

Frustular morphology and polyphyly in freshwater *Denticula* (Bacillariophyceae) species, and the description of *Tetralunata* gen. nov. (Epithemiaceae, Rhopalodiales)

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Background and aims – With the advent of molecular techniques to analyze relationships among diatom genera came the realization that the complex canal raphe system has evolved in at least two separate lineages, the Bacillariales and the Rhopalodiales + Surirellales. One genus with a canal raphe system, *Denticula*, is a small, but morphologically diverse group of species whose systematic position has been debated since its description.

Methods – Presented here is documentation of the valve and girdle band morphology of *D. kuetzingii*, *D. tenuis*, *D. valida*, *D. rainierensis*, *D. vanheurckii* and *Epithemia zebra* var. *denticuloides*, all freshwater taxa, based on light and scanning electron microscopy.

Key results – Observations suggest that *Denticula* is polyphyletic, with species previously placed in *Denticula* allied with several different groups within the Bacillariales. Another group of species, represented here by *D. vanheurckii*, appears more similar morphologically to the Rhopalodiales, especially the genus *Epithemia*. A new genus, *Tetralunata*, is proposed for *D. vanheurckii* and its allies. This new genus is restricted to Java and Bali.

Conclusion – This work emphasizes the utility of evaluating morphological features within an established phylogenetic context and is part of a larger study to investigate relationships among the Bacillariales using morphological and eventually, molecular data.

Key words – Bacillariales, canal raphe, *Denticula*, diatom, *Epithemia*, new genus, species flocks, Sumatra, systematics, *Tetralunata*.

INTRODUCTION

Raphid diatoms are a monophyletic lineage (e.g. Medlin et al. 1996, Medlin & Kaczmarska 2004, Sorhannus 2004, Theriot et al. 2010) that possess a slit through their siliceous cell wall called a raphe. The canal raphe system is a specialized type of raphe in which the raphe is separated from the cell interior by struts of silica referred to as fibulae. As a result of the position of the raphe and the fibulae, the raphe is essentially inside a canal with small ‘windows’ called portulae, connecting the raphe canal to the cell interior (Round et al. 1990). Once considered a homologous feature (e.g. Hustedt 1928, Schrader 1973), molecular phylogenies have revealed the canal raphe system has evolved in at least two independent lineages, the Bacillariales Hendey and the Rhopalodiales D.G.Mann + Surirellales D.G.Mann (e.g. Sorhannus 2004, Sims et al. 2006, Bruder & Medlin 2008, Ruck & Theriot 2011). One genus that bears a canal raphe, *Denticula*

Kütz., is of particular interest because its systematic position has been debated.

Denticula was originally described as belonging to the Fragilariaceae Grev. (Kützing 1844), a group of diatoms with a longitudinal sternum but lacking a raphe system. *Denticula* was placed in this group primarily based on the presence of costae, and was recognized as separate from *Diatoma* Bory because of its growth as individual cells and not in the zig-zag colonies typical of the latter genus (Kützing 1844). The original description of *Denticula* was broad and included some species that have since been transferred to a wide range of genera, including *Diatoma*, *Rhabdonema* Kütz., and *Cymatopleura* W.Smith (Fourtanier & Kociolek 2011). Additional authors (Schütt 1896, Van Heurck 1896, Mann 1907, Schönfeldt 1907) continued to classify *Denticula* as an araphid genus into the 20th century. Van Heurck (1896) questioned the position of *Denticula* within the fragilarioid diatoms and suggested that it may have a closer affinity with *Nitzschia* Hassall based on the presence of a keel,

a thickened elevated structure for the raphe on some valves, but still included it in the Tabellariineae cohort Van Heurck (most closely related to *Diatoma*). During its classification as an araphid diatom, *Denticula tenuis* Kütz. was designated the type species of the genus by Mann (1907), a status often attributed to *D. elegans* Kütz. by Boyer (1927), a later authority (Fourtanier & Kociolek 2011). While earlier workers included only freshwater taxa in the genus, Boyer (1927) included marine species as well.

A second approach was forwarded by Grunow (1862), who disagreed with Kützing (1844) and moved *Denticula* into the Bacillariaceae Ehrenb., a group that included *Nitzschia*, *Tryblionella* W.Smith, *Bacillaria* J.F.Gmelin, and *Homeocladia* C.Agardh. Grunow argued the characteristic feature uniting this group was the strongly eccentric keel. Boyer (1927), Simonsen (1979), and Round et al. (1990) agreed with Grunow's classification. However, Grunow (1862) described Bacillariaceae as a family separate from the Epithemiaceae Grunow and the Surirellaceae Kütz., whereas Boyer (1927) and Simonsen (1979) placed the nitzschoid group closer to the Surirellaceae. Boyer (1927) suggested nitzschoid diatoms were closely related to *Epithemia*, but closer to *Surirella* and that *Denticula* may be separate from all of these based on their presence of thickened siliceous ribs ('costae'), even mentioning *Denticula* had a potential affinity to *Diatoma* because of their similar 'striation' patterns. Based on his study of marine species (Simonsen & Kanaya 1961), Simonsen (1979) erected a new genus for the marine taxa of *Denticula*, *Denticulopsis* Simonsen, and placed this new genus and *Denticula* within the Bacillariaceae. Subsequently many of these marine species, recent and fossil, have been transferred into new genera within the Bacillariales (i.e., *Crucidenticula*, *Neodenticula*; Akiba & Yanagisawa 1985). Bessey (1899) and Simonsen (1979) indicate the Bacillariaceae may have evolved from the Naviculaceae Kütz., with no direct relationship to the Epithemiaceae or Surirellaceae, a theory later supported by molecular phylogenies (e.g. Sorhannus 2004, Bruder & Medlin 2008, Ruck & Theriot 2011). Round et al. (1990) agreed with Grunow (1862) and separated the Bacillariales (including *Denticula*) from the Rhopalodiales and the Surirellales.

A third perspective was offered by Hustedt (1914, 1928), who studied four *Denticula* taxa (i.e., *D. tenuis*, *D. thermalis* Kütz., *D. valida* (Pedicino) Grunow in Van Heurck, *D. vanheurckii* Brun) and, based on the shared features of raphe structure and presence of transapical fibulae that partition the valve, he transferred *Denticula* into the Epithemioideae. Karsten (1928), Patrick & Reimer (1975), and Krammer & Lange-Bertalot (1988) followed Hustedt's classification. Karsten (1928) based his agreement with Hustedt's classification on examination of a single taxon, *D. vanheurckii*. Krammer & Lange-Bertalot (1988) examined a greater number of taxa (seven) in their own work and based their classification on Geitler (1977), who studied the cytoplasmic and sexual (auxosporulation) characteristics of only two *Denticula* species, *D. tenuis* and *D. vanheurckii*. Geitler (1977) found variability within *Denticula*, mainly the presence of cyanobacterial endosymbionts and one plastid in *D. vanheurckii* (allying

this lineage with *Epithemia* and *Rhopalodia*) and the lack of endosymbionts and two plastids in *D. tenuis*. Krammer & Lange-Bertalot (1988) followed Geitler (1977) by considering *D. tenuis* an aberrant taxon within the Epithemiaceae. A molecular study later revealed the presence of endosymbionts within *Epithemia* and *Rhopalodia* to be the result of a single evolutionary event (Nakayama et al. 2011).

In each of these proposals regarding the systematic position of *Denticula*, the genus has been regarded as monophyletic. Sims (1983), Mann (1989), and Round et al. (1990) hint at an alternative idea, where some species of *Denticula* may be closely related to the Bacillariales, and others (specifically *D. vanheurckii*) more closely related to the Rhopalodiales. Specific observations in support of this scenario have not been published.

Today, *Denticula* is considered a relatively small genus with only ~ 100 taxa (Fourtanier & Kociolek 2011) with few taxa (eight) reported from Europe and the United States (Patrick & Reimer 1975, Krammer & Lange-Bertalot 1988), but considerably higher taxon richness (nineteen) in Sumatra (Hustedt 1938) and Java (Brun 1891). *Denticula* species are often found in the littoral margins of lakes, ponds and streams with high conductivity, with particular species inhabiting either cold mountain streams or hot springs (e.g. Kützing 1844, Patrick & Reimer 1975, Krammer & Lange-Bertalot 1988).

Morphologically, *Denticula* species are united by a linear to lanceolate valve outline, symmetry about the apical and transapical axis, and a canal raphe system subtended by large internal fibulae that either partially (e.g. *D. kuetzingii* Grunow) or entirely (e.g. *D. tenuis*) extend across the valve face from margin to margin, forming partitions within the valve (Round et al. 1990). Some authors have included taxa with either partial or entire fibulae in *Denticula* (e.g. Krammer & Lange-Bertalot 1988), whereas others have placed taxa with only partial fibulae in different subgroups (Schönfeldt 1907, Grunow 1862) or other genera (*Nitzschia*, Lange-Bertalot & Krammer 1993). In addition to fibula features, raphe characteristics (i.e., position, presence/absence of a keel, and whether the raphe is continuous or interrupted) and valvocopula structure are variable and often used for species identification (Krammer & Lange-Bertalot 1988). Limited investigation of cytoplasmic characteristics has revealed the presence of endosymbiotic cyanobacteria in one taxon (*D. vanheurckii*), but not others (*D. tenuis*) and either one or two chloroplasts (Geitler 1977).

The goal of this study is to document with light and scanning electron microscopy the valve and cingulum structure of several *Denticula* taxa, including the generitype. These observations are expected to cast light on the systematic position of *Denticula* species as compared to *Epithemia* (Rhopalodiales) and *Nitzschia* (Bacillariales). Included in this study is an examination of Hustedt's material from Sumatra containing species that many authors (Sims 1983, Mann 1989, Round et al. 1990) have mentioned as being closely related to *Epithemia*.

Table 1 – List of diatom samples examined.

Information includes: collection locality, accession number, geographic location, date of collection, temperature, conductivity, and pH measurements. ¹JPK: Kociolek Collection at the University of Colorado, Museum of Natural History Diatom Herbarium; BRM: Alfred-Wegener-Institute für Polar- und Meeresforschung. ²Latitude and longitude are reported in decimal degrees. ³n.d.: no data.

Locality	Collection accession number(s) ¹	Latitude, longitude ²	Collection date	Temperature (°C)	Conductivity (mS cm ⁻¹)	pH
Coldbrook Creek, California	JPK 2724	34.29411, -117.8396	9 Jun. 2008	17.4	285	8.7
Blue Lake, Utah	JPK 8512	40.50257, -114.03359	14 Apr. 2012	6.78	26.24	8.08
Blue Lake, Utah	JPK 8535/ JPK 8535-Dent3208	40.50257, -114.03359	14 Apr. 2012	8.7	15.37	7.22
Indonesia - Sumatra, Ranaumeer, Nordufer, Banding Agung, Potamogeton	JPK 9297/ BRM AS555	n.d. ³	28 Jan. 1929	n.d.	n.d.	n.d.
Indonesia - Sumatra, Danau di Atas, Moos an Felsblock	JPK 9298/ BRM AS756	n.d.	17 Mar. 1929	n.d.	n.d.	n.d.
Indonesia - Sumatra, Tobasee, Bucht von Meat, Potamogeton von der Oberfläche	JPK 9299/ BRM AS869	n.d.	10 Apr. 1929	n.d.	n.d.	n.d.
Indonesia - Sumatra, Tobasee, Pangururan-Beken, Nordufer, Potamogeton	JPK 9300/ BRM AS871	n.d.	12 Apr. 1929	n.d.	n.d.	n.d.
Sheep Lakes, Colorado	JPK 9144/ JPK 9144-Dent1	40.40422, -105.61983	9 Sep. 2012	n.d.	n.d.	n.d.

