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Auxosporulation, morphology of vegetative cells and perizonium of *Fallacia tenera* (Hust.) D.G. Mann (Bacillariophyceae)

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Abstract

Specimens of *Fallacia tenera* were collected from the surface sediment at in a river estuary in Japan. Auxosporulation occurred in a rough culture. Morphological structures of vegetative cells and auxospores were observed in detail. The vegetative cells have one H-shaped chloroplast. The striae were interrupted by two depressed lateral sterna internally and partly covered by a finely porous conopeum on the external surface. The lateral sterna and porous conopea formed two more or less curved longitudinal canals connecting with the exterior via opening pores on both sides of a terminal fissure. This combination of characteristics is unique to the genus *Fallacia*. The cingulum was composed of three bands, such as an open valvocupula and two comparatively thin pleurae. The two pleurae could be distinguished by the shape of their ligulae. The second band had a triangular ligula, whereas the ligula of the third band is arc-shaped. The auxosporulation was type IA1a in Geitler's classification. Two paired gametangia formed two anisogametes in each of them. Two auxospores formed in the thecae of the gametangia after a trans physiological anisogamy. The perizonium of the auxospore consisted of a set of transverse bands and five longitudinal bands. The primary transverse band was about twice wider than the secondary ones. The circular incunabular scales were present on the two terminals of the auxospore and on the surface of the primary transverse band. The primary longitudinal band had an acute terminal and was flanked by secondary longitudinal bands. Each side had two secondary longitudinal bands. All longitudinal bands were immediately beneath the transverse bands. Morphological comparison between *Fallacia* and *Pseudofallaica*, and the taxonomic position of *F. tenera* is also discussed.

Key words: Auxospore, Auxosporulation, Conopeum, Depressed later sterna, Diatom, *Fallacia tenera*, Incunabula scales, Perizonium, *Pseudofallacia*, Sexual reproduction.

Introduction

The genus *Fallacia* A.J. Stickle & D.G. Mann in Round *et al.* (1990: 667) includes many small taxa formerly assigned to *Navicula* sect. *Lyratae* and sect. *Bacillares* (Hustedt 1961–1966). Most *Fallacia* species are epipelic and epipsammic, living among coastal and estuarine intertidal sediments (Sabbe *et al*. 1999, Round *et al*. 1990). The genus *Fallacia* is characterized by a single H-shaped plastid, lyre-shaped hyaline lateral areas and finely porous conopeum partly or completely covering the striae (Round *et al.* 1990). The taxonomy of this genus has been studied by Sabbe *et al.* (1999) and Garcia (2003). There also have other reports of the genus *Fallacia* (Witkowski 1991, 1993, Witkowski *et al.* 2000, Procopiak & Fernandes 2003, Mann & Stickle 2009, Rakowska 2010). Diatoms in the genus *Fallacia* have a well-developed finely porous conopeum, elevated silica structure on the mantle and intricate structure of the valve. We still know little about those structures and their variation in many *Fallacia* species. In addition, the morphology of many *Fallacia* species has never been studied in detail.

Recently, a new genus *Pseudofallacia* Y. Liu, Kociolek & Q. X. Wang (2012: 624) was proposed based on the morphological study of *Pseudofallacia occulta* (Krasske) Y. Liu, Kociolek & Q. X. Wang (2012: 625). Four species of *Fallacia* were transferred to this new genus, including *Fallacia tenera* (Hust.) D.G. Mann in Round *et al.* (1990: 669) (Liu *et al.* 2012). *Pseudofallacia* differs from *Fallacia* mainly in the structure of lateral canals on both sides of the raphe. The canals in *Pseudofallacia* are formed by the depressed part of valve plane, which continue to

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the raphe sternum. By contrast, the depression will rise up to the general valve plane in *Fallacia*. In addition, the areolae are present in the canals of *Pseudofallacia*, whereas in *Fallacia* areolae are not present there. Currently there are over one hundred taxa in *Fallacia* (Guiry in Guiry 2013), but most of them lack thorough morphological observation by SEM and TEM. The morphological study reported here is a first step towards evaluation of the taxonomic and phylogenetic relations within the species in this genus.

The ultrastructure of a perizonium in pennate diatoms has been reported in many species covering araphid (Sato *et al.* 2004, Sato *et al.* 2008a, b) and raphid diatoms (Mann 1982, Toyoda *et al.* 2005, Poulíčková & Mann 2006, Toyoda *et al.* 2006, Kaczmarska *et al.* 2007, Poulíčková *et al.* 2007, Mann & Poulíčková 2009, Mann *et al.* 2011, Idei *et al*. 2013). It also has been suggested that the ultrastructure of the perizonium has phylogenetic significance at higher taxonomic levels (Medlin & Kaczmarska 2004). Currently, the information is still insufficient to review the phylogenetic relationship in most specific taxonomic groups. Auxosporulation and ultrastructure of perizonium and incunabula in *Fallacia* have never been reported.

In this study, a strain of *Fallacia tenera* (Hust.) D.G. Mann in Round *et al.* (1990: 669) was isolated from a river mouth in the coastal area in Japan. The vegetative cells, sexual reproduction, auxosporulation and ultrastructure of a frustule and an auxospore were observed in order to reexamine the morphology of frustule, reveal the auxosporulation and the structure of incunabula and perizonium.

Materials and methods

Surface sediments with benthic diatoms were collected on 31 May 2012 from a small river mouth on Ubara beach, Chiba Prefecture, Japan (35°8'17"N, 140° 16' 22" E) by using a glass tube as described by Round (1953). Epipelic diatoms were harvested on a cover slip according to Round *et al*. (1990). Clonal cultures of *F. tenera* were obtained by isolating a single cell from the cover slip and its transfer to F/2 medium (20 psu, the salinity of the sampling site). Those cultures were maintained at 20–23°C, with 20–30 μmol photons•m^{-2}•S^{-1} from cool-white fluorescent tubes; The photoperiod was 14:10 light:dark (L:D). Sexual reproduction and auxosporulation occurred in a rough culture derived from numerous cells isolated from natural population (the cells harvested on a cover slip).

The method for observation of the cultured materials was described by Poulíčková *et al*. (2007). Cleaned frustules were prepared by the bleach solution method (Nagumo & Kobayasi 1990) and mounted on a glass slide with Mountmedia (Wako, Osaka, Japan). Nikon Optiphot-2 light microscope (LM), with differential interference contrast (DIC) was used to make LM observations. The size for vegetative cells, gametangia and initial cells was measured on LM photographs by using ImageJ software (Rasband, W.S. *et al.*1997–2012). Data set was analyzed by R software (R Development Core Team 2008) graphed by the package "simba" (Jurasinski 2007).

For EM observation, vegetative cells were fixed with 2.5% glutaraldehyde, dropped on cover slips, 13-mm diameter for scanning electron microscope (SEM) observations, and treated by 70% nitric acids as described by Mann & Poulíčková (2009) or by boiling in a mixture of concentrated sulphuric and nitric acids. Auxospores were isolated from the mixed culture, transferred onto a cover slip (3-mm diameter or 13-mm diameter for SEM) and treated with NaClO followed by rinsing with distilled water repeatedly. Cleaned samples were dried in air and coated with osmium for SEM observations. Hitachi S-4000 and S-5000 were used for SEM observations; a JEOL-2000EX was used to make transmission electron microscope (TEM) observations.

