## TWO SHORT-STRIATED SPECIES OF *STAUROSIRELLA* (BACILLARIOPHYCEAE) FROM INDONESIA AND THE UNITED STATES\*

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Abstract. The paper presents a detailed morphological analyses by light (LM) and scanning electron microscopy (SEM) of two short-striated diatoms of the genus *Staurosirella* D. M. Williams & Round. The first was encountered in the periphyton of a river in Oregon (U.S.A.) and is described here as a new species, *S. krammeri* sp. nov. The second diatom was found in a lake plankton collection made during the Wallacea-Expedition in the early 1930s; it was originally described as *Fragilaria lapponica* f. *lanceolata* Hustedt, but based on its ultrastructure we propose its transfer to *Staurosirella* at species level as *S. lanceolata* comb. nov. et stat. nov. These two taxa are compared by LM and SEM with the generitype *Staurosirella lapponica* (Grunow) D. M. Williams & Round as well as other morphologically related species in the light of available published material.

Key words: Bacillariophyceae, diatoms, Staurosirella, new taxa, Indonesia, U.S.A.

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#### INTRODUCTION

The genus Staurosirella comprises a group of species characterized by 'punch-hole' (sunken) striae with developed volae, wide valvocopulae, and a valve mantle edge parallel to the valve face/ mantle junction (Williams & Round 1988; Morales & Manoylov 2006b). The species are colonial and can be found in benthic habitats, where a single cell is attached to different substrates by means of a mucilage stalk and the rest of the cells comprising the colonies are attached end to end or form ribbon-like filaments (Morales 2005). Other species inhabit the plankton, where they produce stellate colonies that attach by secreting mucilage, probably embedding mineral particles from the environment to harden the connection (Morales 2003, 2005). Different species probably

are able to alternate between benthic and planktic habitats by forming ribbon-like or stellate colonies, respectively. Morales (2005) reported this alternation of colonial types in *S. confusa* E. A. Morales and proposed that such alternation could be ecologically advantageous, allowing a taxon to exploit two different niches. Morales (2005) also presented micrographs showing *S. leptostauron* (Ehrenberg) D. M. Williams & Round producing both colonial types.

The ecology of even the most common species within *Staurosirella* is still difficult to characterize despite the tremendous number of reports in the literature. This difficulty arises from a lack of consistency in the taxonomy applied by different authors, which in turn is rooted in the lack of clear monographic/taxonomic works on the different taxa. For example, the identity of one of the taxa most commonly reported from around the world,

 $<sup>^{\</sup>ast}$  Dedicated to Dr. Kurt Krammer on the occasion of his  $85^{\rm th}$  birthday

*S. pinnata* (Ehrenberg) D. M. Williams & Round, remains unclear because a study of type material has not been published. A myriad of specimens of all shapes, forms and sizes appear in the literature under this name. For instance, Morales *et al.* (2001) reported it from a eutrophic lake with neutral pH just above sea level in the U.S.A., while Maidana and Seeligmann (2006) gave it from an alkaline oligotrophic river at 4000 m a.s.l. in the Andes. As many of these reports are from quite disparate habitats, it is possible that a number of different entities are currently classified as *S. pinnata* and that detailed studies by LM and SEM are needed to sort them out.

A series of papers have been published, mainly from North America, in an effort to clarify the taxonomy of species within *Staurosirella* from a morphological standpoint (Morales *et al.* 2001; Morales 2002, 2003, 2005; Morales & Manoylov 2006a, 2006b). These publications also contain several new taxa, and many others remain to be described as new (E. A. Morales, pers. obs.); the diversity of the group has yet to be determined, even for North America. Taxonomical revisions of known taxa and descriptions of new ones, employing detailed analyses combining LM and SEM, are needed for a better understanding of their ecology and biogeography. They can then be used more reliably as indicators of habitat and water quality.

Here we present detailed LM and SEM analyses of the Indonesian type material of *Fragilaria lapponica* f. *lanceolata* Hustedt and propose the combination *Staurosirella lanceolata* comb. nov. et stat. nov. We also describe a new taxon under the name *Staurosirella krammeri* sp. nov. from a river in the state of Oregon (U.S.A.), and compare the two taxa with morphologically related species from the literature.

