DR. SHINYA SATO (Orcid ID : 0000-0001-6649-6975)

DR. SHINYA YOSHIKAWA (Orcid ID : 0000-0002-6629-1539)

Article type : Regular Article

Ontogenetic analysis of siliceous cell wall formation in *Triparma laevis* f. *inornata* (Parmales, Stramenopiles)

Kazumasa Yamada

Faculty of Environmental & Symbiotic Sciences, Prefectural University of Kumamoto, 3-1-100 Tsukide, Kumamoto 862-8502, Japan

Hirotaka Katsura

Department of Marine Science and Technology, Fukui Prefectural University, 1-1 Gakuen-cho, Obama City, Fukui 917-0003, Japan

Mary-Hélène Noël

National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan

Mutsuo Ichinomiya

Faculty of Environmental & Symbiotic Sciences, Prefectural University of Kumamoto, 3-1-100 Tsukide, Kumamoto 862-8502, Japan

Akira Kuwata

Tohoku National Fisheries Research Institute, 3-27-5 Shinhama-cho, Shiogama, Miyagi 985-0001, Japan

Shinya Sato

Department of Marine Science and Technology, Fukui Prefectural University, 1-1 Gakuen-cho, Obama 917-0003, Japan

Shinya Yoshikawa[1]

Department of Marine Science and Technology, Fukui Prefectural University, 1-1 Gakuen-cho, Obama City, Fukui 917-0003, Japan

[1]Author for correspondence: Email syoshika@fpu.ac.jp, Phone +81-770-52-9608, Fax +81-770-52-9608

Running title: Siliceous plate development in Parmales

Editorial Responsibility: R. Wetherbee (Associate Editor)

ABSTRACT

Triparma laevis f. *inornata* is a unicellular alga belonging to the Bolidophyceae, which is most closely related to diatoms. Like diatoms, *T. laevis* f. *inornata* has a siliceous cell wall. The cell wall of *T. laevis* f. *inornata* consists of four round plates (three shields and one ventral plate) and one dorsal and three girdle plates. But, unlike diatoms, *T. laevis* f. *inornata* cells can grow when concentrations of silica are depleted. We took advantage of this ability, using TEM to study the ontogeny of the siliceous plate, pattern center formation and development. Two types of pattern centers (annulus and sternum) were observed in the early and middle stage of plate formation. During their formation, the annuli were initially crescent-shaped but eventually their ends fused to make a ring. Only outward silica

deposition of the branching ribs occurred on the growing annulus until it became a ring, resulting in an unfilled circle inside the annulus. The pattern center of the shield plate was always an annulus, but in ventral plates both annulus and sternum were observed. The annuli and sterna in *T. laevis* f. *inornata* round plates were very similar to the annuli and sterna in diatom valves. These results suggested that the round plates of Parmales are homologous to diatom valves. This information on the plate ontogeny of *T. laevis* f. *inornata* provides new insights into the evolution of the siliceous cell wall in the Parmales and diatoms.

KEY WORDS: annulus, diatom, pattern center, scale, sternum

INTRODUCTION

The Order Parmales is a group of small (2-5 um) unicellular marine phytoplankton. Members of this order are distributed widely from tropical to polar waters (Booth and Marchant 1987, Guillou 2011, Ichinomiya et al. 2016). Although members of the Parmales were assumed to be related to diatoms on the basis of morphological similarities of their cell walls (Mann and Marchant 1989), they were tentatively classified into the Chrysophyceae (Booth and Marchant 1987). Ichinomiya et al. (2011) established a culture of *Triparma laevis* f. *inornata* NIES-2565, the first cultured strain of any Parmales, and showed it was placed within a clade of

Bolidomonas, a flagellated sister group of diatoms but entirely lacking in siliceous cell components (Guillou et al. 1999). Given the phylogenetic relationship, Ichinomiya et al. (2016) proposed an emended description of the Bolidophyceae: the members of the genus *Bolidomonas* have now been included in the genus *Triparma*, and thus this currently includes non-flagellated siliceous forms and flagellated non-siliceous forms.

