

The relevance of canaliculi in the identification of *Frustulia* species (*Amphipleuraceae*, *Bacillariophyceae*)

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The type material of *Frustulia krammeri* Lange-Bertalot & Metzeltin (in Metzeltin & Lange-Bertalot 1998: 96, pl. 37: figs 1–3, pl. 120: fig. 4) from the oligo-dystrophic canyon lake Julma Ölkky in Finland was provisionally named ‘*Frustulia* nov. spec. 1 Julma Ölkky’ by Lange-Bertalot & Metzeltin (1996: pl. 37: figs 1–3; pl. 120: fig. 4; see Kusber & Jahn 2002). Differentiating features of *F. krammeri* from *F. saxonica* Rabenhorst (1853: 50, pl. VII [7]: *Frustulia*: fig. 1) were wider valves with broadly rounded ends, more convergent striae, and the presence of narrow non-perforated areas (appearing hyaline in the light microscope) between the terminal node and the apex (Lange-Bertalot & Metzeltin 1996: pl. 37: figs 1–3; pl. 120: fig. 4). In their description of *F. krammeri*, Metzeltin & Lange-Bertalot (1998: 96, pl. 37: figs 1–3, pl. 120: fig. 4) termed these hyaline apical regions canals ‘canaliculi’.

The application of the name *Frustulia krammeri* to populations in North America originally included in *F. rhomboides* has not been straightforward. A morphometric study of samples from the periphyton of lakes and ponds in the Ocala National Forest (Florida USA) showed that morphological variability (combination of length, width, valve shape, striae density, and areolae density) of taxa identified as *F. krammeri* partially overlaps with *F. saxonica* (Siver & Baskette 2004). The presence of canaliculi was considered to be a variable character in *F. krammeri*: “Striae may encircle the valve apices (Figs 43, 44), be reduced to one or two areolae (Fig. 24), or be lacking altogether (Figs 27, 28, 34, 35); this feature was found to vary on the same valve” (Siver & Baskette 2004: 636). Accordingly, Siver & Hamilton (2011) distinguished specimens from waterbodies on the Atlantic Coastal Plain as *F. krammeri* (without canaliculi, Siver & Hamilton 2011: pls 137–140) and *F. cf. krammeri* (with long helictoglossae and without canaliculi, Siver & Hamilton 2011: pl. 141: figs 1–5, or with canaliculi, pl. 142: figs 1, 3, 4).

Molecular data from *Frustulia* isolates from Fennoscandia (Urbánková & al. 2016) and North America (Bouchard & al. 2019) resulted in the separation of two lineages morphologically similar to *F. krammeri*. Bouchard & al. (2019) described one of the lineages as *F. gibsonae* A.J.Bouchard, P.B.Hamilton, J.R.Starr & Savoie based on the shape and size of single cells used for sequencing and more detailed morphological examination of natural populations whose cells were indirectly (i.e. morphologically only) assigned to these lineages. Morphological differentiation (length, width, striae density, raphe measurements) of *F. gibsonae* from *F. krammeri* was not discrete (Bouchard & al. 2019, fig. 31) and variability in valve shapes partially overlapped with the other species analysed (Bouchard & al. 2019, fig. 32). Cells identified by Bouchard & al. (2019) as *F. gibsonae* were with canaliculi (Bouchard & al. 2019 - figs 3, 10, 16, 21–26, 29, 30) or without canaliculi (Bouchard & al. 2019, figs 19, 20), and cells identified as *F. krammeri* were without canaliculi (Bouchard & al. 2019, figs 2, 8, 14).

Examination of clonal strains and multilocus reconstructions of the phylogeny of the genus *Frustulia* (Kulichová & Urbánková 2020, 2023) indicated that the presence of canaliculi is most

likely a conserved character. Within the two well-supported clades of *Frustulia*, all morphologically examined strains have canaliculi on at least one of the apices: (i) *F. cf. magaliesmontana sensu* Beier & Lange-Bertalot (2007) and *F. pseudomagaliesmontana* K.E.Camburn & D.F.Charles, and (ii) *F. erifuga* Lange-Bertalot & Krammer (see Van de Vijver & al. 2024 for the current taxonomic change), *F. curvata* Kulichová & Urbánková, *F. gondwana* Lange-Bertalot & Beier, and *F. gibsonia* forming a common lineage with *F. krammeri sensu* Urbánková & al. (2016). *Frustulia krammeri sensu* Bouchard & al. (2019) resolved to a different clade lacking canaliculi together with species such as *F. crassinervia* (Brébisson ex W.Smith) Lange-Bertalot & Krammer, *F. gaertnerae* Lange-Bertalot, *F. maoriana* Lange-Bertalot & Beier, *F. saxonica* Rabenhorst, and *F. septentrionalis* Lange-Bertalot.