#### MATERIALS AND METHODS

Two samples were used for this work (Table 1). One is a periphyton composite sample (from rocks, macrophytes and snags) from the North Umpqua River, Oregon, U.S.A., preserved with formaldehyde (4% final concentration) and analyzed by the Phycology Section, Patrick Center for Environmental Research (PCER), Academy of Natural Sciences of Philadelphia (ANSP). Collection methods followed protocols developed for the United States Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) (Fitzpatrick *et al.* 1998; Moulton *et al.* 2002). Environmental data were retrieved from Tanner *et al.* (2006).

The second sample was provided by Friedel Hinz from the Hustedt Collection, Alfred-Wegener-Institut für Polar-und Meeresforschung. Material number AS1322 was elected as the lectotype of *F. lapponica* f. *lanceolata* 

Locality, sample type	Country, year of collection	Accession No., Collection	Coordinates
Lake Mahalona, plankton with littoral forms	Indonesia (Sulawesi), 1931–1932	Material No. AS1322, Slide No. 397/39 A, Hustedt Collection, Alfred Wegener Institute, Germany	N/A
North Umpqua River, per- iphyton	U.S.A., Oregon, 2001	ANSP G.C 64866, U.S.A.	43°18′39″N, 122°09′13″W
Little Kijik River, per- iphyton	U.S.A., Alaska, 2001	AK00005, Phycology Sec- tion, ANSP, U.S.A.	60°18′28″N, 154°17′17″W
Campbell Creek, periphyton	U.S.A., Alaska, 2001	GSN92067, Phycology Sec- tion, ANSP, U.S.A.	61°10'1.92"N, 149°46'14.16"W
Lake o' Pines, sediments from 7.5 m along a core	U.S.A., Wisconsin, 1995	E. A. Morales personal col- lection	45°46′28″N, 90°42′55″W
Clear Lake, sediment sample from 67–68 cm along a core	U.S.A., Minnesota, 2003	Saint Croix Watershed Research Station, Science Museum of Minnesota	43°37.595′N, 95°4.865′W

Table 1. Samples used in this study.

by Simonsen (1987). The material from Celebes (now Sulawesi, Indonesia), almost all plankton with littoral forms, was sampled by Prof. Dr. R. Woltereck during the Wallacea-Expedition in 1931–1932 (Hustedt 1942). Hustedt's material AS1322 was originally collected from Lake Mahalona, a natural body of water in south Sulawesi Province near the town of Soroako. This sample was analyzed at the Department of Environment and Agro-biotechnologies, Public Research Centre-Gabriel Lippmann, Belvaux, Luxembourg.

For comparison, SEM images of *Staurosirella lapponica* (*Fragilaria lapponica* Grunow *sensu* Krammer & Lange-Bertalot 1991) from the archives of the ANSP were also used in this work (Figs 45–50). These images were made from material from four sites in the United States of America (Table 1): the Little Kijik River and Campbell Creek (South Fork), Alaska; Lake o' Pines, Wisconsin; and Clear Lake, Minnesota.

For LM analyses of all U.S.A. material, subsamples were digested with nitric acid using the microwave method, washed by rinsing and decanting with distilled water, and air-dried aliquots were mounted on glass slides using Naphrax® (Charles et al. 2002). A Zeiss® Universal microscope equipped with differential interference contrast (DIC) and a Spot Insight QE Model No. 18.2 Color Mosaic digital camera were used for analysis of specimens. A subsample from the Sulawesi material (AS1322) was cleaned by oxidation with hot hydrogen peroxide (30% v/v) and hydrochloric acid. Then the sample was rinsed by decanting using distilled water, and air-dried aliquots were mounted on permanent glass slides using Naphrax®. A Leica® DM-RB light microscope equipped with a Leica® DC500 camera were used for analysis of this Indonesian material.

For SEM study of all U.S. material, aliquots of clean material were air-dried onto 15 cm<sup>2</sup> pieces of aluminum foil. The foil was trimmed to smaller pieces and mounted on aluminum stubs with double-sided tape. The stubs were then coated with gold-palladium using a Polaron Sputter Coater for ca. 1.5 min at 1.8 kV (coating 40 nm thick) and analyzed with a Leo-Zeiss 982-DSM electron microscope. For SEM analysis of the Indonesian sample, aliquots of clean slurries were mounted on stubs, coated with gold (coating 40 nm thick) using a Modular High Vacuum Coating System (BAL-TEC MED 020) and viewed with a Leica® Stereoscan 430i.