MATERIAL AND METHODS

Material

Samples examined include: recent collections from Colorado; the Southern California Coastal Water Research Project (SCCWRP) from 2004 to 2008 (www.sccwrp.org); a larger survey of the flora of Blue Lake, Utah; and the Friedrich Hustedt collection. Collection information including water chemistry data (when available) is presented in table 1. All materials discussed have been placed in the Kociolek Collection at the University of Colorado, Museum of Natural History Diatom Herbarium, Boulder, Colorado and are available upon request. Two of the taxa, *D. kuetzingii* and *D. valida*, were isolated and cultured (cultures available upon request) from the Colorado site and one of the Utah sites, respectively (see site information below) prior to examination. Morphology of these isolates was confirmed with field material to ensure that culturing conditions did not cause aberrant morphologies (both culture and field material are pictured). These two taxa were isolated and grown in WC and modified WC [same nutrients, but with artificial sea salt Instant Ocean® (Blacksburg, Virginia) to increase conductivity] media, respectively in 12:12 light:dark conditions.

The Colorado site was located along the edge of Sheep Lakes within Rocky Mountain National Park at 2590 m above sea level. Sheep Lakes are a series of small ponds ~ 3 km from the Fall River entrance to the park. The sample from this site was composed of epiphytes collected from *Chara* spp.

The California site is located in Coldbrook Creek, a tributary off the North Fork of the San Gabriel River, in the An-

geles National Forest northwest of Los Angeles, California. The site is ~ 85 km from the Pacific Ocean at 1021 m above sea level with an average depth of 9 cm and a discharge of 0.078 m³ s⁻¹. Water chemistry data and a composite sample of all available habitats were collected in the summer of 2008 by SCCWRP.

The two Blue Lake, Utah sites were located on the littoral edge of Blue Lake and surrounding ponds (see table 1 for exact localities). Blue Lake is a natural warm spring located 26 km south of Wendover, Utah at 1300 m above sea level. Temperature, conductivity, and pH were measured near shore using a YSI 556 multiprobe (YSI Incorporated, Yellow Springs, Ohio) at the time of sampling. The two samples analyzed here were a composite sample from the benthos (JPK 8512) and a sample of epiphytes on grass (JPK 8535).

Samples from Sumatra were analyzed by Hustedt (1935, 1938) as part of his evaluation of the freshwater diatoms of Java, Bali and Sumatra (table 1). We are indebted to Dr. Friedel Hinz for supplying four samples from the Hustedt Collection for this analysis. The Sumatran material was collected originally by Ruttner (1931) as part of a 10-month German expedition (1928–1929) to document the biota of lakes and rivers in Bali, Java, and Sumatra. The four epiphytic samples analyzed for this study were all collected from lakes in Sumatra, including: one sample (JPK 9297/BRM AS555) of epiphytes collected from *Potamogeton* sp. is from the north shore of Lake Ranau; one sample (JPK 9298/BRM AS756) of epiphytes from moss growing on a rock from Lake Diatas; and two samples (JPK 9299/BRM AS869; JPK 9300/BRM AS871) of epiphytes from two sites in Lake Toba (both collected from *Potamogeton* sp.).

Table 2 – Characters and character states utilized in the cladistic analysis.

Character	State (1) description	State (0) description	State (2) description
1. Areolae lunate	Lunate areolae present (see Results; figs 5H & 7C)	Lunate areolae absent (e.g. <i>Surirella</i> , <i>Amphora</i> , <i>Navicula</i>)	
2. ‘Entire’ fibulae	‘Entire’ fibulae are thickened fibulae that extend entirely across the internal valve face (see Results; figs 6A & 7E)	Fibulae are marginal (See Results; e.g. <i>Surirella</i>) or absent (e.g. <i>Amphora</i>)	
3. Frustule symmetry along the apical axis	Frustule is asymmetrical along the apical axis (fig. 7A & E)	Frustule is symmetrical along the apical axis (fig. 5A & D)	
4. Keel	A thickened, raised structure holding the raphe (keel) is present (see Results; e.g. <i>Rhopalodia</i>)	Absence of a keel (e.g. figs 5D & 7B)	
5. Raphe canal	Frustule has a specialized raphe in a canal (figs 6A & 7E)	Raphe is not in a canal (e.g., <i>Amphora</i>)	
6. Raphe position	Raphe is positioned along the ventral margin of the frustule (fig. 7A, B & E)	Raphe is positioned along the center of the frustule (fig. 5A & D–F)	
7. Raphe shape	Raphe is slightly arched (figs 5A & D–F, 7A & B)	Raphe is straight (e.g. <i>Navicula</i>)	Raphe is biarcuate (e.g. <i>Epithemia</i>)
8. Raphe continuity	Raphe is continuous (e.g. <i>Surirella</i>)	Raphe is interrupted or discontinuous (figs 5E & 7C)	
9. Valvocopula structure	Valvocopulae with extensions that cover and envelope the ‘entire’ fibulae (e.g. fig. 6C)	Valvocopulae simple bands	Valvocopulae a narrow scalloped band with corrugations that clasp the ‘entire’ fibulae (e.g. <i>Epithemia</i>)

Methods

Each sample was boiled in nitric acid. Cleaned materials were rinsed with filtered water and settled systematically until neutral pH was achieved. For light microscopy (LM) observations, cleaned materials were dried onto coverslips, mounted to slides with Naphrax®, and examined using an Olympus BX-51 LM (Olympus America Inc., Center Valley, Pennsylvania). Photomicrographs were captured using an Olympus DP 71 digital camera. For scanning electron microscopy (SEM) observations, cleaned material was dried on coverslips that were mounted on aluminum stubs with double-sided carbon tape. Dried material was sputter coated with 1 nm of gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK) and examined using a JEOL JSM 6480LV low vacuum SEM at an acceleration voltage of 15 kV or a JEOL JSM 7501 field emission SEM (JEOL Ltd, Tokyo, Japan) at an acceleration voltage of 3–5 kV.

Morphological investigation of specimens of *Denticula vanheurckii* and *Epithemia zebra* var. *denticuloides* suggested these taxa belonged to the Rhopalodiales, while the remaining *Denticula* spp. studied here (*D. kuetzingii*, *D. rainierensis*, *D. tenuis*, *D. valida*) are closely allied with the Bacillariales. To examine the phylogenetic relationships within the Rhopalodiales and determine whether *D. vanheurckii* and *E. zebra* var. *denticuloides* represent a monophyletic lineage from Indonesia, we performed a cladistic analysis. Other taxa included in the analysis were chosen due to their morphological similarity to these taxa (*Epithemia*, *Rhopalodia*, and *Surirella*). *Amphora* and *Navicula*

were chosen as outgroups based on their systematic position as close relatives to the Rhopalodiales and Surirellales in recent molecular phylogenies (e.g. Sorhannus 2004, Bruder & Medlin 2008, Ruck & Theriot 2011). Character states of *Epithemia*, *Rhopalodia*, *Surirella*, *Amphora*, and *Navicula* were determined by descriptions and images presented in Sims (1983) and Round et al. (1990). Nine unordered and equally weighted characters, described below, were utilized, two of which were multi-state characters (table 2). Coding of the character states analyzed is presented in table 3. The data matrix was analyzed using the branch and bound procedure in PAUP* 4.0b10 to determine the most parsimonious trees.

Table 3 – Data matrix for the cladistic analysis.

9 = not applicable.

Taxa	Character numbers and states								
	1	2	3	4	5	6	7	8	9
<i>Denticula vanheurckii</i>	1	1	0	0	1	0	1	0	1
<i>Epithemia zebra</i> var. <i>denticuloides</i>	1	1	1	0	1	1	1	0	1
<i>Epithemia</i>	1	1	1	0	1	1	2	0	2
<i>Rhopalodia</i>	1	1	1	1	1	0	0	0	0
<i>Surirella</i>	0	0	9	1	1	9	9	1	0
<i>Amphora</i>	0	0	1	0	0	0	9	0	0
<i>Navicula</i>	0	0	0	0	0	0	0	0	0

Terminology

Despite past (Anonymous 1975, Ross et al. 1979) and recent (Cox 2012) efforts to standardize descriptions and features, terminology used to describe diatom morphology generally, and *Denticula* in particular, is often confusing and non-uniform. In the context of *Denticula* specifically, this inconsistency in terminology can be attributed to the great morphological variability within the genus and the variable systematic position of the genus (and traditional usage of terms associated with each of these groups). In an effort to clarify these terms, provided here are definitions, references, synonyms, and figures illustrating terminology previously applied to *Denticula* and/or similar species that were used in this study.

Areolar strut. Small strut of silica projecting inward from the walls of the areolae that often support volae (Sims 1983). Fig. 6B, black arrowheads; fig. 7G, arrow.

Cribrum. A thin layer of silica across an areola and perforated by small, regularly arranged pores (Anonymous 1975). Fig. 1C, arrow.

Deck. A plate of silica corresponding to the deck of a ship if the valve were a ship, sometimes isolated from the valve (*sensu* Yanagisawa & Akiba 1990). No evidence was found that the ‘deck’ is a separate plate of silica from the valve in this group of species, and as such the use of this term should be discouraged for these groups. Evidence contradicting the occurrence of a deck is presented in figure 3F.

Domed cap. External small cap of silica covering an areola that is supported internally by areolar struts originating from the margins of the areola (Sims 1983). Fig. 5H, arrow.

Keel. Thickened raised structure holding the raphe (Anonymous 1975). Fig. 2B, arrowheads.

Lunate areolae. Complex alveolate areolae with ‘domed caps’ externally that are supported internally by areolar struts. Externally, volae extend from ‘domed caps’ leaving semicircular slit-like openings, usually 4–5 per areola. Fig. 5H.

Marginal ridge. Hyaline ridge of silica on the valve face, often opposite the raphe (Round et al. 1990). Fig. 1B & D.

Fibula. Internal strut of silica that provides structure and support for the raphe canal (Round et al. 1990). Fibulae may extend: transapically for a short distance, which we term ‘marginal’ (e.g. *Nitzschia perminuta* (Grunow) M. Peragallo; Krammer & Lange-Bertalot 1988); part of the way across the valve face interior, termed ‘partial’ (fig. 1E); or entirely across the valve, termed ‘entire’ (e.g. fig. 2C). Although the term costae is often used to describe fibulae that extend farther across the valve than marginally (in *Epithemia* and *Rhopalodia* by Round et al. 1990), the term ‘costae’ is vague and has been used to describe structures that are not homologous in disparate groups of diatoms (e.g. *Diatoma*, *Meridion*, *Epithemia*, *Rhopalodia*). Additional synonyms for fibulae include: keel puncta, pseudosepta (*sensu* Yanagisawa & Akiba 1990, Simonsen & Kanaya 1961), and *Fibularwände* (Krammer & Lange-Bertalot 1988).

Portula. The round to elliptical opening created by fibulae widening along the apical plane closer to the valve. Fig. 2C *versus* fig. 4F.

