A comparison of the morphology and ultrastructure of the diatoms (Bacillariophyceae) *Discostella stelligera* and *D. elentarii* from two lakes in Fiordland, New Zealand

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**Abstract** Morphological and ultrastructural variability of the diatoms, *Discostella stelligera* and *Discostella elentarii*, were examined from lakes Te Anau and Manapouri, located in Fiordland on the South Island of New Zealand. The objectives of this study were to examine and compare the size variability, frustule morphology, and ultrastructural detail of these taxa, which were recently collected from locations of a different origin than the type localities. To support the current descriptions of the newly erected genus of *Discostella* and recently described species, *D. elentarii*, information on morphological characteristics across valve patterns was compared. A principal components analysis of the external valve features of *D. stelligera* and *D. elentarii* measured by light microscopy did not separate the two taxa. This close similarity in external morphology highlights the need to optically dissect the valves underneath the light microscope to clearly differentiate between the convex/concave nature of the *D. stelligera* valves compared with the flat valve of *D. elentarii*. New external and internal morphological details of *D. elentarii* were documented with a field emission scanning electron microscope.

**Keywords** *Cyclotella*; *Discostella stelligera*; *D. elentarii*; diatoms; field emission microscope; Lake Te Anau; Lake Manapouri; New Zealand; morphology; ultrastructure

**INTRODUCTION**

The diatom genus *Cyclotella* (Kützing) Brébisson has been a recent focus of systematists, and clades of related species have been transferred to newly erected genera (Skabichevskii 1975; Håkansson 2002). The stelligeroid taxa of *Cyclotella* have been transferred to a new genus, *Discostella* (Houk & Klee 2004). *Cyclotella sensu lato* has been characterised by the presence of two distinct patterns on the valve face consisting of marginal, radially arranged striae, and a distinctly different pattern in the central area (Lowe 1975). *Cyclotella sensu lato* has fultoportulae located in a ring near the margin and, in some species, also in the central area (Lowe 1975). The rimoportulae are few and located on the costae or at the edge of the central area (Round 1990). Within the genus *Cyclotella*, there are c. 15 species of stelligeroid taxa, which are distinguished from other *Cyclotella* by the presence of a stellate pattern in the central area (Houk & Klee 2004). These taxa have marginal fultoportulae and one rimoportula located at the valve edge between two costae. The external expression of the rimoportula is more centripetal to the valve margin than that of the fultoportulae (Lowe 1975; Houk & Klee 2004). The genus *Discostella* was erected to accommodate a clade of species whose placement of the fultoportulae and rimoportulae were inconsistent with the current description of *Cyclotella* (Houk & Klee 2004).

*Discostella stelligera* (Cleve et Grunow) Houk et Klee, the type species from Lake Rotoaira, New Zealand.
Zealand, maintains the two morphologically distinct patterns of the valve face that consist of marginal radially arranged striae and a distinctly different central area (Houk & Klee 2004). These morphological patterns of *D. stelligera* are further characterised by a concentrically undulate valve face with a stellate pattern composed of alveoli or external ridges. Stellate patterns composed of alveolae that are open to the inside are always present on valves that are convex, whereas concave central areas lack alveolae and have an ill-defined or “ghost” stellate pattern. The frequency and mechanisms of regulation for the development of these morphological patterns (i.e., degree of development or pattern of the alveolate stellate pattern) are not known.

Inside the valve, the collared fultoportulae are located between every one to four marginal costae with satellite pores situated laterally on either side of the process. The single rimoportula is located between two costae and is externally expressed with a simple opening (Houk & Klee 2004).

*Discostella elentarii* (Alfinito et Tagliaventi) Houk et Klee, a recently described stelligeroid species, was first collected from Lake Monowai on the South Island of New Zealand (Alfinito & Tagliaventi 2002). *Discostella elentarii* has flat valves with a large, colliculate central area with scattered punctae. A pore at the end of a shortened stria marks the external expression of the fultoportulae located every one to two striae. The interior of the valve is smooth in the centre with costae and alveoli in the marginal area. The fultoportulae are located between the costae and are surrounded by two satellite pores. There is one sessile rimoportula per cell. Some cells have a faint stellate pattern in the central region of the valve interior that does not penetrate the siliceous layer (Alfinito & Tagliaventi 2002).

