilemmaphora uniting the latter three taxa. The somatic kine-

ties invariably consist of dikinetids with cilia only at the anterior basal bodies in the oligotrichids, hypotrichs, and euplotids, a kinetid type not found in *S. meunieri*.

Ultrastructural data on kinetids are scarce in oligotrichids and base on studies of the strombidiids *Strombidium sulcatum* (Fauré-Fremiet and Ganier 1970), *S. inclinatum*, and *Novistrombidium testaceum* (Modeo *et al.* 2003). While the dikinetidal basal bodies of euplotids, hypotrichs, and *S. meunieri* are arranged in parallel as typical in ciliates (Grimes and Adler 1976, Görtz 1982), those of the oligotrichids diverge distally, forming an angle of 45–50°. In oligotrichids, short (0.7–3 µm long) and stiff/immobile cilia are usually associated with the anterior basal bodies of the longitudinal ventral kinety and with the left basal bodies of the curved girdle kinety, which are homologous to the anterior ones in the longitudinal rows (Agatha 2004, 2011; Agatha and Strüder-Kypke 2014). They may have short and distinctly curved cilia associated with the posterior ventral or right girdle basal bodies, which are usually only visible in transmission electron micrographs (Fauré-Fremiet and Ganier 1970, Modeo *et al.* 2003). Information on the fibrillar associates is lacking.

The dikinetid ultrastructure in the dorsal kineties of hypotrichs (Grimes and Adler 1976, Fleury *et al.* 1985, Lynn 2008) and euplotids (Ruffolo 1976, Görtz 1982, Wicklow 1983, Lenzi and Rosati 1993, Morelli *et al.* 1996, Lynn 2008, Modeo *et al.* 2013) is much better known. Their dikinetids have (i) a tangential transverse ribbon, a single postciliary microtubule, and a rather short, stiff/immobile cilium (bristle) associated with the anterior basal bodies and (ii) a divergent postciliary ribbon associated with the posterior basal bodies (Grimes and Adler 1976, Ruffolo 1976, Görtz 1982, Morelli *et al.* 1996, Lynn 2008). Euplotids and hypotrichs, however, differ in the kinetodesmal fibrils (permanent vs. transient), condylocilia at the posterior basal bodies (present vs. absent), and parasomal sacs (present vs. absent). The dikinetids in the tintinnid *S. meunieri* (this study) share a permanent kinetodesmal fibril with those in euplotids, but the absence of parasomal sacs with those of hypotrichs.

The position of the condylocilia in dikinetids of *S. meunieri* deviates from that in euplotids (at the anterior vs. posterior basal bodies) indicating that such club-shaped cilia developed several times independently and might represent either a pre-stage for or the remains of a common cilium depending on the presence/absence of a certain protein (Dave *et al.* 2009). In euplotids, the condylocilia contain nine microtubular doublets, and an

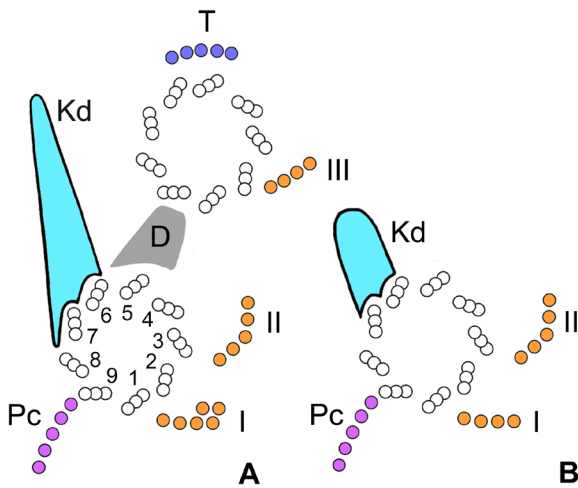


Fig. 8. Schematics of somatic kinetids in *Schmidingerella meunieri*, namely of a dikinetid (A) and a monokinetid (B). The postciliary ribbons consist probably of four or five microtubules. The structure of ribbon III is somewhat variable, ranging from one layer containing four microtubules to two layers with six microtubules as in ribbon I. 1–9, triplets 1–9; I–III, extraordinary microtubular ribbons I–III; D, desmose; Kd, kinetodesmal fibril; Pc, postciliary ribbon; T, transverse ribbon.

