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Six new *Frustulia* species (Bacillariophyta) in Tierra del Fuego peatbogs, Patagonia, Argentina

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Abstract: During a survey of the freshwater aquatic diatom flora of two peat bog areas in Tierra del Fuego, southern Argentina, six taxa belonging to the genus *Frustulia* that could not be identified were observed. Comparison with the type material of *Frustulia (Navicula) crassinervia* separated the Tierra del Fuego populations showing some resemblance as a new species: *F. australocrassinervia* sp. nov. The other five taxa are likewise described as new based on light and scanning electron microscopy observations and comparison with all known *Frustulia* species worldwide. *Frustulia delicatula* sp. nov., *F. ellipticolanceolata* sp. nov., *F. fuegiana* sp. nov., *F. patagonica* sp. nov. and *F. yaganiana* sp. nov. Comments are made on their taxonomic position and how they can be distinguished from other *Frustulia* species in this genus. Brief notes on the ecology and distribution of the seven taxa are added.

Key words: Argentina, *Frustulia*, Tierra del Fuego, peatbogs, morphology, taxonomy

Introduction

The genus *Frustulia* Rabenhorst was originally described in 1853 as a freshwater genus abundantly present in oligotrophic, acidic habitats. Members of the genus are characterized by their linear, lanceolate to even rhombic valve outline, internally covered, small simple areolae and the presence of a pair of thick parallel, longitudinal ribs, internally fused with the helictoglossae and the central nodule (Round et al. 1990; Lange–Bertalot 2001).

The diversity within the genus *Frustulia* has for a long time been underestimated, mainly because of force–fitting (Tyler 1996) many independent taxa into *Frustulia rhomboides* (Ehrenberg) De Toni, an erroneous combination of *Navicula rhomboides* Ehrenberg with *Frustulia* (Lange–Bertalot & Jahn 2000; Lange–Bertalot 2001). For instance Hustedt (1959) recognized 8 taxa whereas Patrick & Reimer (1966) discussed only three freshwater taxa. In 2001, Lange–Bertalot published a first elaborate study on *Frustulia*, recognizing 17 different taxa in Europe and four from North America and Israel. Since then, more than 50 taxa were described from different parts of the world including Europe (Urbankova et al. 2015), New Zealand (Beier & Lange–Bertalot 2007; Urbankova et al. 2015), Asia (Blanco et al. 2012), South America (Metzeltin & Lange–Bertalot 1998; 2007; Rumrich et al. 2000), North America (Bahls 2014; Edlund & Brandt 1997; Graeff et al. 2012; Lange–Bertalot & Sterrenburg 2004; Siver & Baskette 2004; Siver et al. 2009), New Caledonia (Moser et al. 1995, 1998), the sub–Antarctic Region (Van de Vijver et al. 2002; Van de Vijver & Gremmen 2006) and the Falklands/Malvinas (Flower 2005), making it evident that *Frustulia* should not be considered as species–poor genus.

The present study reports on the *Frustulia* diversity in two peat bogs on Tierra del Fuego (TDF), the insular southernmost section of Argentinean Patagonia. Diatom records from TDF are rather scarce. The oldest records date from Cleve (1900) who studied several samples from the Rio Grande and Isla Desolación. The most elaborate study was produced by Frenguelli (1923, 1924a,b,c, 1953) investigating diatom samples from all over TDF. Two other comprehensive studies were produced by Cleve–Euler (1948) and Krasske (1949) both reporting on freshwater diatoms from several *Sphagnum*–peatbogs. The PhD thesis of Mataloni (1994) illustrates several diatom taxa from TDF peatbogs. Finally, in Rumrich et al. (2000), a few samples collected on TDF were included in their monograph on diatoms from the Andes. More taxonomically specific studies were published by Maidana (2000) on the Achnanthaceae and by Guerrero

et al. (2001), discussing the families Epithemiaceae and Surirellaceae.

The number of *Frustulia* taxa reported from TDF in these studies is very low. The most commonly reported taxon is *Frustulia saxonica* Rabenhorst (under the name of *F. rhomboidea* var. *saxonica* (Rabenhorst) Pfitzer) and some of its infraspecific taxa such as *F. undulata* Cleve–Euler and var. *capitata* Mayer (Mataloni 1994). It was recorded in almost every report on TDF diatoms. Frenguelli (1924a) reported *Frustulia interposita* var. *incomperta* Lewis (as *Vanheurckia interposita* var. *incomperta* (Lewis) Frenguelli), a species described in 1865 by Lewis as *Navicula incomperta* Lewis from the White Mountain Range near Boston, USA. The species was later also reported by Cleve–Euler (1948) but a comparison of the drawing in Cleve–Euler (1948) with Lewis (1865) indicates that this record is most certainly an incorrect identification since the valves are clearly rhombic–lanceolate and not linear.

In this new study, we analysed the *Frustulia* diversity in two contrasting peat bogs near Ushuaia City, Rancho Hambre and Andorra Valley. Six *Frustulia* taxa could not be identified using the currently available literature and based on extensive light and scanning electron microscopy observations, and using the currently available literature, and are thus described here as new to science. Since some taxa showed a resemblance to *F. crassinervia* (Brébisson) Lange–Bertalot et Krammer, the type material of the latter was investigated.

Material and Methods

For light microscope (LM) observation, diatom samples were prepared following the method described in Van der Werff (1955). Subsamples of the original material were oxidized using 37% H_2O_2 and heating to 80 °C for about 1 h. The reaction was further completed by the addition of saturated $KMnO_4$. Following digestion and centrifugation (three times 10 min at 3700 g), the material, free of organic matter, was further diluted with distilled water for sample mounting to avoid excessive concentrations of diatom valves on the slides. A subsample from the organic–free material was mounted in Naphrax®. The slides were analyzed using an Olympus BX53 microscope, equipped with differential interference contrast (DIC) optics (Nomarski) and Colorview I Soft Imaging System. Diatom samples and slides are stored at BR (Botanic Garden Meise). For scanning electron microscopy (SEM), aliquots of the oxidized suspensions were filtered through 5–µm pore size polycarbonate filters which were cut in small pieces, fixed on aluminum stubs after air–drying and sputter coated (Cressington 208HR, Watford, UK) with PtPd (10 nm). Observations and photomicrographs were performed with a Jeol® JSM–7100F SEM at 1 kV at the Botanic Garden Meise (Belgium).

Smith (1853) described the species *Navicula crassinervia* Brébisson in W. Smith based on material he received from de Brébisson in September 1852. The species was transferred in 1996 to the genus *Frustulia* by Lange–Bertalot et Krammer Original material of the latter species sampled by de Brébisson in Falaise (France) on 23 July 1852 was found in the W. Smith collection of the Botanic Garden Meise. Despite the slight difference in the date (July vs. September), this material is believed to represent the type of the species and selected to study the type of *Frustulia* (*Navicula*) *crassinervia* and hence selected for comparison in our study.

Identifications and species comparisons are based primarily on Bahls (2014), Beier & Lange–Bertalot (2007), Edlund & Brandt (1997), Flower (2005), Lange–Bertalot (2001), Lange–Bertalot & Sterrenburg (2004), Metzeltin & Lange–Bertalot (1998, 2007), Moser et al. (1995, 1998), Rumrich et al. (2000), Siver & Baskette (2004), Siver et al. (2009), Van de Vijver et al. (2002) and Van de Vijver & Gremmen (2006). Terminology follows mostly Lange–Bertalot (2001) except for valve outline terminology that is based on Hendey (1964) and raphe morphology that follows Round et al. (1990).

Study Site

Two *Sphagnum magellanicum* Bridel dominated ombrotrophic peat bogs were sampled in 2014 and 2016: Rancho Hambre and Andorra. Both are located in low valleys (altitude 130–200 m a.s.l.) among the ridges of the Andes, within the southernmost main watershed of Tierra del Fuego (Iturraspe & Urciuolo 2000). The Rancho Hambre peat bog (54°47'S, 68°19'W) is almost 50 km away from Ushuaia city, within the protected area of Tierra Mayor Valley. The area is cold–temperate with a monthly mean air temperature of 4.2 °C and a monthly mean precipitation of 60 mm (Gonzalez Garraza et al. 2012). In turn, the Andorra peatbog (54°45'S, 68°20'W) is composed of four raised bogs and a fen, and located in the Andorra Valley, about 6 km north–west of Ushuaia, within the "Glaciar Vinciguerra y Turberas Asociadas" Ramsar protected site. The central area of both peatbogs has a characteristic dome shape, and most of their surfaces are covered by small pools with low conductivity, nutrient and pH values. In both of them, sediment samples were collected from different pools (both vegetated and clear) along transects crossing the entire peatbogs. Several types of samples were collected: sediment, floating mosses and submersed mosses. For each sample, pH, temperature (°C) and conductivity ($\mu S.cm^{-1}$) were measured in situ.

Results and Discussion

A total of eight *Frustulia* taxa was observed in the samples. Two of them had been previously described (Rumrich et al. 2000; Lange–Bertalot 2001): *F. vulgaris* (Thwaites) De Toni and *F. kosmolliana* Lange–Bertalot & Rumrich. Six taxa could not be identified using the currently available literature. One of the taxa showed a clear resemblance to *Frustulia crassinervia* (Brébisson in W. Smith) Lange–Bertalot et Krammer. The Tierra del Fuego population was therefore compared with the type population of this species to analyse its conspecificity. Although only small differences could be found, the Tierra del Fuego populations were described as a new species: *Frustulia australocrassinervia* sp. nov. Based on extensive analysis of light and scanning electron microscopy observations, and after comparison with almost

all previously described *Frustulia* taxa worldwide, it is clear that the other five taxa are also new for science: *Frustulia delicatula* sp. nov., *F. ellipticolanceolata* sp. nov., *F. fuegiana* sp. nov., *F. patagonica* sp. nov. and *F. yaganiana* sp. nov.