The cell wall of *T. laevis* consists of three round plates of equal size (shield plates), one large round plate (ventral plate), one dorsal plate and three girdle plates (Fig. 1; Booth and Marchant 1987, Konno et al. 2007). The ventral plates have characteristic ornaments, circular ridges, which enable us to distinguish the ventral and shield plates. Mann and Marchant (1989) noted that the central ring (annulus) and the pattern of the branching ribs seen in shield plates of *T. columacea* and *T. retinervis* were very similar to those of incunabular scales in the auxospore and vegetative valves of centric diatoms. Homology assessment of mature valves in diatoms has effectively been made using ontogeny information; that is, superficially similar structures have developed through totally different processes, which strongly indicates morphological convergence. Examples include the spine-like extrusion of *Proboscia* vs *Rhizosolenia* (van de Meene and Pickett-Heaps 2004) or the canals of *Diploneis* vs *Fallacia* (Idei et al. 2018). Although ontogenetic study of the Parmales has long been needed, the absence of a culture strain has hindered such an approach.

The culture strain *T. laevis* f. *inornata* is becoming a model strain for the Parmales, with growing research into its physiology (Yamada et al. 2014), cell biology (Yamada et al. 2016, Yamada et al. 2017) and organellar genome architecture (Tajima et al. 2016). We have already demonstrated that this strain can grow at the same pace even if the cells developed incomplete (smaller, thinner or malformed) plates or were devoid of any plates because of limitations in or the absence of silica (Yamada et al. 2014, 2016). We also established that the regeneration of normal siliceous plates from initially naked cells could occur when sufficient silica was available (Yamada et al. 2014, 2016). Because the plate formation of *T. laevis* f. *inornata* can readily be induced by experimental control of silica concentrations in the culture medium, this strain is an ideal model to examine the ontogeny of the siliceous cell wall.

Information on valve ontogeny in centric diatoms has accumulated (e.g., Mann 1984, Mayama and Kuriyama 2002, Tiffany and Hernandez-Becerril 2005, Tiffany 2008, Sato 2010), however, to our knowledge little has been learned about the formation of the annulus itself (Li and Volcani 1985a,b), possibly because the deposition of such a tiny structure requires relatively small amounts of silica, and also because the process occurs rapidly during the overall process of valve ontogeny.

In this study, we observed plate ontogeny in *T. laevis* f. *inornata*, with a special emphasis on its earliest phase: the formation of the pattern center. We employed a strategy in which the culture medium contained depleted or very low amounts of silica to stop or slow down the

process, respectively. This is a strategy inapplicable to diatoms as their valve formation would be arrested entirely in the absence of a source of silica.

MATERIALS AND METHODS

Cell culture

Cells of *Triparma laevis* f. *inornata* NIES-2565 were cultured in modified Aquil medium (Ohki et al. 1986) under a 12-h light (daylight-type fluorescence lamps, 60 μmol · m^{-2} · s^{-1})/12-h dark photoperiod at 5°C. The cells were grown with silica-replenished or silica-depleted culture media containing 100 μM or 1 μM sodium metasilicate nonahydrate ($Na_2SiO_3 \cdot 9H_2O$), respectively, as the initial concentrations. Polycarbonate culture vessels were used to avoid any contamination of silica from glassware. Almost all cells grown with the silica-depleted medium became naked (i.e., no siliceous cell wall formed) but their growth rate was comparable to the cells grown with the silica-replenished medium (Yamada et al. 2014).

Induction of cell wall regeneration

To induce cell wall regeneration, silicate was added to silica-depleted cultures to a final concentration of 100 μM. The wall-regenerating cells were collected at intervals for observation with TEM.

TEM observations

The early and middle stages of plate ontogeny were investigated with cells harvested from the silica-depleted culture and cells were harvested within 16 h after silica replenishment, except for the early stage of girdle plate and the middle stage of dorsal plate formation. The other plates were observed in cells grown in the silica-replenished medium. The cells were collected through centrifugation at 2,000*g* for 15 min at 5°C. The cell pellet was boiled with concentrated sulfuric acid and potassium nitrate for 10 min to remove organic matter, and then washed several times with distilled water (Sato et al. 2004). Cleaned material was mounted on a formvar-coated copper mesh grid and viewed under TEM (JEM-1011, JEOL, Tokyo, Japan or HT-7700, Hitachi, Tokyo, Japan).

SEM *observations*

The cells were observed using SU1510 (Hitachi Hitachi-Technology, Tokyo, Japan). Preparation of specimens was as described previously (Yamada et al. 2014).