Valvocopula. Girdle band closest to the valve. In some taxa the valvocopulae have extensions that adjoin transapically to envelop the fibulae, sometimes referred to as septa (Patrick & Reimer 1975, Round et al. 1990) or septum-like plates/structures (Gotoh 1985, Round et al. 1990). The structure of these extensions is often used as a taxonomic character (Sims 1983, Gotoh 1985). Fig. 2D *versus* fig. 7E.

Vimine. Crossbar between the virgae that separate areolae within a stria (Cox & Ross 1981). Fig. 6B, white arrowhead.

Virga. Bar of silica between the striae (Cox & Ross 1981). Fig. 6B, arrow.

Vola. Flap-like areola covering that originates from the edge of areolae (Sims 1983). Fig. 5H, arrowheads.

RESULTS

Denticula kuetzingii Grunow (Grunow 1862)

Figure 1, appendix 1A–H

Synonyms – *Denticula denticula* (Grunow) Schönfeldt (1907) – *Nitzschia denticula* Grunow, in Cleve & Grunow (1880); see Schönfeldt (1907) and Lange-Bertalot & Krammer (1993) who suggested these synonymies.

LM: description – *Frustules* with nitzschoid symmetry. In valve view, valves narrowly lanceolate to elliptical to linear with convex to parallel sides and narrowly rounded to acute apices, 13–33 µm long and 4–5 µm wide (fig. 1A, appendix 1A–G). *Striae* parallel in the center to convergent to the apices, but evenly spaced throughout the valve, 16–18 striae in 10 µm. *Canal raphe* continuous and eccentric with no obvious keel, fibulae number 5–8 in 10 µm. In LM, fibulae appearing well defined near the canal raphe, merging with the valve face across the apical axis (appendix 1C–F), but possibly visible across the entire valve if focused on their base (appendix 1B). If focusing on the top of the valve, a small round nodule visible along the fibulae close to the canal raphe (appendix 1A & G, arrows). In girdle view, cells rectangular and the small round nodules on each fibula more obvious (appendix 1H, arrow).

SEM: description – *Canal raphe* raised slightly above the valve face lying at the junction of the valve face and the mantle (fig. 1B). *Canal raphe* continuous from pole to pole (fig. 1B) with hooked distal raphe ends (fig. 1C). Opposite the raphe, a hyaline marginal ridge (fig. 1B), more obvious in girdle view (fig. 1D, arrow). *Striae* consisting of round areolae each occluded by a delicate cribrum (fig. 1C). Viewed from the interior, raphe system subtended by fibulae widening toward the valve face to form round portulae (figs 1E, arrows). Round nodule present on each of the fibulae close to the raphe side of the valve (fig. 1E, arrowheads). Fibulae farthest from the valve face close to the canal raphe and gently decreasing in height to ~2/3 the way across the transapical axis where they appear only slightly wider and almost the same height as the slightly thickened virgae (fig. 1E). Fibulae maintaining a similar width across the valve and occurring every 2–3 striae. Virgae slightly thickened across the valve face except ~3 µm from each apex (fig. 1E). Vimines linking the fibulae and virgae apically expanded at their attachment point to form round areolae. Vimines less thickened than the virgae (except towards the apices) and giving the striae the

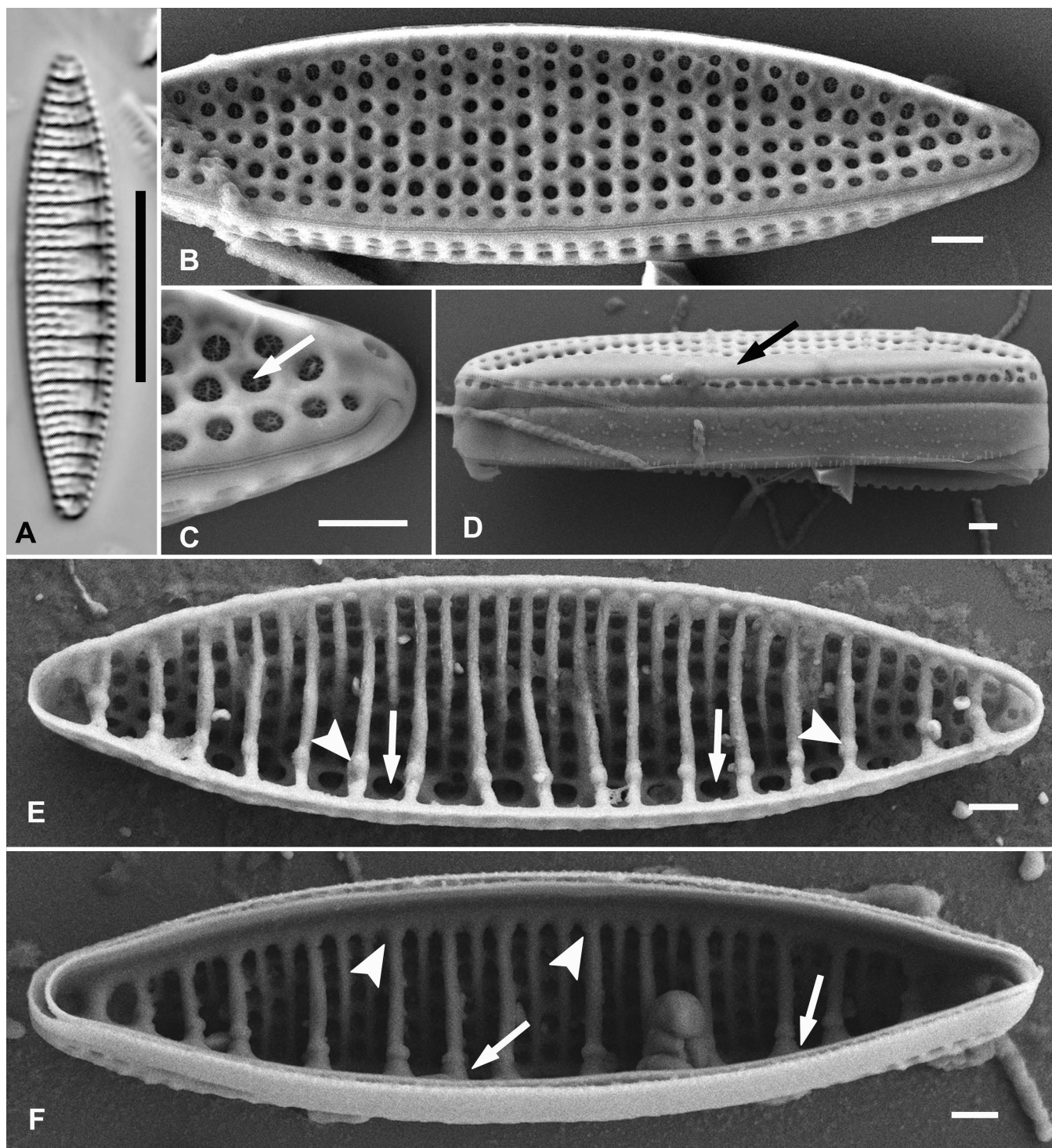


Figure 1 – *Denticula kuetzingii* from field material (A) and a culture (B–F) from Sheep Lakes, Colorado, USA, LM (A) and SEM (B–F): A, valve view; B, external view of whole valve with continuous raphe and marginal ridge; C, external view of apex showing hooked distal raphe end and cribrum (arrow); D, external view of one valve in girdle view showing the marginal ridge (arrow); E, internal view of whole valve with portulae (arrows) and round nodules (arrowheads) on each of the fibulae; F, internal view of whole valve with valvocopula showing septum extensions adjoining the round nodules on the raphe side of the valve (arrows) and just grasping the fibulae or thickened virgae on the opposite side of the valve (arrowheads). Scale bars = 10 µm in A; 1 µm in B–F.

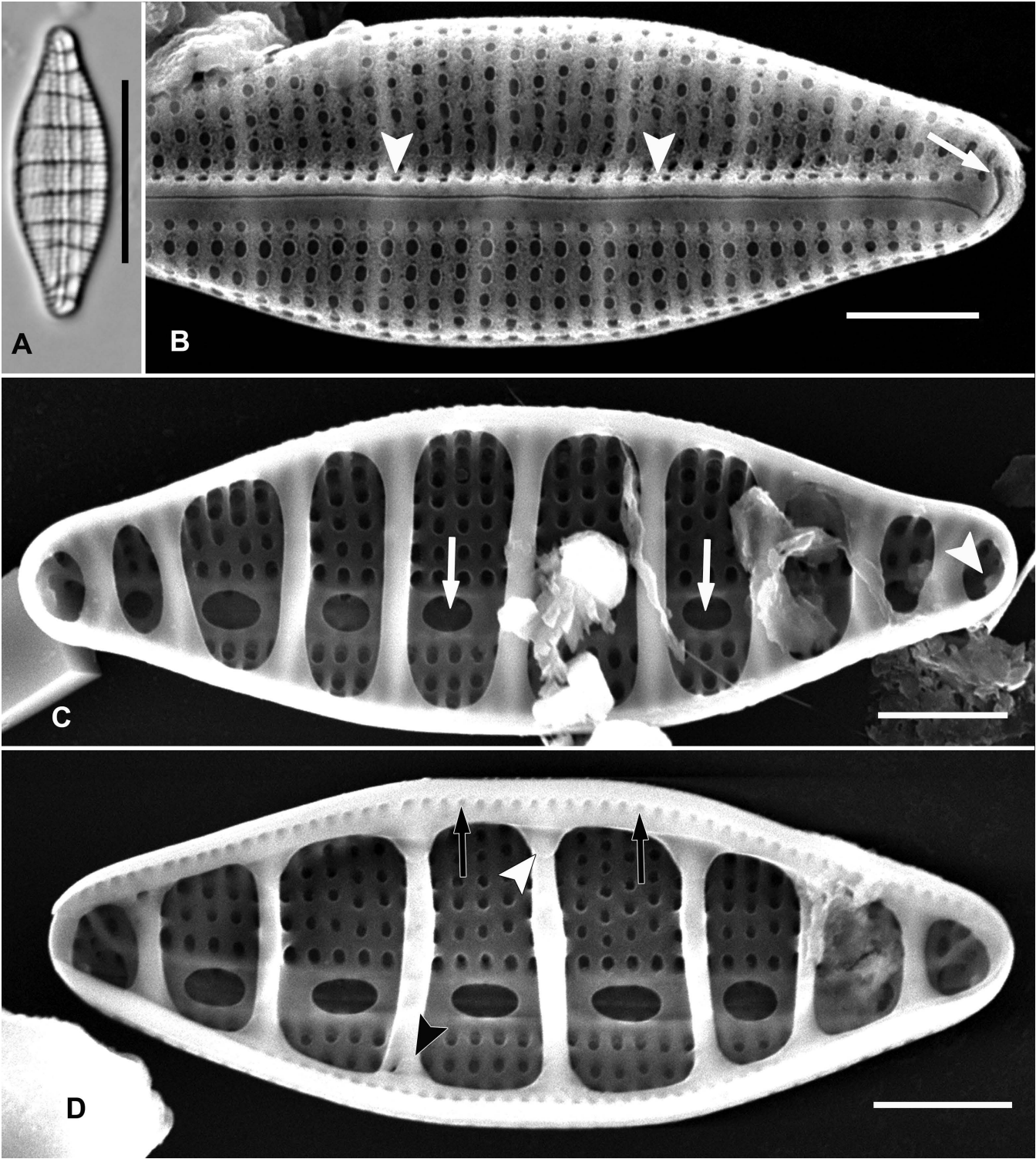


Figure 2 – *Denticula tenuis* from Coldcreek, California, USA, LM (A) and SEM (B–D): A, valve view; B, external view of whole valve with hooked distal raphe end (arrow) and keel (arrowheads); C, internal view of whole valve with portulae (arrows) and distal helictoglossa (arrowhead); D, internal view of whole valve with valvocopula showing areolae (arrows) and scalloped extensions (arrowheads). Scale bars = 10 μ m in A; 2 μ m in B–D.

appearance of occurring in a shallow canal. Valvocopula an open band with septum extensions adjoining the round nodules on the raphe side of the valve (fig. 1F, arrows) and just grasping the fibulae or thickened virgae on the opposite side of the valve (fig. 1F, arrowheads).