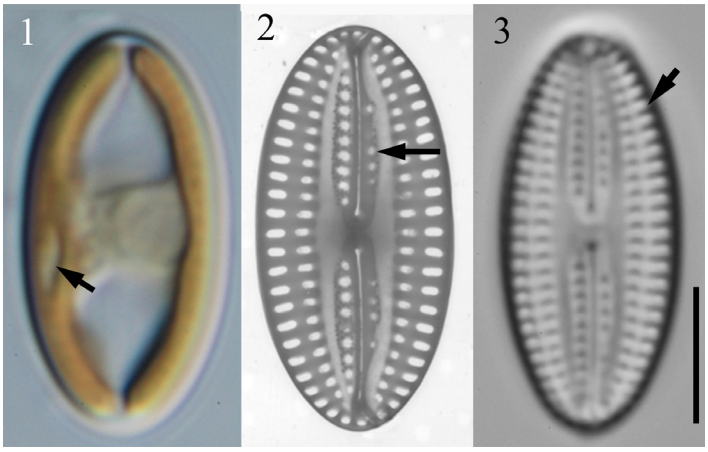
Terminology follows Anonymous (1975), Ross *et al.* (1979), Sims & Paddock (1979), Round *et al*. (1990) and Kaczmarska *et al*. (2013).

Results

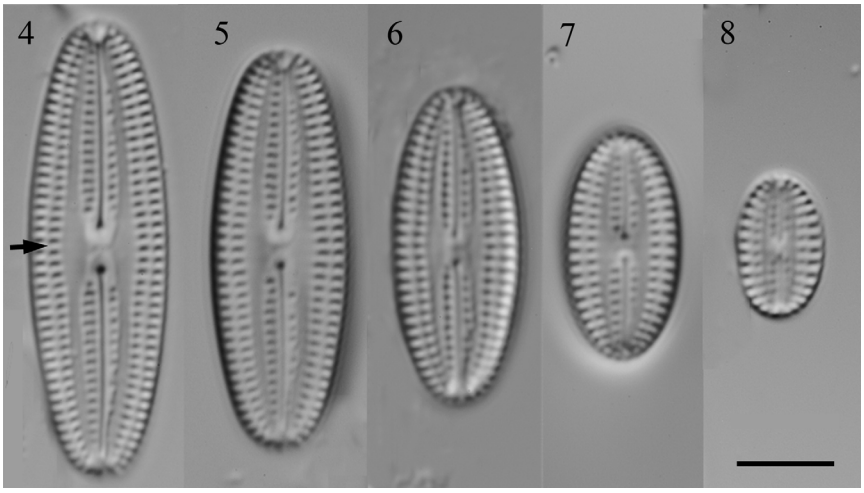
Morphology of vegetative and initial cells

Vegetative cells were solitary or could form short straight chains under artificial cultural conditions, which were not found in natural populations. Each cell contained a single H-shaped chloroplast consisting of two plates connected in the center (Fig. 1). One pyrenoid (Fig. 1, arrow) was present in one of the two plates. Valves were naviculoid to elliptical with bluntly rounded poles, 8.5–24.1 μm long and 5.4–8.6 μm wide. The raphe was straight and slightly arched near the apices (Fig. 3). Terminal fissures curved toward the secondary side of valve. Two

hyaline curved narrow lateral areas went through the valve longitudinally and connected with the slightly transapical convex central area. The striae were radiate especially near the poles, 17–20 in 10 µm. Striae were interrupted into three longitudinal lines of areolae on half valve face. Two lines of areolae are adjacent to the raphe branch on both sides (Fig. 2, arrow), and areolae in the line on the secondary side of the raphe were smaller, fewer or even missing. In initial cells, the center of outline and middle parts of lateral area were more convex than normal vegetative cells (Fig. 4). Size diminution resulted in smaller valves becoming elliptic or even circular (Figs 4–8).

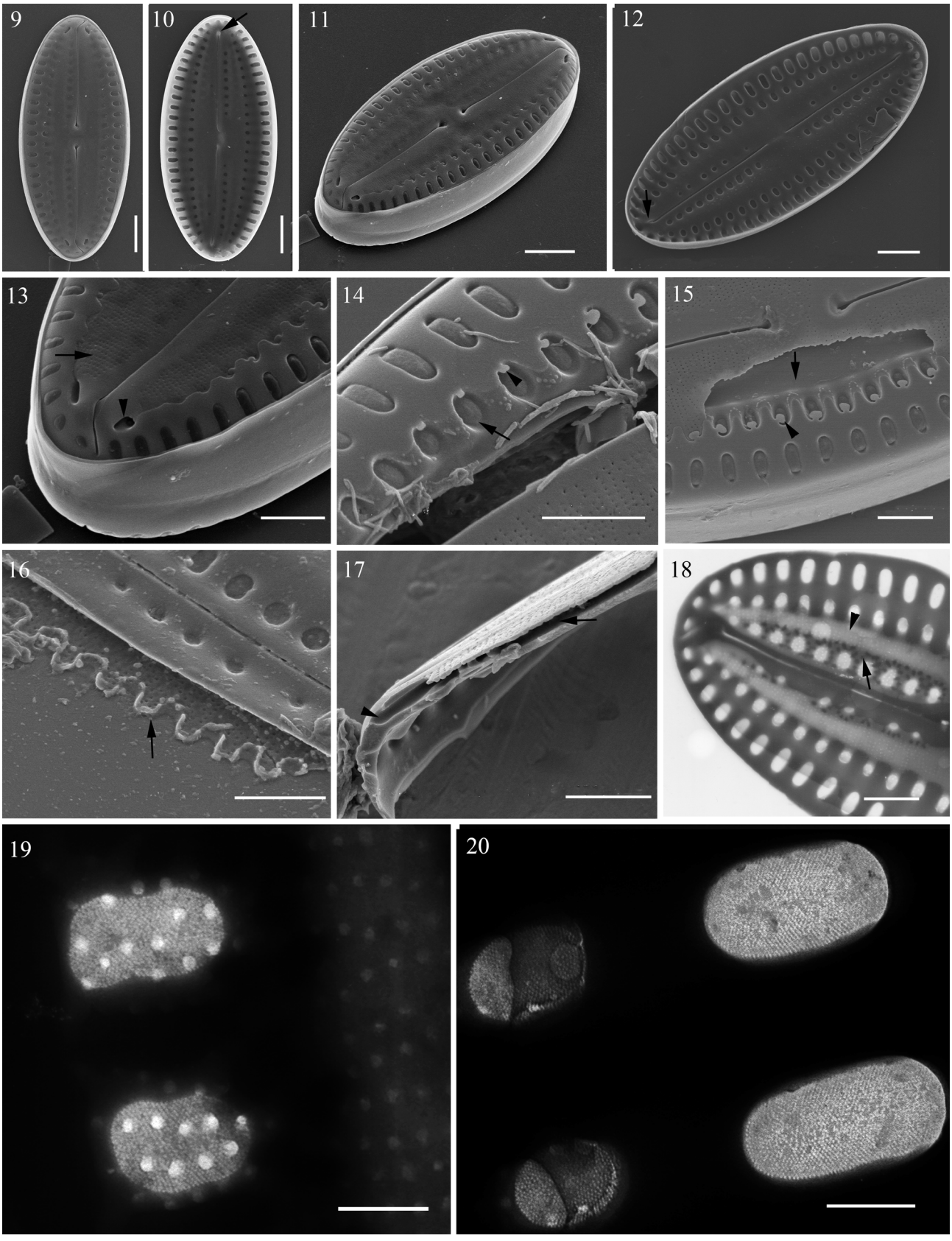


FIGURES 1–3: The vegetative cell and a valve of *Fallacia tenera*. Scale bar = 5 µm.
Fig. 1. H-shaped plastid with a pyrenoid (arrow). Fig. 2. TEM image of *F. tenera*. Note the asymmetric longitudinal line of areolae (arrow). Fig. 3. Cleaned frustule of *F. tenera*. Note the distal longitudinal line of areolae (arrow).