axosome, but no central microtubules (Ruffolo 1976, Görtz 1982, Morelli et al. 1996); thus, they are similar to those in *S. meunieri*, except for the apparent lack of microtubular doublets. The function of the condylocilia is unclear.

The lumen of the euplotid kinetosome contains a row of electron-dense granules (“core granules”) about 35 nm across (Ruffolo 1976), whereas that of the kinetosomes in *S. meunieri* contains two, rarely three scattered electron-dense granules about 50 nm across as recognisable in basal bodies of the oral ciliature.

The dikinetids are clockwise inclined with approximately the same angle in relation to the kinety axis in *S. meunieri*, the euplotids, and the hypotrichs [inferred from illustrations in Ruffolo (1976) and Grimes and Adler (1976)] and their cilia protrude from ciliary pits; data on oligotrichids are not available.

In contrast to euplotids, hypotrichs, and oligotrichids, the tintinnid *S. meunieri* has not only more kinetid types (three vs. one) in its somatic kineties, but also mobile cilia of different lengths ranging from the common (3–6 μm long) cilia to the elongated (up to 15 μm long) cilia in the dorsal kinety and the anterior dikinetids of the right and left ciliary fields.

Phylogenetic inferences

The preliminary data on the ultrastructure of somatic kinetids shall cladistically be analysed by discussing the character states and their distribution among the taxa (Fig. 9, Table 1). The euplotids are used as out-group for the following argumentation.

Kinetid structure. Dikinetids with cilia only at the anterior basal bodies are regarded as the plesiomorphic character state (coded 0). Following the “Kinetid Transformation Hypothesis”, the posterior dikinetidal basal bodies became ciliated (coded 1). Then, the anterior cilia were lost (coded 2) and finally the anterior basal bodies, resulting in ciliated monokinetids (coded 3) (Agatha and Strüder-Kypke 2014). Probably, this process happened three times independently in choreotrichids.

Orientation of dikinetidal basal bodies. The basal bodies of a ciliate dikinetid are parallel to each other (coded 0), except for the diverging ones of the oligotrichids (coded 1).

Condylocilia. The presence of condylocilia associated with the posterior dikinetidal basal bodies in euplotids (coded 1) and with the anterior dikinetidal basal bodies in tintinnids (coded 2) is considered an apomorphic character state that developed several times independently.

Kinetodesmal fibril. Since kinetodesmal fibrils are usually permanent (coded 0), their transient nature in hypotrichs is regarded as apomorphy (coded 1) like their apparent absence in strobilidiids (coded 2).

Postciliary ribbon at anterior basal body. A single postciliary microtubule originates at the anterior basal body in euplotids and hypotrichs (coded 0), but is absent in the other taxa under consideration (coded 1).

Postciliary ribbon at posterior basal body. The presence of divergent postciliary microtubules at the posterior basal bodies is regarded as plesiomorphic character

state (coded 0), and their absence as an apomorphy of the strobilidiids (coded 1).

Long stacked postciliary ribbons. The “false postciliodesmata” of tintinnids are long, stacked postciliary ribbons which are apparently also stained with protargol. Since they are neither known from strobilidiids nor from oligotrichids, hypotrichs, or euplotids, their presence is considered an apomorphy of the tintinnids (coded 1).

Transverse ribbon. While the euplotid outgroup, the hypotrichs, and tintinnids have single-layered transverse ribbons (coded 0), the leftwards directed microtubular ribbons of the strobilidiid kinetids are two-layered; thus, they probably represent another structure or a derived character state (coded 1).

