J. Mar. Biol. Ass. U.K. (2000), **80**, 989–994
Printed in the United Kingdom

A study on a new species of *Tontonia* (Ciliophora: Oligotrichida) from the East China Sea and adjacent sea areas

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Morphometric characteristics and spatial distribution of *Tontonia grandis* sp. nov. were investigated in the northern East China Sea and the adjacent sea areas. *Tontonia grandis* has a large conical body shape, 113 μm (70–155 μm) in length by 55 μm (40–113 μm) in width, a caudal appendix, multiple macronuclei and 14 (13–16) anterior and 28 (24–33) ventral polykinetids. Some cells have numerous cortical platelets on the surface and others do not. These two forms showed different horizontal distribution, and hence the presence of cortical platelets might be influenced by the environment inhabited. However, various forms of caudal appendix were observed, which constitute a continuous series of their developmental stages. This result might suggest that the caudal appendix is replaced by new growth and repeats its developing and sloughing spontaneously.

INTRODUCTION

The oligotric ciliates are important components in the marine microbial food webs which sometimes dominate among the ciliate community in the surface ocean (Montagnes et al., 1988a; Lynn et al., 1991). Since their species character is difficult to distinguish under normal microscopic observation, such as the Utermöhl method and Sedgwick–Rafter method, they have been described recently with the protargol impregnation method (e.g. Montagnes & Lynn, 1987; Skibbe, 1994). This staining method displays their accurate morphometrics and will reveal their diverse assemblages in the ocean.

Genus *Tontonia*, belonging to order Oligotrichida, frequently occurs in the ocean. *Tontonia* ciliates have a caudal appendix and this peculiar character enables us to distinguish them from the other oligotric ciliates such as *Strombidium*. *Tontonia* was originally described by Fauré-Fremiet (1914) and seven *Tontonia* species have been reported to date. Among them, two species, *T. appendiculariformis* Fauré-Fremiet, 1914 and *T. caudata* Lohmann, 1908, are difficult species to identify, because their morphological descriptions were not based on impregnated materials. On the other hand, five other species have been described recently in detail: *T. antarctica* Pets et al., 1995; *T. gracillima* Fauré-Fremiet, 1924 *sensu* Lynn et al., 1988; *T. poopsia* Montagnes & Lynn, 1988; *T. turbinata* Song & Bradbury, 1998; and *T. simplicidens* Lynn & Gilron, 1993. The accurate morphometrics of these five species have been derived from impregnated materials. Protargol staining would help to clarify the diverse *Tontonia* species in the ocean.

During the study of planktonic ciliates in the East China Sea, a new *Tontonia* species occurred in the water column. Its morphometrics are distinguishable from the previously described species. This study provides a description and ecological characters of this *Tontonia* as a new species.

MATERIALS AND METHODS

Water was collected with a rosette multisampler attached with a CTD (conductivity–temperature–depth probe) from 10 to 16 May, 1997 on the 97th cruise of T/S 'Nagasaki-maru' (Figure 1). Surface water was taken in the East China Sea (12 stations), the Korea Strait (one station) and the Yellow Sea (four stations). Water column sampling between surface and 43–100 m depth was also done at four stations, stations 6–9, along 32°N in the East China Sea (16 samples). Salinity and temperature were recorded simultaneously. The 100 ml water was fixed immediately on-board with Bouin's solution at a final concentration of 5%. After returning to the laboratory, the samples were stained and mounted using the QPS (quantitative protargol staining) method (Montagnes & Lynn, 1987). Cells were observed and counted under ×400 and ×1000 magnification using a microscope (Zeiss Axioplan). For the metric and morphometric measurements, longitudinally oriented specimens were examined. The detection limit on cell counting is 10 cells l^{-1}.

RESULTS

Tontonia grandis *sp. nov.*
(Figures 2–4, Table 1)

Diagnosis

Body generally conical, 113 μm (70–155 μm) in length by 55 μm (40–113 μm) in width, with a caudal appendix; multiple macronuclei, 14 (13–16) anterior and 28 (24–33) ventral polykinetids.