FIGURES 4–8: Morphological variation of *Fallacia tenera*. Scale bar = 5 µm.
Fig. 4. Initial cell with a slightly convex center (arrow). Figs 5–7. Outline of vegetative cells from naviculoid to elliptical. Fig. 8. Vegetative cell finally become to circle and probably could not have sexual reproduction.

In SEM, the external valve face was flat with a shallow mantle (Figs 11, 13). On the internal valve face the small helictoglossae were present at the polar terminals of the raphe, slightly curved to the primary side of the valve (Figs 10, 12, arrows). The distal longitudinal lines (Fig. 3, arrow) of areolae in LM were in fact a circle composed of areolae on the mantle around the valve face (Fig. 11). The other two longitudinal lines of areolae were on the valve face, interrupted by a depressed lateral sternum (Figs 10, 12). The sterna appeared as hyaline areas in LM. The areolae on the valve face were covered by a finely porous conopeum that extended from the raphe sternum to the valve mantle (Fig. 13, arrow). The edge of mantle also possessed "pegs" between two protrusions, which probably could fasten the connection of the conopeum and the mantle (Figs 14, 15, arrowheads). The conopeum and the inner edge of mantle all possessed undulate margins connected like a zipper (Fig. 16, arrow). The lateral depressed sterna (Fig. 15, arrow) and the finely porous conopa (Fig. 13, arrow) comprised two longitudinal canals (Fig. 18, arrowhead) that connect with the exterior by pores (Figs 13, 17, arrowhead) on both sides of terminal fissure. Around the areolae adjacent to the raphe, siliceous ribs were present between the valve and the conopeum to support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FIGURES 9–20: The fine structure of *Fallacia tenera* in SEM and TEM. All Scale bars = 1 μm except Figs 9, 10, 18 (2 μm) and Figs 19–20 (200 nm). Figs 9–10. Plan view (Figs 19–20) of external valve face and internal valve face. Figs 11–12. View of external and internal valve face at 30°tilt. Fig. 13. The detail of a frustule terminal. Note the finely porous conopeum (arrow) and two pores (arrowhead) lies beside the terminal fissures. Fig. 14. The broken valve shows a round areola (arrow) which should be covered by a conopeum and a "peg" (arrowhead), a silica flip. Fig. 15. The broken valve showing the depressed sterna (arrow). And also the "peg" (arrowhead) extend from the edge of the valve mantle. Fig. 16. The undulate margin of the conopeum. Fig. 17. The lumen between the conopeum and the valve (arrow), connecting outside through the terminal pores (arrowhead). Fig. 18. TEM photograph of a valve clearly shows the hyaline canal (arrowhead) and some silica structures (arrow) supporting the conopeum. Figs 19–20. The pattern of the areolae in longitudinal lines on the valve surface (fig. 19. Areolae are between the raphe and the canal, fig. 20, left) and on the mantle of valve. (fig. 20, right). They all belong to hexagonal array of hymen.

the conopeum (Fig. 18, arrow). The two longitudinal lines of areolae next to the raphe are asymmetric to one another (Fig. 18). The areolae were occluded by hymenes with a hexagonal array (Figs 19–20). The cingulum was composed of three non-perforated bands (Fig. 31). One open valvocopula bore an undulate advalavar edge of the pars interior (Fig. 22), and pars exterior with a curved down part of edge (Fig. 21, double arrows) at the pole opposite the opening. Two open pleurae had a relatively large ligula and a very narrow strip extending along the whole inner surface of the valvocopula. The two pleurae differ in the shape of their ligula, the width of strips and their orientations. The ligula of pleura 1 (second band) is arc shaped (Figs 21, 25, 26, arrowheads) and on the inner side of the closing pole of valvocopula, filling the missing parts formed by the curving down edge of the pars exterior of the valvocopula. The triangular ligula of the pleura 2 (third band) (Figs 23−24, arrows) was present on the inner side of the opening of valvocopula to fill the gap. The strip of the third band (Fig. 29, arrow) is thicker than the strip of the second band (Fig. 29, arrowhead). The terminals of those strips were present on the poles opposed to the lingua (Fig. 28, arrows, band three & Fig. 30, arrowheads, band two).

Auxosporulation

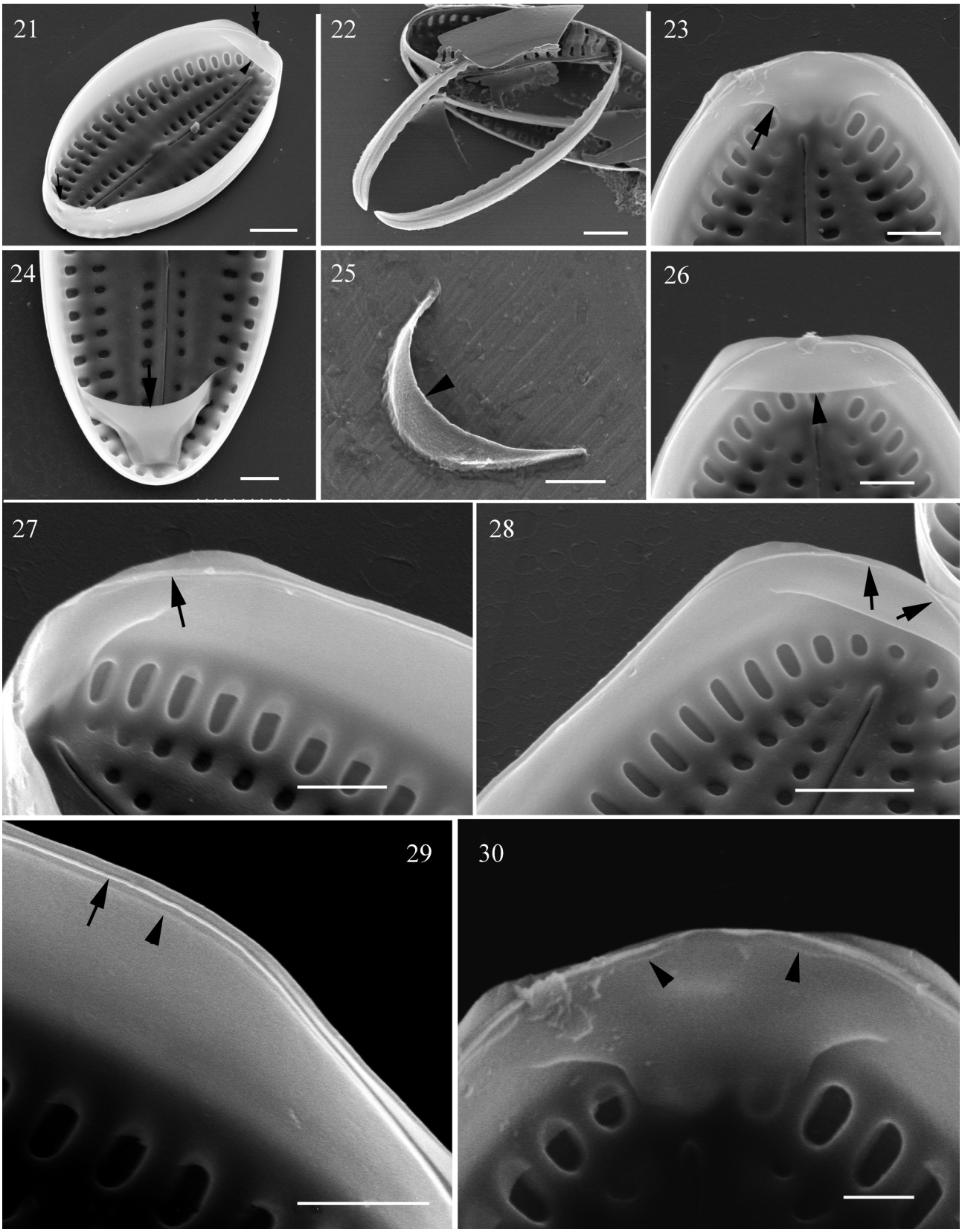
Auxosporulation occurred in the rough cultures after introduction of fresh media. The size range of vegetative cells, gametangia and initial cells was measured (Table1) and illustrated in Fig. 46. *Fallacia tenera* became capable of sexual reproduction when it reached its cardinal point, which in this study is 15.33 μm in length. It is about 63.5% of the upper size of the initial cell size range. The average length of gametangia (mean ± s = 11.62 ± 1.84 μm) is 53% of the average length of initial cells (21.92 ± 1.19 μm). The length of the two pairing cells (gametangia) usually is in different size, including a longer cell measured 11.39–15.33 μm (13.02 ± 0.94) and a shorter cell measured 8.84–13.59 μm (10.22 ± 1.40).