Using light microscopy (LM), it can be difficult to differentiate between *D. stelligera* and *D. elentarii* given the ill-defined stellate pattern on the external concave valve face of *D. stelligera* and the faint stellate pattern on the internal valve face of *D. elentarii*. The purpose of the present work was to describe and compare the size variability, frustule morphology, and ultrastructural detail of *D. stelligera* and *D. elentarii* to better differentiate between them. This study was centred on LM and scanning electron microscopy (SEM) observations of recent collections from two fiord lakes, and differs from previous studies of these taxa that have focused on collections from the type localities. Observations included the use of a field emission SEM, which enabled close examination and documentation of fine ultrastructural detail of *D. elentarii* that previously had not been described for this genus. This work supports recent name changes for *D. stelligera* and *D. elentarii*, and provides a modern record of two additional New Zealand locations for these taxa. The descriptions of the two fiord lakes are provided to contribute knowledge of the habitat of these two newly transferred taxa.

**MATERIALS AND METHODS**

**Study sites**

Diatom samples were collected from lakes Te Anau (45°12’S, 167°47’E) and Manapouri (45°31’S, 167°27’E) on the South Island of New Zealand (Fig. 1). Previous studies of *D. stelligera* focused on collections made from Lake Rotoaira (39°01’S, 175°42’E), a caldera on the North Island, and previous studies of *D. elentarii* focused on collections from
Lake Monowai (45°87’S, 167°45’E) of the South Island in the same Fiordland region as lakes Te Anau and Manapouri (Fig. 1).

Lake Te Anau originated from the union of four glacial valleys resulting in highly complex basin morphologies (Carter & Lane 1996). It is the largest lake on New Zealand’s South Island, and it occurs at 203 m a.s.l. (Jolly 1968; Glasby et al. 1991). The lake has a surface area of 347 km², with a long axis of 60 km. Lake Te Anau has three fiord arms, the North Fiord, Middle Fiord and South Fiord. There are 26 islands within the lake and eight named basins, six of which are over 250 m deep (Jolly 1968; Glasby et al. 1991). Lake Te Anau can be classified as an oligotrophic, warm monomictic lake (Jolly 1968; Glasby et al. 1991). The lake is relatively clear with an average Secchi disc reading of 10 m (Wells et al. 1998). The main inflows are located at the head of the lake and in the three fiord arms, and the main outflow is into the Waiau River, which flows to Lake Manapouri. The catchment surrounding Lake Te Anau covers an area of 2998 km² and is composed of 43% native forest, 37% tussock, 12% lake, and 4% lowland scrub (Jolly 1968; Glasby et al. 1991). The western part of the catchment contains primarily metamorphic rock with some granite and sandstones, whereas the eastern part of the catchment is primarily gravels (Jolly 1968; Glasby et al. 1991).

Lake Manapouri is smaller than Lake Te Anau (surface area 143 km²) with the greatest breadth and length being 9.6 and 19.3 km, respectively (Ellwood et al. 2001). It is a deep (max. 444 m), glacial lake fed primarily by the Waiau River, which drains Lake Te Anau (Ellwood et al. 2001). Lake Manapouri has an average Secchi disc reading of 6.5 m (Wells et al. 1998) and can be classified as a warm, monomictic lake. The catchment surrounding Lake Manapouri is c. 3000 km² and is composed of tussock-covered hills or native forestland (Ellwood et al. 2001).

Sample collection and analysis

Periphyton was collected from littoral areas from various arms of Lake Te Anau on 18 February 2002, and Lake Manapouri on 21 and 22 February 2002. Macrophytes were shaken in a bag with water to remove epiphyton. Epilithon was scraped off rocks using a spoon, and epipsammon and epipelon were carefully removed using a pipette. Samples were air dried onto aluminum foil and processed in the laboratory.

Diatoms were cleaned in preparation for LM (Round et al. 1990) and SEM (Postek et al. 1980). Twenty-five ml of sample were removed and boiled in nitric acid in a 1:1 sample to acid ratio until the volume returned to the original volume of 25 ml. The samples were rinsed with distilled water and allowed to settle for 8 h, followed by decanting. This rinsing process was repeated until the pH of the suspension was neutral (c. 10 rinses). The cleaned material was then concentrated, air dried onto coverslips and processed for either LM or SEM analysis.