RESULTS AND DISCUSSION

The cell wall of *Triparma laevis* f. *inornata* consisted of various plates that could be divided morphologically into two types: (1) round (circular or disc-shaped, including shield and ventral plates); and (2) non-round (trifurcate dorsal and trapezoidal girdle plates; Fig. 1). Based on the outline of each plate it was possible to distinguish the two morphological types even during their morphogenesis. Within the round type, however, the shield and ventral plates were so similar that they were indistinguishable, particularly while they were small during early ontogeny. Therefore, unless otherwise noted, hereafter we use "round" refer to both the shield and ventral plates. Furthermore, for clarification we arbitrarily divided round plate ontogeny into three stages (early, middle and late), as follows.

Round plate early stage – pattern center formation

The earliest stage of round plate formation was investigated from cells cultured under silica-depleted conditions and from cells taken 4 h after replenishment of silica in the culture medium. Two types of pattern center, annulus (Fig. 2) and sternum (Fig. 3), were observed. The earliest ontogenetic stage of the annulus detected in this study was a structure with a somewhat curved axis, forming a crescent shape; ribs emerged sporadically from this, remaining at a distance from one another and growing asymmetrically only in an outward direction (Fig. 2, A and B). Although the earliest structures seemed to be extremely fragile, we were able to rule out the possibility that they were artificial (i.e., that they were broken fragments produced during sample preparation). It was relatively straightforward to check that silica deposition was occurring at its ends, because growing tips were fuzzy, possibly indicating the presence of silica nanoparticles (e.g., Fig. 2A), unlike the sharp ends of broken parts (not shown). Subsequently, the curved axis elongated further to become C-shaped and one-directional outgrowth of the ribs continued (Fig. 2, C–E). While the ribs radiated to form a circular outline, they occasionally branched (Fig. 2, A-F). Further elongation of the axis resulted in fusion of both ends to form a ring—the annulus—with well-developed branching ribs and an as-yet unfilled circular gap inside the annulus (Fig. 2F). Thick silica deposition lines on the annulus could be found, giving the appearance of one or two straight or curved dark bar(s) during TEM observations (Fig. 2, C–F). Some short ribs emerged from the

annulus, growing centrifugally but to lesser extent, with little or no bifurcation/branching (Fig. 2F).

In addition to early stages in the formation of annulus, we also found early stages in the ontogeny of round plates with linear pattern centers (sterna; Fig. 3). In the earliest stages of round plates with a sternum, ribs appeared to radiate from the center but an annulus was not observed (Fig. 3, top). In later stages, ribs which were located in the middle parts of the sternum extended vertically in both directions (Fig. 3, bottom).

Round plate middle stage – filling the outward part of the plates

Observations of the middle stage ontogeny of round plates were based on cells 6 h after replenishment of silica to the medium. The two types of pattern center were again observed. During this stage, silica deposition resulted in the fusion of neighboring ribs in both types (Fig. 4, A and B), to form a plain surface. In the case of circular plates, expansion of the branching ribs continued outwards so that the annulus remained an open area (Fig. 4A).

Round plate late stage – inward growth and three-dimensional development

Cells grown in the silica-replenished medium were harvested during the stationary phase to observe later-stage ontogeny. At this stage, silica deposition had ceased around the periphery, leaving the plate margins thin and only poorly defined. Secondary centrifugal thickening had occurred, probably starting from the pattern center, masking the gaps originally present between the bases of the ribs (Fig. 4, C and D). Further silica deposition on the pattern center resulted in the three-dimensional development of the plate-specific projections, making them more readily distinguishable: a small central projection and a discontinuous circular ridge were characteristic for the shield (Fig. 5A) and ventral plates (Fig. 5B), respectively. At the same time, centripetal silica deposition took place to fill the circular space within the annulus, and no rib-like structures were left visible (Fig. 5A).

Non-round (girdle and dorsal) plate formation

Primary sterna with ribs growing perpendicularly toward both sides were formed (Fig. 6). In the early stage, the ribs of the girdle plate, which were narrower in width than those in round plates, particularly in the basal parts, branched further into finer ribs with fringes peripherally (Fig. 6A). Subsequent silica deposition formed a trapezoidal edge to the girdle plate, with the sternum bent into an arch running through the middle of the plate (Fig. 6B). In the late stage,

the sternum became thicker as silica deposition continued, as did the plate overall (Fig. 6C). Eventually the plate flattened to mask the entire original rib framework, as in the round plate ontogeny. Finally, three-dimensional development outwards from the sternum occurred to create the high crest of the plate (Fig. 6C, see also Fig. 1, A–C).