TABLE 1: Cell length for each life stage of *Fallacia tenera* (μm)

		Initial cell	Vegetative cell	Gametangia	Longer gametangia	Shorter gametangia
Length	Min	19.46	10.28	8.84	11.39	8.84
	Mean	21.92	14.02	11.62	13.02	10.22
	Max	24.11	21.32	15.33	15.33	13.59
	s	1.19	2.09	1.84	0.94	1.40

FIGURES 21–30: The fine structure of a complete cingulum. Bars in Figs 21, 22, 28 are 2 μm. Bars in Figs 23–27 and Fig. 28 are 1 μm. The bar in Fig. 30 is 500 nm.

Fig. 21. A complete theca, two ligulae (arrow and arrowhead) of pleurae could been found at the two terminals. Fig. 22. An open valvocopula with undulate margin. Fig. 23. The internal view of a ligula (arrow) of the pleura 2 (the third band). Fig. 24. The external view of the ligula (arrow) of the pleura 2 (the third band). Fig. 25. The external view of a ligula of the pleura 1 (the second band). Fig. 26. The internal view of the ligula (arrowhead) of the pleura 1 (the second band). Fig. 27. The linear strip (arrow) of the pleura 2 (the third band). Fig. 28. The two terminals of the linear strips (arrows) of the pleura 2 (the third band). Fig. 29. The linear strip (arrow) of pleura 2 (the third band) and the linear strip (arrowhead) of pleura 1 (the second band). Fig. 30. The two terminals (arrowheads) of pleura 1 (the second band).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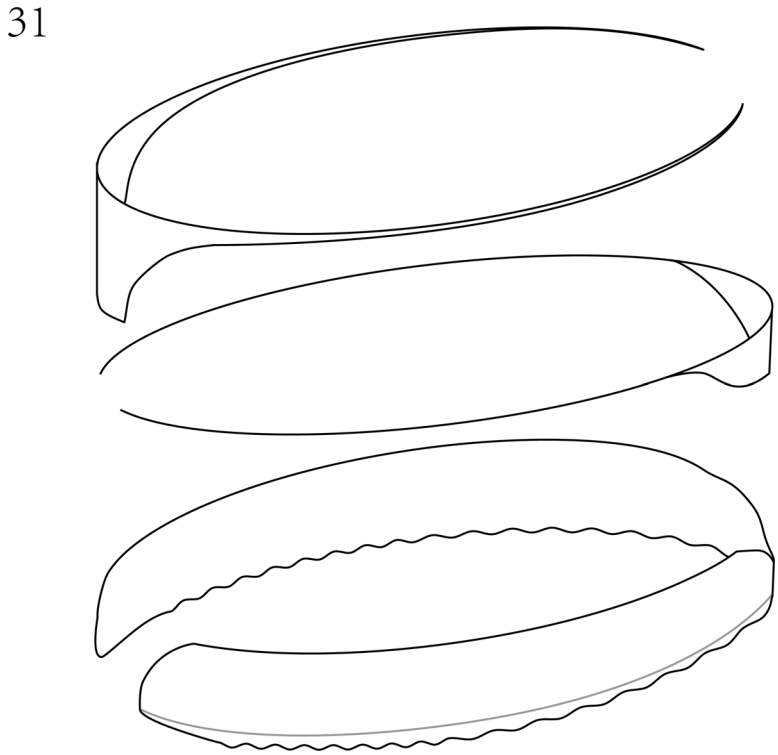


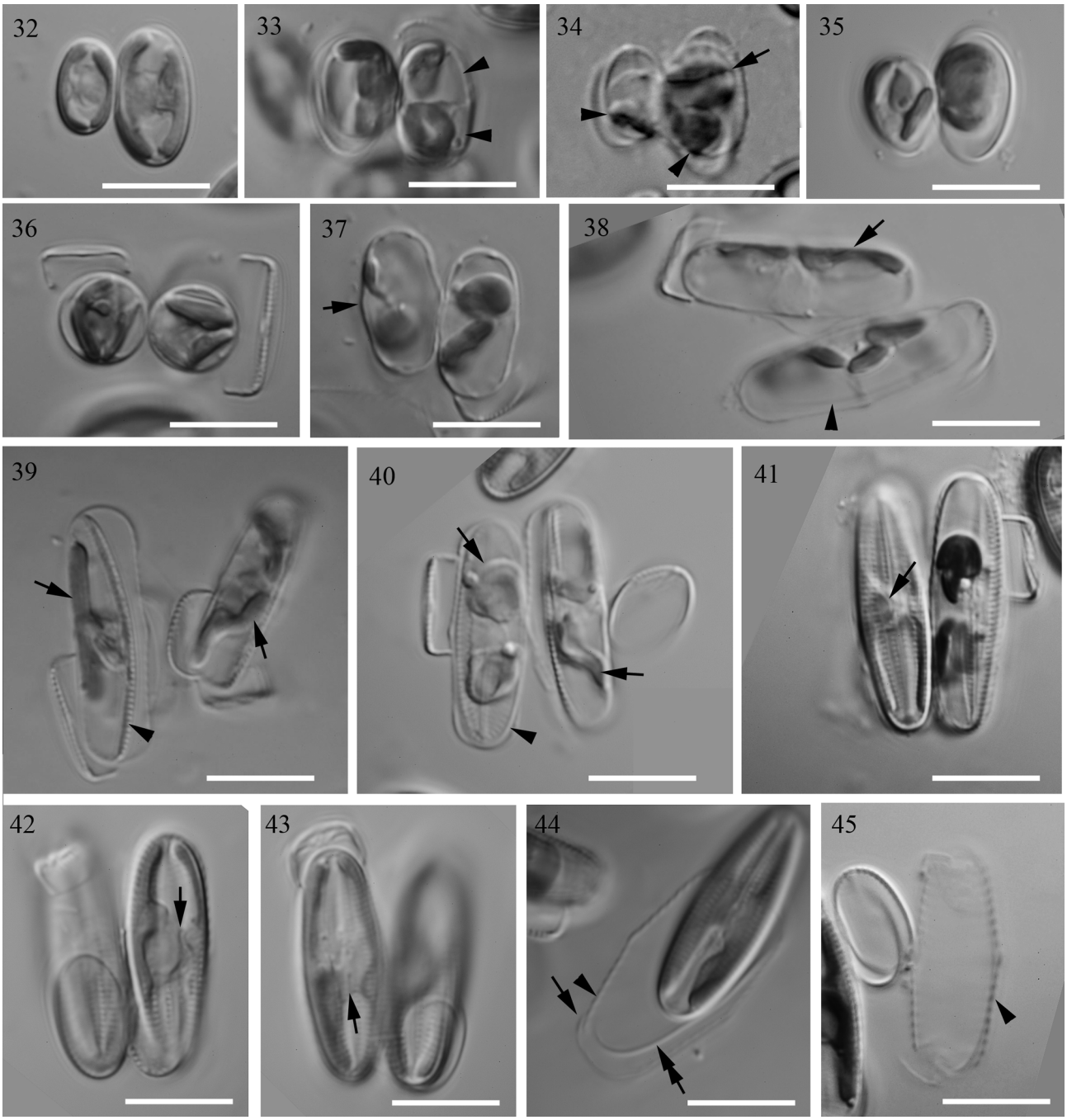
FIGURES 31. The illustration of the composition of a cingulum. An open valvocopula, a pleura with an arc shaped ligula and a pleura with an triangular ligula (bottom to top).