Frustulia crassinervia (Brébisson in W. Smith) Lange–Bertalot et Krammer (Figs 1–9)

Light microscopy (Figs 1–6): Valves lanceolate to elliptic–lanceolate with convex, distinctly undulating margins and clearly protracted, rostrate to even subcapitate apices. Valve dimensions (n=12): length 44–51 µm, width 11.5–12.5 µm, length/width ratio 3.8–4.1. Longitudinal ribs parallel, straight to weakly undulating, not widening near the proximal raphe endings, fused and only slightly constricted near the central nodule. Ribs fused with the helictoglossae forming short porte–crayon structures at the apices. Transapical striae parallel throughout the entire valve, circumducting the apices, 32–34 in 10 µm. Longitudinal striae clearly wavy, 30–35 in 10 µm, equidistant as the transapical striae.
Scanning electron microscopy (Figs 7–9): Striae composed of apically elongated, slit–like areolae, except for one row of rounded to transapically elongated areolae bordering the axial area (Fig. 7). Several longitudinal rows of areolae in the central area smaller, rounded (Fig. 7). External proximal raphe endings expanded, crescent moon shaped, weakly deflected (Fig. 7), ca. 3.5 µm separated from each other. Distal raphe endings straight, crescent moon shaped, terminating ca. 3.0 µm from the valve apex. Internally, longitudinal ribs not expanded near the central area, fused with the central nodule (Fig. 8). Near the apices, ribs fused with the linear helictoglossae forming a short, porte–crayon structure (Fig. 9).

Taxonomical remarks: Smith described *Frustulia crassinervia* originally in 1852 as *Navicula crassinervia* based on material he received from de Brébisson collected in Falaise (France). The species was rapidly recombined as a variety of *Navicula rhomboides* in 1880 (as *N. rhomboides* var. *crassinervia* (Brébisson) Grunow in Van Heurck) and later transferred to *Frustulia rhomboides* var. *crassinervia* by Ross in 1947. Lange–Bertalot et Krammer in Metzeltin & Lange–Bertalot (1996) proposed to separate *F. crassinervia* from *Navicula rhomboides* and transferred the species to the genus *Frustulia*, but this time in the rank of an independent species. The separation between *F. crassinervia* and *F. saxonica*, both often found together in acidic, oligotrophic habitats caused some confusion, mainly because the type of both taxa were never studied. Lange–Bertalot & Jahn (2000) analysed the type material of *F. saxonica*, unfortunately only based on LM observations but an analysis of the type of *F. crassinervia* was never made. Recently, the former *Frustulia rhomboides*–complex was thoroughly investigated using molecular, morphological and morphometric methods (Veselá et al. 2012; Urbanková & Veselá 2013; Urbanková et al. 2016). *Frustulia saxonica* and *F. crassinervia* were both included in these studies but the authors failed to separate them and grouped them systematically as *F. crassinervia–saxonica* species complex.

The analysis of the type of *F. crassinervia* allows now for a better delimitation of the latter compared to *F. saxonica*. *Frustulia saxonica* morphotype I has typically rhombic valves with a length of 65–105 µm and a valve width of 15–19 µm, clearly higher than observed in the *F. crassinervia* type population. *Frustulia saxonica* morphotype II on the other hand has comparable valve dimensions (length 35–76 µm, width 10.5–14 µm) with a similar, though usually slightly more rhombic, valve outline. A difference can be noted however in the shape of the margins that are always typically undulating in *F. crassinervia* and never in *F. saxonica*.

Frustulia australocrassinervia sp. nov. (Figs 10–20)

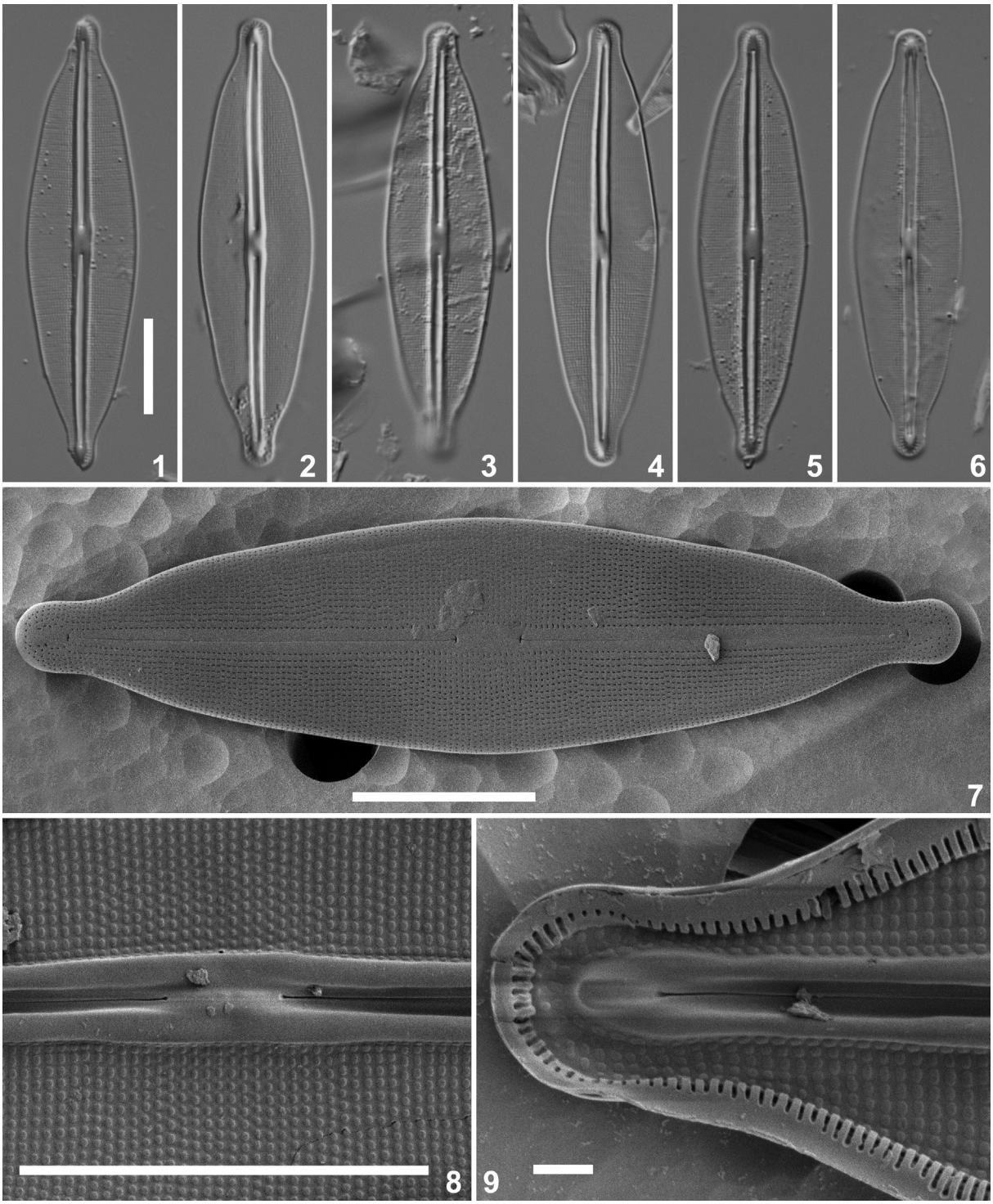
Light microscopy (Figs 10–18): Valves lanceolate with (weakly) undulated, convex margins and protracted, narrow, rostrate apices. Valve dimensions (n=25): length 30–60 µm, width 8.5–11.5 µm, length/width ratio 3.5–5.5. Longitudinal ribs only slightly widening before fusion with the constricted central nodule. At the apices, ribs fused with the helictoglossae forming short but protruding porte–crayon structures. Transapical striae parallel near the central area, becoming convergent towards the apices, 30–32 in 10 µm. Longitudinal striae wavy near the central area, more parallel near the apices, well visible in LM, 25–32 in 10 µm.
Scanning electron microscopy (Figs 19–20): Axial area narrow, linear. External proximal raphe endings small, T–shaped, almost straight, 2.2 –3.5 µm separated from each other (Fig. 19). Distal raphe endings T–shaped, terminating 1.8–2.7 µm from the valve apex (Fig. 20). Striae composed of apically elongated, almost elliptical areolae (Fig. 20). Areolae near the central area smaller and almost round (Fig. 19). Striae circumradiating the apices composed of one, maximum 2 areolae (Fig. 20). Longitudinal ribs not widened, fused with the central nodule (Fig. 20). Short but distinct linear porte–crayon structure present at the apices (Fig. 20).

Holotype: BR–4492 (Botanic Garden Meise, Belgium)
Isotypes: PLP–329 (University of Antwerp, Belgium)
Type locality: Rancho Hambre, Tierra del Fuego, Argentina, sample RH–VP4–SED (Leg. V. Casa; coll. date 23/11/2016).
Etymology: the specific epithet refers to the resemblance to *F. crassinervia* and its geographical distribution in the southern hemisphere.

Ecology and distribution: *Frustulia australocrassinervia* is usually found in samples from sediments and mosses from small to large pools with an acid pH (3.6 to 5.5)

and low conductivity (<50µS.cm^{-1}) and DOC ranging between 0.06 to 21.23 mg.l^{-1}.