Ontogeny of the dorsal plate greatly resembled that of the girdle plate (Fig. 7). The earliest stage we observed was a trifurcate sternum, with branching ribs growing centrifugally (Fig. 7A). Interestingly, however, the heavily silicified ridges present on the mature dorsal plate (cf. Fig. 1A) appeared first not along the central axis of the sternum but to one side of it and at a slight angle (Fig. 7B): this offset remained visible in later stages (Fig. 7B, see also Fig. 1A). During development of the thickenings into the triradiate keel of the mature plate, centrifugal silica deposition of the basal portion occurred and obscured the original rib patterns to make a solid plate (Fig. 7, B and C). Heavy deposits also developed on the edge of the plate, outlining the margin, leaving slightly broadened apices that were less defined (Fig. 7C).

Incomplete correspondence between ontogeny and final form in round plates

In previous studies the round plates of *Triparma laevis* f. *inornata* have been classified into two types, shield and ventral, from their relative size and the ornamentation found on the

mature plates (Booth and Marchant 1987, Konno et al. 2007). In this study, we revealed that the ontogeny of the round plates was of two types: (1) the center of the plate starts as a crescent-shaped structure and evolves into an annulus; and (2) the center of the plate begins as a linear sternum from which symmetrical silicification occurs. When plates were detached from cells and mixed together, shield and ventral plates in the process of formation were indistinguishable from each other. Indeed, although the ventral plate was larger than the shield plates from a single cell, plate sizes varied greatly from one cell to another, even in a clonal culture strain. The only way to identify that a round plate was a shield plate rather than a ventral plate was to look for the presence or absence of a circular ridge. If an almost-mature round plate possessed circular ridges, it was a ventral plate; and, if not, it was a shield plate.

To help interpret the ontogeny of the ventral plate, we examined 26 isolated semi-mature round plates. We found that half of them lacked any circular ridges and these were therefore identified as being shield plates. All of them had an annulus as a pattern center and would therefore all have developed from a crescent primary shape during plate ontogeny. The other 13 round plates possessed circular ridges and must therefore have been ventral plates. Their pattern centers could be either an annulus (7 plates; Fig. 8) or a linear sternum (6 plates; Fig. 4D). Based on our observations to date, as well as a literature survey (Booth and Marchant 1987, Konno et al. 2007, Konno and Jordan 2012, Yamada et al. 2014, 2016, Ichinomiya et al. 2016, Malinverno et al. 2016), the morphological criterion to discriminate

ventral from shield plates— the presence of a discontinuous circular ridge—is valid. It seems therefore that the ventral plates can have two different types of pattern center—annulus or sternum—while the shield plates have only the annulus as pattern center. As one cell bears only one ventral plate and three shield plates this means that, within a culture established from a single cell, some cells had ventral plates with an annulus as the pattern center and others had ventral plates with sternum. The biological significance and meaning of this heterogeneity of ventral plate ontogeny remains unclear.

Comparison with diatoms (evolutionary implications)

Triparma and centric diatoms resemble each other in that an annulus is visible at the pattern enter, at some stage during ontogeny if not in the mature plate or valve. However, it seems that the formation of the annulus may differ slightly in the two groups. In *Triparma*, the annulus and the branching ribs are formed simultaneously, judging by the simultaneous presence of an incomplete annulus (crescent or C-shaped) and ribs that are already growing outwards. In contrast, rib growth in centric diatoms begins after completion of the annulus (Li and Volcani 1985a,b). It is still not known whether this difference during the earliest stages of ontogeny represents a phylum-level divergence, since our understanding of the ontogeny from both lineages remains limited; annulus formation has been reported only from *Ditylum*

brightwellii (Li and Volcani 1985a) and *Chaetoceros rostratum* Lauder (Li and Volcani 1985b). The mechanism controlling the earliest stages in annulus formation also remains unclear. Given the fact that the lengths of radiating ribs growing from both apices of the C-shaped annulus were almost equal, it seems that the annulus extension proceeds bidirectionally; that is, silica deposition occurs at both ends within the silica deposition vesicles (SDVs) of *T. laevis* f. *inornata*. Several studies have reported that microtubules and actin filaments are involved in silica deposition within the SDVs in diatoms (e.g., Pickett-Heaps et al. 1990, Tesson and Hildebrand 2010). Our previous TEM study based on ultrathin sections, however, showed neither microtubules nor actin filaments around the SDVs during cell wall morphogenesis in *T. laevis* f. *inornata* (Yamada et al. 2016), although their absence could be an artefact of sample preparation (particularly for actin, as it can be difficult to properly fix it for TEM; Murata et al. 2002).