For LM, coverslips were permanently mounted onto glass slides with Naphrax® mounting medium and analysed under oil immersion at 1000× using an Olympus BX51 photomicroscope with high resolution Nomarski DIC optics. Digital images were captured with a monochromatic camera (Spot®) attached to the microscope and recorded on computer. Size variability and external valve features were measured digitally using SPOT® software (v. 4.01, Diagnostic Instruments, Inc.) calibrated against a stage micrometer and recorded for 53 D. stelligera valves and 211 D. elentarii valves. The density of D. stelligera valves was much lower than that for D. elentarii valves, thus an unequal number of valve measurements were obtained for the two taxa. Measurements included the valve diameter, the diameter of the central area (from valve centre to the inner start of the striae), striae length, number, and density, the ratio of striae length to radius of the central area, and the ratio of the centre radius to the valve radius. Striae density was calculated in two ways. First, the conventional striae/10 µm was calculated. Next, the number of striae/90° was calculated. This second calculation provides a standardised summary of the striae density to ensure that the size of the cell does not alter the proportion of the valve from which the striae are counted. Summaries of the morphological characteristics were calculated using JMP (v. 5.1). A principal components analysis (PCA; Primer 5 v. 5.2.9) (Legendre & Legendre 1998) was performed on standardised morphological measurements based on a correlation matrix to test whether the external morphological variables other than the flex of the central area (flat, concave, convex) could distinguish between D. stelligera and D. elentarii or if different size dependent trends were present for each taxa. A PCA was performed on measurements that applied to all valves (no inner stellate measurements included), first for both taxa considered together and then for each taxon separately. A PCA was also applied to D. stelligera data including measurements of the inner stellate pattern.

For SEM, coverslips were mounted on specimen stubs and either sputter-coated with a gold/palladium alloy (10 nm) and analysed using a Hitachi S2700
Although different size-dependent trends were not apparent for *D. elentarii* and *D. stelligera*, variance of the second PCA axis was explained by slightly different morphological characteristics. Part of the variance of the second PCA axis for both taxa was explained by striae length (µm), and striae length (µm)/centre radius (µm) (Table 2). Additional variance of the second PCA axis for *D. elentarii* was explained by cell diameter and total number of striae, the latter having a higher loading value and a negative relationship (Table 2). In contrast, additional variance of the second PCA axis for *D. stelligera* was explained by centre radius (µm)/total radius (µm), and striae density (Table 2). When the measurements of the inner stellate pattern of *D. stelligera* were included in the PCA (PCA-D), the variance was spread over three axes with the presence or absence of the centre alveolus explaining most of the variance of the third PCA axis (Table 2).

Spines were documented in one or more rows on the valve face of *D. elentarii*, and their arrangement varied from no spines to spines encircling the entire valve face (Fig. 24–27). In contrast, spines were not observed on valves of *D. stelligera* (Fig. 28–30). Every valve of *D. elentarii* examined using FEVP contained a fimbriate margin (Fig. 38–40). The fultoportulae were expressed externally as a round opening on the margin of the striae (Fig. 41, 42); however, the external expression of the rimoportula was not observed. External expression of the punctae located on the margin of each costa, including those around the fultoportulae, was observed using both SEM and FEVP (Fig. 40, 41). The central area of *D. elentarii* was flat with punctae scattered throughout (Fig. 24, 25). These punctae have not been previously documented, and it is believed that they are now visible owing to the high resolution achieved using FEVP.

