FRUSTULAR MORPHOLOGY AND TAXONOMIC AFFINITIES OF NAVICULA COMPLANATOIDES (BACILLARIOPHYCEAE)

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Abstract

Live and prepared cells of the marine pennate diatom Navicula complanatoides Hust. were examined with light and electron microscopy. It has narrowly lanceolate valves (26–35 μm long, 4–5 μm wide) and girdles 10–24 μm in depth. Striae are parallel at the center of the valve (24–28 in 10 μm), becoming slightly convergent toward the apices.

Electron microscopy revealed that the external valve surface presents a longitudinally ribbed appearance (20–28 parallel ribs at its maximum width), whereas internally, rectangular areolae are occluded by ricae. The raphe slit lies in a narrow axial area, and one side of the raphe sternum is deeper and folds over the other, obscuring the internal opening. Internally, the central virga on one side of the raphe and two virgae on the other are somewhat broader. A conspicuous pore (stigma) is present between the two broadened virgae.

The girdle consists of valvocopulae, copulae, and pleurocae. There are 16–20 bands per cingulum. The valvocopulae and copulae are hollow tube-like structures, with inner and outer portions contrasting in morphology. They decrease in diameter in an abvalvar direction. There are four pleurae. These are flat bands which facilitate overlap of the epicingulum and hypocingulum.

Fundamental features of the valve and girdle reveal the distinctness of this species within Navicula. The areolae, external longitudinal ribs, and raphe structure suggest affinities with Pleurosigma, Gyrosigma, and Haslea. It is hypothesized that they share a derived state which indicates a recent common ancestor for these taxa. N. complanatoides and related species of the Naviculae microstigmataceae are distinctive enough to merit their own genus within the Naviculaceae.

Key index words: diatoms; frustule; girdle bands; Navicula; Naviculaceae; phylogeny; taxonomy; valve morphology

Navicula Bory is the largest diatom genus (Hendey 1964). Diatom morphologists have long recognized the diversity of form within it, and since the works of Kützing (1833, 1844), common practice has been to divide it into a number of sections containing species that appear similar with light microscopy. Patrick (1959) described the historical development of these sections and proposed raising them to the rank of subgenera. In spite of this taxonomic formalization, the sections established by Cleve (1894, 1895), with modifications by Hustedt (1961–1966) and Hendey (1964), are deeply embedded in contemporary diatom taxonomy and still enjoy common usage.

Despite recent work, Navicula remains a systematically problematic group. The prevailing disarray will not be rapidly rectified, if only because so many species await detailed examination. Furthermore, consensus is lacking among diatomists as to the level of structural difference sufficient to warrant specific, generic or higher ranking taxonomic status. Cox (1979a:170) stated that “recognition of species and genera is at present very much the result of individual worker’s understanding of a particular group and his personal opinion as to the importance or otherwise of particular points of difference.” There is also little agreement about which characters are important in determining relationships among groups and the kind of relationship to be reflected in the classification.

Taxonomic changes have often resulted from detailed examination of sections or subgenera within Navicula. For example, work on Navicula ostrearia (Gaillon) Bory (Robert 1973, Neuville et al. 1975) and Navicula trompii Cleve (Helmcke and Krieger

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1954) supports Simonsen’s (1974) separation of the former Sect. Fusiformes Cleve from Navicula as a new genus, Haslea. Similarly, Karayeva (1978a) has removed Sect. Lyraeae Cleve from Navicula and formed the genus Lyrella.

The position of section Naviculaceae microstigmataeae (sensu Hustedt 1962) warrants more attention. Hustedt (1962) lists 37 species divided into five groups. Group 3 (Hustedt 1962: 286, 335–341) is the smallest, containing only five species: N. hyalosira Cleve, N. hyalosiraella Hust., N. complanata Grun., N. planatula Hust. (= N. poretzkjæae Koretkevich, see Giffen 1973), and N. complanatoides Hust. Information on this species is largely limited to data from floristic surveys of benthic diatoms, e.g. N. poretzkjæae (Giffen 1967, 1970, 1975, Edsbagge 1968, van der Werff 1970, McIntire and Overton 1971, Hendey 1974, others cited in McIntire and Moore 1977: 340), N. complanata (Hustedt 1955, Giffen 1967, 1965, Riznyk 1975, Hendey 1974, Bacon and Taylor 1976, Ehrlich 1978), and N. complanatoides (Giffen 1967, van der Werff 1970). Where abundances were noted, these species were listed as rare or extremely rare, with two exceptions (Edsbagge 1968, Giffen 1973). Writing on N. complanata from Beaufort, North Carolina, U.S.A., Hustedt (1955: 31) stated that “the species is insufficiently known since specimens are rarely found and can be observed only with difficulty . . . . Since the taxonomy of the species belonging to this group is still very uncertain, I have united the specimens observed with N. complanata, hoping that the taxonomy can be cleared up in the future by more material.” Similarly, Ehrlich (1978: 4) had difficulty placing a species described in detail from light and electron microscopy.

Until their structure is elucidated using SEM and TEM, it is unlikely that the taxonomy can be clarified. In this study putative N. complanatoides is described in detail from light and electron microscopy. We have not compared it to the type material, however. It is the first complete description of a species from Hustedt’s (1962) Naviculaceae microstigmatacae Group 3. SEM and TEM data have been published by Ehrlich (1978, 1981) and Karayeva (1978b, N. complanatoides as Proschkinia complanatoides), but in both cases treatment is superficial and does not fully document frustular structure.