We recommend focusing on ultrastructural features such as canaliculi when identifying *Frustulia* cells. The shape and size of the frustules can help in identification, but cannot be used for taxon diagnosis (Williams 2020). When examining local populations, care should be taken that morphologically similar or indistinguishable species may live in sympatry (viz Urbánková & al. 2016). We here propose to synonymise *F. gibsonia* with *F. krammeri sensu* Urbánková & al. (2016) as they form a common phylogenetic lineage with highly similar DNA sequences of *rbcL* and SSU rRNA genes (Kulichová & Urbánková 2020). The identity of the lineage as *F. krammeri* is supported by common features of the strains with the type material: they are of appropriate size and shape, have canaliculi, and originated from Finland (Figs 1–4). *Frustulia krammeri sensu* Bouchard & al. (2019), as found in Canada, is a genetically and morphologically different species that will be described at a later date.

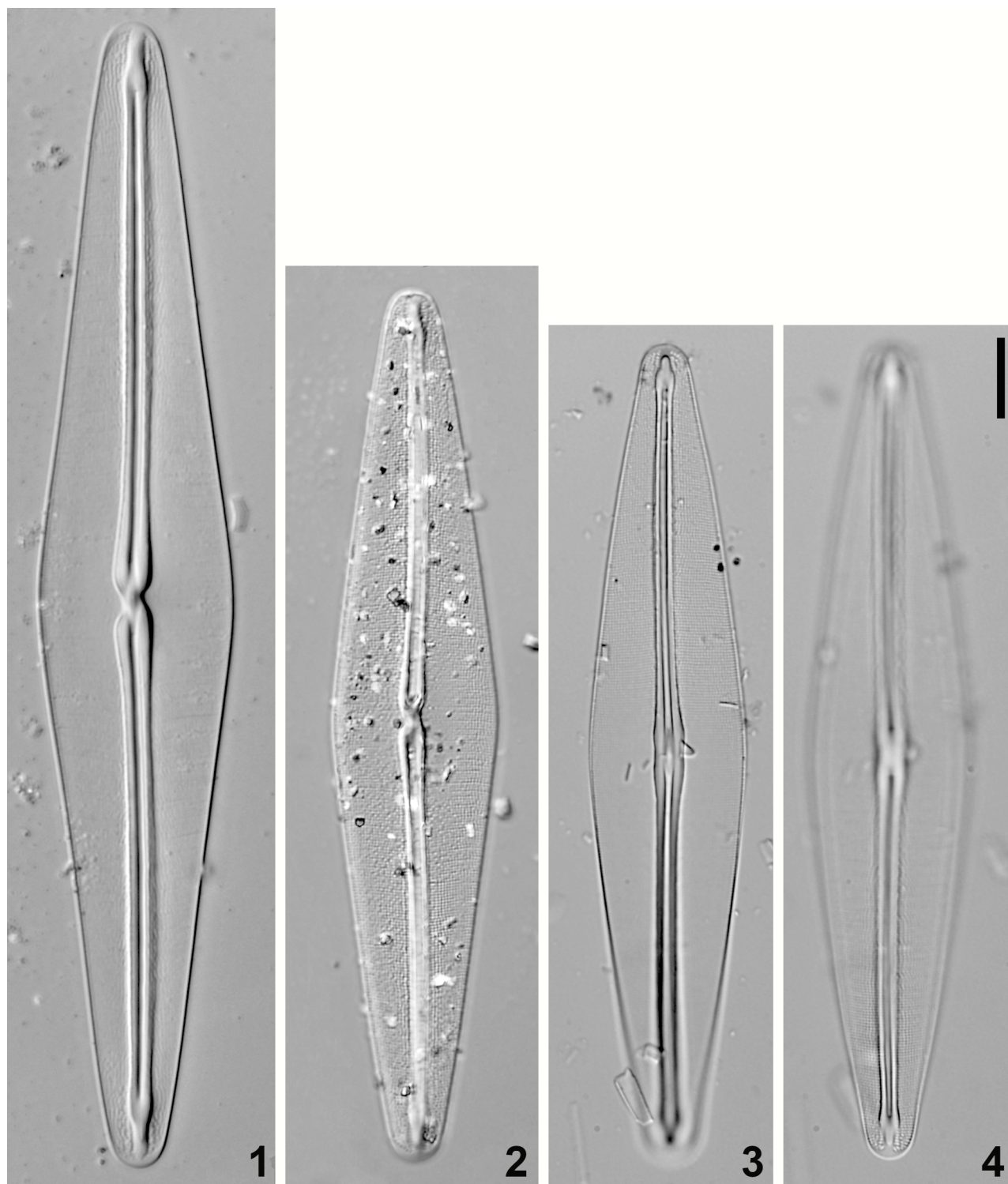
Frustulia krammeri Lange-Bertalot & Metzeltin 1998: 96, pl. 37: figs 1-3, pl. 120: fig. 4
Heterotypic synonym: *Frustulia gibsonia* A.J.Bouchard, P.B.Hamilton, J.R.Starr & Savoie 2019: 212, figs 3, 10, 16, 21-26, 29, 30

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Figs 1-4: *Frustulia krammeri* Lange-Bertalot & Metzeltin from a Finnish sample (Urbánková & al. 2016 - site 25). Fig. 1: strain JB5. Fig. 2: strain JC8. Figs 3, 4: differently focused valve from the field sample. Scale bar = 10 μ m.