Material examined – JPK 9144, JPK 9144-Dent1 (see table 1).

Remarks – This taxon is similar to *N. amphibia* Grunow, *N. denticuloides* Hust., *N. robusta* Hust., *N. semirobusta* Lange-Bert., and *N. subdenticula* Hust. (e.g. members of the subgenus *Denticuloidea* of *Nitzschia sensu* Lange-Bertalot 1993) due to both the degree the fibulae extend across the transapical axis and the structure of the valvocopulae (Lange-Bertalot & Krammer 1993).

Denticula tenuis Kütz. (Kützing 1844)

Fig. 2, appendix 1I–O

LM: description – Frustules with nitzschiod symmetry. In valve view, valves lanceolate with cuneate to rostrate apices, 11–19 µm long and 4–6 µm wide (fig. 2A, appendix 1I–N). Striae parallel at the center of the valve to slightly radiate towards the apices, but evenly spaced throughout the valve, striae 25–29 in 10 µm. Canal raphe continuous, slightly eccentric from the apical axis, and raised on a keel above the valve surface, fibulae number 5–7 in 10 µm. Fibulae extending across the entire transapical axis (appendix 1J, L, & N). In girdle view, frustules rectangular and fibulae not appearing capitate (appendix 1O).

SEM: description – Continuous raphe situated within a hyaline area on a keel with hooked distal raphe ends (fig. 2B). Striae consisting of elliptical areolae surrounded by slight siliceous ridges (fig. 2B). Areolae smaller and more rounded closest to the raphe (fig. 2B). From the interior, canal raphe system subtended by large fibulae widening to the valve face to form round to elliptical portulae (fig. 2C, arrows). Fibulae becoming wider transapically towards the valve margins. Fibulae spaced every 5 striae towards the center of the valve to every 2 striae towards the apices (fig. 2C). Virgae slightly thickened at the margins and similar width to the vimines towards the raphe (fig. 2C). Internally, distal raphe ends terminating as helictoglossae prior to the hook of the external terminal fissures (fig. 2C, arrowhead). Valvocopula an open band with a single row of areolae (fig. 2D, arrows) and only slightly scalloped extensions that meet up with each of the fibulae (fig. 2D, arrowheads).

Material examined – JPK 2724 (see table 1).

Denticula valida sensu Patrick & Reimer (1975)

Fig. 3, appendix 1P–U

LM: description – Frustules with nitzschiod symmetry. In valve view, valves lanceolate to linear with convex to parallel sides with the raphe side often having a slight constriction indicating an intermissio, and rounded to acute apices, 18–37 µm long and 5–7 µm wide (fig. 3A, appendix 1P–T). Striae parallel in the center to slightly convergent to the apices, but evenly spaced throughout the valve, 19–22 striae in 10 µm. Canal raphe eccentric with no obvious keel, fibulae number 3–4 in 10 µm. Fibulae extending entirely across the transapical axis and parallel throughout the valve with

a slightly larger gap between fibulae towards the valve constriction. If focusing on the fibulae, two humps visible on the fibulae towards the valve margins with a slightly more silicified area between them, giving the fibulae the appearance of having a small bow tie (appendix 1T). In girdle view, cells rectangular with capitate fibulae (appendix 1U, arrow).

SEM: description – Raphe situated between the valve and the mantle (fig. 3B) with simple proximal raphe ends (fig. 3C, arrow) and hooked distal raphe ends (fig. 3D). In the external view, striae consisting of elliptical to rectangular areolae increasing in size and density towards the margin opposite the raphe (fig. 3B & C). Striae extending transapically across the valve and onto the mantle (fig. 3B). From the interior, the raphe system subtended by fibulae that widen toward the valve face to form round portulae (fig. 3E, arrows). Moving across the transapical axis, each fibula thin at the attachment point to the mantle, drastically thickening to form a hump, becoming slightly more narrow again to a second hump and finally becoming thinner again to the attachment point on the opposite mantle (fig. 3F). Fibulae spaced slightly closer together towards the apices, often with 4–6 striae between them (fig. 3F). Virgae slightly thickened at the margin opposite the raphe and similar width to the vimines towards the raphe (fig. 3F). Proximal raphe ending in a small central nodule (fig. 3G). Internally, distal raphe ends terminating as helictoglossae prior to the hook of the external terminal fissure (fig. 3H). Valvocopula a closed band with only slightly scalloped extensions that meet up with each of the fibulae (fig. 3I).

Material examined – JPK 8535, JPK 8535-Dent3208 (see table 1).

Remarks – The identity of this taxon requires further research. The narrow concept of *D. valida* described by Patrick & Reimer (1975) best fits our specimens. Although an examination of the material pictured in Krammer & Lange-Bertalot (1988) indicates some of our specimens to be similar to their concept of *D. valida* (which includes *D. kittoniana* Grunow in Van Heurck), their description of the fibulae structure (a single knob-shaped protuberance, as in *D. elegans* Kütz.) does not agree with the specimens presented here.

Denticula rainierensis Sovereign (Sovereign 1963)

Fig. 4, appendix 2A–F

LM: description – Frustules with nitzschiod symmetry. In valve view, valves are linear to lanceolate with acute to narrowly rounded apices, 9–20 µm long and 2–3 µm wide (fig. 4A, appendix 2A–E). Striae are hyaline in LM, but fibulae are visible, 7–11 in 10 µm. Fibulae extending entirely across the transapical axis and parallel to slightly convergent towards the apices. Each fibula appearing to have two round nodules at each margin. Canal raphe eccentric and often not visible in the LM, but proximal raphe ends indicated by a slight constriction in larger valves (fig. 4A, appendix 2A–B). In girdle view, frustules rectangular and fibulae appearing capitate (appendix 2F, arrow).

SEM: description – Raphe situated between the valve face and the mantle (fig. 4B) with simple proximal raphe ends (fig. 4C, arrow) and hooked distal raphe ends (fig. 4D, arrow). Raphe separated from the rest of the valve by a small

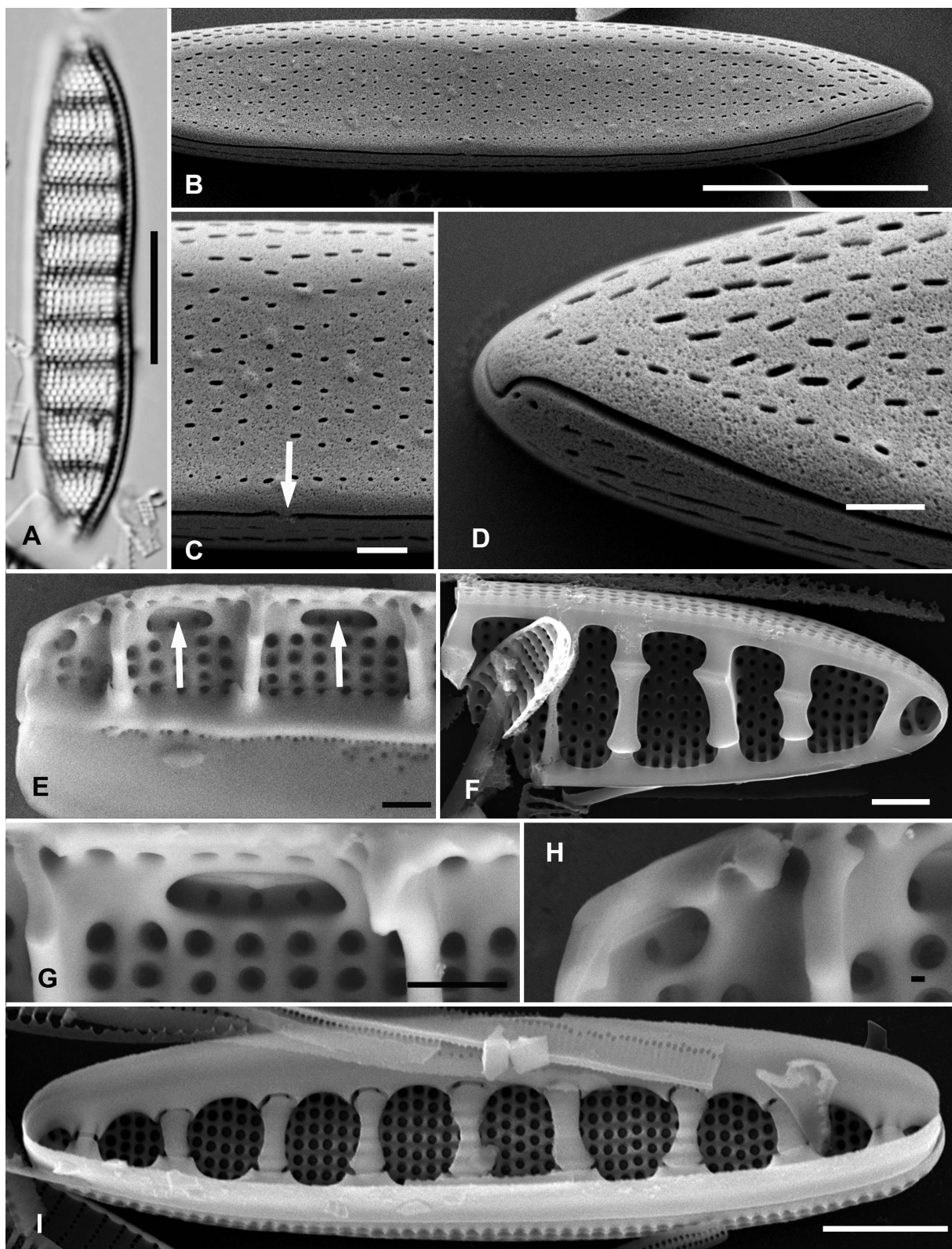


Figure 3 – *Denticula valida* from field material (A–D, F, I) and culture (E, G–H) from Blue Lake, Utah, USA, LM (A) and SEM (B–I): A, valve view; B, external view of whole valve; C, external view of simple proximal raphe ends (arrow); D, external view of hooked distal raphe end; E, internal view of valve in girdle view showing portulae (arrows); F, internal view of valve showing bowtie structure of the fibulae; G, internal view of valve in girdle view showing central nodule; H, internal view of valve in girdle view showing distal helictoglossa; I, internal view of valve showing scalloped extensions of the valvocopula adjoining the fibulae. Scale bars = 10 μ m in A–B; 1 μ m in C–E & G; 2 μ m in F; 100 nm in H; 5 μ m in I.