Parasomal sac. These organelles are a widespread feature; therefore, their absence is considered a synapomorphy of the hypotrichs and Oligotrichea (coded 1). Although data on oligotrichids are not available, this is the most parsimonious assumption.

Extraordinary microtubular ribbons. The connective ribbon I, the posterior oblique ribbon II, and the anterior oblique ribbon III have not previously been described, but had been shown in a TEM micrograph of the tintinnid *Ptychocylis minor* by Hedin (1976); hence, these ribbons are regarded as tintinnid apomorphies (coded 1). However, it cannot be excluded that

they already evolved in the last common ancestor of the Oligotrichea or of the choreotrichids and had secondarily been lost in the strobilidiids (see below).

The present analyses support the previous observations that the structure of somatic kinetids in the spirotrichs is apparently less conserved than in other groups of ciliates (Lynn 2008); particularly, the choreotrichids demonstrate a considerable variability. Further, some of the discovered differences in the kinetid ultrastructures corroborate the genealogies inferred from genetic and previous cladistic analyses (Agatha and Strüder-Kypke 2007, 2014; Santoferrara *et al.* 2017).

Owing to the scarcity of data on the ultrastructure of somatic kinetids in oligotrichids and choreotrichids, the first occurrence of the three extraordinary microtubular ribbons remains uncertain. Four alternatives for their origin shall be discussed below: (i) they are restricted to tintinnids with a ventral kinety; (ii) they occurred first in the ancestor of all tintinnids; (iii) they are specific for choreotrichids and had secondarily been lost by strobilidiids; or (iv) they evolved in the ancestor of the Oligotrichea with a secondary loss in strobilidiids.

In contrast to most ciliate classes, the ultrastructure of the somatic kinetids of Oligotrichea might provide a phylogenetic signal on family level, the systematically problematic rank; yet, more data are required.

Table 1. Kinetid ultrastructure in euplotids, hypotrichs, oligotrichids, and choreotrichids, and the distribution of the character states. Please, note that euplotids, hypotrichs, oligotrichids, and strobilidiids possess invariably one type of somatic kinetid, while the somatic ciliature of the other choreotrichids, including tintinnids, comprises both monokinetids and dikinetids with one or two cilia. For coding see text

Characters	Euplotids	Hypotrichs	Oligotrichids	Choreotrichids						
				Others			Strobilidiids	Tintinnids		
Kinetid type	0	0	0	0 ^a	1 ^a	2 ^a	3 ^b	1	2	3 ^b
Orientation of basal bodies	0	0	1	?	?	?	–	0	0	–
Condylolium in dikinetid*	1	0	0	?	?	?	–	0	2	–
Kinetodesmal fibril*	0	1	?	?	?	?	2	0	0	0
Postciliary ribbon at anterior basal body	0	0	?	?	?	?	–	1	1	–
Postciliary ribbon at posterior basal body	0	0	?	?	?	?	1	0	0	0
Long stacked postciliary ribbons	0	0	?	?	?	?	–	1	1	1
Transverse ribbon	0	0	?	?	?	?	1	0	0	–
Parasomal sac	0	1	?	?	?	?	1	1	1	1
Microtubular ribbons I & II	0	0	?	?	?	?	0	1	1	1
Microtubular ribbon III	0	0	?	?	?	?	–	1	1	–

* unordered character state

^a in a single species, up to two kinetid types may be found

^b homoplasy as indicated by differences in the fibrillar associates

– not applicable in monokinetids which presumably represent the previously posterior dikinetidal basal bodies

? unknown character state

Some thoughts about evolutionary constraints

The discovery of new microtubular ribbons associated with the somatic kinetids in the loricate choreotrichids raises questions about the evolutionary constraints that might have fostered their development. These, however, depend on the first occurrence of the three extraordinary ribbons.