Mann and Marchant (1989) proposed homology between the shield plates of the Parmales and the valves and auxospore scales of the centric diatoms, based on morphological similarities of the pattern centers among them (Li and Volcani 1985a,b, Booth and Marchant 1987). In this study, we conducted detailed analyses of the ontogeny of siliceous plates, especially on the round plates in *T. laevis* f. *inornata*. The presence of the annulus and branching ribs in the shield plate of *T. columacea* was already known (Booth and Marchant 1987, Ichinomiya et al. 2011) as these structures are left unmasked even in the mature plates

of this species, unlike *T. laevis* f. *inornata*, in which silica deposition finally obscures the course of development. Ours, however, is the first study confirming the fact that the annulus acts as a pattern center during morphogenesis of the shield plates in the Parmales, and that shield plates and centric diatom valves and auxospore scales are ontogenically equivalent.

In addition, we demonstrated that the Parmales possess not only the annulus but also the sternum as pattern centers. Research during the last 20 years has shown that diatom auxospores also have both circular and elliptical scales and that these have different pattern centers - annuli and sterna respectively, e.g., in *Ellerbeckia arenaria* (Schmid and Crawford 2001), *Diploneis papula* (Idei et al. 2013), and *Hydrosera triquetra* (Idei et al. 2015). It is entirely reasonable therefore to assume that the presence of the annulus and sternum as pattern centers is synapomorphic for the Parmales and diatoms: that is, the sternum cannot be regarded as an autapomorphy of pennate diatoms, since their last common ancestor already had both pattern centers, annulus and sternum.

ACKNOWLEDGEMENTS

We thank the two anonymous reviewers for valuable comments and suggestions. This work was supported by KAKENHI Grant Numbers 14J05973 (Grant-in-Aid for Japan Society for the Promotion of Science (JSPS) Fellows), JP17H03724 (Grant-in-Aid for Scientific

Research B) and 17K07515 (Grant-in-Aid for Scientific Research C). We thank Elaine Monaghan, BSc (Econ), from Edanz Group for editing a draft of this manuscript.

REFERENCES

Booth, B. C. & Marchant, H. J. 1987. Parmales, a new order of marine chrysophytes, with descriptions of three new genera and seven new species. *J. Phycol.* 23:245–60.

Guillou, L., Chrétiennot-Dinet, M. J., Medlin, L. K., Claustre, H., Goër, S. L. D., & Vaulot, D. 1999. *Bolidomonas*: a new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). *J. Phycol.* 35:368–81.

Guillou, L. 2011. Characterization of the Parmales: Much more than the resolution of a taxonomic enigma. *J. Phycol.* 47:2–4.

Ichinomiya, M., Lopes dos santos, A., Gourvil, P., Yoshikawa, S., Kamiya, M., Ohki, K., Audic, S., De Vargas, C., Noël, M.H., Vaulot, D. & Kuwata, A. 2016. Diversity and oceanic distribution of the Parmales (Bolidophyceae), a picoplanktonic group closely related to diatoms. *ISME J.* 10:2419–34.

Ichinomiya M., Yoshikawa S., Kamiya M., Ohki K., Takaichi S. & Kuwata A. 2011. Isolation and characterization of Parmales

(Heterokonta/Heterokontophyta/stramenopiles) from the Oyashio region, western north Pacific. *J. Phycol.* 47: 144–51.

Idei, M., Sato, S., Nagumo, T. & Mann, D. G. 2018. Valve morphogenesis in *Diploneis smithii* (Bacillariophyta). *J. Phycol.* 54. 171–86.

Idei, M., Sato, S., Watanabe, T., Nagumo, T., & Mann, D. G. 2013. Sexual reproduction and auxospore structure in *Diploneis papula* (Bacillariophyta). *Phycologia* 52:295–308.

Idei, M., Sato, S., Nagasato, C., Motomura, T., Toyoda, K., Nagumo, T., & Mann, D. G. 2015. Spermatogenesis and auxospore structure in the multipolar centric diatom *Hydrosera*. *J. Phycol.* 51:144–58.

Konno, S. & Jordan, R.W. 2012. Parmales. *In* Encyclopedia of Life Sciences (eLS). John Wiley & Sons, Ltd., Chichester, UK.