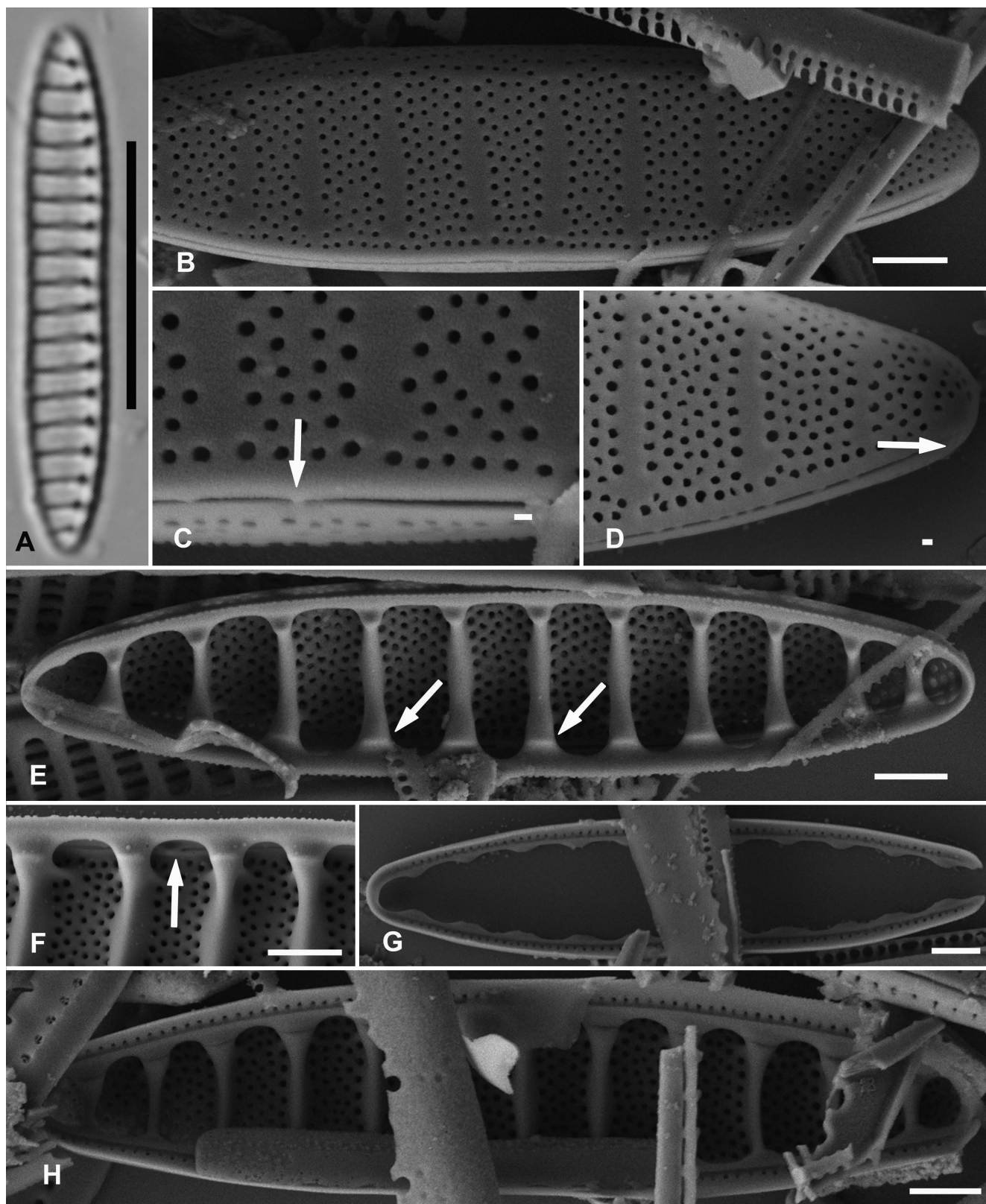


Figure 4 – *Denticula rainierensis* from Blue Lake, Utah, USA, LM (A) and SEM (B–H); A valve view; B, external view of whole valve; C, external view of simple proximal raphe ends (arrow); D, external view of hooked distal raphe end (arrow); E, internal view of whole valve with slightly wider humps (arrows) indicating the margin bearing the raphe; F, internal view of proximal raphe ends (arrow) and open canal raphe; G, detached valvocopula with areolae and scalloped extensions; H, internal view of whole valve showing valvocopula with scalloped extensions adjoining the fibulae. Scale bars = 10 μ m in A; 1 μ m in B & E–H; 100 nm in C–D.

hyaline area and a single row of simple areolae (fig. 4B–D). Striae composed of small areolae becoming larger closer to the hyaline areas, ~ 50 in 10 µm (fig. 4B). Hyaline areas consistent with the placement of the fibulae internally (fig. 4B & E). Striae and hyaline areas continuing onto the distinct mantle (fig. 4C). From the internal view, canal raphe subtended by large fibulae widening only slightly towards the valve (fig. 4E). Canal raphe largely open to the valve interior without portulae (fig. 4E & F). Fibulae becoming slightly wider transapically with two humps on each fibula near the valve margins (fig. 4E), with ~ 5 striae between them. Slightly wider humps indicating the margin bearing the raphe (fig. 4E, arrows). Striae composed of simple areolae with no difference in thickness between virgae and vimines (fig. 4E & F). Internal proximal raphe ends terminating in a small central nodule (fig. 4F, arrow). Valvocopula an open band with a single row of areolae (fig. 4G) and scalloped extensions that meet up with each of the fibulae (fig. 4H). Valvocopulae slightly asymmetrical in regards to the septum extensions in order to adjoin with the raphe (fig. 4G & H).

Material examined – JPK 8512 (see table 1).

***Denticula vanheurckii* Brun (Brun 1891)**

Figs 5 & 6, appendix 2G–M

LM: description – In valve view, valves linear-lanceolate to slightly asymmetrical along the apical axis with rounded to rostrate apices, 20–105 µm long and 6–11 wide µm (fig. 5A, appendix 2G–L). Alveolate striae parallel throughout the valve, 11–14 in 10 µm. Fibulae extending entirely across the valve face and parallel throughout the valve with a greater density towards the apices, fibulae number 2–3 in 10 µm. Each fibula appearing to have a suture towards the apical axis (appendix 2K, arrow). Canal raphe interrupted in the center and separated into two distinct branches with the proximal raphe ends bent to one side of the valve. Portulae easily discernable as small round openings along the canal raphe (appendix 2G & I). In girdle view, frustule slightly trapezoidal to rectangular with capitate fibulae (appendix 2M, arrow). Valvocopulae closed bands with internal protrusions that each meet at a central suture (fig. 5B) and adjacent closed copulae with shorter, grasping internal protrusions (fig. 5C).

SEM: description – Raphe contained within a narrow axial area (fig. 5D). Proximal raphe ends slightly bent to one side and widening into a simple areolae (fig. 5E). Distal raphe ends simple (fig. 5F). A cross section of the raphe showing the raphe fissure to be a key and slot type (Krammer & Lange-Bertalot 1986) (fig. 5G, arrow). Striae composed of lunate areolae, a type of complex alveolate pores (see terminology above, fig. 5H). Externally, striae appearing as a regular series of ‘domed caps’ (fig. 5H, arrow) supported internally by areolar struts. Volae extending from the ‘domed caps’ to fill the space between the struts, caps, and areolar walls leaving semicircular slit-like openings, usually 4–5 per areola (fig. 5H). Elaboration of the volae relatively simple. Volae absent in some specimens (fig. 5D). Striae continuing from the apical axis onto the distinct mantle (fig. 5I). Internally, canal raphe subtended by large fibulae crossing the valve transapically from margin to margin (fig. 6A). Fibulae connecting with a large hyaline area above the canal raphe, only opening to the valve interior through a series of

small round portulae with a larger elliptical portula around the central nodule (fig. 6A, arrows). Virgae thickened with thinner vimines expanding at their attachment point to form round areolae (fig. 6B). Areolar struts, often four, extending to the external surface of the valve and serving as an attachment point for ‘domed caps’ and associated volae (fig. 6B, black arrowheads). Often 4–8 striae occurring between fibulae. Proximal raphe ends simple with no widening. A small central nodule apparent (fig. 6B). Valvocopulae closed bands with extensions covering and enveloping each of the large fibulae and terminating along the apical axis in serrated sutures (fig. 6B & C). Adjacent copulae with extensions grasping the valvocopulae (fig. 6C, arrowhead).

Material examined – JPK 9297; JPK 9298; JPK 9299; JPK 9300 (see table 1).

***Epithemia zebra* var. *denticuloides* Hust. (Hustedt 1935)**

Fig. 7, appendix 2N–R

LM: description – In valve view, valves dorsiventral with a convex dorsal margin and a straight to slightly concave ventral margin with narrowly rounded to rostrate apices, 22–35 µm long and 5–6 wide µm (fig. 7A, appendix 2N–R). Alveolate striae parallel the entire length of the valve, 14 in 10 µm. Fibulae parallel throughout the valve, fibulae number 2–4 in 10 µm. Each fibula appearing to have a suture towards the apical axis (appendix 2Q, arrow). Canal raphe straight close to the ventral margin with proximal raphe ends indicated by a slight constriction in some specimens (appendix 2O–Q). Portulae easily discernable as small round openings along the canal raphe (fig. 7A, appendix 2N).

SEM: description – Raphe contained externally within a narrow axial area (fig. 7B). Proximal raphe ends widening into simple areolae and very slightly bent towards the dorsal side of the valve (fig. 7C). Distal raphe ends simple (fig. 7D). Striae consisting of lunate areolae, a type of complex alveolate pores (fig. 7C, rectangle). Striae appearing as a regular series of ‘domed caps’ supported by areolar struts with relatively simple volae extending from the ‘domed caps’ to fill the space between the struts, caps, and areolar walls leaving semicircular slit-like openings, usually four per areola (fig. 7C). Striae continuing from the apical axis onto the mantle (fig. 7D). Internally, the canal raphe subtended by large fibulae crossing the valve transapically from margin to margin (fig. 7E). Fibulae widening slightly along the transapical plane towards the valve and connecting with a large hyaline area above the canal raphe, only opening to the valve interior through a series of small round to elliptical portulae with a larger portula around the central nodule (fig. 7F, arrows). Virgae thickened with thinner vimines expanding at their attachment point to form round to rectangular areolae (fig. 7F). Areolar struts, usually four, extending to the external surface of the valve and serve as an attachment point for the volae (fig. 7G). Striae number 4–6 between the fibulae. Internal proximal raphe ends simple with no widening. A small central nodule apparent (fig. 7F). Valvocopulae closed bands with extensions covering and enveloping each of the large fibulae and terminating along the apical axis in serrated sutures (fig. 7E & G).