MATERIALS AND METHODS

Material was collected from a 70 L marine aquarium containing synthetic seawater (Instant Ocean, Aquarium Systems) maintained at 24 ± 2°C, a specific gravity of 1.020–1.050, and a pH of 7.9–8.3. A 2.5–3 cm thick mat of the filamentous green alga Derbesia marina (Lyngbya) Kjellman supported a rich flora of attached microalgae. The origin of the Derbesia and other algae in the aquarium is unknown, but they may have been introduced with shipments of fish and invertebrates originating at locations in the Caribbean and tropical Indo-West Pacific.

Samples were obtained by vigorously stirring D. marina filaments in a beaker. A brown suspension of diatoms and dino- fflagellates relatively rich in N. complanatoides was obtained. Samples were thus examined immediately by bright-field microscopy (BFM) and Nomarski interference microscopy (NIM) or fixed for at least 24 h in 2% glutaraldehyde in aquarium water at 4°C. Osmium tetroxide was removed by hydrogen peroxide, potassium dichromate treatment (van der Werff 1955) or by boiling in 50% nitric acid for 1 h. Cleaned material was examined with BFM, NIM, or TEM (air-dried onto Formvar coated, carbon reinforced 150 mesh copper grids). Cleaned material was also air-dried onto glass coverslips for SEM. Glutaraldehyde fixed cells and hydrogen peroxide cleaned cells were washed several times in distilled water, settled onto polylysine (Polycryscines Inc.) coated coverslips (Sanders et al. 1975), dehydrated in a 10% graded acetone series (10 min each) and processed in a Denton DCP-1 (Cherry Hill, NJ) critical point drying (CPD) apparatus using liquid CO2. After CPD the coverslips were attached to aluminum SEM specimen stubs and coated with 30 nm Au/Pd in a Denton DV 515 vacuum evaporator. Additional coating was applied in 10 nm increments with a Technics Hummer II (Alexandria, VA) diode sputterer as needed to prevent charging (Rosowski et al. 1981).

A Zeiss research microscope fitted with a 35 mm camera was used for BFM and NIM; specimens were photographed with Kodak Plus-X film (ASA 125). Ultrastructural analysis was performed with a Philips 201 TEM operated at 60 kV, using Kodak 4489 film, and with a Cambridge Stereoscan S410 SEM operated at 20 or 30 kV, angles of tilt (AOT) from 0–85°, and Kodak 4127 or Polaroid 55/PN film. Fusules were measured at 1000 × using an ocular micrometer in the Zeiss microscope or on the SEM at various magnifications and 0° AOT.

To assist in interpretation of valvar structures from various angles, a model of the axial area was constructed from Perma- plast modeling compound at a scale of approximately 1:2000. The size and shape of all components was estimated from SEM micrographs. The model represents the axial area near the center of the valve, including portions of the raphes sternum, three internal transapical interstriae (virgae), and three exterior longitudinal interstriae (vimines). The left hand side of the model was constructed from dark Perma-plast and the right hand from light. It was mounted on a small stand and photographed from several angles (particularly those which were difficult to interpret in SEM micrographs of N. complanatoides valves) with a Pentax Super Program 35 mm camera with a 28 mm macro lens and Kodak Plus-X film.

Whenever possible, descriptive terminology followed von Stosch (1975) and Ross et al. (1979) and, for raphes terminology, Cox (1977), Krammer (1982), and Mann (1984). Transapical interstriae and longitudinal interstriae are called virgae and vimines, respectively, after Cox and Ross (1981).

RESULTS

Light Microscopy

Living cells of N. complanatoides were actively motile on microscope slide wet mounts. Measurements of 60 cells indicated valves 26–55 μm long and 4–5 μm wide, and girdles 10–24 μm deep. Each cell had a central nucleus measuring 3–5 μm in diameter and two deeply lobed chloroplasts, one pressed against each girdle wall (Figs. 1, 2). In valve view, lobes of a chloroplast were arranged closer to one apex in the focal plane of one valve, and near the opposite apex in the opposing valve plane. The second chloroplast was inversely arranged giving the two plastids a diagonal symmetry. Pyrenoids were not observed in the chloroplasts, but no staining was attempted. Cells in various stages of division were
NOTE: Abbreviations used in figures: AD = air-dried, NA = nitric acid cleaned, PPD = hydrogen peroxide/potassium dichromate cleaned, CPD = critical point dried, AOT = angle of tilt, NIM = Nomarski interference microscopy, TEM = transmission electron microscopy. Figures are scanning electron micrographs at 20 kV and 0° AOT unless stated otherwise.

FIGS. 1-8. Figs. 1–3. Girdle views of glutaraldehyde fixed cells. NIM. N = nucleus, C = chloroplast, Scale = 10 μm. Fig. 1. One plate-like chloroplast in the focal plane. Fig. 2. Same cell as in Figure 1 but with the other chloroplast in focus. Fig. 3. Dividing cell with two new valves (arrows) formed within the frustule. Fig. 4. N. complanatoides valves. Note the forked structure (black arrow) and robust raphe sternum (white arrow). TEM, PPD, AD. Scale = 10 μm. Fig. 5. Valve view of intact frustule. Note opened raphe slit running the length of the valve (arrows). PPD, CPD. Scale = 5 μm. Fig. 6. Exterior of isolated valve. The medial axial ribs appear to fuse a short distance from the central area (arrows). NA, AD. Scale = 5 μm. Fig. 7. Oblique view of an epicingulum. The terminal fissure is barely visible (white arrow). PPD, CPD. Scale = 5 μm. Fig. 8. Central area of a valve face. VM = valve margin, E = elevation, S = striae bordering the axial area, arrow = point of overlap of the medial axial ribs. NA, AD. Scale = 1 μm.
often observed including those with daughter cells within the mature theca (Fig. 3).