Taxonomical remarks: *Frustulia australocrassinervia* presents slight differences with the type population of *F. crassinervia*. The latter is clearly larger (valve width 11.5–12.5 µm vs. 8.5–11.5 µm in *F. australocrassinervia*), has more crescent moon shaped raphe endings (compared to the T–shaped endings in *F. australocrassinervia*) and shorter apices whereas *F. australocrassinervia* have very protracted, elongated apices. Other features including stria density, valve outline and areola structure showing smaller, more rounded areolae near the central area, are however similar. Nevertheless, we consider the differences

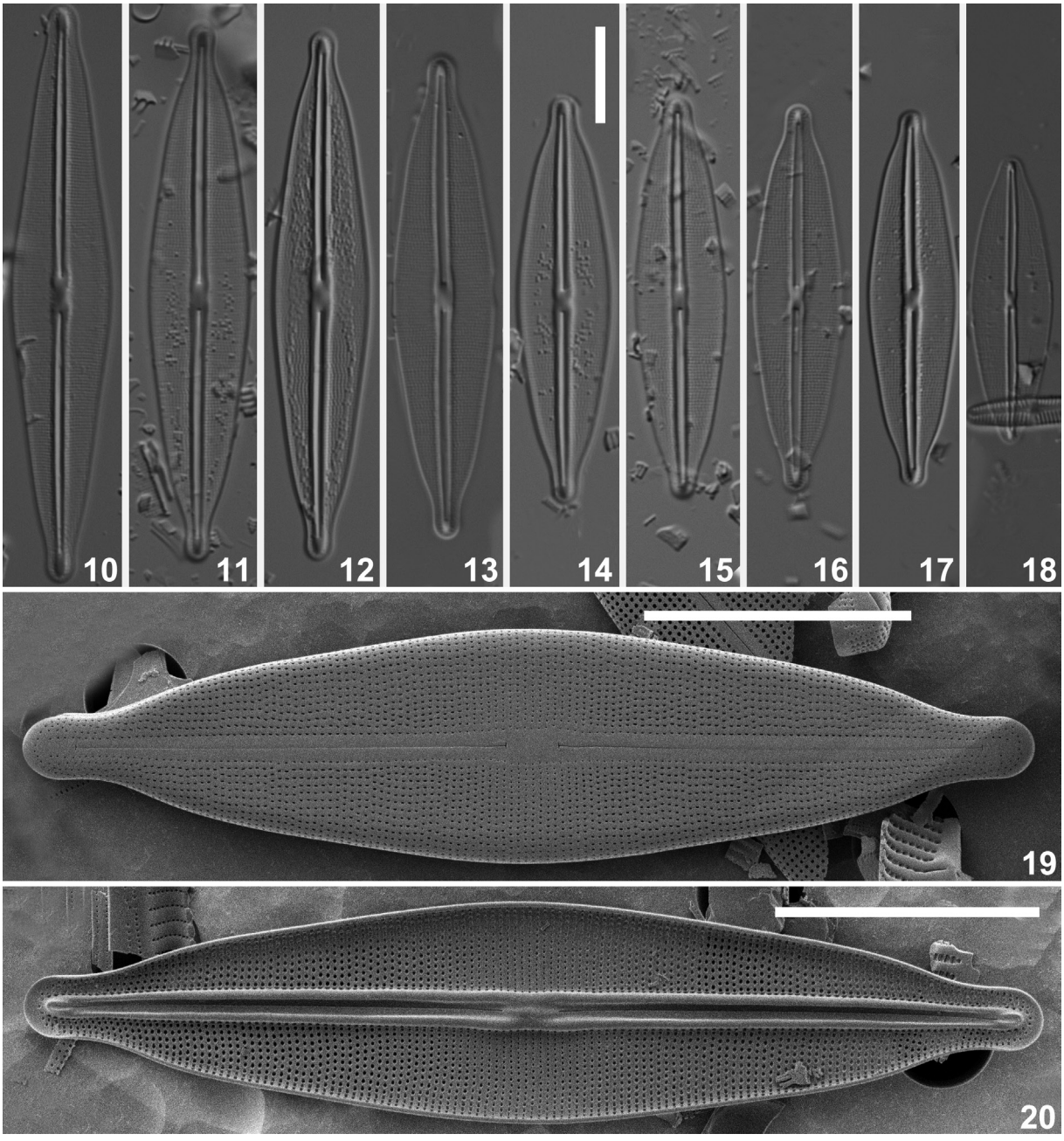


Figs 1–9. *Frustulia crassinervia.* Type population from Falaise (France): (1–6) LM views; (7) SEM external view; (8) SEM internal view of the central area showing non–constricted longitudinal ribs; (9) SEM internal view of the apex with the weakly developed porte–crayon structure and circumradiating striae. Scale bar represents 10 µm except for Fig (9) where scale bar = 1 µm.

sufficiently large to separate *F. australocrassinervia* from *F. crassinervia* and therefore, the new species is formally described. Another similar taxon is *Frustulia pseudoundosa* Flower, described in 2005 from the Falkland Islands (Flower, 2005). The latter is slightly larger (valve width 10–12 µm), has a higher stria density (ca. 38 in 10 µm) and a large subfascia that is lacking in *F. australocrassinervia*. Rumrich et al. (2000) shows a unknown *Frustulia* taxon (Rumrich et al. 2000, plate 96, Figs 1–4) from Central–Chile, that shows a resemblance to *F. australocrassinervia*. The unknown *Frustulia* has less protracted valve apices and a larger central nodule.

***Frustulia delicatula* sp. nov. (Figs 21–30, 38–43)**

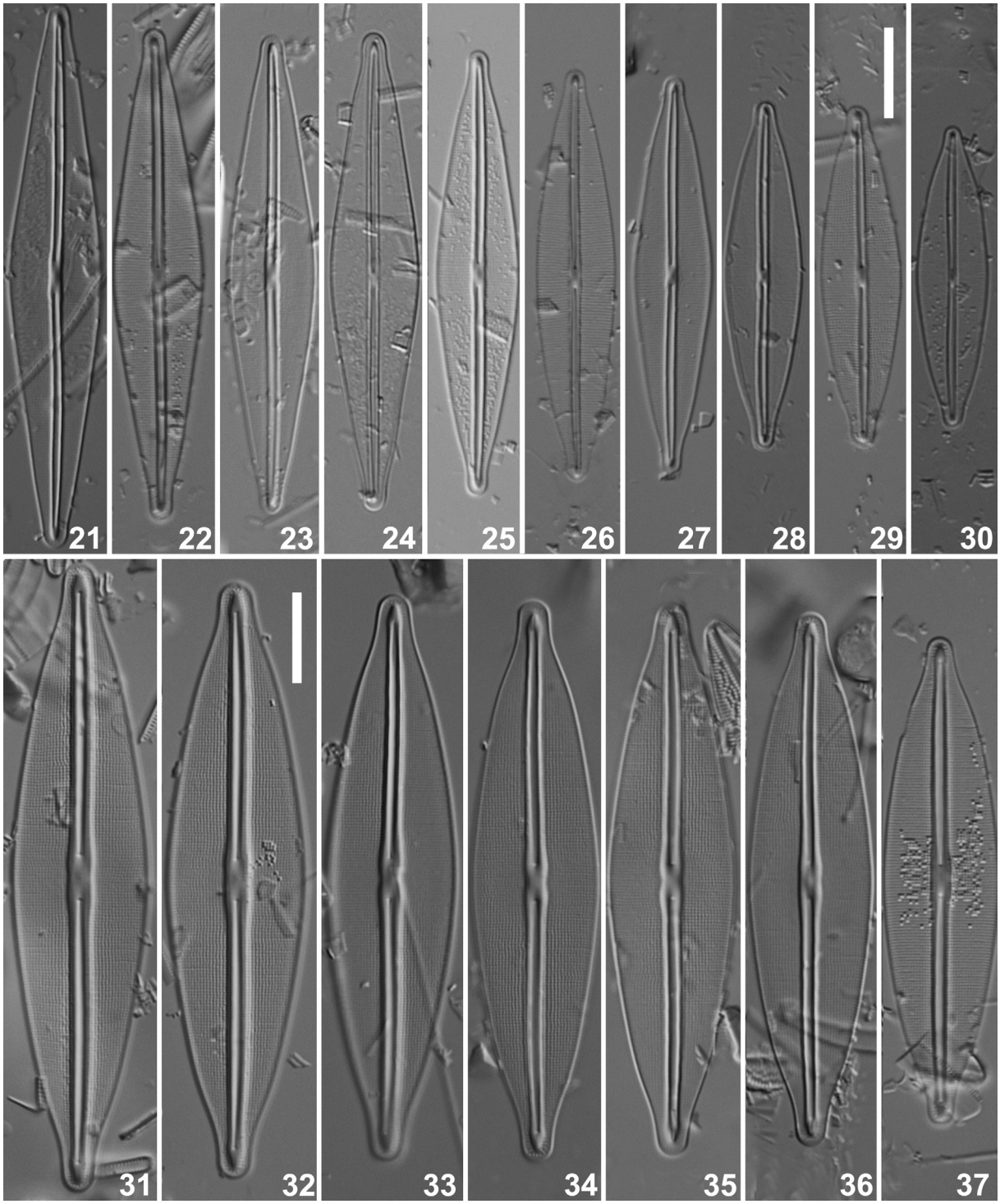
Light microscopy (Figs 21–30): Valves strictly rhombic to weakly rhombic–lanceolate with more convex margins in smaller valves and slightly to non–protracted, short, subrostrate apices. Valve dimensions (n=25): length 30–63 µm, width 7–10 µm, length/width ratio (3.6) 4.0–5.9. Axial area very narrow, linear. Longitudinal ribs parallel, never widening near the central area, fused with the central nodule. Ribs fused with helictoglossa almost entirely lacking porte–crayon structures. Transapical striae only weakly discernible in LM, slightly radiate becoming more convergent near the apices, 30–35 in 10



Figs 10–20. *Frustulia australocrassinervia* sp. nov. Type population from Rancho Hambre, (sample RH–VP4–SED): (10–18) LM views; (19) SEM external view; (20) SEM internal view. Scale bar represents 10 µm.

µm, continuing around the apices. Longitudinal striae not discernible in LM.