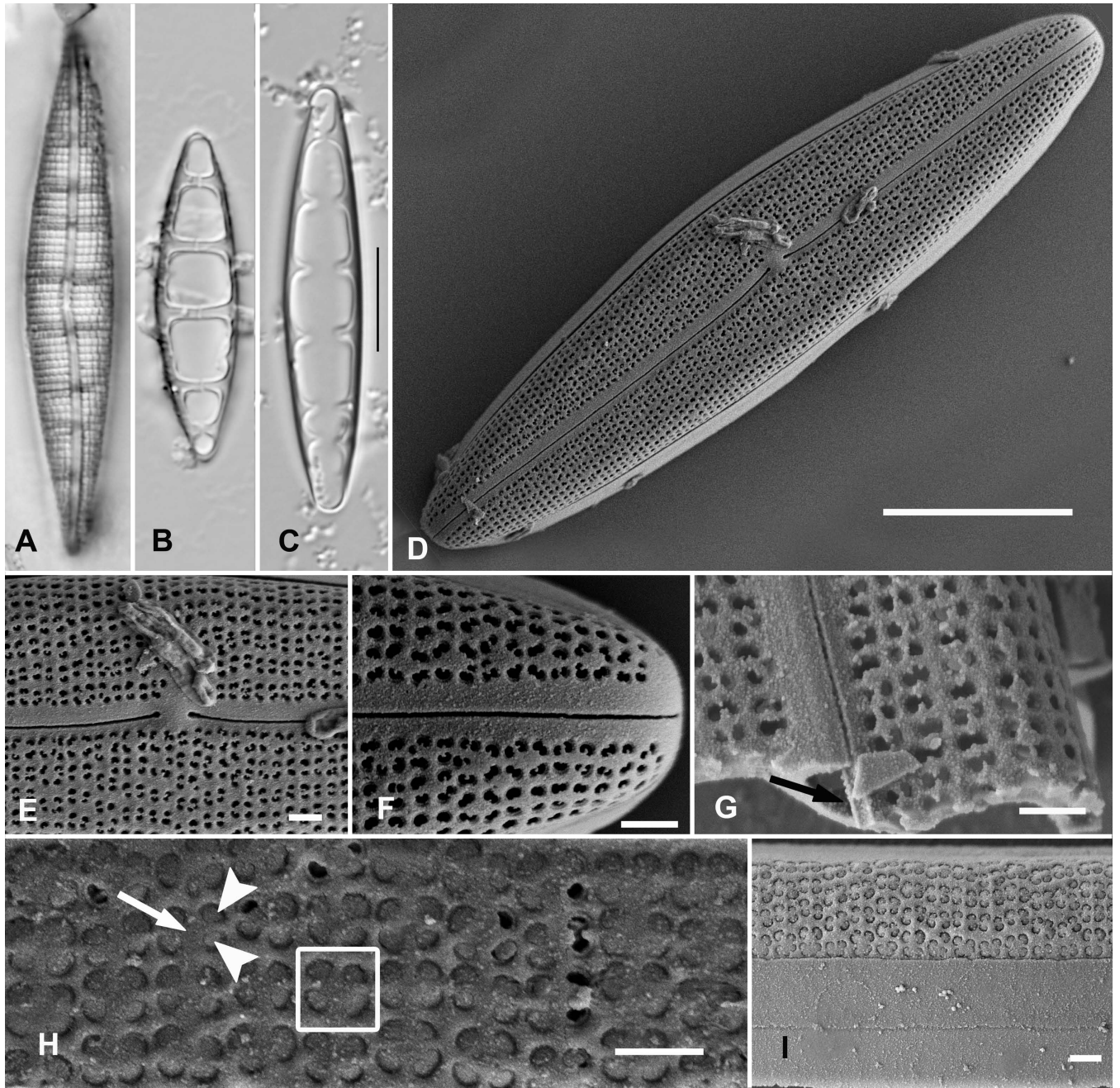


Figure 5 – *Denticula vanheurckii* from Lake Diatas (A), Lake Toba (B–C, H), and Lake Ranau (D–G, I) in Sumatra, LM (A–C) and SEM (D–I): A, valve view; B, valve view of valvocopulae showing closed band and extensions meeting along the transapical axis; C, valve view of copulae adjacent to valvocopulae showing closed band with short extensions; D–I, external views; D, whole valve; E, proximal raphe ends; F, distal raphe end; G, tongue and groove construction of the raphe (arrow); H, areolae showing the ‘domed cap’ (arrow) supporting the four volae (arrowheads) per areola (outlined); I girdle view. Scale bars = 10 µm in C–D; 1 µm in E–I.

Material examined – JPK 9298; JPK 9299; JPK 9300 (see table 1).

Remarks – Aside from symmetry differences, this taxon resembles *D. vanheurckii* with regard to valve and cingulum morphology.

Cladistic analysis identified one most parsimonious cladogram with 14 steps, a consistency index of 0.73, and retention index of 0.64 (fig. 8). Synapomorphies uniting the in-group included the presence of a canal raphe and frustules

with asymmetry along the apical axis. *Surirella* was diagnosed by the continuous raphe around the entire periphery of the valve and the presence of a keel. Synapomorphies uniting the Rhopalodiales included lunate areolae and ‘entire’ fibulae extending across the internal valve face. *Rhopalodia* was defined by the presence of a keel. An alternative interpretation, equally parsimonious, of the evolution of the keel (character 4) is that it diagnoses all of the canal raphe forms considered in this lineage, and was subsequently lost in *Epithemia* spp.

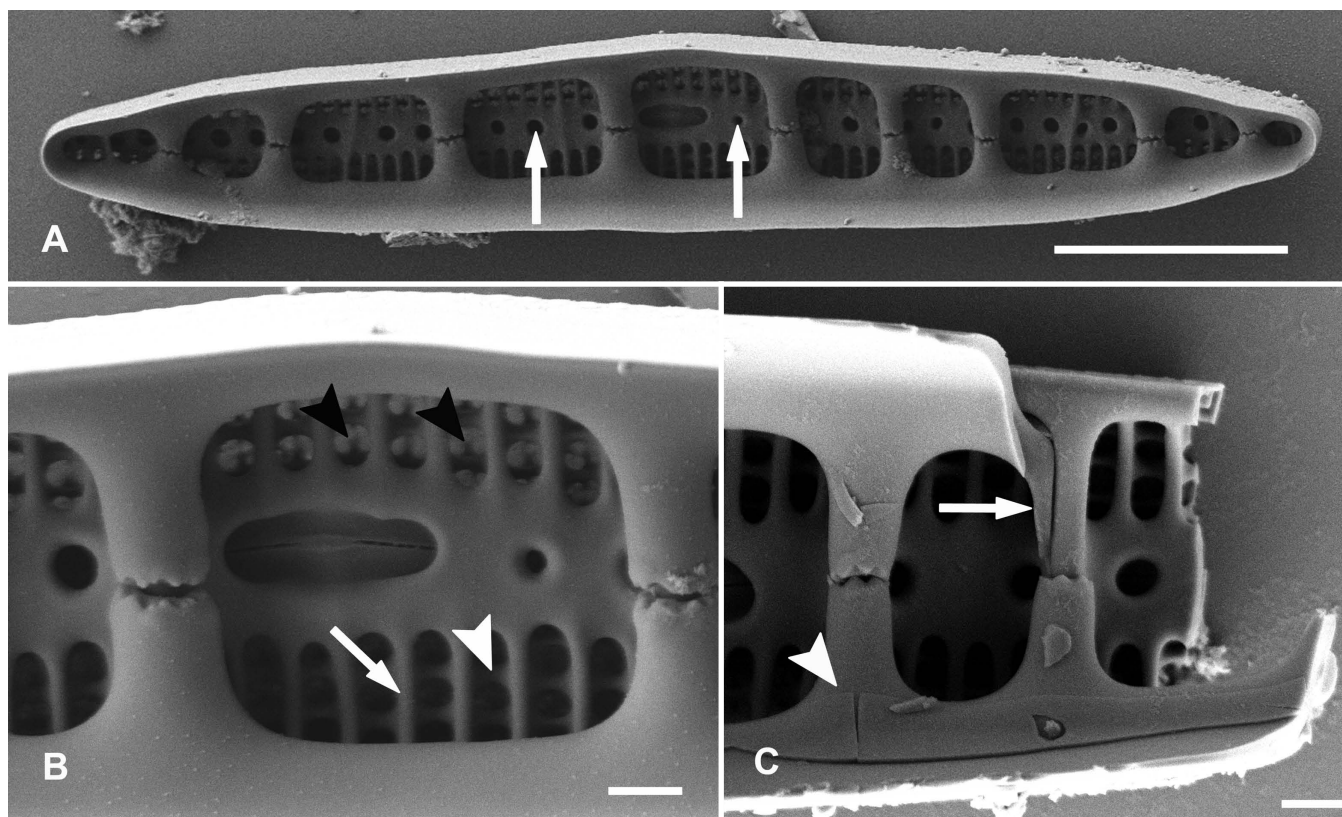


Figure 6 – Scanning electron micrographs of the internal view of *Denticula vanheurckii* from Lake Toba in Sumatra: A, whole valve with valvocopula showing portulae (arrows); B, proximal raphe ends and areolar structure showing virgae (arrow), vimines (white arrowhead), and areolar struts (black arrowheads); C, broken valve showing valvocopula enveloping fibulae (arrow) and adjacent copula barely grasping the valvocopula (arrowhead). Scale bars = 10 µm in A; 1 µm in B–C.

+ *D. vanheurckii* clade. The *Epithemia* spp. + *D. vanheurckii* clade was defined by the raphe shape and valvocopulae structure, two features that were subsequently modified in *Epithemia*. *Epithemia zebra* var. *denticuloides* was most closely related to *Epithemia*, not *D. vanheurckii*. The slightly arched raphe and unique valvocopulae structure expected to unite *E. zebra* var. *denticuloides* and *D. vanheurckii* were shown to be symplesiomorphic characters, and most likely ancestral to *Epithemia*. The synapomorphy distinguishing *D. vanheurckii* and its allies from *Epithemia* (including *E. zebra* var. *denticuloides*) is its symmetry along the apical axis.

DISCUSSION

As presently circumscribed, the genus *Denticula* includes a wide range of morphological diversity. Aside from their more or less symmetry about both the apical and transapical axes, and presence of fibulae extending partially or entirely across the valve face, few additional features serve to unite the species within *Denticula* that would indicate they represent a natural group. In fact, not only may this assemblage of species be allied with different genera, but also their morphological differences are great enough to ally them with different orders within the diatoms.

The first taxon examined, *Denticula kuetzingii*, is more similar to other *Denticula* species along what appears to be a morphological gradient between *Nitzschia* and *Denticula*

based on the degree the fibulae extend across the valve face and the extensions on the valvocopulae (Lange-Bertalot & Krammer 1993). Lange-Bertalot & Krammer (1993) suggest other taxa in this category include: *N. amphibia* Grunow, *N. denticuloides* Hust., *N. robusta* Hust., *N. semirobusta* Lange-Bert., and *N. subdenticula* Hust. This was a change of opinion by Lange-Bertalot & Krammer (1993), since their earlier treatment of the Bacillariales allied *Denticula* with Epithemiales (Krammer & Lange-Bertalot 1988). In fact, Lange-Bertalot & Krammer (1993) referred to *D. kuetzingii* as *Nitzschia denticula* and described a subgenus, *Denticuloidea* (Lange-Bertalot 1993), which includes these ‘border-line’ taxa. This debate is not new; many authors have placed *D. kuetzingii* in *Denticula* (Grunow 1862, Schönfeldt 1907) or *Nitzschia* (Cleve & Grunow 1880, Boyer 1927). A recent molecular study by Ruck & Theriot (2011) places *D. kuetzingii* firmly within the Bacillariales. Although the morphology discussed here agrees with the conclusions of Ruck & Theriot (2011) (i.e. *Denticula sensu stricto* is closely allied with *Nitzschia*), placement of *Denticula* within the Bacillariales requires further examination because of the absence of the generitype of *Denticula* in current molecular analyses.