In all cases the digital images were captured and the plates were assembled using Adobe Photoshop CS4. Morphological terminology follows Anonymous (1975), Ross *et al.* (1979), and Round *et al.* (1990). The variation ranges of valve dimensions are given from measurements of up to 50 valves of each taxon from LM and SEM images.

#### **RESULTS AND DISCUSSION**

# *Staurosirella krammeri* E. A. Morales, C. Wetzel & Ector, *sp. nov.* Figs 1–18 & 33–38

Frustula aspectu cingulari rectangularia superficie curvata, catenas formantes per spinas. Valvae isopolares rhombicae apicibus anguste rotundatis in speciminibus majoribus vel ellipticae apicibus late rotundatis in speciminibus minoribus. Latitudo 4-14 µm, longitudo 3-5 µm, striae 12-14 in 10 µm. Sternum amplum lanceolatum. Limbus valvaris acutus margine parallelo ad valvae et limbi juncturam. Striae alternantes respectu axis valvaris nonnunguam parallelae in area centralis valvae, radiantes ad apices. Striae uniseriatae non interruptae e valva ad limbum sed tantum aliquot areolae in limbo valvaris. Areolae apicale elongatae. Volae delicatae crescentes ex ambitu interno areolae. Spinae cavae spatulatae costis elevatis in valvae et limbi junctura. Costae elevatae interius valvis conferens striis aspectu perforatio similis. Areae porellarum apicales nullae nonnunquam substitutae per striam incipientem praecipue in speciminibus minoribus. Rimoportula nulla. Copulae clausae amplae non perforatae. Valvocopulae pars interioris depressae in margine limbis valvae fimbris. Plasti non visae.

TYPE: U.S.A. Oregon, Douglas County, North Umpqua River, Lat. 43°18'39" Long. 122°09'13". 2005, United States Geological Survey [HOLOTYPE: Slide ANSP GC 64866, Diatom Herbarium, Academy of Natural Sciences, Philadelphia (ANSP); ISOTYPE: Slide HCUCB D-4, Herbario Criptogámico Universidad Católica Boliviana, Cochabamba, Bolivia].

ETYMOLOGY: The species epithet is dedicated to Dr. Kurt Krammer for his valuable contribution to diatom research.

Frustules rectangular in girdle view, with evident curved surface, forming chains with the aid of spines. Valves isopolar, rhomboid with narrowly rounded apices in larger specimens, becoming elliptical with broader rounded apices in smaller ones. Length 4–14  $\mu$ m, width 3–5  $\mu$ m, striae density 12–14 in 10  $\mu$ m. Sternum wide, lanceolate. Valve mantle steep, with edge parallel to valve face/valve mantle junction. Striae on each side of valve axis aligned alternate to one another, sometimes parallel in middle area of valve, becoming radial toward apices. Striae uniseriate, uninter-



**Figs 1–32.** 1–18. *Staurosirella krammeri* E. A. Morales, C. Wetzel & Ector *sp. nov.* from type material (North Umpqua River, Douglas County, Oregon, U.S.A.). 19–32. *Staurosirella lanceolata* (Hustedt) E. A. Morales, C. Wetzel & Ector *comb. nov et stat. nov.* from lectotype material for *Fragilaria lapponica* f. *lanceolata* Hustedt (Hustedt Collection, Alfred-Wegener-Institut für Polar-und Meeresforschung, Material number AS1322). All LM micrographs. Scale bars = 10 μm.

rupted from valve face to valve mantle although only a few areolae are located on valve mantle. Areolae apically elongated. Volae delicate and arising from the inner longer edge of each areola. Spines hollow, spatulate, located on raised costae at the valve face/mantle junction. Costae also raised in valve interior, giving the characteristic 'punch-hole' appearance to the striae. Apical pore fields absent and sometimes replaced by a weakly developed stria, especially in smaller specimens. Rimoportula absent. Girdle bands closed, wide, without perforations. Pars interior of valvocopula recessed to accommodate valve mantle edge and bearing fimbriae. Plastids not observed.