Deposition of type material

A holotype of *Tontonia grandis* on a slide of protargol impregnated cells has been deposited in the Faculty of Fisheries, Nagasaki University, Nagasaki, Japan.

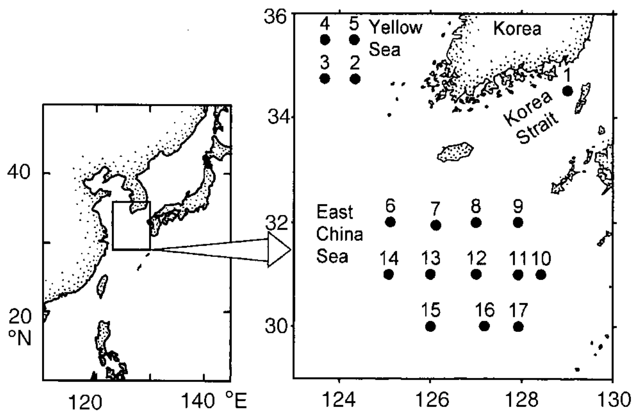

Figure 1. Sampling stations on the cruise of T/S 'Nagasaki-maru' from 10 to 16 May, 1997. Numerals indicate station number.

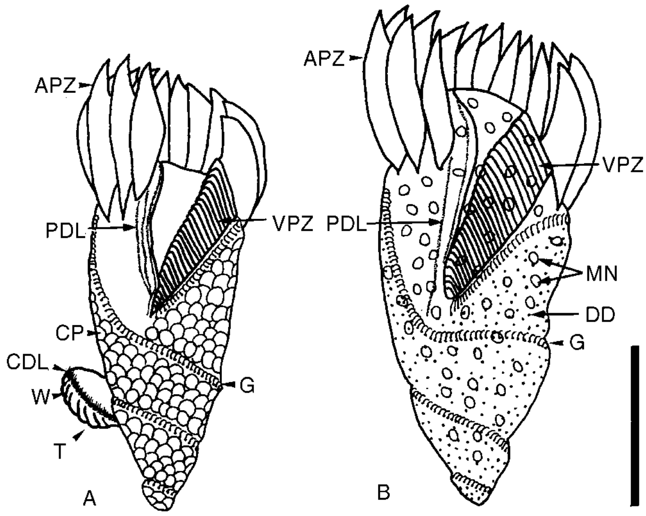

Figure 2. Schematic figures of protargol stained *Tontonia grandis*: (A) plated-form; and (B) aplated-form. Macronuclei in (A) and tail in (B) are omitted. APZ, anterior polykinetid zone; CDL, ciliated dark-stained line; CP, cortical platelet; DD, dark-stained dot; G, girdle; MN, macronuclei; PDL, paroral dark-stained line; T, tail; VPZ, ventral polykinetid zone; W, wrinkle. Scale bar: 50 μm.

Etymology

The specific epithet, *grandis* (Latin= large), refers to the large size of this species.

Description

Cell shape rather constant, twisted conical due to the body whorls, with a caudal appendix. Oral groove opened longitudinally down, about 1/2 of the body length. Cell surface slightly swelling to produce a series of bulges associated with whorls. Ventral polykinetid zone (VPZ) separated to anterior polykinetid zone (APZ). APZ of equal length surrounding anterior end; VPZ lying on left side of oral groove. Paroral dark-stained lines, which should be paroral membrane and usually consists of two or three lines, on the right side of the oral groove. Girdle (G) of monokinetids with short stubby cilia forming a sinistral (when viewed from anterior) helix of 3.3 (3.0–3.5) whorls. It originates posteriorly left of the oral groove, runs to the left lateral side along the left margin of the oral groove, turns around horizontally at posterior base of APZ on dorsal side, curves posteriorly on right lateral side, continues to ventral surface and spirals sinistrally to near posterior apex. Multiple macronuclei, ovoid and sometimes elongate shaped, located throughout the cytoplasm. Trichites often numerous inserting anterior to G and extending internally posteriorly. A dark-stained L-shaped line, ridge-like structure stained by protargol, immediately anterior to G of the second spiral on the dorsal side. No ventral kinety observed. Cortical platelets sometimes clearly observed, round or elliptical shape of 4–6 μm diameter overlapping in the manner of anterior-outside and posterior-underside, covering the cell surface from G of the first spiral to posterior apex of cell, except for G, caudal appendix and periphery of L-shaped line. A comma-shaped caudal appendix developing from the L-shaped line frequently observed, 29 μm (15–43 μm) long and 18 μm (12–28 μm) wide equipped with wrinkles and a dark-stained line composed of ciliated several rows.