Two cells engaged in sexual reproduction, usually of different sizes, were paired parallel with the girdle (Fig. 32). Each gametangium formed two gametes (Fig. 33, arrowheads). After a trans physiological anisogamy (Fig. 34), two zygotes were formed in the frustules of the two gametangia respectively (Fig. 35), and released (Fig. 36). Then the two zygotes elongated into two auxospores. In the young auxospores, the convex primary transverse perizonial band (Fig. 37, arrow) and two chloroplasts in each auxospore were present. In mature auxospores, the two chloroplasts began to appress to the auxospore wall (Fig. 38, arrow). The longitudinal perizonial bands were present in the auxospores (Fig. 38, arrowhead). Each auxospore could produce one initial cell. While the initial cell was forming in auxospore, the two chloroplasts contracted and appressed to one side of the auxospore, lying one towards each pole of auxospore (Figs 39–40, arrows). The newly formed theca of the initial cell (Figs 39–40, arrowheads) could be seen clearly in some auxospores. After the formation of the initial cell frustule, the two chloroplasts rotated to transapical axis, elongated and appressed to both sides of the frustule. In the meantime, the two chloroplasts still keep their identity except the hyaline connection (Figs 41–43). One of the two chloroplasts appears to extend along the hyaline connection towards the other one (Fig. 41, arrow). Finally, an H-shaped chloroplast was formed before the initial cell emerged from the wall of the auxospore. The initial cell broke the incunabular cap and escaped via the pole of the auxospore (Figs 44–45). The incunabular cap (Fig. 44, arrow), transverse perizonial bands (Figs 44–45, arrowheads) and longitudinal perizonial bands (Fig. 44, double arrows) could be observed. The mucilage capsule around the auxospore was not found in several auxosporulations. In addition, the expansion of auxospores is more or less parallel to the gametangia. The thecae of gametangia were not always present near a terminal of auxospore. Some could be seen near the primary perizonial transverse band (Fig. 40) or two theca were at two terminals of the auxospore separately (Fig. 42, left auxospore)

The ultrastructure of perizonium and incunabula

The perizonium of *F. tenera* consisted of a set of transverse and longitudinal bands. The transverse bands comprised a moderately broader primary band that was about twice the width than secondary bands (Figs 48, 50, arrow), and a series of narrow secondary bands on both sides of primary band, overlapping each other (Figs 48, 50). The primary band was a closed band (Fig. 49 double arrows). The secondary bands were open bands. All the secondary bands have splits that formed a suture longitudinally (Fig. 49, arrow).

The longitudinal bands were immediately beneath a series of transverse bands, and consisted of five bands including one primary band (Fig. 52, double arrows) and four semilanceolate secondary bands. Two were on each



FIGURES 32–45: The sexual reproduction, the auxospore development and the formation of initial cells. All scale bars = 10 µm. Fig. 32. Two paired cells in different size. Fig. 33. Two gametes (arrowheads) were formed in one gametangia. Fig. 34. The trans anisogamy? was occurred. One zygote (arrow) was formed in a theca (right) of gametangia and two unfused gametes (arrowheads). Fig. 35. Two zygotes were formed after the gametes fusing. Fig. 36. Two zygotes were released from thecae of gametangia. Fig. 37. Younger auxospores paired more or less parallel to each other. Note the slightly convex center (arrow). Fig. 38. Matured auxospores. Two chloroplasts (arrow) appressed to the wall of auxospores. The longitudinal bands also could been found (arrowhead). Figs 39–40. Formation of initial cells. A theca was present in the left auxospore (arrowhead) in fig. 39. During the formation of initial cells, the two chloroplasts show strongly contraction (arrows) Fig. 41. In new formed initial cells, two elongate chloroplasts apressed to the girdle. One chloroplast projects to another one along the hyaline connection between them (arrow). Figs 42–43. The initial cells completely formed in the two auxospores. The two chloroplasts nearly fused (arrow) in the right initial cell (fig. 42). The H-shaped chloroplast (arrow) already formed in the left initial cell (fig. 43). Fig. 44. The initial cell were escaping from the perizonium. Note the transverse perizonial bands (arrowhead), cape (=incunabular cap, arrow) and probably longitudinal band (double arrows). Fig. 45. The empty perizonium with two broken poles. Note the primary transverse perizonial band and seven to nine secondary perizonial bands on each side of it.