Some features of cleaned valves were observed but not photographed. The raphe sternum was asymmetrical and thicker at the center of the valves than toward the apices. The raphe slit could not be seen. A narrow Y-shaped structure, formed by the central virga on one side of the raphe and two on the other, appeared to contain a large pore in its fork. Terminal nodules were evident as small bright spots a few µm medial to the valve apices. Parallel transapical striae (ca. 25 in 10 µm) could be detected only at the valve center, and faint longitudinal striae were also visible.

**SEM and TEM**

Because of the fragility of the frustules, air-dried specimens were invariably broken and useful only for studying broken edges in cross-section. In CPD samples, however, the integrity of the frustules was maintained, and the natural configuration of their components could be investigated.

**Valve structure.** *Navicula complanatoides* valves are narrowly lanceolate to rhombic in outline with apiculate apices (Fig. 4); exterior surfaces are marked by numerous longitudinal striae (Figs. 5, 6). The axial area is narrow but quite distinct and is separated from the remainder of the valve face by a wide stria on either side (Figs. 7, 8). An additional 9–13 very narrow, longitudinal striae are present on either side of the axial area totalling 20–28 such striae at the valve center (ca. 8–9 in 1 µm). The longitudinal striae end successively as they intersect the valve margin toward the apices (Figs. 7, 9). Subcylindrical ribs averaging 90 nm in diameter are present between the longitudinal striae (Figs. 8, 10, 11).

Internally, parallel transapical striae are recessed into the basal siliceous layer and separated by narrower virgae (Figs. 12–14). There are 24–28 transapical striae in 10 µm except at the valve apices where they become more dense (38 in 10 µm), slightly convergent (Fig. 4), and indistinct (Fig. 15). The striae are composed of apically oriented rectangular poroid areolae occluded internally by ricae (Fig. 13). Externally, the areolae open into the slit-like striae between the longitudinal ribs on the valve face. The perforations through each rica are very small (8–10 nm, as measured on uncoated TEM specimens) and roughly circular. About 4–5 longitudinal rows of 12–14 perforations comprise each rica.

The virgae form two characteristic features of the valve interior. The first is the Y-shaped structure crossing the central area and visible with light microscopy. Electron microscopy provides more detail (Figs. 12, 16) and demonstrates the presence of a pore (stigma) in the fork of the structure. The pore is bordered by a collar-like margin and is partially occluded (Fig. 12). An external opening is not visible but may be in a longitudinal stria or under a prominent elevation on the valve face (Fig. 8).

The second noteworthy feature is the way in which the virgae medially curve in away from the valve toward the internal edge of the raphe sternum (Figs. 11, 14). This inward curvature results in unusual chambers running along either side of the sternum (Figs. 10, 11, 14). These chambers open externally through the wide longitudinal striae bordering the axial area (Figs. 8, 11) and internally through large ricate areolae (Fig. 13).

The raphe sternum and raphe slit also are quite unusual. The raphe slit is between the left and right halves of the sternum (Fig. 10) but is not apparent in all valves. This has caused some difficulty in interpretation. Each side of the raphe sternum bifurcates externally (Fig. 10) to form the longitudinal ribs in the axial area. Either three (Figs. 6, 8) or four (Figs. 5, 7) such ribs may be visible depending on the position or specimen. In those specimens with four axial ribs the raphe slit is visible between the two medial ones. However, the slit is only visible near the central area in those valves with three ribs apparent. As Figures 8 and 11 suggest, the two medial ribs may overlap one another, obscuring the raphe slit and one rib in surface views. It is likely that overlap of the medial ribs is the natural configuration and that partial collapse of the girdle during preparation exerts a force pulling the raphe slit open (Fig. 9).

In Figure 11 we have interpreted structures a and b (and arrow) as connected to the left half of the raphe sternum and c and d as connected to the right. The left medial rib (b) overlaps the right (c, Fig. 11). Since the left half of the sternum has broken off behind the right it is not visible in the figure. Figure 14 shows a similar case. The broken left half of the sternum is visible with its club-shaped inner extremity (black arrow), but the broken end of the right half is hidden in shadows. Only the inner edge of the right half of the sternum is visible; it wraps over the left half (Fig. 14, white arrow). The raphe slit is visible between the two (Fig. 14, SL). Because one side of the sternum wraps over the other, the raphe slit is difficult to observe internally (Fig. 12) except near the valve apices where the sternum ends in small straight helictoglossae (Fig. 15). Presumably it is the helictoglossae which form the "terminal nodules" as viewed with BFM.

The Peima-plast model of the raphe system supports our interpretation. Figure 17 is analogous to Figure 11 in both viewing angle and labeling. When the model is rotated several degrees to the right (Fig. 18), the left half of the raphe sternum and raphe slit become visible (arrow). The other end of the same model (Fig. 19) is analogous to a valve with no overlap of the medial axial ribs (e.g. Fig. 10) and is made possible by constructing the axial ribs with variable overlap over the length of the valve (model—Fig. 20, cf. Fig. 8).