Ancestor of Oligotrichea. The obvious change in life style (benthic to planktonic) in concert with modifications in the cell shape (dorsoventrally flattened to globular or obconical), position (ventral to apical) and function (feeding to feeding plus locomotion) of the adoral zone, and the mode of movement (crawling by ventral cirri on the substrate to swimming in the water column) might provide clues for this innovation in the monophyletic Oligotrichea.

Additionally or alternatively, the dorsalisation process suggested by Foissner et al. (2004) might have caused the ultrastructural changes in the kinetids. Owing to the hypothesised reduction of the flattened ventral side with the cirri and the membranellar zone anchored by various microtubular ribbons on the one hand and an expansion of the dorsal side with a few longitudinal somatic kineties on the other hand, the emerging globular, oligotrichous cells of the Oligotrichea possessed less stabilising microtubular ribbons than the hypotrich sister group. As a substitute, the microtubular ribbons I, II, and III and the overlapping postciliary microtubular ribbons perhaps evolved. Whether these structures – if present – provide enough rigidity in the oligotrichids with only a girdle and a ventral kinety is questionable. Likewise, a stronger anchoring of the somatic cilia paralleling a change in their function is not a plausible

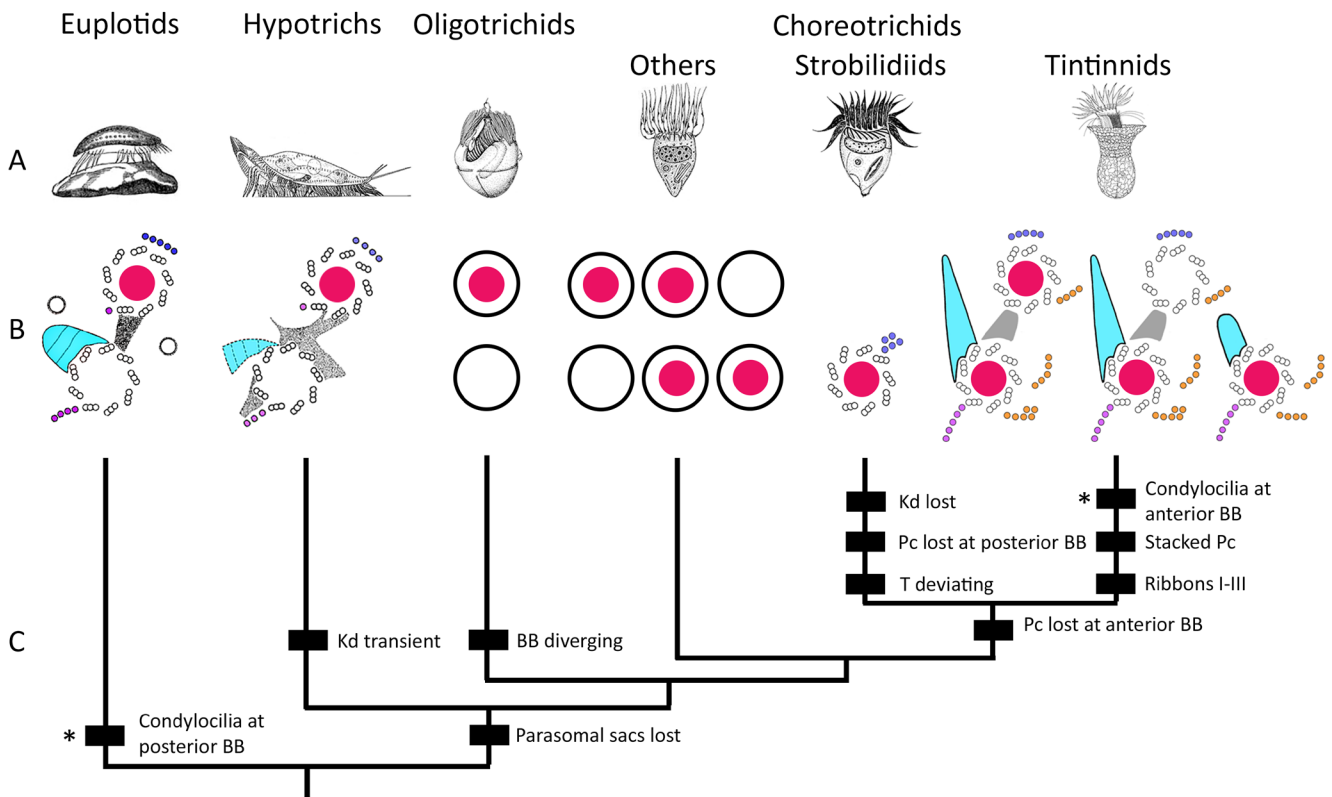


Fig. 9. Tree displaying the phylogenetic relationships based on morphological and genetic data of euplotids, hypotrichs, oligotrichids, and choreotrichids. (A) Overall morphologies of the taxa [illustrations: euplotid after Ehrenberg (1830); hypotrich from Deitmer et al. (1984); oligotrichid from Montagnes (1996); choreotrichids from Petz et al. (1995), Petz and Foissner (1992), and Gruber et al. (2018)]. (B) Kinetid structures. For strobilidiids, inferred from micrographs and description in Grim (1987). (C) Molecular genealogy with apomorphies (black squares) in the kinetid ultrastructures mapped on the branches with the most parsimonious placement based on the current state of knowledge. Black circles represent basal bodies and red dots associated common cilia as inferred from protargol-stained material. Asterisks mark homoplasy. Dark blue, transverse ribbon; grey, desmose; light blue, kinetodesmal fibril; orange, extraordinary microtubular ribbons; purple, postciliary ribbon; BB, basal body; Kd, kinetodesmal fibril; Pc, postciliary ribbon; T, transverse ribbon.