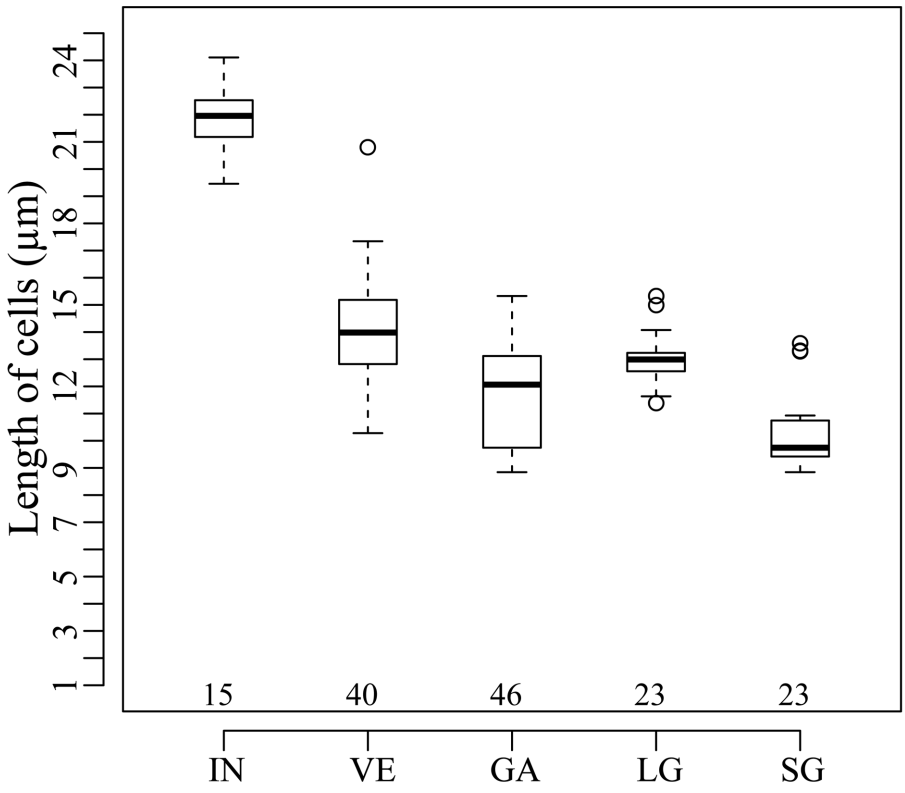


FIGURE 46. The length ranges of cells in each life stages of *Fallacia tenera*.
IN: Initial cells, VE: Vegetative cells, GA: Gametangia (paired parental cells), LG: the longer gametangium of the two paired gametangia, SG: the shorter gametangium of the two paired gametangia. The numbers on the horizontal axis are the number of observations.

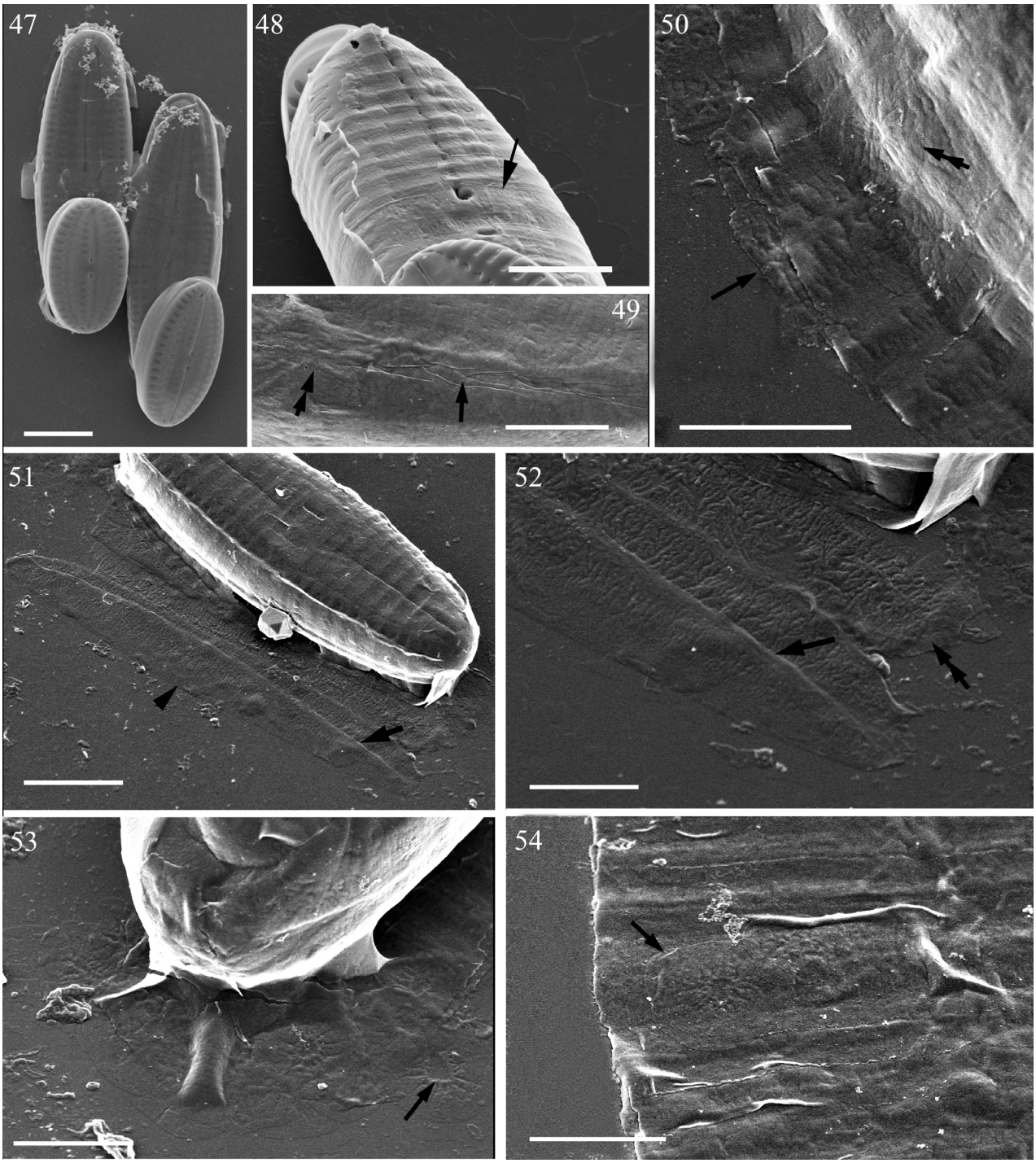
side of the primary band (Fig. 51). The primary longitudinal band was bifacial and lanceolate with fimbriate margin. The secondary longitudinal bands were unifacial and possess fimbriate margin at one side (Fig. 51). The distal lateral edges of secondary bands were more or less convex (Fig. 51, arrowhead).

Concentric normal scales (incunabular scales) were detected on the two terminals and the surface of transverse primary band (Figs 53–54).

Discussion

Morphology and taxonomy of Fallacia tenera

Fallacia tenera was first reported by Hustedt under the name *Navicula uniseriata* Hust. in Schmidt *et al.* (1934: pl. 392). He changed the name to *N. tenera* Hust. in Schmidt *et al.* (1936: pl. 405 footnote) because of it being a later homonym with *N. uniseriata* Østrup (1913: 8). The formal description of *N. tenera* wasn't given until in the diatom flora studies of Java, Bali and Sumatra (Hustedt 1937). The specimens in this study agree with the description given by Hustedt, except the longitudinal uniseriate areolae adjacent to the raphe. In Hustedt's description, there is only one longitudinal line of areolae on the concave side of raphe (the primary side of the valve), and no areola on the convex side (the secondary side of the valve). In our sample the areolae could be visible on both sides, although those areolae on the convex side are variable and invisible in times. When we compared our specimens with the photographs of lectotype illustrated by Simonsen, one small and indistinct isolated areola could be found on the convex side of the raphe (Simonsen 1987, pl. 255, fig. 7, 9 under the name *Navicula unistriata*). It also was present in specimens from South Africa and Westerchelde estuary, the Netherlands (Schoeman & Archibald 1976–1980, Sabbe *et al.* 1999). Variation in the areolae also occurred in our monoclonal culture, it appears to be caused by



FIGURES 47–54: The incunabula and perizonium of *Fallacia tenera.* Scale bars = 2 µm, except Figs 47, 50, 51 (5 µm).
Fig. 47. Paring of two auxospores with two parental cells valves in different size. Fig. 48. Perizonium investing the initial cell, note the primary transverse band (arrow). Fig. 49. "Suture" of secondary transverse perizonial bands (arrow) and closed primary band (double arrows). Fig. 50. Detial of primary transverse band (arrow) with an incunabular scale (double arrows) on the surface. Fig. 51. The longitudinal series of perizonial bands. Note the semilanceolate secondary bands (arrow) with a slightly convex outline in the center (arrowhead). Fig. 52. The detail of fig. 51. Showing the one side fimbriate margin of secondary longitudinal bands and lanceolate primary longitudinal band (double arrows). Figs 53, 54. Incunabular scales (arrow) on the terminal and on the primary transverse band.