Externally, the central area is somewhat depressed into the valve (Figs. 21, 23) and contains a prominent
FIGS. 9-14. Fig. 9. End view of an intact frustule lying on the girdle plane. Arrows = terminal fissures, V = valves, GB = girdle bands. CPD, 85° AOT. Scale = 5 µm. Fig. 10. View of a broken valve. RS = left and right halves of the raphe sternum. VM = valve margin, arrow = raphe slit. NA, AD, 70° AOT. Scale = 1 µm. Fig. 11. View of a broken valve. a, b = axial ribs left of the raphe, c, d = axial ribs right of the raphe, arrow = internal edge of left side of the raphe sternum. The raphe slit (not visible) runs between b and c (see Fig. 25 for clarification). VC = valvocopular lumen. PPD, CPD, 70° AOT. Scale = 1 µm. Fig. 12. Central area of valve interior. F = forked structure, S = stigma, RS = larger side of the raphe sternum folded over the smaller one. PPD, AD. Scale = 1 µm. Fig. 13. Ricate areolae (RA) of the valve face. TA = internal transapical interstriae (virgae), S = longitudinal striae bordering the axial area and opening into the chambers beside the raphe sternum. VM = valve margin. TEM, PPD, AD. Scale = 200 nm. Fig. 14. Oblique interior view of a broken valve. Black arrow = left side of raphe sternum, white arrow = internal extremity of right side of the raphe sternum, TA = transapical interstriae (virgae), SL = raphe slit. CPD, 80° AOT. Scale = 1 µm.
elevation derived from one of the axial ribs (Fig. 8). The proximal ends of the raphe fissure are slightly enlarged and curve toward this elevation (Fig. 8). The distal raphe fissures form strongly recurved hooks (Figs. 7, 9), which are deflected in the same direction on opposite ends of a valve but opposite that of the proximal raphe termini (Fig. 7). In the few frustules checked, distal raphe fissures of opposing valves curved in opposite directions (Fig. 9).

**Girdle structure.** The girdle of *N. complanata* is deep (16–20 bands per epicingulum) and composed of valvocopulae, copulae, and pleurae (Figs. 22–24). Each copula is hollow, forming an unoccluded semi-tubular running the length of the frustule (Figs. 22, 23, 25). In cross-section each is U-shaped with the opening toward the valve. Although exterior “walls” and floors (abvalvar walls) are unornamented, the interior walls exhibit regularly spaced slits, forming comb-like edges (Figs. 22, 23, 25). The teeth of the combs are abutted against and fused to the abvalvar wall of the next girdle element in the advalvar direction (Fig. 23). Exterior walls of the copulae are not attached to adjacent elements along most of their length, although they may abut each other (Figs. 24, 26, 27). A prominent collar-like ridge runs along the exterior abvalvar edge of each copula (Figs. 22, 23, 25), but it does not appear to overlap the succeeding element.

The valvocopulae do not appear to differ from the copulae in structure but are wider in most frustules. In general, bands decrease in diameter in an abvalvar direction (Fig. 22). The valvocopulae and copulae are split rings (not completely encircling the girdle) opening alternately at opposite apices (Figs. 24, 26, 27). The gap in each copula is closed by a ligula on the adjacent abvalvar copula, and the ends of the copula appear to fuse to the ligula (Figs. 26, 27). Copular overlap is facilitated by a flattening of the semi-tubular elements at the poles, but the elements do not overlap along most of the length of the frustule. The valve mantle overlaps neither the inner nor outer valvocopular wall; instead the comb-like teeth of the inner wall fuse directly to the valve margin (Figs. 11, 15), whereas the exterior wall often appears free (Figs. 9, 24).

Overlap of the epicingulum and hypocingulum is facilitated by four pleurae on the abvalvar edge of the epicingulum (Figs. 26, 27). The pleurae are flat split rings, but unlike the copulae, they are flat (not semi-tubular) so that the hypocingulum can fit inside.

**DISCUSSION**

**Basal Siliceous Layer**

The valve structure of *N. complanata* is unlike that of other *Naviculae microstigmatae* which have been examined by SEM and TEM (Cox 1978). Areolae of *N. delognei* Van Heurck and *N. pseudocomoides* Hendey are round perforations through a simple layer of silica which open internally in transapical depressions between the virgae and are occluded by ricae with hexagonally arranged pores (Cox 1978). The ricae perforations in *N. complanata* are sometimes hexagonally arranged, but irregularities are frequent.

The type of striation and pore structure in *N. complanata* is reminiscent of that in *Haslea Simonsen* and *Gyrosigma Hassall*, with some similarities to the *Naviculae lineolatae* Cleve. Striae of *Naviculae lineolatae* are cross-lineate (Patrick 1959), but there is no tendency for the external slits to continue over several areolae (cf. *Navicula tripunctata* (Müll.) Bory, Cox 1979b). Ricae occlude the internal openings (Cox 1979b). Some *Gyrosigma* species, e.g. *G. littorale* (Cox 1979c) appear intermediate between *N. tri-
Valve structure of Haslea species is very similar to that of N. complanatoides. Simonsen (1974) emphasized the intersecting longitudinal and transapical striae pattern in *Navicula trompii* Cleve (TEM data, Helmcke and Krieger 1954, plates 173–175) and based the new genus *Haslea* on the *Naviculae fusiiformes* Cleve. Cox (1979c) has investigated valve structure in *H. crucigera* (Wm. Smith) Simonsen, and Neuville et al. (1975) have documented that in *H. ostrearia* (Gaillon) Simonsen. Both are structurally similar to *H. trompii*, and the reconstruction of *H. ostrearia* given by Neuville et al. (1975:402) seems to be representative of the genus. As in *N. complanatoides* (valve reconstruction, Fig. 28), *Haslea* valves are composed of a network of intersecting ribs (vrgae and vimines), with internal rical occlusions to the areolae. Externally, strips of silica which are broader than the vimines, overlie the latter, forming continuous longitudinal slits along the surface. Cross-sections of valves show that the external strips are flat in *H. ostrearia* (Neuville et al. 1975) but subcylindrical in *N. complanatoides*. *Haslea* and *Gyrosigma* have a peripheral stria which forms a continuous line around the valve perimeter (Cox 1979c). This is lacking in *N. complanatoides*.