Konno, S., Ohira, R., Komuro, C., Harada, N. & Jordan, R.W. 2007. Six new taxa of subarctic Parmales (Chrysophyceae). *J. Nannoplankton Res.* 29:108–28.

Li, C. W. & Volcani, B.E. 1985a. Studies on the biochemistry and fine structure of silica shell formation in diatoms. VIII. Morphogenesis of the cell wall in a centric diatom, *Ditylum brightwellii. Protoplasma* 124:10–29.

Li, C. W. & Volcani, B.E. 1985b. Studies on the biochemistry and fine structure of silica shell formation in diatoms. IX. Sequential valve formation in a centric diatom, *Chaetoceros rostratum*. *Protoplasma* 124:30–41

Malinverno, E., Maffioli, P. & Gariboldi, K. 2016. Latitudinal distribution of extant fossilizable phytoplankton in the Southern Ocean: Planktonic provinces, hydrographic fronts and palaeoecological perspectives. *Mar. Micropaleontol.* 123:41–58.

Mann, D.G. 1984. An ontogenetic approach to diatom systematics. *In* Mann, D.G. [Ed.] *Proceedings of the 7th International Diatom Symposium* O. Koeltz, Koenigstein, pp.113–144.

Mann, D.G. & Marchant, H.J. 1989. The origins of the diatom and its life cycle. *In* Green, J.C., Leadbeater, B.S.C. & Diver W.L. [Eds.] *The chromophyte algae: problems and perspectives* Clarendon Press, Oxford, UK, pp. 307–323.

Mayama, S. & Kuriyama, A. 2002. Diversity of mineral cell coverings and their formation processes: a review focused on the siliceous cell coverings. *J. Plankton Res.* 115:289–95.

Murata, T., Karahara, I., Kozuka, T., Giddings Jr, T. H., Staehelin, L. A., & Mineyuki, Y. 2002. Improved method for visualizing coated pits, microfilaments, and microtubules in cryofixed and freeze‐substituted plant cells. *J. Electron Microsc.* 51: 133-36.

Ohki, K. Rueter, J.G. & Fujita, Y. 1986. Cultures of the pelagic cyanophytes *Trichodesmium erythraeum* and *T. thiebautii* in synthetic medium. *Mar. Biol.* 91:9–13.

Pickett-Heaps, J. D., Schmid, A. M. M. & Edgar, L. A. 1990. The cell biology of diatom valve formation. *Progr. Phycol. Res.* 7:1–168.

Sato, S., Nagumo, T. & Tanaka, J. 2004. Auxospore formation and the morphology of the initial cell of the marine araphid diatom *Gephyria media* (Bacillariophyceae). *J. Phycol.* 40:684–91.

Sato, S. 2010. Valve and girdle band morphogenesis in a bipolar centric diatom *Plagiogrammopsis vanheurckii* (Cymatosiraceae, Bacillariophyta). *Eur. J. Phycol.* 45:167–76.

Schmid, A. M. M. & Crawford, R. M. 2001. *Ellerbeckia arenaria* (Bacillariophyceae): formation of auxospores and initial cells. *Eur. J. Phycol.* 36:307–20.

Tajima, N., Saitoh, K., Sato, S., Maruyama, F., Ichinomiya, M., Yoshikawa, S., Kurokawa, K., Ohta, H., Tabata, S., Kuwata, A. & Sato, N. 2016. Sequencing and analysis of the complete organellar genomes of Parmales, a closely related group to Bacillariophyta (diatoms). *Curr. Genet.* 62:887–96.

Tesson, B. & Hildebrand, M. 2010. Extensive and intimate association of the cytoskeleton with forming silica in diatoms: control over patterning on the meso-and micro-scale. *PLoS ONE* 5:e14300.

Tiffany, M. A. 2008. Valve development in *Aulacodiscus*. *Diatom Res.* 23:185–212.

Tiffany, M. A. & Hernandez-Becerril, D. U. 2005. Valve morphogenesis in the diatom family Asterolampraceae H. L. Smith. *Micropaleontology* 51:217–58.

van de Meene, A. M. & Pickett-Heaps, J. D. 2004. Valve morphogenesis in the centric diatom *Rhizosolenia setigera* (Bacillariophyceae, Centrales) and its taxonomic implications. *Eur. J. Phycol.* 39:93–104.