Staurosirella krammeri belongs in Staurosirella because it shares many features with other taxa currently placed in this genus. The type of striae, recessed into the valve due to the raised nature of the costae ('punch-hole' appearance) present in the new taxon (Figs 33–37), is also observed in the generitype S. lapponica (Figs 45–48, 50; Haworth 1975). S. leptostauron, S. martyi (Héribaud) E. A. Morales & Manoylov and others likewise show this same type of striae (Morales & Manoylov 2006a). Positioning of the spines on the costae at the valve face/mantle junction is also observed in other representatives of Staurosirella (compare Figs 33, 34, 37 with spiny taxa in Morales & Manoylov 2006b). Another characteristic of *S. krammeri*, the valve mantle edge being parallel to the valve face/mantle junction (Figs 33–37), is also noted in several species currently allocated in *Staurosirella*, such as *S. rhomboides* (Grunow) E. A. Morales & Manoylov and *S. subrobusta* E. A. Morales (Morales & Manoylov 2006a, and *S. lapponica* presented here [Figs 46–48, 50]). The wide valvocopula bearing fimbriae in *S. krammeri* (Fig. 38) has been reported as a unifying feature for *Staurosirella* species (Morales 2006).

Staurosirella krammeri is unique in combining the rhomboid to elliptical valve shape with the short striae (Figs 1-17). Another feature so far reported only for this taxon is the lack of apical pore fields (Figs 33-37). Populations of other species in Staurosirella may or may not produce this structure [e.g., the case of Staurosirella incognita E. A. Morales & Manoylov (Morales & Manoylov 2006b, compare Figs 21 & 22 in that reference)], but the complete lack of it has not been reported in the literature before. In view of the habitat in which this diatom was found (periphyton), it is unclear how it accomplishes attachment to its substrate. Short ribbon-like chains have been seen in digested material (Fig. 18), but reliable observations of live/preserved material could not be made because the sample is particularly rich in araphid diatoms, including unknown taxa with a shape and size range similar to that of S. krammeri, and



**Figs 33–38**. *Staurosirella krammeri* E. A. Morales, C. Wetzel & Ector *sp. nov*. SEM images of type material (North Umpqua River, Douglas County, Oregon, U.S.A.). 33 & 34 – Details of valve face outer surface showing characteristics of sternum, costae, striae and spines. 35 & 36 – Details of valve interior. Note that the external and internal views do not reveal the presence of apical pore fields. 37 – Tilted view of complete frustule with upper valve sunken into the cingulum. 38 – Valvocopula with developed fimbriae and recessed pars interior. Scale bars: 33–35 & 37 – 2 µm; 36 – 1 µm; 38 – 5 µm.

all produce filamentous colonies. The attachment (or lack thereof) of *S. krammeri* to its substrate needs further study.

Short striated variants of araphid species re-

ported in the literature are more common among taxa within *Pseudostaurosira* D. M. Williams & Round. Only in a few cases do the reports belong to taxa in *Staurosira* Ehrenberg or *Stau*- *rosirella*. It is remarkable how many of these reported variants do not bear formal names, are ascribed to taxa commonly not producing short-striated valves, or have been misidentified (e.g., Lange-Bertalot & Metzeltin 1996, Pl. 111, fig. 7; Metzeltin *et al.* 2005, Pl. 15, figs 10–12; Wojtal 2009, Pl. 52, fig. 2).

Perhaps the most commonly reported shortstriated taxon within Staurosirella is S. lapponica (Figs 45-50). This species clearly differs from S. krammeri. Staurosirella lapponica typically has elongated elliptical valves with parallel sides (Figs 45-48). LM micrographs of lectotype material presented by Krammer and Lange-Bertalot (1991, Pl. 134, figs 1 & 2) show short pointy striae clearly composed of apically elongated areolae. These striae appear wider than in S. krammeri (compare with Figs 1-17 here). The valve dimensions of S. lapponica reported by Krammer and Lange-Bertalot (1991) are length 10-30(40) µm, width 3-6 µm and striae density 6-10 per 10 µm, indicating that S. krammeri tends to have shorter valves and much higher striae density (12-14 per 10 µm). Additionally, S. lapponica has open girdle elements (Fig. 49), in contrast to the closed copulae in S. krammeri (Fig. 38). Yet another difference lies in the structure of the spines, which are well developed and dichotomously branched in S. lapponica (Fig. 50); S. krammeri has less developed spatulate spines (Figs 33-37).