**Raphe Structure**

Raphe morphology is useful in assessing relationships among taxa. Schrader (1973) and Cox (1977, 1979c) used SEM to document the internal and external paths taken by the raphes of many species. Schrader (1973) found great constancy in raphe structure within many of the genera examined, *Navicula* being an exception. He recommended that the *Naviculae fusiiformes* be removed from *Navicula* based on its atypical internal raphe structure which resembled that of *Pleurosigma* and *Gyrosigma*. Cox (1977) also emphasized the variation in raphe structure in *Navicula* and interpreted the variety as indicating the need for reassessment of the genus.

The external raphe path of *N. complanatoides* is similar to that in the *Naviculae lineolatae*, *Pleurosigma*, *Gyrosigma*, and *Haslea* in that all curve at the poles. However, these taxa differ with respect to the direction of curvature and the appearance of the central raphe endings (Schrader 1973, Cox 1977, 1979c). More similarities between these taxa become apparent when the internal raphe paths are examined. They all have the raphe slit in a ridge which is bordered by additional ribs developed to different degrees. In *N. ramosissima* (C.A. Ag.) Cleve (Sect. *Lineolatae*) one rib extends almost the entire length of the raphe system (Cox 1977). In *Pleurosigma angulatum* (Quekett) W. Smith there is a short rib on both sides of the raphe near the center of the valve (Schrader 1973, Cox 1977, 1979c), and in *Gy*...
**NAVICULA COMPLANATOIDES MORPHOLOGY**

269

**Figures 21–23.** Fig. 21. Stereo-pair of the central area of a valve face. Note the depression from which the elevation (E) rises. Arrows = raphe slit. PPD, CPD, AOT; left = 7°, right = 0°. Scale = 1 μm. Fig. 22. Abvalvar margin of an epicingulum in transverse section. The upper girdle wall has collapsed onto the lower. EW = exterior wall, IW = interior wall, L = lumen. PPD, CPD, 50 kV, 70° AOT. Scale = 1 μm. Fig. 23. Same frustule as Fig. 22, abvalvar margin with attached valve. Note comb-like edge of the inner girdle wall (white arrow). VC = valvocopula, D = depression in valve face, arrow = raphe slit. PPD, CPD, 50 kV, 70° AOT. Scale = 2 μm.

**roisigna** the two ribs may differ in the extent of their development from species to species. The two ribs are developed equally in *G. balticum* (Ehr.) Cleve (Schrader 1973) and *G. fasciola* (Ehr.) Cleve (Cox 1979c) but unequally in *G. attenuatum* (Kütz.) Rabb. (Cox 1977) and *G. litorale* (W. Smith) Cleve (Cox 1979c). This increase in asymmetry of the ribs is carried further in *Haslea crucigera* (W. Smith) Simonsen (Cox 1977) where the larger rib actually covers the raphe. In *N. complanatoides* these ribs (comprising the raphe sternum) are much deeper than in the above taxa, but once again their development is asymmetrical. The larger one covers the raphe and wraps over the smaller one in the central area, with the overlap decreasing toward the poles until the raphe is visible near the valve apices. *N. pseudocomoides* (Sect. Microstigmataceae) has a very different raphe structure which lacks the ribs altogether (Cox 1977).

Krammer (1981, 1982) examined the raphe slits in broken valves of many members of the Achnanthaceae and Naviculaceae and illustrated the known types. He stated that all raphes examined thus far are variations on four basic designs. The only species shown by Krammer (1982) in which the raphe slit is formed by two deeply intruding ribs of the sternum (termed raphe costae) is from the genus *Mastogloia* Thwaites. He considered it a variant of the key and slot design typical of the Naviculaceae (Krammer 1982). Unfortunately, information about the raphe slit in cross-section is not available for all the taxa appropriate for comparison to *N. complanatoides*. TEM investigations of Neuville et al. (1975) show that the raphe in *Haslea ostrearia* is of the key
and slot variety. There is one deep and one shallow raphe rib, and each has a key and slot such that the raphe slit follows two sharp bends forming an “S.” Both ribs in *N. complanatoides* deeply intrude into the frustule but the single key and slot is weakly developed so that the raphe slit is straight except for the slight hook at the internal edge of the ribs.

The raphe structure described here for *N. complanatoides* is at odds with that proposed by Karayeva (1978b). She noted similarities between internal views of the raphe of *N. complanatoides* and *N. tubilifera* Geissler and Gerloff (Geissler and Gerloff 1963, 1964) and considered them identical. Based on TEM micrographs of intact valves, Geissler and Gerloff (1964) presented a reconstruction of *N. tubilifera* in cross-section. They showed the raphe as a slit in a hollow tube, without openings to the interior. Such an axial canal-raphe is structurally similar to that in the Epithemiaceae and Surirellaceae (Paddock and Sims 1977) but is unknown in the Naviculaceae. Geissler and Gerloff presented no solid evidence in support of their claim, nor did Karayeva (1978b) provide new evidence to support this for *N. complanatoides*. However, believing *N. complanatoides* had a canal-raphe and realizing that such a species did not fit in the Naviculaceae, Karayeva (1978b) erect-
ed a new genus, Proschkinia, in its own suborder. The genus Proschkinia was to contain N. tubilifera, N. complanatoides, and N. bulnheimii Grun., all former members of the Naviculaceae microstigmataeae. Our work, however, suggests that the primary character (axial canal-raphe) separating Proschkinia from the naviculoid genera does not exist.