Morphological variation

This species can be divided into two forms based on the existence of cortical platelets (Figures 2 & 3, Table 1): plated-form, having round or elliptical cortical platelets on cell surface; aplated-form, lacking the platelets. Aplated-form possesses numerous dark-stained dots instead of the cortical platelets on the same part of cell surface. The division between these two forms was not always strict, because some specimens have fewer platelets covering only a part of the cell surface (Figure 4C). The body length, body width, oral length and number of ventral polykinetids are slightly larger in the aplated-form, though their ranges are overlapping, respectively (Table 1).

A caudal appendix is not always a complete shape; some individuals lack the appendix (atailed-form). Between both atailed- and tailed-forms, various transitional forms are observed and they constitute a series of vicissitudes: almost flat around L-shaped line on dorsal side, small protuberance from L-shaped line, ovoid with a non-ciliated dark-stained line, comma-shaped with a ciliated dark-stained line and wrinkles on one side or both sides, comma-shaped with narrow connection to body, and detached free form (Figure 5). These continuous variations were recognized both on the plated-form and aplated-form.

Comparison

The above description is based on the observation of over 50 specimens from the East China Sea and the Korea Strait. This new species is similar to *Tontonia appendiculariformis* Fauré-Fremiet, 1914 in body size, a large and long oral groove, a caudal appendix, a dark-stained mark at the root of appendix and multiple macronucleus. However, *T. grandis* has a large number of anterior polykinetids (APk) (13–16) than *T. appendiculariformis*; the latter has at most seven APks ('quatre lignes ciliaires' and 'trois éléments vibratiles'; Fauré-Fremiet, 1914). The APZ of *T. grandis*, surrounding the most anterior end of peristome, differs from that of *T. appendiculariformis*, situating only on the left edge of anterior part. Body shape

https://doi.org/10.1017/S0025315400003052 Published online by Cambridge University Press

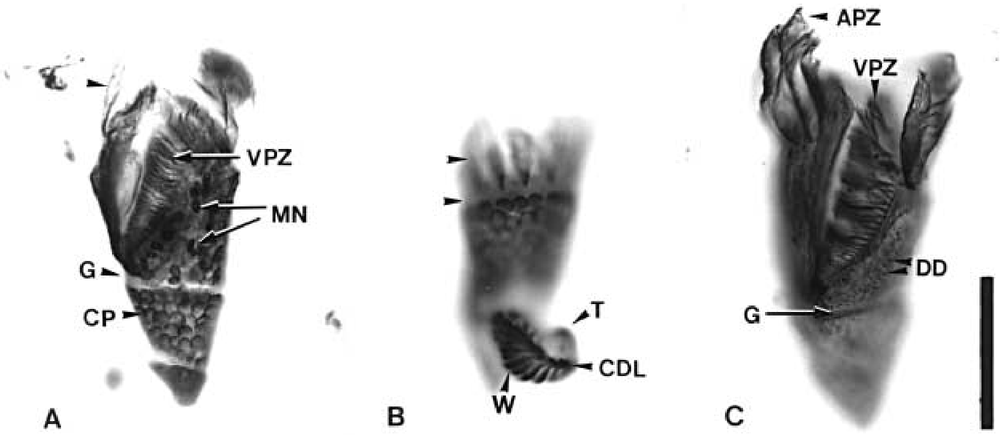

Figure 3. Micrographs of protargol stained *Tontonia grandis*: (A) ventral view of plated-form indicating a sinistral girdle and cortical platelets; (B) dorsal view of plated-form indicating a tail; (C) ventral view of aplated-form indicating dark-stained dots. APZ, anterior polykinetid zone; CDL, ciliated dark-stained line; CP, cortical platelet; DD, dark-stained dot; G, girdle; MN, macronuclei; VPZ, ventral polykinetid zone; W, wrinkle. Scale bar: 50 μm.