Round et al. (1990), reviewing the morphology of *D. tenuis*, suggested its affinity within the Bacillariales. This placement of *D. tenuis*, the generitype of *Denticula*, is suggested by the chloroplast arrangement, presence of a keel, raphe structure, areola structure, and girdle structure (Mann

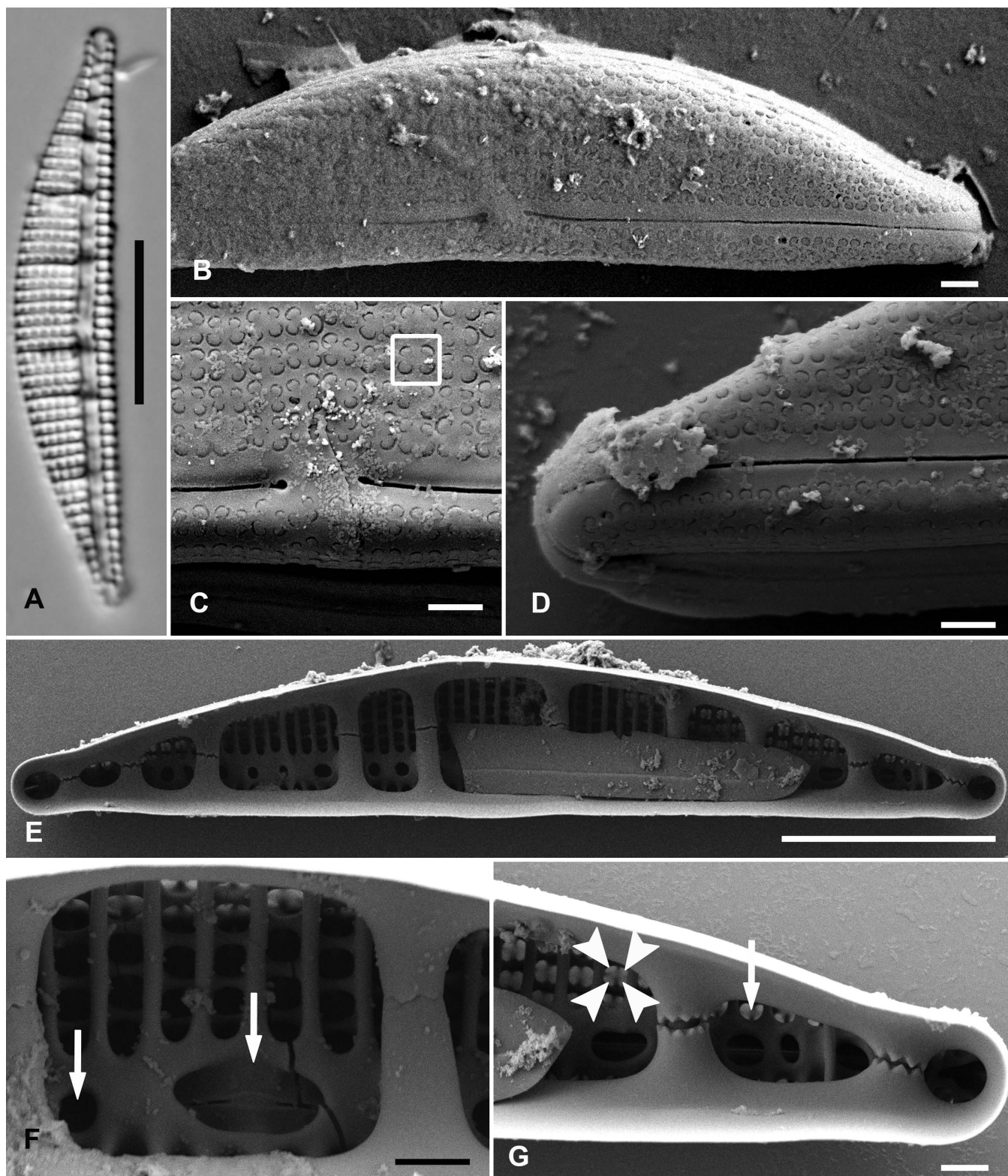


Figure 7 – *Epithemia zebra* var. *denticuloides* from Lake Toba in Sumatra, LM (A) and SEM (B–G): A, valve view; B–D, external views; B, whole valve; C, proximal raphe ends and areolae (rectangle); D, distal raphe ends; E–G, Internal views; E, whole valve with closed valvocopula band; F, proximal raphe ends and portulae (arrows); G, distal raphe ends and areolar struts (arrow) with four volae (arrowheads). Scale bars = 10 µm in A, E; 1 µm in B–D, F–G.

1989, Round et al. 1990). *Denticula tenuis* may be most closely related to *N. sinuata* (Thwaites in W. Smith) Grunow in Cleve & Grunow and its relatives (Mann 1989, Round et al. 1990), a group that had been included previously in the genus *Grunowia* Rabenhorst (1864). Should these species be shown to form a natural group (*sensu* Kociolek 1997) and be recognized within the Bacillariales at the level of genus, *Denticula* Kütz. (Kützing 1844) would have nomenclatural priority over *Grunowia*. Also included here would be *Denticula valida* and its allies (e.g. *Denticula elegans* Kütz., *Denticula kittoniana* Grunow in Van Heurck). This group includes species that possess a raphe with or without an intermissio. The ecological breadth of this putative group is great, with species that are found in cold mountain lakes and streams (e.g. *D. tenuis*; Schönfeldt 1907, Patrick & Reimer 1975), to high conductivity environments (e.g. *N. sinuata* var. *tabellaria*; Krammer & Lange-Bertalot 1988), to those able to tolerate elevated temperatures (e.g. *D. valida*; Patrick & Reimer 1975). *Denticula* as circumscribed here is firmly within the Bacillariales. Further work is warranted to determine whether the species discussed here form a monophyletic group.

Denticula rainierensis, by virtue of lacking a keel, the organization of its striae, and position of the raphe on the mantle rather than the valve face, differs significantly from the previous two groups discussed above. *Denticula rainierensis* is very similar to *D. subtilis* Grunow, differing primarily in

valve metrics and the presence/absence of portulae (Johansen et al. 1994, Lange-Bertalot & Krammer 1993). *Denticula rainierensis* and *D. subtilis* are most commonly reported from inland hot or warm springs and estuaries, respectively (Sovereign 1963, Wojtal 2013, Krammer & Lange-Bertalot 1988). In terms of morphology, these species appear to be related to *Fragilariopsis* and *Neodenticula*, being comparable to species in these genera by their striae and raphe structure (Round et al. 1990, Poulin et al. 2010). In phylogenies of the Bacillariales based on partial nuclear-encoded large subunit (LSU) rDNA, *Fragilariopsis* and *Neodenticula* form a natural group, part of a lineage that also includes *Pseudonitzschia*, with *Nitzschia frustulum* (Kütz.) Grunow in Cleve & Grunow being a sister taxon to this lineage (Lundholm et al. 2002, Poulin et al. 2010). *Denticula rainierensis* and *D. subtilis* differ from *Fragilariopsis* by having parallel striae at the apices and internal protrusions on the valvocopulae (Johansen et al. 1994, Round et al. 1990). In contrast to *Neodenticula*, these two species have a shallower mantle and an interrupted raphe (Poulin et al. 2010). A more robust phylogenetic analysis of this group of species is required before proposing their placement in the classification system of the Bacillariales.

Denticula vanheurckii was described originally from Java (Brun 1891), and Hustedt (1935, 1938) described eighteen taxa with similar morphology from Sumatra. This species has a raphe system quite similar to naviculoid diatoms, with two branches positioned in the middle of the valve face. A keel is lacking. In terms of valve morphology, the complex arrangement and structure of the areolae firmly place this species group within the Rhopalodiales. Similar complicated areolar structure can be seen in *Epithemia* species (Sims 1983, pers. obs.) and a modified version in *Rhopalodia* (Round et al. 1990). In addition, *D. vanheurckii* has a complex series of closed and open girdle bands, additional support for its placement within the Rhopalodiales (Sims 1983, Round et al. 1990). In contrast, the Bacillariales have either open or closed girdle bands, but both states do not occur within the same species (Round et al. 1990).

The relationship of these Sumatran species with members of the Rhopalodiales is underscored by Geitler's (1977) report of the presence of blue-green endosymbionts in *D. vanheurckii*. Symbionts in other members of the Rhopalodiales have been well documented (Lowe et al. 1984, DeYoe et al. 1992), and a recent phylogeny of the Rhopalodiales and the symbionts themselves, suggest the endosymbionts were acquired by a common ancestor of these diatoms and have been retained throughout host speciation (Nakayama et al. 2011). *Denticula vanheurckii* differs from other members of the Rhopalodiales by its valve symmetry and position and structure of its raphe. In terms of the raphe structure, *D. vanheurckii* is similar to *Nagumoea* Kociolek & Witkowski, a canal-bearing genus that has two distinct raphe branches per valve positioned in the middle of the valve and lacking a keel (Witkowski et al. 2011). Structure of the valvocopula (enveloping the fibulae *versus* simply undulated) and areolae (complex alveolate *versus* simple pores) distinguish the two. Witkowski et al. (2011) suggested *Nagumoea* to be more closely related to the Bacillariales, but further

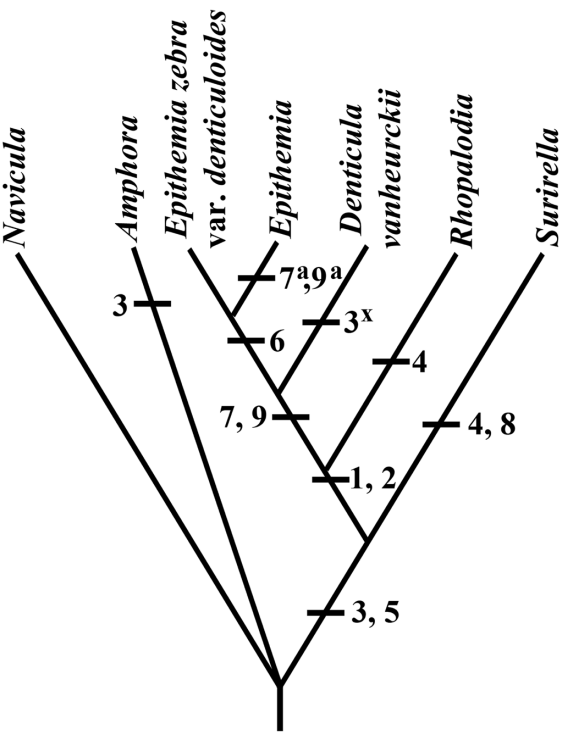


Figure 8 – Most parsimonious hypothesis of the relationships of the Rhopalodiales and Surirellales based on the character matrix presented in table 3. Horizontal bars indicate synapomorphies and numbers refer to characters described in table 2. x = an evolutionary reversal and a = the most derived state of a multistate character.

research is needed to verify the systematic position of this enigmatic genus.