Girdle Structure

N. complanatoides also differs in girdle structure from other members of the Naviculaceae microstigmataeae. N. delognei and N. pseudocomoides do not have the hollow semi-tubular copulae found in N. complanatoides. Cox (1978) showed that their girdle elements are flat, overlapping bands with rows of pores similar to those in the valves. Presumably the girdle bands are split rings, with openings alternating at opposite apices, as with the flat bands of N. confervacea (Kutz.) Grun. (Rosowski 1980) and N. tripunctata (Cox 1979b).

It remains to be determined if the other four species in Hustedt's Naviculaceae microstigmataeae Group 3 have the same girdle structure as N. complanatoides. Ehrlich (1978) showed SEM figures of the girdle of N. complanata but did not comment on its structure. Externally it resembles our specimens and the girdle elements are probably hollow. Karayeva (1978b) showed TEM of a girdle segment from N. complanatoides, but the structure is unclear. With the exception of N. hylalosira, drawings and micrographs (Hustedt 1962) show very similar girdles in Group 3 species. In N. hylalosira, however, the girdle bands are wide and curved, with bands from the epicingulum and hypocingulum intersecting at an angle (Hustedt 1962, Fig. 1447). The figure bears strong resemblance to that of N. pseudocomoides drawn by Cox (1977, Fig. 1H), perhaps indicating a fundamental difference between Group 3 species. Since many of the Naviculaceae microstigmataeae have deep girdles it will be interesting to discover if they are of the N. pseudocomoides or N. complanatoides type.

Tubular girdle bands have not been recorded elsewhere in the Naviculaceae, but several other genera do possess modified copulae. In Striatella unipunctata (Lyngbye) Agardh, examined with SEM by Roth and de Francisco (1977), copulae are not tubular, but each does have an inwardly projecting septum. It is possible to envisage such a structure as the progenitor of tubular bands. Paddock and Sims (1980) have shown the presence of hollow bands in two Undatella species and believe that a third also has such bands. Their precise structure and integration were not determined, but struts crossing the cell interior from one side of a band to the other were found. The complex girdle of Rhabdonema arcuatum (Lyngbye) Kütz. includes hollow bands which are rectangular in cross-section and internally partitioned into a number of chambers (Pocock and Cox 1982). Each band possesses a collar-like margin on its abvalvar edge and overlaps the succeeding band. Although the collar-like margins on the abvalvar edges of N. complanatoides bands often appear not to overlap the succeeding bands, they may have been pulled apart during specimen preparation. As in N. complanatoides, R. arcuatum has flat pleurae facilitating overlap of the epicingulum over the hypocingulum.

All these species with stout septa or hollow girdle bands are marine littoral diatoms with deep girdles. Previous authors (e.g. Paddock and Sims 1980, Pocock and Cox 1982) have suggested that such modified bands are means of strengthening a deep girdle against the rigors of the littoral environment. It may be obvious that such deep girdles need to be strengthened; more intriguing questions are: Was the evolution of deep girdles via selection for that trait or the result of selection on a correlated trait? If deep girdles were selected for, what is their adaptive value? In any case, the presence of collar-like margins on tubular bands and the retention of flat pleurae in otherwise tubular girdles, in two unrelated species, may be indicative of shared constraints in the evolution of novel frustule designs.

Taxonomic Affinities

Our specimens clearly belong in Group 3 of the Naviculaceae microstigmataeae (sensu Hustedt 1962) on the basis of light microscopy. The five species of this group are separated on the basis of striae density, width and number of copulae, and fasciae and stigmat characteristics, and our specimens best fit the description of N. complanatoides. Material examined by Ehrlich (1978) was placed in N. complanata and differs from our material primarily in girdle depth. Figures of N. complanatoides presented by Karayeva (1978b) are not very clear but TEM and SEM views are very similar to our specimens, supporting our identification. However, our electron microscope study has revealed valve and girdle structures which are unknown elsewhere in Navicula. These suggest that N. complanatoides (and perhaps other members of this species group) do not belong in Navicula and should be removed.

Identification of homologous characters between what appear to be closely related taxa can help gen-
erate hypotheses concerning phylogenetic relationships of *N. complanatoides*. Structure of the basal siliceous layer and raphe may be indicative of the correct systematic position of the species. Unlike valve size, shape, and striae densities, which may change during cell diminution or under different growth conditions (Germain and Le Cohu 1981, Lange-Bertalot and Runrich 1981, Theriot and Stoemer 1984 and papers cited therein), changes in the fundamental construction of diatom valves may be expected to be more constrained. Similarities in valve construction between *Navicula lineolatae*, *Pleurosigma*, *Gyrosigma*, *N. complanatoides* and *Haslea* suggest a close affinity between these taxa. The development of continuous longitudinal striae from cross-lineate striae, and the increasing size and asymmetry of the raphe ribs from the *N. lineolatae* to *Pleurosigma*, *Gyrosigma*, and *N. complanatoides* and *Haslea*, is hypothesized to represent change in homologous characters from the ancestral to the derived state. Presence of a peripheral stria unites *Pleurosigma*, *Gyrosigma*, and *Haslea*, but their exact relationship with *N. complanatoides* is unresolved. *N. complanatoides* is the most derived with respect to girdle structure.