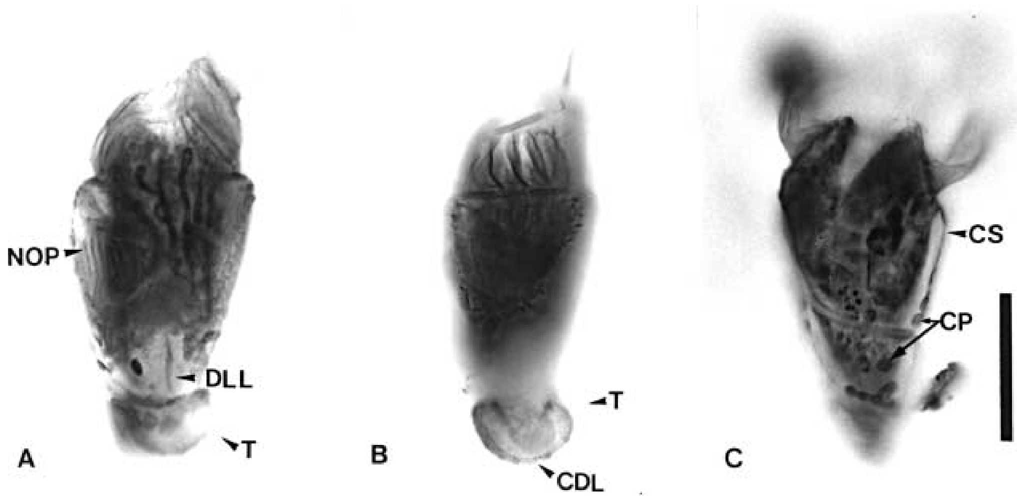

Figure 4. Micrographs of protargol stained *Tontonia grandis*: (A) dorsal view indicating a new oral part, a tail and a dark-stained L-shaped line; (B) the same cell as (A) in shallower microscopic focus indicating a tail and a ciliated dark-stained line; (C) ventral view indicating sparsely arranged cortical platelets. CDL, ciliated dark-stained line; CS, distended cell surface; CP, cortical platelet; DLL, dark-stained L-shaped line; NOP, new oral part; T, tail. Scale bar: 50 μm.

Table 1. *Morphometric characters for plated-form and aplated-form in* Tontonia grandis *sp. nov.*

	Length ±SD (μm)	Width (μm)	Oral length (μm)	APk number	VPk number	Helix of G number
Plated form	103.5 ±14.0	48.8 ±4.9	53.9 ±5.2	13.8 ±0.7	26.9 ±1.5	3.3 ±0.1
N	37	37	32	34	34	35
Range	70–138	40–62	43–65	13–15	24–31	3.0–3.5
Aplated form	126.9 ±16.0	63.2 ±2.1	68.0 ±5.6	13.9 ±0.8	30.1 ±1.6	3.3 ±0.1
N	27	27	27	27	27	27
Range	104–155	48–113	55–80	13–16	27–33	3.0–3.5

Length, maximum cell length (excluding cilia of anterior polykinetids); width, maximum cell diameter; APk, anterior polykinetids; VPk, ventral polykinetids; G, girdle; N, sample size.

(conical vs ovoid) and peristomial collar (flat vs scooped-out on dorsal side) are also different. Cortical platelets do not cover the anterior end in *T. grandis*. A spiralled G is not recognized in *T. appendiculariformis*.