Another taxon similar to S. krammeri is Fragilaria canariensis Lange-Bertalot (Lange-Bertalot 1993, Pl. 14, figs 1-6). The latter has apical pore fields on both valve apices, and striae that in some individuals seem more developed on the valve mantle than on the valve face, features not observed in S. krammeri. Also, it is possible (although not completely clear from Pl. 14, fig. 5 in Lange-Bertalot 1993) that the girdle elements are open in F. canariensis, while they are closed in S. krammeri. What is clear from the figure in Lange-Bertalot (1993) is that the girdle elements are curved toward the apices, becoming much shallower in these regions. The girdle elements in S. krammeri possess an abvalvar edge completely parallel to the valve mantle edge (Figs 33-37). Another difference regards the spines, which are single, hollow and spatulate in *S. krammeri* (Figs 33–37), while those of *F. canariensis* originate as two lumps on a single costae, then fuse to form a small spatulate structure. Whether the latter spines are hollow cannot be determined from the material presented by Lange-Bertalot (1993). Regarding dimensions, *S. krammeri* produces larger valves (4–14  $\mu$ m) than *F. canariensis* (4–7  $\mu$ m), but they greatly overlap in width and striae density.

Another taxon that produces short striae is *Staurosira fernandae* García-Rodríguez, Lange-Bertalot & Metzeltin in Metzeltin & García-Rodríguez (Metzeltin & García-Rodríguez 2003, Pl. 5, figs 1, 2). It differs from *S. krammeri* in many regards: (1) it has smaller valves, (2) which are elliptical and have broadly rounded apices which never assume a rhomboid shape, (3) it has reduced apical pore fields on both apices, and 4) it does not present conspicuously raised costae in either internal or external views (see also Metzeltin *et al.* 2005, Pl. 15, Figs 1–9).

Staurosirella krammeri is only known from the type locality, the North Umpqua River in the western United States. The water at the collection site was low in electrolyte content (53  $\mu$ S · cm<sup>-1</sup>) and slightly alkaline (pH 7.6), with low concentrations of nitrogen (0.008 mg/l) and phosphorous (0.07 mg/l). *S. krammeri* appears to thrive at low temperature (7.9°C) and occurs in high-discharge waters that are well oxygenated.

*Staurosirella lanceolata* (Hustedt) E. A. Morales, C. Wetzel & Ector, *comb. nov. et stat. nov.* Figs 19–32 & 39–44

BASIONYM: *Fragilaria lapponica* f. *lanceolata* Hustedt, Süßwasser-Diatomeen des indomalayischen Archipels und der Hawaii-Inslen; Internationale Revue der gesamten Hydrobiologie und Hydrographie **42**(1/3): 25; Figs 20–23. 1942.

The frustules of this taxon are rectangular and usually deep in girdle view (Figs 19–32). The cells form short chains, probably with the aid of cementing substances since the spines are poorly developed (up to two spherical lumps on each costa toward the valve face/mantle junction, Fig. 40) or are absent altogether (Figs 39 & 40). The valves



**Figs 39–44**. *Staurosirella lanceolata* (Hustedt) E. A. Morales, C. Wetzel & Ector *comb. nov. et stat. nov.* SEM images from lectotype material (Hustedt Collection, Alfred-Wegener-Institut für Polar-und Meeresforschung, Material number AS1322). 39 & 40 – Valve exterior showing features of sternum, costae, striae and apical pore fields. Note that spines can be absent (39) or weakly developed (40), and the blunt ends of striae on valve face. 41 & 42 – Valve interior depicting short striae and developed apical pore fields. Note that the valves are thick, as shown by the broken edge of the mantle toward the striae in 42. 43 & 44 – Girdle views showing details of valve mantle, mantle edge and cingulum. White arrows point out that the copulae are open. Also note pointed ends of striae. Scale bars = 1  $\mu$ m.