Scanning electron microscopy (Figs 38–43): Striae composed of small, rounded to weakly apically elongated areolae (Figs 38, 41). Areolae in the central area almost not different from the rest of the valve, occasionally somewhat smaller and slightly more rounded (Fig. 41). External proximal raphe endings weakly expanded, drop–like to t–shaped, straight, 1.3–3.5 µm separated from each other (Fig. 41). Distal raphe endings T–shaped, terminating 1.4–2.3 µm from the valve apex (Fig. 43). Internally, longitudinal ribs only weakly widening near the fusion with the central nodule (Figs 39, 40). At the apices, longitudinal ribs fused with the helictoglossae forming a weakly elongated porte–crayon structure (Fig. 42). Areolae covered internally by small, oval individual



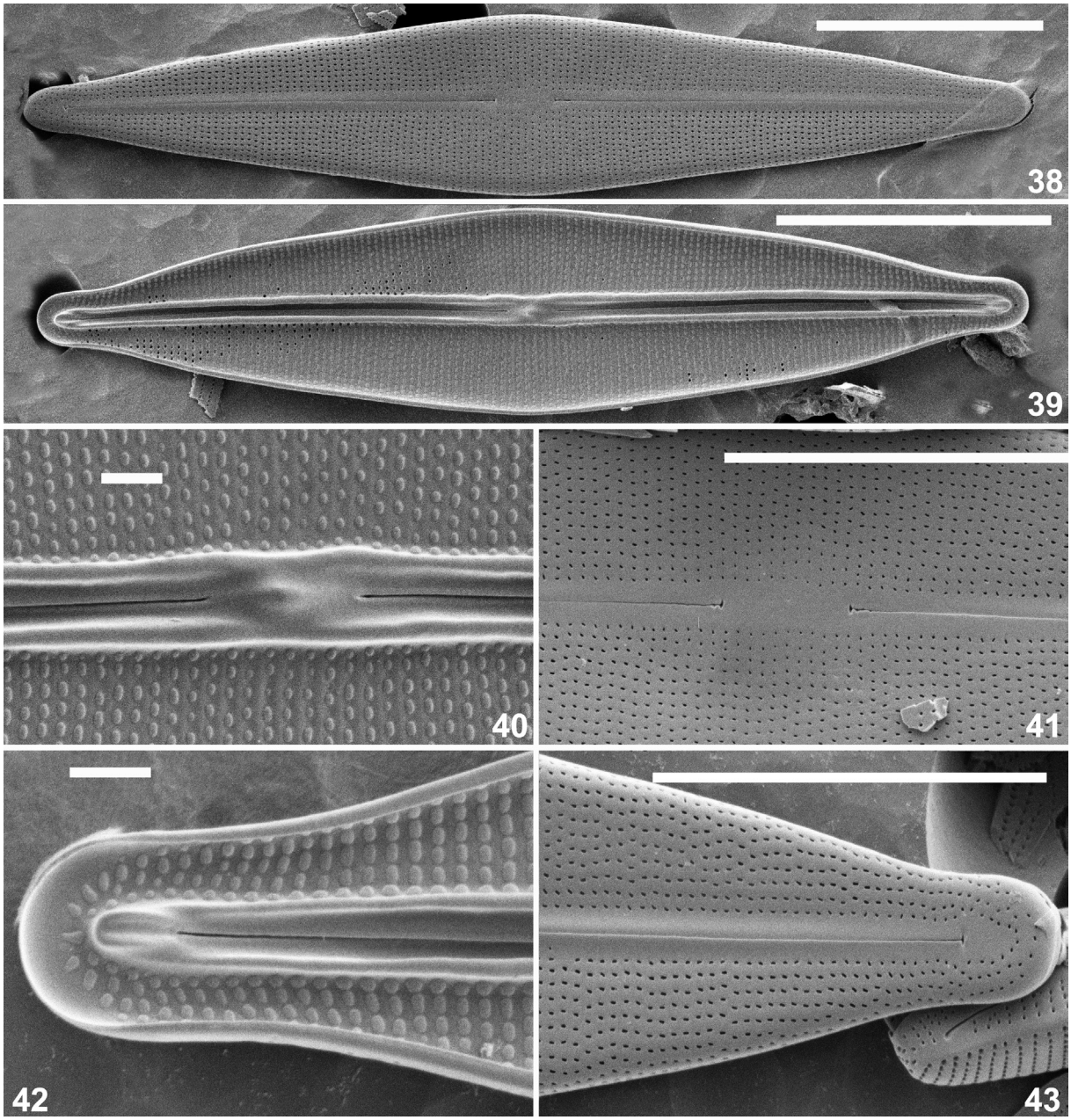
Figs 21–30. *Frustulia delicatula.* Type population from Rancho Hambre, Tierra del Fuego (sample RH–VP2–SED) and Figs (31–37) *Frustulia ellipticolanceolata*. Type population from Rancho Hambre, Tierra del Fuego (sample RH–E5–SED). LM views. Scale bar represents 10 µm.

hymenes (Fig. 42).

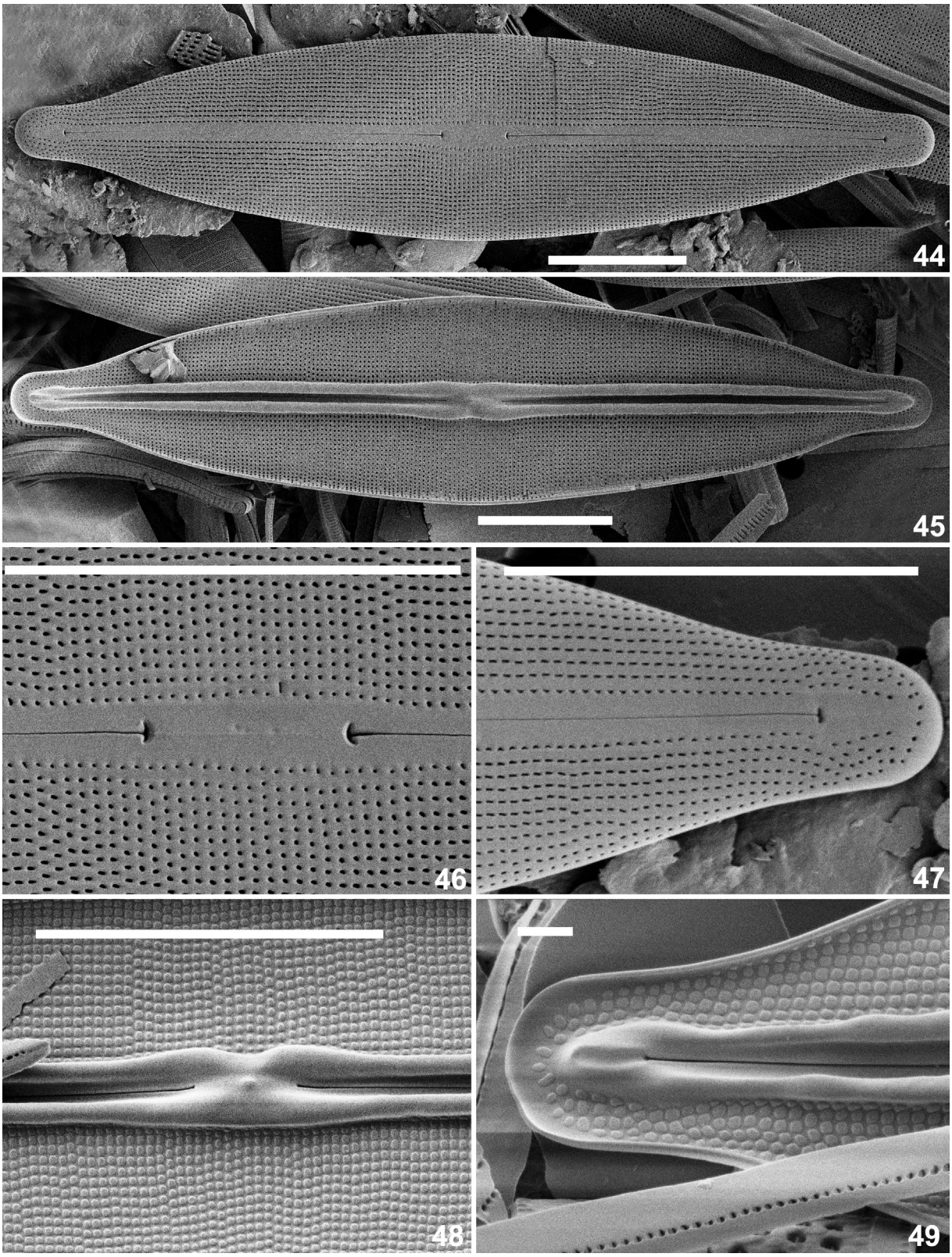
Holotype: BR–4475 (Botanic Garden Meise, Belgium)
Isotypes: PLP–320 (University of Antwerp, Belgium)
Type locality: Rancho Hambre, Tierra del Fuego, Argentina, sample RH–VP2–SED (Leg. V. Casa; coll. date 23/11/2016).
Etymology: the specific epithet refers to the fine, delicate structure of this new *Frustulia* species that is difficult to discern in LM.

Ecology and distribution: *Frustulia delicatula* is not a rare species in the TDF peatbogs and was typically found in several types of waterbodies from small to large ponds, moats and rivers of the Rancho Hambre and Andorra Valley peat bogs. Valves were present in sediment and moss samples. pH varies between 3.6 and 7.1, while the conductivity values remain very low (<100µS.cm^{-1}).