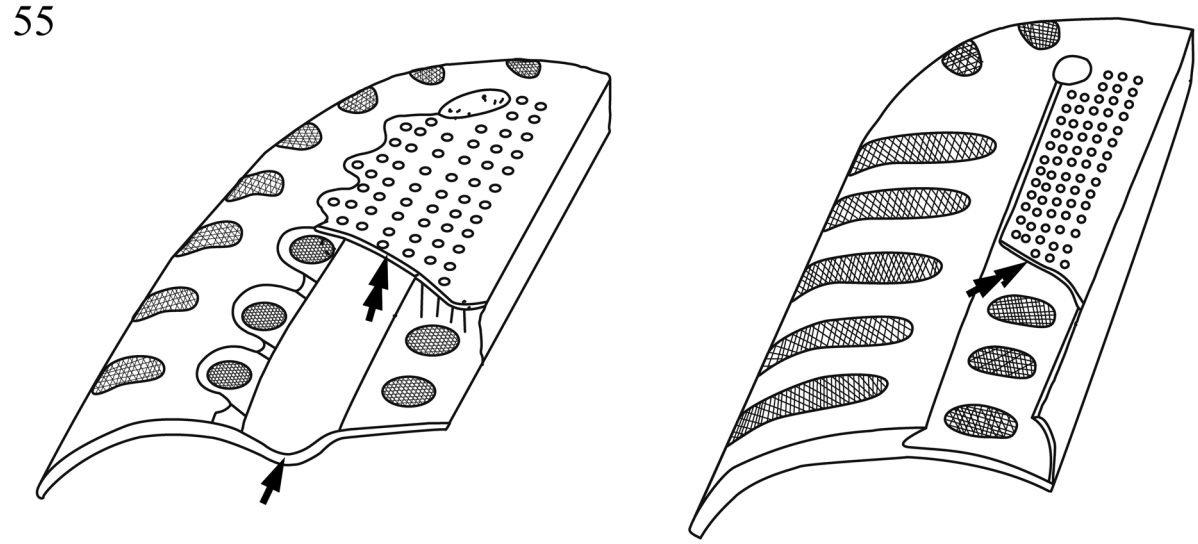


FIGURE 55. Comparison of general valve patterns of *Fallacia tenera* (left) and *Pseudofallacia occulta* (right). Section view of half valve. Showing *F. tenera* possess longitudinal depressed sternum (arrow). The conopeum (double arrows) covers two longitudinal row of areolae in *F. tenera*, while in *P. occulta* covers one longitudinal line.

environmental factors rather than genetics. Because of this variation, Schoeman & Archibald (1976–1980) united *Navicula dissipata* Hust. in Schmidt *et al.* (1936: pl. 403) with *N. tenera*. Since *N. dissipata* agrees with the description of *F. tenera*, except *N. dissipata* bears areolae presented on both side of raphe and the large cell size. Nevertheless, there is an additional feature that may distinguish *F. tenera* from *N. dissipata*. Schoeman & Archibald (1976–1980) mentioned that "the inner puncta of the central striae are weakly developed or absent" in the type specimen of *N. dissipata*. That was not present in the samples from South Africa and in this study. Curiously, those constricted inner puncta of the central striae were present on the specimen of *Fallacia* cf. *teneroides* illustrated by Sabbe *et al.* (1999). Currently, there are still not sufficient data of *N. dissipata* to compare those two taxa in detail, especially the unknown morphological varying degree of *N. dissipata*.

Quantitative data on the *F. tenera* population on which the current study is based suggest it differs slightly from the reports from Ranu Klindungan (type location), South Africa and Westerschelde (Table 2). The differences in the size range of cells from different locations could be due to the presence of cells in different stages of the life cycle. Since we observed the whole life cycle, the range of cell size in this study covers others except the report from South Africa, in which the length and width of specimens is even larger than the initial cells reported in this study. Interestingly, the length and width of South African material agree with the description given by Hustedt (1961–1966) of *N. dissipata*. The differences could probably be due to Schoeman & Archibald's (1976–1980) synonymizing *N. dissipata* with *F. tenera.* Or perhaps they invested huge size materials which have high phenotypic plasticity from different locations in South Africa. With respect to the sampling sites, the type location is a freshwater lake, whereas meso- and polyhaline raches in the Westerschelde estuary. The sampling site in this study is ecologically similar to Westerschelde estuary. Because the specimens in this study differ slightly from the species from Westerschelde and Ranu Klindungan in striation density only, without considering the differences in length and width, such differences might be caused by phenotypic plasticity.

TABLE 2: Comparison of *Fallacia tenera* reported in different locations

	Fallacia tenera				*Navicula dissipata*
	Ubara, Japan (this study)	Ranu Klindungan (Holotype)	South Africa	Westerschelde	Nordasot
Length (μm)	10.3–16.2 (8.5–24.1)*	12–14	9–27	9.7–10.6	9–27
Width (μm)	5.9–7.1 (5.4–8.6)*	5–6.5	4–9	5–5.4	5–7
Striation density (per 10 μm)	17–20	16–18	13–22	19–21.5	14–17

*the length measured including initial cells.

Recently, *F. tenera* was transferred into *Pseudofallacia* based on presence of longitudinal ribs instead of lyre-shaped canals and one large areola per striae (Liu *et al.* 2012). According to morphological characters of *F. tenera* observed in this study, it does possess the typical characteristics of *Fallacia*. The lyre-shaped canal, which does not have areolae in it, probably is the most important characteristics distinguish the two genera. The presence of this canal has been detected in our material with certainty. The canal appears as a finely porous, hyaline area under TEM. It was also visible in the figures presented by Schoeman & Archibald (1976–1980). Although they did not mention the depression of sterna and longitudinal canals in their description of *F. tenera* ("longitudinal costa" in their article), it is still reasonable to believe the specimens from South Africa have the same structure as ours. Since depressed sterna could not been well observed in valve view in SEM, this feature has been only clearly illustrated in TEM micrographs given by Schoeman & Archibald (1976–1980). And in most articles "rib" or "costa" was used to describe this feature. Use of those terms probably mislead Liu *et al.* (2012) to transfer this species to *Pseudofallacia*

The presence of one areola per striae and the slightly elongate areolae on the mantle uncovered by the conopeum more or less resembles *Pseudofallacia*. The areolae on valve mantle are usually less than twice longer than others in *F. tenera*, by contrast the elongate areolae of *Pseudofallacia ocullata* usually more than three times longer than the areolae in canals. Those elongate areolae also appear to be more linear and take nearly two third width of the valve in *Pseudofallaica*. In addition, Areolae uncovered by a conopeum are also present on many *Fallacia* species like *Fallacia gemmifera* (Simonsen) D.G. Mann (1990: 668), *Fallacia litoricola* (Hust.) D.G. Mann (1990: 668) (unpublished observations), *Navicula hodgeana* R.M. Patrick & Freese (1961:189)(this taxon will be transferred to *Fallacia*) and *Fallacia pygmaea* (Kütz.) A.J. Stickle & D.G. Mann (1990: 668) (Garcia, 2003, fig. 9, 42) etc., although in some species like *F. litoricola* and *F. pygmaea* those areolae were only present at the two terminals. Thus areolae present on the valve mantle appear homologous with *Fallaica* species rather than *Pseudofallaica* species.