In order to further resolve the relationship of these taxa, other characters, such as those from chloroplasts (Cox 1981) and ontogeny (Mann 1984), should be analyzed. Cox (1981) has shown that there are significant differences between chloroplasts of *Navicula sensu stricto*, members of the *N. microstigmataceae*, *Haslea*, *Pleurosigma*, and *Gyrosigma*. Although *Gyrosigma* and *Haslea* chloroplast morphologies are similar to the typical *Navicula* type, *Pleurosigma* plastids appear quite different. It is most interesting that within the *N. microstigmataceae* two types of plastid morphology were found. *N. elongata* has two deeply lobed "butterfly" chloroplasts, whereas *N. complanata* (a Group 3 species) has two unlobed chloroplasts diagonally opposed at opposite apices. The *N. complanatoides* we examined has intermediate chloroplasts; they are lobed and have diagonal symmetry (though not to the same extreme as in *N. complanata*).

The data suggest *N. complanatoides* is more closely related to *Haslea* and *Gyrosigma* than to the *Naviculaceae lineolatae* and some members of the *Naviculaceae microstigmataceae*. As a consequence, *N. complanatoides* and similar species in the *Naviculaceae microstigmataceae* Group 3 should be removed from *Navicula* and placed in their own genus within the *Naviculaceae*. The genus *Proschkinia* has been published by Karyeva (1978b) for *N. complanatoides* and similar species, but it was not placed in the *Naviculaceae*. Future phylogenetic work on the taxa discussed here would benefit from detailed investigations of additional traits so that more characters can be incorporated into the analysis.

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Bacon, C. B. & Taylor, A. R. A. 1976. Succession and stratification in benthic diatom communities colonizing plastic collectors in a Prince Edward Island estuary. *Bot. Mar.* 19:231–40.

Cleve, P. T. 1994. Synopsis of the Naviculoid diatoms. Part 1. *K. Svenska Vetensk. Akad. Handl.* 26:1–194.

——. 1995. Synopsis of the Naviculoid diatoms. Part 2. *K. Svenska Vetensk. Akad. Handl.* 27:1–219.

Cox, E. J. 1977. Raphe structure in naviculoid diatoms as revealed by the scanning electron microscope. *Nova Hedwigia* Beih. 54:261–74.

——. 1978. Taxonomic studies on the diatom genus *Navicula* Bory. *Navicula grevillii* (C.A. Ag.) Heiberg and *N. complanatae* (Dillwyn) H. & M. Peragallo. *J. Bot. Linn. Soc.* 76:127–43.

——. 1979a. Studies on the diatom genus *Navicula* Bory. *Navicula scopulorum* Breb. and a further comment on the genus *Berkeleya* Grev. *Physcol. J.* 14:161–74.

——. 1979b. Taxonomic studies on the diatom genus *Navicula* Bory: The typification of the genus. *Bacillaria* 2:137–53.

——. 1979c. Symmetry and valve structure in naviculoid diatoms. *Nova Hedwigia* Beih. 64:193–206.

——. 1981. The use of chloroplasts and other features of the living cell in the taxonomy of naviculoid diatoms. In Ross, R. [Ed.] Proceedings of the Sixth Symposium on Recent and Fossil Diatoms. Otto Koeltz Science Publishers, Koennigstein, pp. 115–33.

Cox, E. J. & Ross, R. 1981. The striae of pennate diatoms. In Ross, R. [Ed.] Proceedings of the Sixth Symposium on Recent and Fossil Diatoms. Otto Koeltz Science Publishers, Koennigstein, pp. 267–78.

Edsbacke, H. 1968. Distribution notes on some diatoms not earlier recorded from the Swedish west coast. *Bot. Mar.* 11:54–63.

Ehrlich, A. 1978. The diatoms of the hypersaline Solar Lake (Ne Sina). *Isr. J. Bot.* 27:1–13.

Geller, U. & Gerloff, J. 1963. Elektronenmikroskopische Beiträge zur Phylogenie der Diatomeenrhaphe. *Nova Hedwigia* Beih. 6:339–52.

——. 1964. Eine neue *Navicula-Art* (*Navicula tubulifera* nov. spec.) aus dem Watt von Wilhelmshaven. *Nova Hedwigia* 7:482–8.

Germain, H. & Le Cohu, R. 1981. Variability of some features in a few species of *Gomphonema* from France and the Kerguelen Islands (South Indian Ocean). *In Ross, R. [Ed.] Proceedings of the Sixth Symposium on Recent and Fossil Diatoms. Otto Koeltz Science Publishers, Koennigstein, pp. 167–78.

Giffen, M. H. 1967. Contributions to the diatom flora of South Africa. III. Diatoms of the marine littoral regions at Kidd's Beach near East London, Cape Province, South Africa. *Nova Hedwigia* 13:246–92.

——. 1970. New and interesting marine and littoral diatoms from Sea Point, near Cape Town, South Africa. *Bot. Mar.* 13:87–99.

——. 1973. Diatoms of the marine littoral of Steenberg's Cove in St. Helena Bay, Cape Province, South Africa. *Bot. Mar.* 16:32–48.

——. 1975. An account of the littoral diatoms from Langebaan, Saldanha Bay, Cape Province, South Africa. *Bot. Mar.* 18:71–95.

Helmcke, J-G. & Krieger, W. 1954. Diatomeenschulen im elektro- nennmikroskopischen Bild. *Teil II.* J. Cramer, Weinheim.

Hendey, N. I. 1964. An introductory account of the smaller algae of British Coastal waters. Part V. Bacillariophyceae (Diatoms). *Fisheries Investigation Series IV.* H.M.S.O., London, 317 pp.
NAVICULA COMPLANATOIDES MORPHOLOGY

— 1974. A revised check-list of British marine diatoms. J. Mar. Biol. Assoc. U.K. 54:277–300.

Hostetler, F. 1955. Marine littoral diatoms of Beaufort, North Carolina. Mar. Sta. Bull. Duke Univ. 6:1–67.