Yamada, K., Yoshikawa, S., Ichinomiya, M., Kuwata, A., Kamiya, M. & Ohki, K. 2014. Effects of silica-limitation on growth and morphology of *Triparma laevis* NIES-2565 (Parmales, Heterokontophyta). *PLoS ONE* 9:e103289.

Yamada, K., Yoshikawa, S., Ohki, K., Ichinomiya, M., Kuwata, A., Motomura, T. & Nagasato, C. 2016. Ultrastructural analysis of siliceous cell wall regeneration in the stramenopile *Triparma laevis* (Parmales, Bolidophyceae). *Phycologia* 55:602–09.

Yamada, K., Nagasato, C., Motomura, T., Ichinomiya, M., Kuwata, A., Kamiya, M., Ohki, K. & Yoshikawa, S. 2017. Mitotic spindle formation in *Triparma laevis* NIES-2565 (Parmales, Heterokontophyta). *Protoplasma* 252:461–71.

FIGURE LEGENDS

Fig. 1. Scanning electron micrographs of whole cells of *Triparma laevis* f. *inornata* NIES-2565. (A–C) Cells with siliceous cell wall observed from different directions; dorsal plate (d), shield plates (s), girdle plates (g) and ventral plate (v) are visible. Arrow, arrowhead and double arrowheads indicate keel, wing and circular ridge, respectively. Scale bars = 1 μm.

Fig. 2. Early stages of round plate with annulus in *Triparma laevis* f. *inornata* NIES-2565. Images in (A–C) and (E, F) show embryonic plates formed in silica-depleted culture. (D) Plate formed 4 h after silica replenishment. (A, B) Primordial silica structure, crescent shape. (C–E) Plate primordium elongating and curving to form a C-shaped structures bearing short branching ribs (arrows). (F) Annulus with well-developed branching radial ribs and some intervening, much shorter ribs (arrowheads). Scale bars = 100 nm.

Fig. 3. Early stages of round plate in *T. laevis* f. *inornata* NIES-2565 in silica-depleted culture, with short (top) and long sterna (bottom). Scale bars = 200 nm.

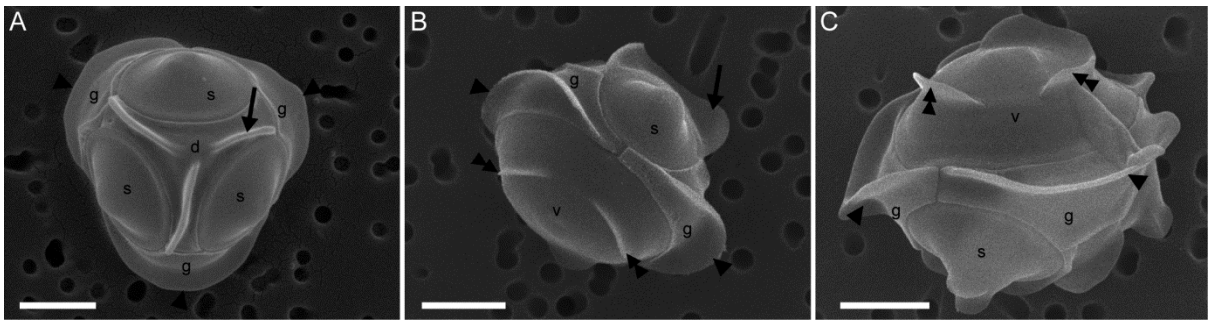
Fig. 4. Middle (A, B) and late (C, D) stages of round plate formation in *Triparma laevis* f. *inornata* NIES-2565. (A, B) Embryonic plates formed 6 h after silica replenishment. (C, D) Embryonic plates formed in silica-replenished culture. (A, C) Plates with annulus. (B, D) Plates with sternum. (A) Branching ribs (small arrow) radiating from annulus (arrow). Silica precipitation was observed basally on the branching ribs (arrowhead) but not within annulus. (C) Plate with almost complete silica precipitation. (B) Radial ribs (small arrow) protruding from sternum (white arrows). (D) Plate with ridge (double arrowheads). Extra silica has been precipitated along sternum. Scale bars = 200 nm.