In comparison with *Tontonia tubinata* Song & Bradbury, 1998, *T. grandis* has a similar morphological character in APk number (13–16 vs 14–16), a spiralled G, a caudal appendix with ciliated kinety, root position of caudal

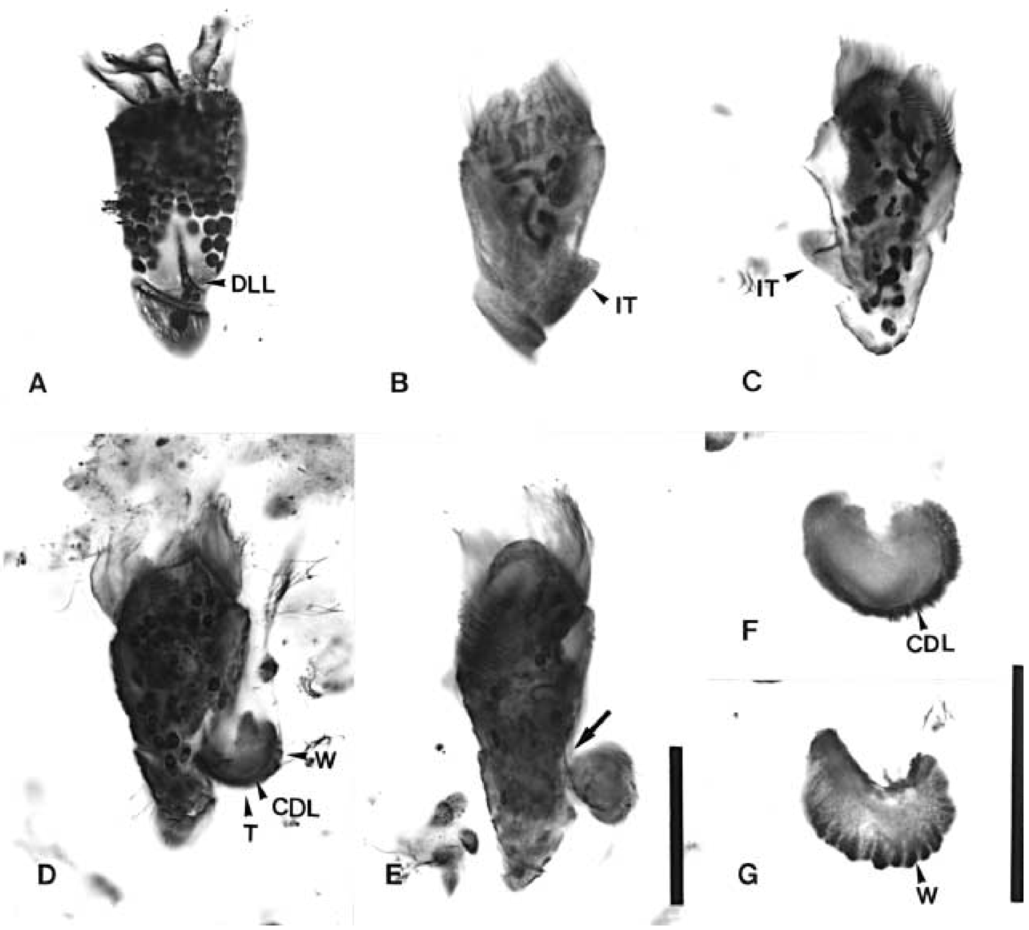

Figure 5. Micrographs of protargol stained *Tontonia grandis*: (A) dorsal view indicating a dark-stained L-shaped line; (B) dorsal-left view indicating an immature tail originated from the line; (C) right lateral view indicating an immature tail with a dark-stained line on the surface; (D) dorsal-left view indicating a matured tail with a ciliated dark-stained line and wrinkles; (E) left lateral view indicating a narrow connection of tail (arrow); (F) separated tail indicating a ciliated dark-stained line; (G) the same cell as (F) in shallower microscopic focus indicating wrinkles. CDL, ciliated dark-stained line; DLL, dark-stained L-shaped line; IT, immature tail; T, tail; W, wrinkle. Scale bar: 50 μm.

appendix and multiple macronucleus. However, it differs in VPk number (24–33 vs 13–17), cell length (70–155 vs 50–70 μm) and cell width (40–113 vs 20–35 μm).