explanation for the oligotrichids with their short, stiff/immobile, and potentially sensory cilia. In these ciliates, however, extrusomes (Modeo *et al.* 2001) and the cortical platelets of the hemitheca might have increased the cell's rigidity and thus counterbalanced the paucity of somatic kineties.

Ancestor of choreotrichids. The choreotrichids comprise the paraphyletic grouping of aloricate taxa and the monophyletic tintinnids. The highly diverse somatic ciliary patterns and kinetid types in the aloricate taxa in combination with usually rather short and probably stiff/immobile cilia again reject the idea that a change in cilia function was responsible for the introduction of the additional fibrillar associates, especially as the kineties are often restricted to the posterior cell portion and are few in numbers. Moreover, *Rimostrombidium lacustre* [reported as *Strobilidium velox* Fauré-Fremiet, 1924 by Grim (1987)] apparently lacks homologous structures in its monokinetids.

Ancestor of tintinnids. The tintinnids are unique planktonic ciliates as they carry a lorica attached to their contractile peduncle. The movements of the adoral membranelles thus not only propel the cell through the water column, but also the more or less voluminous lorica. This might have necessitated a stiffer cell proper. Actually, our data on the tintinnid *S. meunieri* reveal a stabilising network formed by the fibrillar associates of the somatic kinetids which are usually restricted to the anterior portion of cell proper.

Additionally, the few distinctly elongated cilia in tintinnids (the anteriormost cilia in the right and left fields, the dorsal and posterior cilia, the cilia in the posterior portion of the ventral kinety) are supposed to be involved in the formation and cleaning of the lorica as well as in stabilising the cell inside the lorica (Laval-Peuto and Brownlee 1986); therefore, they might have needed a stronger anchoring in the cell cortex.

CONCLUSION

Although new microtubular ribbons and kinetid structures were discovered, the findings of the present study emphasise further the gaps in our knowledge concerning the ultrastructure of somatic kinetids mainly in the oligotrichids and aloricate choreotrichids and prompt future studies on the kinetid evolution and its constraints.

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