Taxonomical remarks: The combination of fine striae, rhombic outline and low valve dimensions, is rarely observed in *Frustulia* taxa. Only a few taxa could be confused with *F. delicatula*: *F. trochus* Siver & Baskette and *F. longiqua* Lange–Bertalot, both described from



Figs 38–43. *Frustulia delicatula*. Type population from Rancho Hambre, Tierra del Fuego (sample RH–VP2–SED): (38) SEM external view of an entire valve; (39) SEM internal view of an entire valve; (40) SEM internal detail of the central area with a weakly constricted central nodule; (41) SEM external detail of the central area showing small, weakly T–shaped proximal raphe endings; (42) Internal detail of the valve apex with a small porte–crayon structure; (43) External detail of the valve apex showing the circumradiating striae at the apex. Scale bar represents 10 µm except for Figs (40, 42) where scale bar = 1 µm.

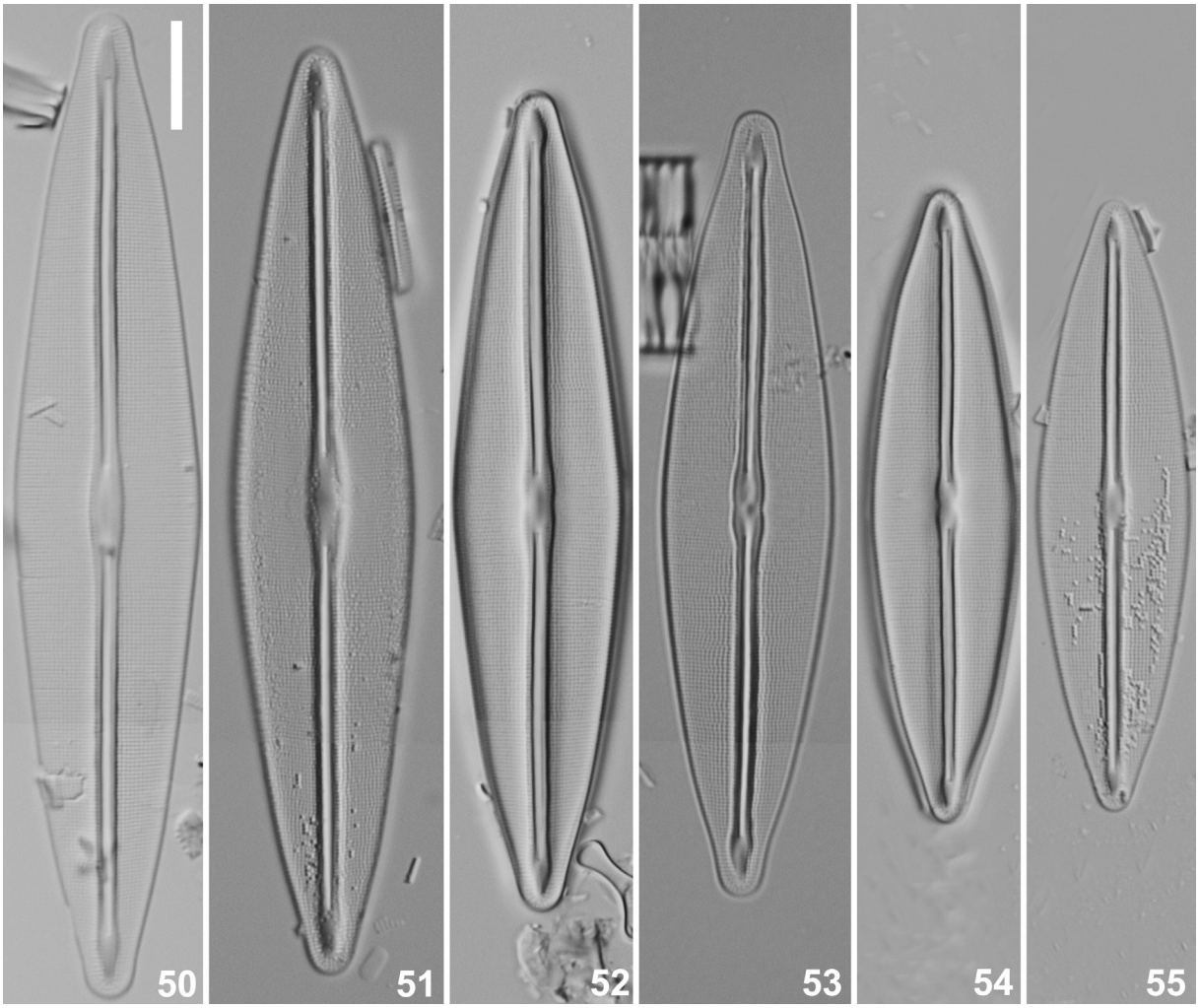


Figs 44–49. *Frustulia ellipticolanceolata*. Type population from Rancho Hambre, Tierra del Fuego (sample RH–E5–SED): (44) SEM external view of an entire valve; (45) SEM internal view of an entire valve; (46) SEM external detail of the central area showing crescent moon shaped proximal raphe endings; (47) External detail of the valve apex showing the circumradiating striae at the apex; (48) SEM internal detail of the central area with a clearly constricted central nodule; (49) Internal detail of the valve apex with the porte–crayon structure. Scale bar represents 10 μm except for Fig (49) where scale bar = 1 μm.

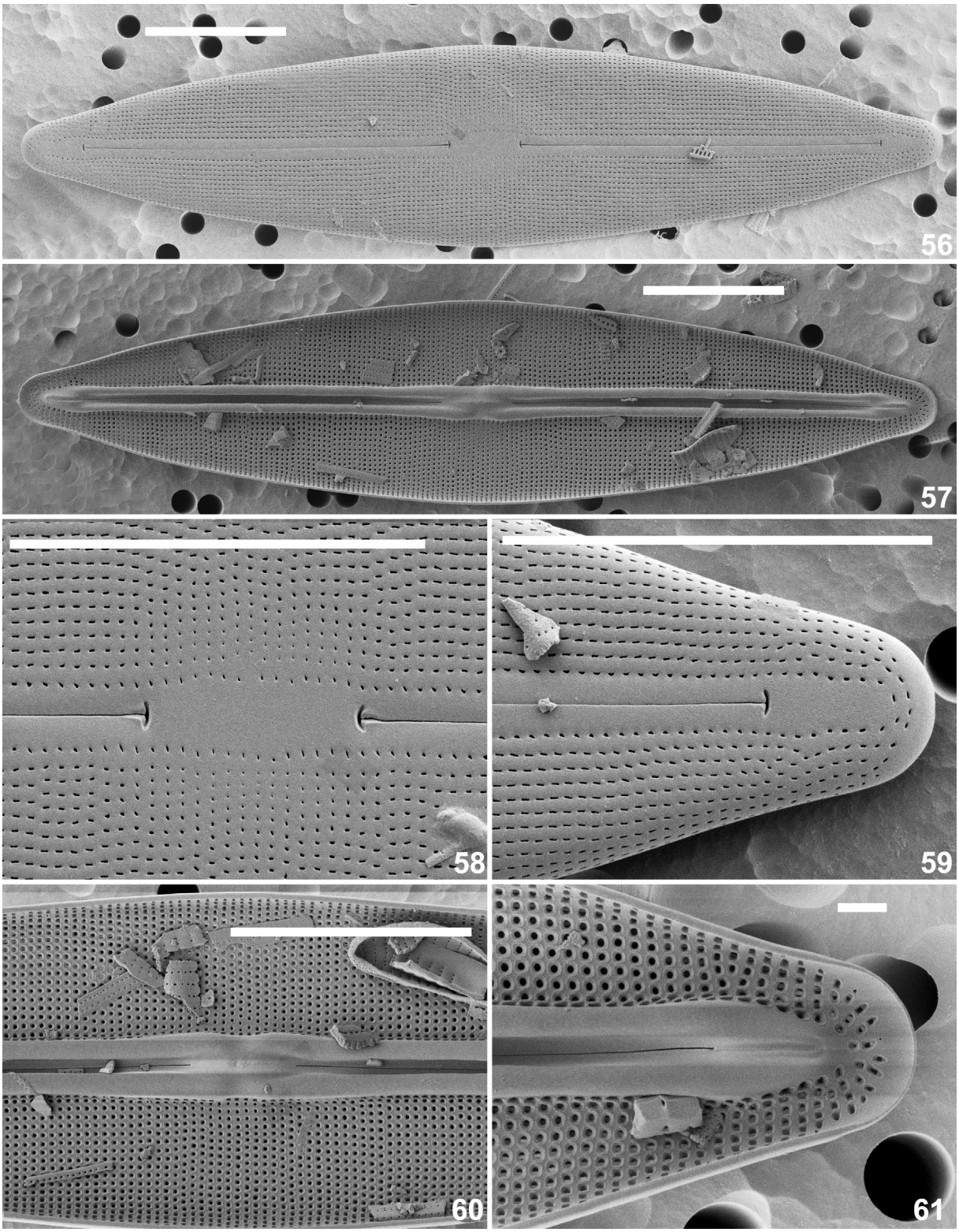
Florida (Lange–Bertalot 2001; Siver & Baskette 2004). The structure of the longitudinal ribs in the new species resembles closely the structure in *F. saxonica* but the latter clearly differs in valve dimensions and valve outline with more convex margins. *Frustulia trochus* has a lower valve width (up to 7.5 µm vs. 8–10 in *F. delicatula*) and a lower valve length (max. 43 µm), a much higher stria density with up to 50 striae making them not discernible in LM whereas in *F. delicatula*, the transapical striae are visible in LM (Siver & Baskette 2004). Similarly, *F. longiqua* is smaller than *F. delicatula* with a higher stria density (Lange–Bertalot 2001). *Frustulia pseudomagaliesmontana* Camburn & Charles has almost linear valves with typically rostrate apices contrary to the rhombic outline with acutely rounded, narrow, subrostrate apices in *F. delicatula* (Camburn & Charles 2000). *Frustulia crassinervia* differs in having typical crescent moon shaped proximal and distal raphe endings, in showing small, rounded areolae in the central area, different from the rest of the valve and in having typically undulated valve margins. In LM, it is possible to observe areolae and hence longitudinal striae in *F. crassinervia* (ca. 30 in 10 µm) whereas in *F. delicatula*, this is not possible (ca. 35–40 in 10 µm). *Frustulia australocrassinervia* differs likewise in having a well–visible striation pattern (ca. 30 striae in 10 µm).