A spiralled G and multiple macronucleus are also the characteristics of *Laboea strobila* Lohmann, 1908. *Laboea strobila*, however, neither possesses a caudal appendix, a generic character of *Tontonia*, nor a trace of appendix such as an L-shaped line. On the other hand, *T. grandis* does not have ventral kinety, the short form of which kinety is one of the generic characters of *Laboea*. Compared with *L. strobila sensu* Montagnes et al., 1988b, *T. grandis* has few helices of G (3.0–3.5 vs 4–5) and more VPk number (24–33 vs 12–22).

Ecological characteristics

Tontonia grandis was observed in the East China Sea and the Korea Strait. The ranges of water temperature and salinity where this species occurred are 14.5–24.9°C and 30.7–34.5 psu, respectively. The maximum abundance of 2220 cells l^{-1} (1920 cells l^{-1} in plated-and-tailed form and 300 cells l^{-1} in plated-and-atailed form) was observed at the surface of station 15. Red tide of *Noctiluca scintillans* was also observed at this station, while another station in this red tide area showed a small abundance of *T. grandis* (30 cells l^{-1} at station 14) (Figure 6). This species generally occurred in the shallower layer (Figure 7).

The environment for the occurrence of plated-form was not necessarily identical to that of aplated-form; the former occurred even in lower temperature (14.5–22.5 vs 18.1–24.9°C) and lower salinity (30.7–34.4 vs 32.6–34.5 psu). Spatial distribution is therefore different between these two forms. Plated-form generally occurred in the western part of sampling area and its maximum abundance was 2220 cells l^{-1} at the surface of station 15 (Figure 6A,B). On the other hand, aplated-form distributed in the eastern part and its maximum was 90 cells l^{-1} at the surface of station 11 (Figure 6C,D).

Appreciable difference was not observed in the inhabited environment between the tailed-form and atailed-form. The former form occurred at a similar temperature range to the latter (14.5–24.9 vs 14.5–22.5°C), while tailed-form occurred even in slightly lower salinity (30.7–34.5 vs 32.2–34.4 psu). Maximum abundance of both forms was observed simultaneously at the surface of station 15 (1920 cells l^{-1} in tailed-form and 300 cells l^{-1} in atailed-form; Figure 6A,B). Vertical distributional pattern was mostly similar between these forms (Figure 7).

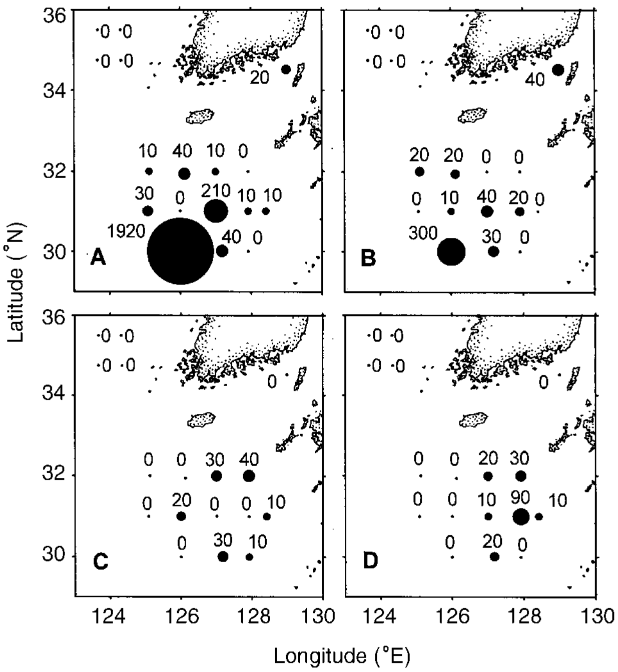

Figure 6. Horizontal distribution of *Tontonia grandis* at the surface of the East China Sea and the adjacent sea areas: (A) plated-and-tailed form; (B) plated-and -atailed form; (C) aplated-and-tailed form; (D) aplated-and-atailed form. Numerals indicate ciliate abundance (cells l^{-1}).