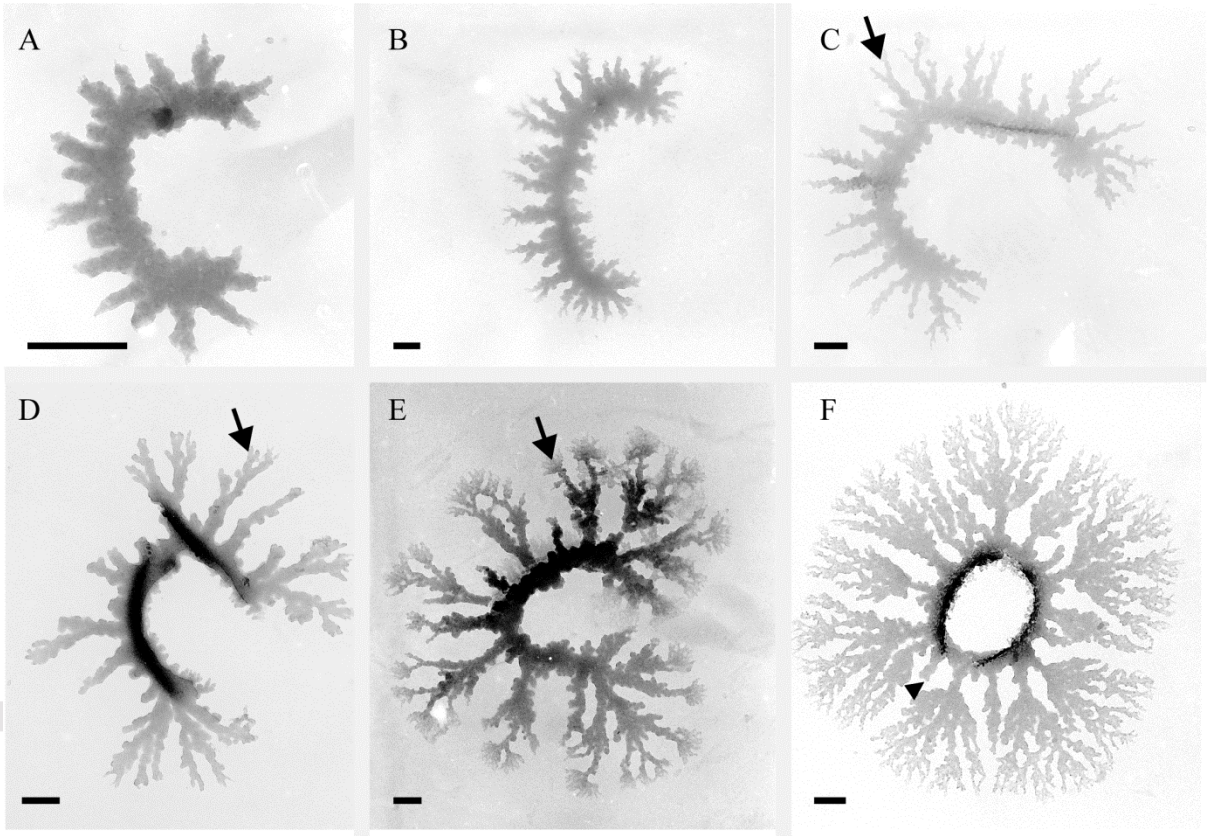
Fig. 5. Complete round plates isolated from *Triparma laevis* f. *inornata* NIES-2565 cells grown in silica-replenished culture. (A) Shield plate with central projection (arrow). (B) Ventral plate with discontinuous circular ridge (arrowheads). Scale bars =1 μm.

Fig. 6. Developmental stages of girdle plate in *Triparma laevis* f. *inornata* NIES-2565 in silica-replenished culture. (A) Early stage of girdle plate formation. Long primary sternum (arrow) and transverse ribs (arrowhead). (B) Middle stage, with fine outline of plate visible (double arrowheads). (C) Late stage, with further silica deposition visible mostly on sternum (arrow). Scale bars = 200 nm.

Fig. 7. Developmental stages of dorsal plate in *Triparma laevis* f. *inornata* NIES-2565. (A) Early-stage, embryonic plates formed 16 h after silica replenishment. Arrow indicates silica deposition on primordium of dorsal plate. (B, C) Middle and late stages of plate formation, respectively. Plates formed in silica-rich medium. White arrows indicate sternum. Arrowheads indicate silica deposition on outline of dorsal plates. Double arrowheads indicate boundary line of further silica deposition in middle stage. Scale bars = 200 nm.

Fig. 8. Late stage of round plate with annulus (arrow) and ridges (arrowheads) in *Triparma laevis* f. *inornata* NIES-2565. Scale bar = 200 nm.





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