are isopolar, narrowly elliptical, with narrowly rounded apices (Figs 21–27). Length 3.5–12.0  $\mu$ m, breadth 3.5–5.5  $\mu$ m (n = 20, since most representatives in the type material were represented by complete frustules lying in girdle view), striae density 8–10 in 10  $\mu$ m. The sternum is wide and lanceolate (Figs 39–42). There is a gradual transition between the valve face and valve mantle (Figs 39, 40, 43 & 44); the edge of the latter is mostly parallel to the valve face/valve mantle junction, although this edge is slightly curved at the apices (Figs 43 & 44). The striae are arranged



**Figs 45–50**. *Staurosirella lapponica* (Grunow) D. M. Williams & Round from different sites in the U.S.A. (45 – Little Kijik River Peninsula Borough County, Alaska; 46 & 47 – Campbell Creek (South Fork), Anchorage, Alaska; 48 & 49 – Clear Lake, Jackson County, Minnesota; 50 – Lake o'Pines, Wisconsin). 46 & 47 – External views showing details of sternum, costae, striae, spines and apical pore fields. 47 & 48 – Internal views with details of sternum, costae and striae. Also visible are developed apical pore fields. 49 – Detached cingulum viewed from top. Notice fimbriae on valvocopula. Arrows indicate open girdle elements. 50 – Girdle view of two neighboring valves still attached by intricate spines. Also note that the mantle edge is parallel to the valve face/mantle junction. Scale bars: 45-47, 49 &  $50-2 \mu m$ ;  $48-5 \mu m$ .

alternately, faintly parallel in the middle area of the valve and becoming radial toward the valve apices (Figs 19–25). The striae are composed of a single row of apically elongated areolae, which run uninterrupted from the valve face to valve mantle (Figs 39–44). There are more areolae on the valve mantle than on the valve face (Figs 39, 43 & 44). Interestingly, the striae are pointed on the valve mantle and blunter on the valve face (Figs 39, 40, 43 & 44). Delicate volae rise from the inner longer edge of each areola (not shown here). Spines are absent from the exteriorly slightly raised costae (Figs 39, 43 & 44) or are only weakly developed as two spherical lumps on each costa at the valve face/mantle junction (Fig. 40). The valves are heavily silicified, and because of this the costae do not appear conspicuously raised when seen from the interior (Figs 41 & 42). However, the striae do have the characteristic 'punch-hole' appearance. Apical pore fields are developed on both valve apices and consist of several rows of round poroids (Figs 39-44). Rimoportulae are absent. The girdle bands are open, with developed ligula and antiligula, and do not have perforations (Figs 43 & 44). The valvocopula is wider and all the girdle elements are curved at the apices (Figs 43 & 44). Fimbriae have not been observed because detached valvocopula could not be found in the analyzed sample. Plastids were not observed.

Staurosirella lanceolata does not resemble S. lapponica (compare Figs 39–44 with 45–50), and that is why we propose a change of its rank to species level. S. lapponica differs in having parallel valve sides, short striae pointed on the valve face and mantle (Figs 45–47 & 50), and longer valves (10–40 against 3.5–12 striae in 10  $\mu$ m in S. lanceolata). Another difference concerns the spines, which are well developed and always seem to be present in S. lapponica (Fig. 50).

During the 1930s the diatom community of Indonesian lakes was surveyed by Hustedt (1942), who stated that Fragilaria lapponica f. lanceolata was restricted to Celebes (Sulawesi), where it was frequently found in Lake Mahalona, but cautioned that its endemism had to be determined by future studies. This taxon has been reported in several subsequent studies. Bramburger et al. (2004) found it in the Malili lakes, also in Sulawesi (Lake Mahalona is part of this lake system). Foged (1957) reported it from Rennell Island (Solomon Islands, Melanesia), while VanLandingham (1967) and Messina-Allen and VanLandingham (1970) found it in lake fossil material from Oregon, U.S.A. Zhang and Qi (1994) found it in brackish pools in Haikou, Hainan Province (China) and proposed

the new combination *Fragilaria lapponica* var. *lanceolata* Z. A. Zhang in Zhang & Qi. Verification of these identifications through detailed analysis by LM and SEM is needed to confirm whether *Staurosirella lanceolata* is indeed distributed in Asia, Melanesia and North America.

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