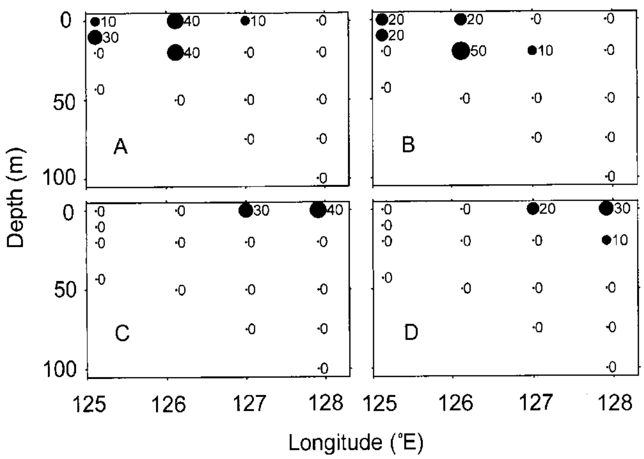

Figure 7. Vertical distribution of *Tontonia grandis* along a 32°N line in the East China Sea: (A) plated-and-tailed form; (B) plated-and-atailed form; (C) aplated-and-tailed form; (D) aplated-and-atailed form. Numerals indicate ciliate abundance (cells l^{-1}).

DISCUSSION

Various tailed forms were observed in *Tontonia grandis* and this variation constituted a series of vicissitudes from atailed-form to detached-form through immature and mature tailed-form (Figure 5). This continuous variation might indicate that the caudal appendix repeats its developing and sloughing routinely in nature. Furthermore, since the tailed-form was predominant in abundance at many stations, the duration of the tailed-form stage might be longer than that of the atailed-form stage in the studied sea areas. However, the caudal appendix has fragile-looking connection in its developed stage. It may be easily damaged and detached by physical stimuli such as sampling procedure and Bouin's fixation. If so, the expectation of tail replacement is not always absolute.

Although the postal protuberance of *Strombidium ovale* looks like a tail and it is recognized as a new daughter cell in the first stage of cell division (Maeda & Carey, 1985), the caudal appendix of *T. grandis* must not fill such a role because it also occurred even when a new oral part was forming around the postal half of the cell body (Figure 4A,B). However, the microgamete of *Tintinopsis beroidea*, which belongs to the suborder Tintinnina, also looks like a tail at the late stage of its formation (Gold & Pollingher, 1971). This tail like microgamate of *T. beroidea* has a specific character in lacking a macronucleus, detaching from the mother cell and having self-motile ability. The latter ability was not recognized in the appendix of *T. grandis*. Live cell observation is indispensable to clarify the role and function of the caudal appendix of *T. grandis*.

Cortical platelets are reported in some other oligotric species, e.g. *Laboea strobila* (Wulff, 1919; Fauré-Fremiet, 1924), *Strombidium inclinatum* (Montagnes et al., 1990), *S. sulcatum* (Fauré-Fremiet & Ganier, 1970), *S. viride* (Kahl, 1932; Foissner et al., 1991), *S. wulffi* (Kahl, 1932) and *Tontonia appendiculariformis* (Fauré-Fremiet, 1914; Fauré-Fremiet, 1924). Among these species, however, the aplated-form is also described in *L. strobila* (Montagnes et al., 1988b) and *S. wulffi* (Lynn et al., 1988). The alternation between plated- and aplated-forms might not be a unique case.

Geographical distribution is different between plated- and aplated-forms in this area; the plated-form occurs mainly in the western part and the aplated-form occurs more in the eastern part. The former is more influenced by the eutrophic coastal water and the latter by the oligotrophic water deflected from the Kuroshio Current. This difference in habitat might have a causal relation to the presence of cortical platelets. Laval-Peuto & Febvre (1986) proposed a hypothesis that cortical platelets on *T. appendiculariformis* are polysaccharide products of enslaved functional plastids. If this hypothesis is correct and applied to *T. grandis*, this species has mixotrophic character in nutritional mode and furthermore the alternation between plated-form and aplated-form might be due to the amount and/or activity of enslaved plastids.

We thank Captain Takagi and staffs of T/S 'Nagasaki-maru' for help of sampling. This study was partially supported by KOSEF-JSPS exchange programme and research grant (10660185) from the Japan Ministry of Education, Science and Culture.

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Submitted 27 April 2000. Accepted 11 September 2000.