**Internal valve morphology**

Collared fultoportulae, surrounded by two satellite pores, occurred between every two to four costae on *D. elentarii* (Fig. 43–45). Punctae were found fairly consistently on the margin of every costa of *D. elentarii* valves, and were only occasionally absent (Fig. 44–47). The punctae of *D. elentarii* were covered with a cribrum that did not appear clearly in previous internal valve micrographs of *Discostella*. It is not known if this cribrum is present in *D. stelligera* as valves were not observed under FEVP because of limited access to the FEVP. No more than one nearly sessile rimoportula was observed on valves...
Fig. 2–22 A series of light micrographs showing the size range and external morphology of *Discostella elentarii* and *D. stelligera*. **Fig. 2–7** *D. elentarii* with a smooth, flat valve face. **Fig. 8–13** *D. elentarii* with ghost striae in the centre area. **Fig. 14–19** *D. stelligera* with a convex valve face with a distinct stellate pattern in the centre. **Fig. 20–22** Valves where the characteristics of these two taxa are less clear. **Fig. 20** A flat valve with a stellate pattern. **Fig. 21** A valve with ghost striae that needs to be optically dissected to determine the flex of the valve. **Fig. 22** *D. stelligera* valve where the stellate pattern is less distinct and optical dissection is required. Scale bar: 10 µm.
Discostella elentarii had an inward projection whose position was uncertain as to whether it occurs on the girdle band or the inside edge of the valve. Closer examination of both the valve margin and single copula of the girdle is required to better understand the location of this structure (Fig. 51, 52). Also on this ambiguous part of the cell, a row of pores was noted (Fig. 53–55). There was no previous record of these pores, and the taxonomic importance is unknown. These pores were only visible using the FeVp, and as no valves of Discostella stelligera were observed under FEVP, it is unknown if they are present on Discostella stelligera.

**DISCUSSION**

Owing to the range of variability in morphology and heterovalvar nature of stelligeroid taxa, it can be difficult to distinguish between taxa with...
Table 2  Principal components analyses (PCA) of the correlation matrix for the morphological characteristics of Discostella stelligera and D. elentarii. PCA component loadings of the morphological characteristics: A, both taxa; B, D. elentarii; C, D. stelligera (no inner stellate measurements); D, D. stelligera (including inner stellate measurements).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<tbody>
<tr>
<td>Eigen values (λ)</td>
<td>6.14</td>
<td>1.29</td>
<td>6.41</td>
<td>1.09</td>
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<tr>
<td>Cell diam. (µm)</td>
<td>0.397</td>
<td>0.133</td>
<td>0.390</td>
<td>0.366</td>
</tr>
<tr>
<td>Striae length (µm)</td>
<td>0.352</td>
<td>0.388</td>
<td>0.355</td>
<td>0.455</td>
</tr>
<tr>
<td>Striae length (µm)/centre radius (µm)</td>
<td>-0.303</td>
<td>0.498</td>
<td>-0.315</td>
<td>-0.419</td>
</tr>
<tr>
<td>Centre radius (µm)/total radius (µm)</td>
<td>0.330</td>
<td>-0.463</td>
<td>0.343</td>
<td>-0.001</td>
</tr>
<tr>
<td>Centre diam. (µm)</td>
<td>0.400</td>
<td>0.005</td>
<td>0.391</td>
<td>-0.061</td>
</tr>
<tr>
<td>Total no. of striae</td>
<td>0.394</td>
<td>-0.025</td>
<td>0.386</td>
<td>-0.680</td>
</tr>
<tr>
<td>Striae/10 µm</td>
<td>-0.218</td>
<td>-0.607</td>
<td>-0.231</td>
<td>-0.061</td>
</tr>
<tr>
<td>Striae/90°</td>
<td>0.394</td>
<td>-0.025</td>
<td>0.386</td>
<td>-0.061</td>
</tr>
<tr>
<td>No. of inner stellate alveoli</td>
<td>0.303</td>
<td>0.104</td>
<td>0.203</td>
<td></td>
</tr>
<tr>
<td>Inner stellate diam. (µm)</td>
<td>0.356</td>
<td>-0.076</td>
<td>0.044</td>
<td></td>
</tr>
<tr>
<td>Presence of centre alveolus</td>
<td>-0.085</td>
<td>0.011</td>
<td>0.912</td>
<td></td>
</tr>
<tr>
<td>Total variance explained (%)</td>
<td>76.8</td>
<td>16.1</td>
<td>80.1</td>
<td>13.6</td>
</tr>
<tr>
<td>Cumulative variance explained (%)</td>
<td>76.8</td>
<td>92.9</td>
<td>80.1</td>
<td>93.7</td>
</tr>
</tbody>
</table>
similar or overlapping morphological characteristics such as those of *D. stelligera* and *D. elentarii*. It is probable that *D. stelligera* and *D. elentarii* are sibling species. *Discostella stelligera* was originally described from New Zealand material (Houk & Klee 2004) but has been reported from many localities in both hemispheres (Houk & Klee 2004). *Discostella elentarii* is thus far endemic to New Zealand and may have recently descended from *D. stelligera*, making separation of the two species difficult. Our initial examination of the stelligeroid valves from lakes Te Anau and Manapouri did not result in a clear differentiation between the two taxa owing to the morphological variability in the central area. Upon further examination, it was clear that the concave/convex nature of the valves is a critical factor in distinguishing between these taxa, especially when ghost striae are present. The PCA analysis of the morphological features demonstrated the overlap in external valve features between *D. stelligera* and *D. elentarii*. Our study emphasises the importance of optically dissecting valves of *D. stelligera* and *D. elentarii* to determine the flat or concave/convex nature of the valve when the stellate characteristics of the centre area appear similar; i.e., on concave *D. stelligera* valves with more of a shadow stellate pattern than a well developed stellate pattern of the convex valve, where the centre alveoli perforate the basal siliceous layer.