Sexual reproduction and Auxosporulation

Kaczmarska *et al*. (2013) described four types of cardinal points. In this study, the length of initial cells and upper sexual size threshold were measured. The minimum size of gametangia is also provided here. Those data measured directly from the pairing gametangia in a rough culture, not from separated monoclonal cultures. Thus we could not identify those cells under 8.84 μm were not paring is due to size or they were sexual incompatible.

The auxosporulation of *F. tenera* belongs to type IA1a of geitler's classification (Geitler 1973). It shows several features that agree with *Fallacia pygmaea*, such as two auxospores per pair of gametangia, presence of a perizonium, expansion more or less parallel to the gametangia, chloroplast contraction during formation of the initial cells (Karsten 1899). The mucilage capsule around the auxospore illustrated by Karsten (1899), however, was not observed in this study. Because of lacking the mucilage, we also could not determine the presence of several single auxospores surrounded by four thecae is type II auxosporulation or caused by our preparation of a slide.

Unfortunately, the detailed process of meiosis and unequal cytokinesis could not been observed in this study, since the cells laid in the valve faces in most cases. Although a few individual cells seemed to undergo unequal cytokinesis, we could not know after that it will die or produce gametes (the separation from another cell caused by the preparation of a slide). The change of chloroplasts during auxosporulation is another interesting point. In every case thus far, the chloroplast inherited from the gametes either survive and retain their identity or undergo controlled senescence and death (anonymous reviewer, pers. Comm.). Based on our observations, the two chloroplasts inherited from the gametes have the tendency to fuse after the formation of initial cells forming an H-shaped chloroplast, although we could not continually focus on one pair of auxospores to observe the whole process (Figs 41–42). In addition, in figures 42–43, the left initial cell shows a completely formed H-shaped chloroplast. The two cells are all at the last stage of initial cell forming. Based on these observations it seems unlikely that one of the chloroplasts will undergo controlled senescence and death.

Ultrastructure of incunabula and perizonium

All of the auxospore envelop and wall components have been reviewed by Kaczmarska *et al*. (2013) recently. In this study, only scales (incunabular scales) and perizonial bands including a set of longitudinal bands and transverse bands were detected. Kaczmarska *et al.* (2013) introduced five types of scales, namely simple scales, distorted scales, slit scales, dendroid scales and spinescent scales. In recent study, two types of scales, simple scales

and distorted scales, were reported in *Diploneis papula* (Schmidt in Schmidt *et al.*) Cleve (1894: 85) (Idei *et al.* 2013). For *F. tenera* only simple scales were found. Similar scales also were reported in *Sellaphora marvanii* (Poulíčková & D.G. Mann in Mann *et al.* 2011:1370), *Nitzschia inconspicua* Grunow (1862: 579) (Mann *et al.* 2013) and *Caloneis linearis* (Grunow) C.S. Boyer (1927: 311) (Ishii *et al*. 2012).

In pennate diatoms, incunabular scales have been reported in araphid diatoms, *Rhabdonema* Kütz. (1844: 126) (Stosch 1982); *Gephyria media* Arn. in Johnston (1860: 20) (Sato *et al.* 2004); *Grammatophora marina* (Lyngb.) Kütz. (1844: 128) (Sato *et al.* 2008c); *Pseudostriatella oceanica* Shin. Sato, D.G. Mann & Medlin in Sato *et al.* (Sato *et al.* 2008b) and *Tabularia parva* (Kütz.) D.M. Williams & Round (1986: 324) (Sato *et al.* 2008a), and in the biraphid diatoms, *S. marvanii, D. papula, C. linearis* and *F. tenera* (this study). In monoraphid diatoms *Achnanthes crenulata* Grunow in Cleve & Grunow (1880: 20) (Toyoda *et al.* 2005) and *Achnanthes yaquinensis* McIntire & Reimer (1974: 174) (Toyoda *et al.* 2006), however, scales were not present. Also, the transverse perizonial bands were not present in the two monoraphid species. Toyoda *et al.* (2005) suggested they were lost during the evolution from biraphid to monoraphid diatoms. Since those silica scales also were present in centric diatoms, which are believed to be the ancestor of pennate diatoms, those isodiametric scales may be the primitive form of incunabula. *Navicula cryptocephala* Kütz. (1844: 95) (Poulíčková & Mann 2006) bear "organic caps" instead of caps consists of isodiametric scales. Although *N. cryptocephala* has similar perizonium with *S. marvanii and F. tenera*, they seem to belong to different lineages.

The set of transverse perizonial bands that are comprised of one open primary band and seven to nine bands on both sides of the primary transverse band. The primary transverse band is nearly two times wider than the secondary transverse band, whereas the primary band in *Rhoicosphenia curvata* (Kütz.) Grunow (1860: 511) (Mann 1982) and *Neidium* cf. *ampliatum* (Mann & Poulíčková 2009) are less than twice wider. In contrast, the primary transverse perizonial band of *S. marvanii*, *Caloneis silicula* (Ehrenb.) Cleve (1894: 51) (Mann 1989) and *Navicula cryptocephala* Kütz. (1844: 95) (Poulíčková & Mann 2006) are much wider than in *F. tenera*. *Pinnularia* cf. *gibba* (Poulíčková *et al.* 2007) has the widest primary band than above. In addition, the prominent suture between primary and secondary band found in *S. marvanii* was also not detected in *F. tenera*.

The longitudinal bands are composed of five bands. A similar arrangement has been reported in *Rh. curvata* (Kütz.) Grunow (1860: 511) (Mann 1982), *D. papula* (Schmidt in Schmidt *et al.*) Cleve (1894: 85) (Idei *et al.* 2013) and *Nitz. inconspicua* Grunow (1862: 579) (Mann *et al.* 2013). The longitudinal bands in *D. papula* have a primary band in the middle flanked on each side by two unifacial bands of different structure (Idei *et al.* 2013). The two bands are secondary and tertiary longitudinal bands. Their shapes (Idei *et al.*2013) differ from *F. tenera*. The shape of complete longitudinal perizonium contracts in the middle, which correspond with the outline of the initial cells (Idei *et al.*2013). Similarly, the outline of a complete longitudinal perizonium (only half could be seen in fig. 48) convexing in the centre also corresponds with the outline of the initial cells of *F. tenera*. Moreover, just like the generally lateral symmetric vegetative cells the longitudinal perizonal bands also arranged lateral symmetricly in *D. papula* (Schmidt in Schmidt *et al.*) Cleve (1894: 85) (Idei *et al.* 2013) and *F. tenera*. In contrast, the longtitudinal perizonal bands in *Nitz. inconspicua* Grunow (1862: 579) (Mann *et al.* 2013) are not laterally symmetric like its vegetative cells. It suggests the longitudinal perizonium closely related to or even determine the shape of initial cells.

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