***Frustulia ellipticolanceolata* sp. nov. (Figs 31–37, 44–49)**

Light microscopy (Figs 31–37): Valves typically elliptic–lanceolate, never rhombic to rhombic–lanceolate, with broadly rounded convex margins and long, protracted, rostrate apices. Valve dimensions (n=20): length 51–69 µm, width 12.5–15.5 µm, length/width ratio 4.0–4.8. Axial area narrow, linear. Longitudinal ribs widening towards the central area, fused with the central nodule. Near the apices, ribs fused with the helictoglossae forming a distinct, though not long, porte–crayon structure. Transapical striae almost parallel near the central area, becoming weakly convergent towards the apices, 30–32 in 10 µm, circumradiating the apices. Longitudinal striae wavy near the central area, becoming more parallel towards the apices, 25–35 in 10 µm.
Scanning electron microscopy (Figs 44–49): Striae



Figs 50–55. *Frustulia fuegiana*. Type population from Rancho Hambre, Tierra del Fuego (sample RH–CP1–SED) LM views. Scale bar represents 10 µm.



Figs 56–61. *Frustulia fuegiana*. Type population from Rancho Hambre, Tierra del Fuego (sample RH–CP1–SED): (56) SEM external view of an entire valve; (57) SEM internal view of an entire valve; (58) SEM external detail of the central area showing crescent moon shaped proximal raphe endings; (59) External detail of the valve apex showing the circumradiating striae at the apex; (60) SEM internal detail of the central area with a clearly constricted central nodule; (61) Internal detail of the valve apex with the relatively long porte–crayon structure. Scale bar represents 10 µm except for Fig (61) where scale bar = 1 µm.

composed of apically elongated areolae, except near the central area where areolae are smaller and rounded (Fig. 44). First row of areolae next to the axial area slightly separated from the other areolae, rounded to weakly elongated (Figs 44, 46). External proximal raphe endings straight, crescent moon shaped, 3.6–4.7 µm separated from each other (Fig. 46). Distal raphe endings crescent moon shaped, terminating 2.8–3.6 µm from the apex (Fig. 47). Internally, longitudinal ribs widening before fusion with the central nodule (Figs 45, 48). At the apices, ribs fused with the helictoglossae forming a rather distinct porte crayon structure (Fig. 49).

Holotype: BR–4476 (Botanic Garden Meise, Belgium)
Isotypes: PLP–321 (University of Antwerp, Belgium)
Type locality: Rancho Hambre, Tierra del Fuego, Argentina, sample RH–E5–SED (Leg. V. Casa; coll. date 23/11/2016).
Etymology: the specific epithet *ellipticolanceolata* refers to valve outline that is typically, strictly elliptic–lanceolate.

Ecology and distribution: The species has been only observed in the sediments of small and shallow pools in Rancho Hambre with low pH (4.2–4.6) and low conductivity values (< 50 µS.cm^{-1}).

Taxonomical remarks: *Frustulia ellipticolanceolata* can be confused with several *Frustulia* species: *F. septentrionalis* Lange–Bertalot, *F. saxonica*, *F. fuegiana* sp. nov., *F. crassinervia* and *F. saxoneotropica* Metzeltin & Lange–Bertalot. *Frustulia septentrionalis* has comparable valve apices but a typical rhombic valve outline and a much larger valve width (17–20 µm vs. 12.5–15.5 µm) (Lange–Bertalot 2001). The newly described *F. fuegiana* (see below) has less developed, only weakly protracted, more cuneately rounded than rostrate apices. The valves of the latter are usually larger for a comparable valve width giving the valves a more slender outlook. *Frustulia saxonica*, with whom the new species most probably was often mistaken, has more rhombic valves with less protracted, only subrostrate apices contrary to the elongated, rostrate apices in *F. ellipticolanceolata* (Lange–Bertalot 2001). Additionally, the proximal raphe endings in *F. saxonica* are T–shaped and never crescent moon shaped. *Frustulia crassinervia* has undulated valve margins with a lower valve width than *F. ellipticolanceolata*. *Frustulia australocrassinervia* has T–shaped proximal raphe endings and narrower valves (valve width 8.5–11.5 µm vs. 12.5–15.65 µm)

Frustulia saxoneotropica has less rostrate, only weakly protracted apices, narrower valves (width 11–13 µm vs. 12.5–15.5 µm in *F. ellipticolanceolata*) and a smaller central nodule. Moreover, the porte crayon structure is less developed than in *F. ellipticolanceolata* (Metzeltin & Lange–Bertalot 2007).

***Frustulia fuegiana* sp. nov. (Figs 50–61)**

Light microscopy (Figs 50–55): Valves lanceolate, never rhombic or rhombic–lanceolate, to weakly elliptic–lanceolate in smaller valves with convex, weakly undulating margins and slightly protracted, cuneately rounded apices. Valve dimensions (n=25): length 44–90 µm, width 12–17 µm, length/width ratio 3.9–5.4. Longitudinal ribs parallel, weakly curved, widening near the central area before fusion with central nodule. Near the apices, longitudinal ribs fused with the helictoglossa, forming a very short porte–crayon structure. Transapical striae parallel almost throughout the entire valve, becoming weakly convergent near the apices, 26–29 in 10 µm, circumradiating the apices. Longitudinal striae slightly wavy especially near the central area, 24–28 in 10 µm.
Scanning electron microscopy (Figs 56–61): Axial area very narrow, linear. Raphe branches straight with crescent–moon–shaped external proximal and distal raphe endings (Figs 56, 58, 59). Areolae apically elongated (Fig. 59) except near the central area close to the raphe endings where areolae showing rounded to transapically elongated foramina (Figs 56, 58). At the apices, only 1–2 areolae circumradiating the apices (Fig. 59). Internally, areolae rounded covered by individual hymenes (Figs 57, 60). Central nodule fused with weakly constricted longitudinal ribs (Figs 57, 60). Weakly shaped linear porte–crayon structure present at the apices (Fig. 61).

Holotype: BR–4477 (Botanic Garden Meise, Belgium)
Isotypes: PLP–322 (University of Antwerp, Belgium)
Type locality: Rancho Hambre, Tierra del Fuego, Argentina, sample RH–CP1–SED (Leg. V. Casa; coll. date 23/11/2016).
Etymology: the specific epithet refers to the geographical distribution of the new species.

Ecology and distribution: *Frustulia fuegiana* has been observed in the sediment and floating and submersed moss vegetation of ponds with low pH values ranging between 3.6 to 4.2 and very low conductivity (<40µS.cm^{-1}) and DOC (<15mg.l^{-1}) values.

Taxonomical remarks: This new *Frustulia* species shows some similarity with *F. saxonica* and it is highly likely that many historical records from Tierra del Fuego actually refer to this new species. Based on the illustrations in Lange–Bertalot & Jahn (2000), the type population of *F. saxonica* Morphotype I shows strictly rhombic valves whereas Morphotype II shows a more rhombic–lanceolate valve outline. *Frustulia fuegiana* on the contrary shows only very rarely valves with a rhombic–lanceolate valve outline whereas most of the valves in the population show a lanceolate to elliptic–lanceolate, more elongated and slender valve outline. Additionally, *F. fuegiana* has a lower stria density (26–29 in 10 µm) than *F. saxonica* (32–33 in 10 µm). Unfortunately, the ultrastructure of the type population of *F. saxonica* is not known, making

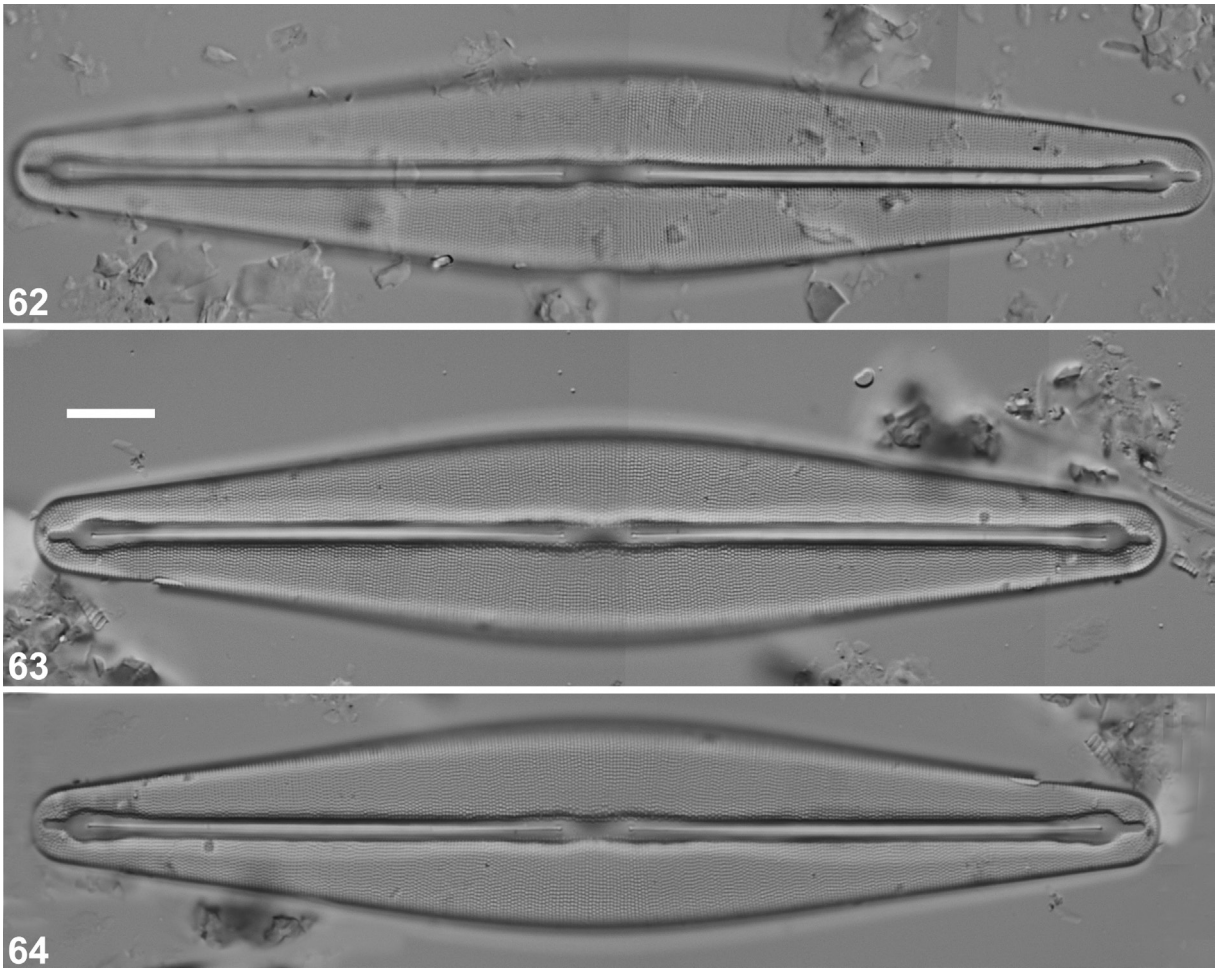
further comparison impossible. Nevertheless, both taxa can be separated without problems.