— 1961–1966. Die Kieselalgen Deutschlands, Österreichs und der Schweiz. In Rabenhorst, L. [Ed.] Kryptogamen-Flora Vol. 7., Part 3, Sect. 1–4, 816 pp.

— 1962. Die Kieselalgen Deutschlands, Österreichs und der Schweiz. In Rabenhorst, L. [Ed.], Kryptogamen-Flora Vol. 7, Part 3, Sect. 2, pp. 161–348.

Karayeva, N. I. 1978a. New genus of the family Naviculaceae West. Bot. Zh. (USSR) 65:1593–6.

— 1978b. Novyi podporyadok diatomovyich rodorsei (a new suborder of diatoms). Bot. Zh. (USSR) 65:1747–50.

Krammer, K. 1981. Zur Deutung einiger Schalenstrukturen bei pennaten Diatomeen. Nova Hedwigia 55:75–105.

— 1982. Observations on the raphe slit of some Bacillariophyceae and ideas on its function. Arch. Hydrobiol. Suppl. 65:177–88.

Küttzing, F. T. 1833. Synopsis Diatomarum. Linnaea 8:529–620.

— 1844. Die Kieselschaligen Bacillarien oder Diatomeen. Kohne, Nordhausen, 192 pp.

Lange-Bertalot, H. & Rumrich, U. 1981. The taxonomic identity of some ecologically important small Naviculae. In Ross, R. [Ed.] Proceedings of the Sixth Symposium on Recent and Fossil Diatoms. Otto Koeltz Science Publishers, Koenigstein, pp. 135–44.

Mann, D. G. 1984. An ontogenetic approach to diatom systematics. In Mann, D. G. [Ed.] Proceedings of the Seventh International Diatom Symposium. Otto Koeltz Science Publishers, Koenigstein, pp. 113–44.

McIntire, C. D. & Moore, W. W. 1977. Marine littoral diatoms: Ecological considerations. In Werner, D. [Ed.] The Biology of Diatoms. University of California Press, Berkeley. Bot. Monogr. 13:333–71.

McIntire, C. D. & Overton, W. S. 1971. Distributional patterns in assemblages of attached diatoms from Yaquina Estuary, Oregon. Ecology 52:758–77.

Neuville, D., Daste, P. & Geneves, L. 1975. Recherches sur l’organisme ultrastructurale et la formation du frustule de la Diatomee Navicula ostraria (Gaillon) Bory. Rev. Gen. Bot. 82:391–417.

Paddock, T. B. B. & Sims, P. A. 1977. A preliminary survey of the raphe structure of some advanced groups of diatoms (Epithemiaceae-Surirellaceae). Nova Hedwigia Beih. (54):291–322.

— 1980. Observations on the marine diatom genus Auriola and two new genera, Undatella and Proboscidea. Bacillaria 3:161–96.

Patrick, R. 1959. New subgenera and two new species of the genus Navicula (Bacillariophyceae). Notulae Naturae 924:1–11.

Pocock, K. L. & Cox, E. J. 1982. Frustule structure in the diatom Rhabdonema arcuatum (Lyngh.) Kütz. with particular reference to the cingulum as seen with the scanning electron microscope. Nova Hedwigia 56:621–41.

Riznyk, R. Z. 1973. Interstitial diatoms from two tidal flats in Yaquina Estuary, Oregon, U.S.A. Bot. Mar. 16:113–38.

Robert, J. M. 1973. La Diatomee Navicula ostraria Bory en Baie de Bourgneuf. Rev. Trav. Inst. Pesch Marit. 37:363–8.

Rosowski, J. R. 1980. Valve and band morphology of some freshwater diatoms. II. Integration of valves and bands in Navicula confervacea var. confervacea. J. Phycol. 16:88–101.

Rosowski, J. R., Hoagland, K. D., Roemer, S. C. & Lee, K. W. 1981. Improving the image of delicate and complex biological surfaces. Scanning 4:181–7.

Ross, R., Cox, E. J., Karayeva, N. I., Mann, D. G., Paddock, T. B. B., Simonsen, R. & Sims, P. A. 1979. An amended terminology for the siliceous components of the diatom cell. Nova Hedwigia Beih. 64:513–33.

Roth, L. E. & de Francisco, A. 1977. The marine diatom, Striatella unispunctata. II. Siliceous structures and the formation of intercalary bands. Cytobiologie 14:207–21.

Sanders, S. K., Alexander, E. L. & Braylan, R. C. 1975. A high-yield technique for preparing cells fixed in suspension for scanning electron microscopy. J. Cell Biol. 67:476–80.

Schrader, H.-J. 1973. Types of raphe structures in the diatoms. Nova Hedwigia Beih. 45:195–217.

Simonsen, R. 1974. The diatom plankton of the Indian Ocean expedition of R/V "Meteor" 1964–1965. "Meteor" Forsch.-Ergebnisse. Reihe D. No. 19:1–107.

Theriot, E. & Stoerner, E. F. 1984. Principal component analysis of character variation in Stephanodiscus nissarum Ehrenb. Morphological variation related to lake trophic status. In Mann, D. G. [Ed.] Proceedings of the Seventh International Diatom Symposium. Otto Koeltz Science Publishers, Koenigstein, pp. 97–111.

van der Werff, A. 1955. A new method of concentrating and cleaning diatoms and other organisms. Int. Ver. Theor. Angew. Limnol. Verh. 12:276–7.

— 1970. In Huls, P. D. G. [Ed.] 1957. Diatomeënflora van Nederland. De Hoef, Utrecht. XVI.1:109.

von Stoch, H. A. 1975. An amended terminology of the diatom girdle. Nova Hedwigia Beih. 55:1–36.