The suite of features related to position and structure of the raphe as well as areolar morphology, diagnose a lineage with *D. vanheurckii* to the exclusion of *Epithemia* and *Rhopalodia*. To include *D. vanheurckii* in *Epithemia* would require the broadening of the concept of *Epithemia* to include valves that are either symmetrical or asymmetrical to the apical axis, placement of the raphe as ventral or central, and the arched or biarcuate shape of the raphe system. Instead of dramatically proposing this new, broad concept of *Epithemia*, we propose here a new genus for *Denticula vanheurckii* and its apically symmetrical allies:

Tetralunata Hamsher, Graeff, Stepanek & Kociolek, **gen. nov.**

Tetralunata vanheurckii (Brun) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula vanheurckii* Brun, Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 31 (part 2, no.1) : 25; pl. 13, fig. 8; pl. 14, fig. 10. 1891 (Brun 1891).

Description – Valves symmetrical, slightly asymmetrical, or strongly asymmetrical about the apical axis. Raphe medial, straight to barely biarcuate, composed of two distinct branches. Externally, raphe in a distinct, wide, straight axial area with proximal raphe ends dilated and deflected in the same direction and distal ends extending onto the mantle; keel lacking. Internally, raphe within a canal containing a large, ovoid central portula and a series of smaller, round portulae extending to the apices. Portulae across a wide hyaline strip that runs the length of the valve. Central nodule small, linear in outline. Fibulae distinct, extending entirely transapically across the valve. Striae distinctly areolate, 4–8 rows of striae between the fibulae. Areolae clover-shaped, with 4–5 c-shaped occlusions. Valvocopula closed with extensions over the fibulae and meeting at the center with small, interdigitating teeth. Additional copulae a complex series of closed and open bands. One large chloroplast present, in addition to spherical bodies that are endosymbiotic blue-green algae.

Etymology – The generic name refers to the ‘four crescents’ shape of the areolae.

Note – Based on the detailed light microscope images provided in Simonsen (1987: plates 345–349), on the taxa described by Hustedt from Lake Toba and environs (Hustedt 1935, 1938), which indicate similarities in valve structure, the following transfers to the new genus are presented below:

Tetralunata spathulifera (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula spathulifera* Hust., Archiv für Hydrobiologie, Supplement 15: 446–447; pl. 36, fig. 1–4. 1938 (Hustedt 1938).

Tetralunata lata (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula lata* Hust., Archiv für Hydrobiologie, Supplement 15: 447–448; pl. 36, fig. 5–9. 1938 (Hustedt 1938).

Tetralunata bicuneata (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula bicuneata* Hust., Archiv für Hydrobiologie, Supplement 15: 446; pl. 36, fig. 10–12. 1938 (Hustedt 1938).

Tetralunata costata (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula costata* Hust., Archiv für Hydrobiologie, Supplement 14: 169–170; pl. 4, fig. 25. 1935 (Hustedt 1935).

Tetralunata elongata (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula elongata* Hust., Archiv für Hydrobiologie, Supplement 15: 448; pl. 37, fig. 1–6. 1938 (Hustedt 1938).

Tetralunata rhynchocephala (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula rhynchocephala* Hust., Archiv für Hydrobiologie, Supplement 14: 170; pl. 4, fig. 26 a, b. 1935 (Hustedt 1935).

Tetralunata tenuis (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. & stat. nov.**

Basionym – *Denticula rhynchocephala* var. *tenuis* Hust., Archiv für Hydrobiologie, Supplement 14: 170; pl. 4, fig. 26 c–f. 1935 (Hustedt 1935).

Tetralunata robusta (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula robusta* Hust., Archiv für Hydrobiologie, Supplement 14: 171–172; pl. 4, fig. 30. 1935 (Hustedt 1935).

Tetralunata dubia (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. & stat. nov.**

Basionym – *Denticula robusta* var. *dubia* Hust., Archiv für Hydrobiologie, Supplement 15: 448–449; pl. 37, fig. 12, 13. 1938 (Hustedt 1938).

Tetralunata vanheurckii (Brun) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula vanheurckii* Brun, Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 31(part 2, no.1): 25; pl. 13, fig. 8; pl. 14, fig. 10. 1891 (Brun 1891).

Tetralunata subrobusta (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. & stat. nov.**

Basionym – *Denticula vanheurckii* var. *subrobusta* Hust., Archiv für Hydrobiologie, Supplement 15: 450; pl. 38, fig. 12–16. 1938 (Hustedt 1938).

Tetralunata angusta (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. & stat. nov.**

Basionym – *Denticula vanheurckii* var. *angusta* Hust., Archiv für Hydrobiologie, Supplement 15: 450; pl. 38, fig. 17, 18. 1938 (Hustedt 1938).

Tetralunata obtusa (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. & stat. nov.**

Basionym – *Denticula vanheurckii* var. *obtusa* Hust., Archiv für Hydrobiologie, Supplement 15: 450; pl. 39, fig. 1–8. 1938 (Hustedt 1938).

Tetralunata amphicephala (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula amphicephala* Hust., Archiv für Hydrobiologie, Supplement 14: 170; pl. 4, fig. 27. 1935 (Hustedt 1935).

Tetralunata lanceolata (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula lanceolata* Hust., Archiv für Hydrobiologie, Supplement 14: 170–171; pl. 4, fig. 28. 1935 (Hustedt 1935).

Tetralunata pelagica (Hust.) Hamsher, Graeff, Stepanek & Kociolek, **comb. nov.**

Basionym – *Denticula pelagica* Hust., Archiv für Hydrobiologie, Supplement 14: 172; pl. 4, fig. 31. 1935 (Hustedt 1935).

Epithemia zebra var. *denticuloides*, which is morphologically similar to *D. vanheurckii*, but is asymmetrical to the apical axis with the raphe positioned towards the ventral margin, was initially expected to be closely related to *D. vanheurckii* and its allies. However, cladistic analysis revealed these characters (raphe shape and valvocopulae structure) to be symplesiomorphies. Therefore, *E. zebra* var. *denticuloides* was not transferred to *Tetralunata*.

Two additional taxa, *Denticula vanheurckii* f. *ventricosa* Hust. (Hustedt 1938: 449; plate 39, fig. 9) and *Denticula vanheurckii* f. *trigona* Hust. (Hustedt 1935: 171; plate 4, fig. 29) require further study for the appropriate rank before being transferred to *Tetralunata*.

Representatives of this new genus appear restricted to Java and Sumatra, a region with many endemic plants and animals (Whitten et al. 1987). It is interesting to consider the biogeographic context of *Tetralunata*, as very few genera of freshwater diatoms have restricted distributions. Examples of other freshwater diatom genera with restricted biogeographic distributions include *Eunophora* Vyverman et al. (restricted to Tasmania and New Zealand, Vyverman et al. 1998), *Gomphocymbella* O.Müller (restricted to East African Rift Valley lakes region, Kociolek & Stoermer 1993), as well as much smaller genera *Bicudoa* (Wetzel et al. 2012) and *Eunotioforma* (Burliga et al. 2013) both reported only from Brazil, and *Tibetiella* (Li et al. 2010) known only from China. A large number of diatom genera have been described from Lake Baikal, Russia (Kulikovskiy et al. 2012), a region that has harbored endemic fossil genera and families of freshwater diatoms (e.g. Khursevich & Chernyaeva 1989, 1994). In In-

donesia, where there are high levels of endemic species in terrestrial ecosystems, there are also well known instances of fish and crustacean adaptive radiations in freshwater lakes of Sulawesi (Herder et al. 2006, 2008, von Rintelen & Cai 2009). These *Tetralunata* species from Sumatra may form a species flock, i.e., many species of a monophyletic group that are geographically constrained and have evolved quickly (e.g. Sullivan et al. 2002). Most other putative examples of species flocks in diatoms include only a few species (Mann 1999, Edlund & Soninkhishig 2009, Seddon et al. 2011), with the exception of the genus *Gomphoneis* in Lake Baikal (Kociolek et al. 2013). While we do not have divergence rates of this species flock from Sumatra, this is an area for future research.

Although *Tetralunata* and other members of the Rhopalodiales share a number of features including fibula and areola structure; their ability to utilize endosymbiotic cyanobacteria; and chloroplast characteristics (Geitler 1977), *Tetralunata* also differs from both *Epithemia* and *Rhopalodia*. Valve morphology of *Tetralunata* differs from *Epithemia* in the following: the ventral to apical instead of biarcuate position of the raphe; a large hyaline area above the canal raphe internally (compare to *Epithemia turgida* (Ehrenb.) Kütz., the generitype; Sims 1983); the lack of thickened ‘secondary’ fibulae that are thicker than the virgae but do not extend across the raphe, the valvocopulae suture along the apical axis, not the dorsal side of the valve; and the valvocopulae extend across the entire valve with adjacent copulae barely grasping the valvocopulae (*Epithemia* is *vice versa sensu* Sims 1983). The differences between *Tetralunata* and *Rhopalodia* are more striking, including: the ventral to apical instead of dorsal position of the raphe; the lack of a keel (some *Rhopalodia* spp.); raphe fissures not bordered by siliceous flanges or ridges; a more elaborate interlock between the valve and valvocopulae; and the absence of any difference in girdle band width between the dorsal and ventral margins (Round et al. 1990).

Tetralunata also differs from the recently described subgenus, *Denticuloidea* (Lange-Bertalot 1993). *Denticuloidea* includes the ‘border-line’ taxa between *Nitzschia* and *Denticula* (e.g. *D. kuetzingii*, *N. robusta*, *N. semirobusta*, etc.) and therefore is within the order Bacillariales. In contrast, *Tetralunata* is within the Rhopalodiales. Whereas both *Tetralunata* and *Denticuloidea* share the presence of fibulae, they differ in areolae and valvocopulae structure, raphe position and shape, and the presence/absence of endosymbionts.

The previous debate about the systematic position of the genus *Denticula* was hindered by acceptance of the group as monophyletic, and the assumed close relationship between canal raphe bearing groups, stated explicitly or implied. Whereas some focused on the raphe and keel structure of certain species, such as *D. tenuis* (e.g. Round et al. 1990), others noted the similarity of *D. vanheurckii* with *Epithemia* (Hustedt 1928, Mann 1989, Krammer & Lange-Bertalot 1988). Although previous authors such as Bessey (1899) and Simonsen (1979) had suggested separate origins of the Bacillariales and the Rhopalodiales + Surirellales, not until this was suggested by molecular data (Sorhannus 2004, Sims et al. 2006, Ruck & Theriot 2011) and the implications of these results detailed by Ruck & Theriot (2011), did diatom sys-

tematists fully grasp the significance. Previously, in the system where canal raphe diatoms were thought closely related or indeed monophyletic, it may have been difficult to reconcile the possibilities of multiple origins of endosymbiotic blue-green algae, or the diversity of forms within a genus such as *Denticula*. In the context of the morphological data presented here, despite the presence of a canal, *Denticula* as previously understood is polyphyletic. This study helps underscore the ability of morphology, in the context of an established phylogeny, to resolve issues of systematic position across what were once thought to be closely related species.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo supp-data>), and consist of: (1) light micrographs of *Denticula kuetzingii*, *D. tenuis*, and *D. valida*; and (2) light micrographs of *Denticula rainierensis*, *D. vanheurekii*, and *Epithemia zebra* var. *denticuloides*.

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