Other *Frustulia* taxa that show some resemblance include *F. crassinervia* (Brébisson) Lange–Bertalot et Krammer, *F. pangaea* Metzeltin et Lange–Bertalot, *F. pangaeopsis* Lange–Bertalot, *F. lakatosii* Metzeltin et Lange–Bertalot and *F. saxoneotropica*. *Frustulia crassinervia* and *F. australocrassinervia* are usually smaller (valve width up to 12.5 and 11.5 µm respectively) with usually weakly to strongly undulated valve margins and rostrate to subrostrate apices, features never seen in *F. fuegiana* (Lange–Bertalot 2001). *Frustulia pangaea* and *F. pangaeopsis* both have larger, more broadly rounded rostrate apices, a more linear–lanceolate (*F. pangaea*) to elliptic–lanceolate (*F. pangaeopsis*) valve outline and lack entirely the typical porte–crayon structure at the helictoglossa (Metzeltin & Lange–Bertalot 1998; Lange–Bertalot 2001). The tropical species *F. lakatosii*, described from Venezuela, has a similar valve outline and similar dimensions but has a lower stria density both in transapically (22–25 in 10 µm) and longitudinally (19–21 in 10 µm) (Metzeltin & Lange–Bertalot 1998). Finally, *F. saxoneotropica* described from Brazil where it was originally identified as *F. (rhomboides* var.) *saxonica* by Hustedt (in A. Schmidt 1930), has narrower valves and a lower stria density (25–27 in 10 µm).

***Frustulia patagonica* sp. nov. (Figs 62–64)**

Light microscopy (Figs 62–64): Valves rhombic–lanceolate, never strictly lanceolate gradually tapering towards the non–protracted, broadly rounded apices. Valve dimensions (n=10): length 110–145 µm, width 20.5–25.5 µm, length/width ratio 5.0–6.0. Longitudinal ribs parallel, straight, weakly thickened around the proximal raphe endings, fused with the central nodule and constricted in the central area. Ribs fused to the helictoglossae forming a very long porte–crayon structure. Transapical striae parallel to weakly radiate throughout the entire valve, circumradiating the apices, 26–27 in 10 µm. Longitudinal striae clearly wavy, 22–26 in 10 µm. **Scanning electron microscopy**: due to the rarity of the taxon, SEM observations were not successful.

Holotype: BR–4478 (Botanic Garden Meise, Belgium)
Isotypes: PLP–323 (University of Antwerp, Belgium)
Type locality: Arroyo Grande, Andorra Valley, Tierra



Figs 62–64. *Frustulia patagonica*. Type population from Andorra Valley, Tierra del Fuego (sample AN–AYO–SED) LM views. Scale bar represents 10 µm.

del Fuego, Argentina, sample AN–AYO–SED (Leg. V. Casa; coll. date 29/11/2016).

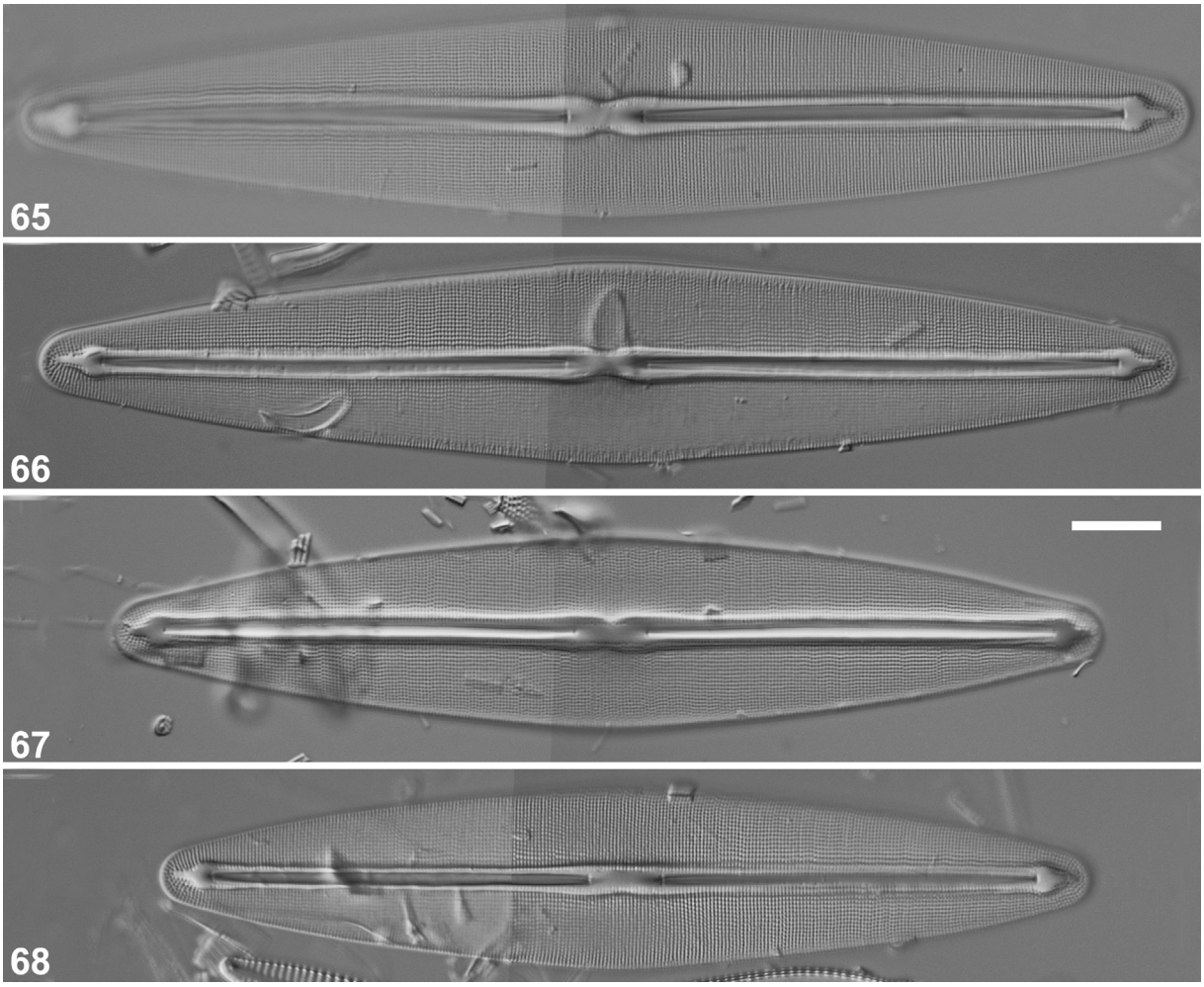
Etymology: the specific epithet *patagonica* refers to the region, Patagonia, where the new species was found.

Ecology and distribution: *Frustulia patagonica* is a rare species, so far only found in the sediments of a small ditch, running next to the Rancho Hambre peatbog and in the Arroyo Grande River in Andorra Valley. The pH of the samples ranges from 5.3 to 6.8 with a low conductivity (<100 µS.cm^{-1}).