The external morphological features of *D. stelligera* and *D. elentarii* were generally similar to those reported in previous studies. The largest valve diameter of *D. stelligera* was lower than that reported by Houk & Klee (2004; 5–40 µm) and

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**Fig. 38–42** External view of *Discostella elentarii* captured by field emission variable pressure scanning electron microscope. **Fig. 38–40** Fimbriate margin on external valve face (a arrows). **Fig. 41, 42** External expression of the fultoportulae (b arrows). **Fig. 40, 41** External expression of the punctae located on the margin of each costa (c arrows). Scale bars: 1 µm.

**Fig. 43–50** Internal view of *Discostella elentarii* captured by field emission variable pressure scanning electron microscope. **Fig. 43–45** Fultoportulae between every second to third costa. **Fig. 46, 47** Collared fultoportulae with satellite pores on either side. **Fig. 48–50** Rimoporal between two costae with satellite pores on either side. Scale bars: 1 µm (Fig. 43–45, 48–50), 500 nm (Fig. 46), 300 nm (Fig. 47).

**Fig. 51–55** Internal morphological characteristics of *Discostella elentarii* captured with field emission variable pressure scanning electron microscope. **Fig. 51, 52** Open girdle with anti ligula. **Fig. 53–55** Pores on girdle band with unknown taxonomic importance. Scale bars: 1 µm (Fig. 51–53, 55), 500 nm (Fig. 54).
similar to that reported by Lowe (1975; 5–25 µm). The range in striae density for D. stelligera (8.6–14.7 striae/10 µm) was similar to that reported in other studies (Houk & Klee 2004, 8.9–11.5 striae/10 µm; Lowe 1975, 10–14 striae/10 µm). The range in valve diameters of D. elentarii (4.0–25.9 µm) was similar to those reported by Alfinito & Tagliaventi (2002, 6–25 µm). The range in striae density (8–14.5 striae/10 µm) extended the upper and lower densities from those previously reported (Alfinito & Tagliaventi 2002, 9–10 striae/10 µm).

The external and internal valve characteristics of D. stelligera and D. elentarii reported in this study were similar to those previously documented. This study provides further documentation of the arrangement and variability in the spines of D. elentarii. In addition to providing high quality images of the fine ultrastructural details, this study introduced a morphological feature not documented before on diatoms. The row of pores on the inside margin of the valves of D. elentarii was visible on every cell examined. Increased development and access to technological advances in microscopy will further our understanding of fine ultrastructural details of diatom morphology such as those observed in this study.

Our results support the findings previously recorded for these closely related species. With the recent separation of Discostella, our study contributes evidence of different and new morphological details of D. elentarii in addition to describing D. stelligera and D. elentarii from recent collections from two new locations of different origin than the type localities. A PCA of the external valve features measured by LM did not distinguish between the two taxa and highlights the need to optically dissect the valves underneath the light microscope to clearly differentiate between the convex/concave valve face of D. stelligera compared with the flat valve face of D. elentarii. The pores on the inside margin of the valves of D. elentarii have not been documented before, and the fimbriate margin and the presence of the cribrum have not been recorded for this species until now.

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