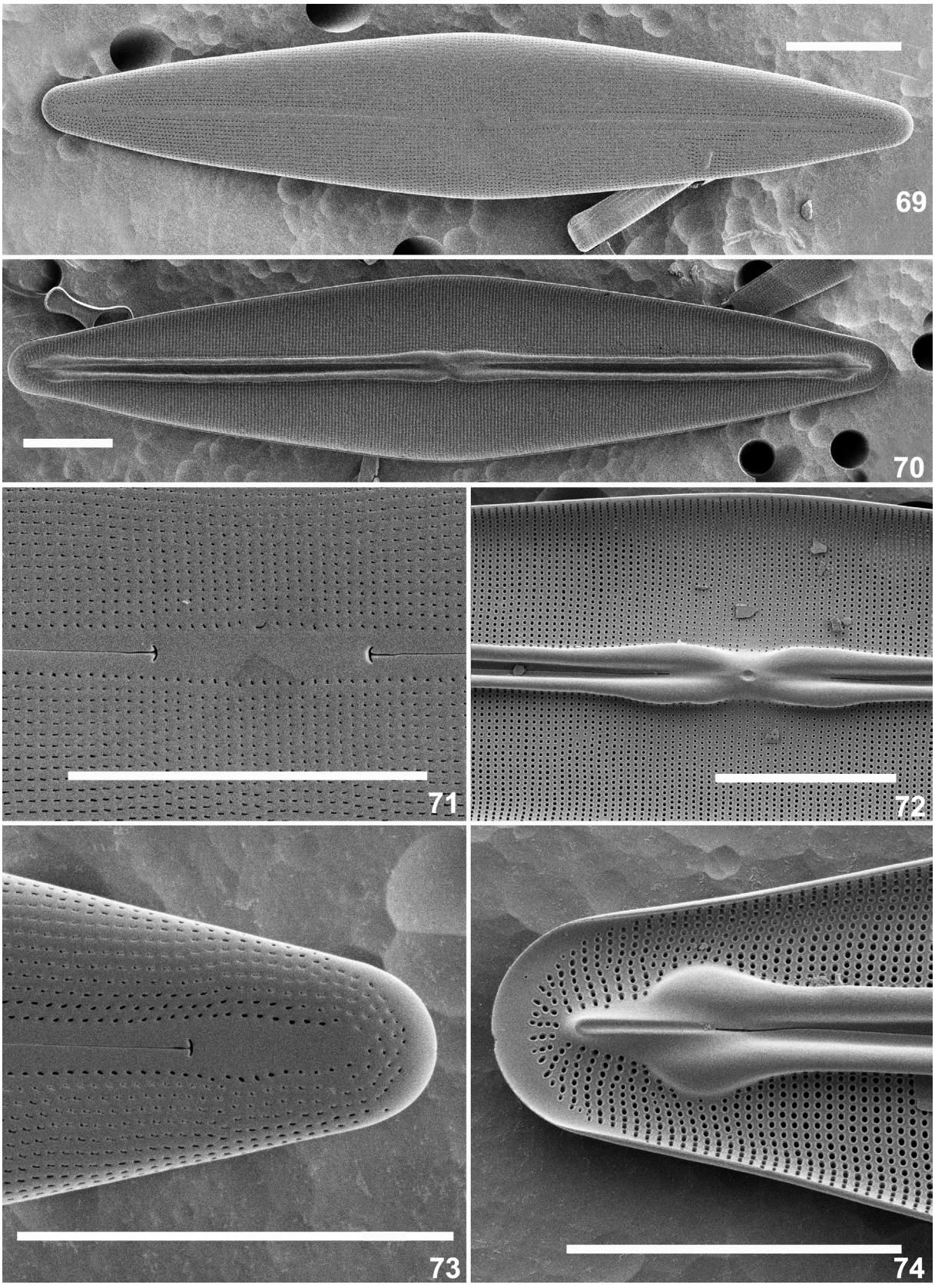
Taxonomical remarks: *Frustulia patagonica* can be confused with several larger–celled *Frustulia* taxa such as *F. australoides* Metzeltin et Lange–Bertalot, *F. bahlsii* Edlund et Brandt, *F. krammeri* Lange–Bertalot et Metzeltin, *F. magna* Metzeltin et Lange–Bertalot, *F. rexii* Graeff et Kociolek and *F. saxonica* morphotype I. *Frustulia krammeri* is probably the most similar species but is slightly smaller (max. valve width up to 24 µm vs. 25.5 in *F. patagonica*), has clearly shorter porte–crayon structures at the apices, a less constricted central nodule and curved longitudinal ribs whereas *F. patagonica* presents straight longitudinal ribs. *Frustulia bahlsii* is much larger with valves up to 193 µm long and 33 µm wide. A more important difference is the shape and length of the helictoglossae lacking any porte–crayon structures presenting a more spatulate outline, contrary to *F. patagonica* that has clearly elongated porte–crayon structures (Edlund & Brandt 1997). *Frustulia magna* shows a comparable valve outline but is clearly larger (valve width 31–33 µm). Both *F. australoides* and *F. rexii* have narrower valves with maximum valve widths up to 22 and 21 µm respectively (Metzeltin & Lange–Bertalot 2007; Graeff et al. 2012), while *F. patagonica* that shows a minimum valve width of 20.5 µm. The apices in *F. australoides* and *F. rexii* are more acutely rounded whereas *F. patagonica* has broadly rounded apices. Finally *F. saxonica* morphotype I shows a comparable outline but much lower valve width and a higher stria density (29–32 in 10 µm) (Lange–Bertalot 2001).

***Frustulia yaganiana* sp. nov. (Figs 65–74)**

Light microscopy (Figs 65–68): Valves lanceolate to



Figs 65–68. *Frustulia yaganiana*. Type population from Rancho Hambre, Tierra del Fuego (sample RH–E6–SED) LM views. Scale bar represents 10 µm.



Figs 69–74. *Frustulia yaganiana*. Type population from Rancho Hambre, Tierra del Fuego (sample RH–E6–SED): (69) SEM external view of an entire valve; (70) SEM internal view of an entire valve; (71) SEM external detail of the central area showing crescent moon shaped proximal raphe endings; (72) SEM internal detail of the central area with a clearly constricted central nodule; (73) External detail of the valve apex showing the circumradiating striae at the apex; (74) Internal detail of the valve apex with the very long porte–crayon structure. Scale bar represents 10 μm.

rhombic–lanceolate with convex margins tapering gradually towards the almost non–protracted, obtusely rounded apices. Valve dimensions (n=12): length 75–145 µm, width 21.0–25.5 µm, length/width ratio 4.7–5.7. Axial area narrow, linear. Longitudinal ribs parallel, complete and rather robust, widening near the central area before fusion with the central nodule. Clear constriction in the ribs visible at the central area, forming a structure shaped like a figure eight. Ribs fused to the helictoglossa at the apices forming an elongated porte–crayon structure. Transapical striae parallel throughout the entire valve, 24–27 in 10 µm, continuing in reduced form (2–3 areolae) around the apices. Longitudinal striae slighty wavy throughout their entire length, 24–26 in 10 µm.

Scanning electron microscopy (Figs 69–74): Axial area narrow, linear. Raphe branches straight with crescent–moon–shaped external proximal and distal raphe endings (Figs 69, 71). Areolae apically elongated (Fig. 73) except near the central area close to the raphe endings where areolae showing smaller and more rounded (Fig. 71). At the apices, number of areolae per stria reduced up to 3–4 circumradiating the apices (Fig. 73). Internally, areolae rounded covered by individual hymenes (Fig. 70). Central nodule clearly constricted, fused with expanded longitudinal ribs (Figs 70, 72). Long, well–developed porte–crayon structure present at the apices (Fig. 74).

Holotype: BR–4479 (Botanic Garde Meise, Belgium)
Isotypes: PLP–324 (University of Antwerp, Belgium)
Type locality: Rancho Hambre, Tierra del Fuego, Argentina, sample RH–E6–SED (Leg. V. Casa; coll. date 23/12/2016).
Etymology: The specific epithet *yaganiana* refers to the Yagan–tribe that lived for centuries in the southern part of Tierra del Fuego.

Ecology and distribution: The species is quite rare and most likely often confused with other larger–celled *Frustulia* taxa such as *F. krammeri* which obscure sits distribution. So far, the only large confirmed population was found at the type locality. The species has been recorded only in some large unvegetated ponds from Rancho Hambre with acid pH (4.1–5.5) and low specific conductance values (<45 µS.cm^{-1}) and DOC (<20 mg.l^{-1}).

Taxonomical remarks: Several large–celled Frustulia taxa show some resemblance to *F. yaganiana*: *F. krammeri*, *F. bahlsii*, *F. erifuga* Lange–Bertalot et Krammer, *F. amphipleuroides* (Grunow) Cleve–Euler, *F. australis* Gerd Moser, Lange–Bertalot et Metzeltin, *F. australoides*, *F. altimontana* Metzeltin et Lange–Bertalot and *F. chilensis* Lange–Bertalot et Rumrich. The most similar species is *F. australoides*, described in 2007 from Guadeloupe but the latter can be separated by its lower valve length (up to 115 µm) a more rhombic outline, convergent (instead of parallel) striae near the apices and a less developed porte–crayon structure (Metzeltin & Lange–Bertalot 2007). Likewise, *F. krammeri* has a more rhombic–lanceolate valves compared to the more rhombic–lanceolate outline in *F. yaganiana* with typical convex margins. Moreover, *F. krammeri* has a different central area lacking the typical eight–shaped fusion and constriction of the longitudinal ribs. The striae do not continue around the apices in *F. krammeri* whereas in *F. yaganiana*, the striae clearly continue. *Frustulia bahlsii* and *F. australis* both have a typical strictly rhombic valve outline and a shorter porte–crayon structure at the apices. *Frustulia bahlsii* has a larger valve width (24–33 µm vs. 21–25 in *F. yaganiana*) given the valves always a more robust outlook where valves of *F. yaganiana* always appear more elongated and slender (Edlund & Brant 1997). *Frustulia erifuga* shares the more lanceolate valve outline with *F. yaganiana* but has a lower valve width (13–19 µm vs. 21–25 in *F. yaganiana*) and a different central area and more bluntly rounded apices compared to the narrowly rounded apices in *F. yaganiana* (Lange–Bertalot 2001). *Frustulia amphipleuroides* presents typically two isolated pores in the central area, that are always absent in *F. yaganiana*. Moreover, the longitudinal ribs are interrupted near the central area leaving a large hyaline space between both sides of the ribs, a feature never observed in *F. yaganiana* (Lange–Bertalot 2001). *Frustulia altimontana*, described from tropical South America, has a comparable valve outline but lacks the long porte–crayon structures and the well–developed eight–structure in the central area (Metzeltin & Lange–Betralot 1998). Finally, *F. chilensis* has a lower valve lenght (up to 120 µm vs. 75–145 in *F. yaganiana*) for a comparable width giving the species a less elongated outlook with more bluntly rounded apices (Rumrich et al. 2000).

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