

## Article

# *Frustulia tunariensis* sp. nov. (Bacillariophyceae) from the Andes of Bolivia, South America

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**Abstract:** *Frustulia tunariensis* sp. nov. is originated from a high-altitude peatland in the Tunari Cordillera, a branch of the Andean range in Bolivia. The new taxon is distinguished by the thick longitudinal ribs, the globose polar nodule with faint helictoglossa that does not produce an apical extension, and by the high areola and stria density, not found in any of the morphologically closely related taxa. Features of the folded valvocopula, such as the presence of a siliceous membrane as pars interior, and poroids present in the tube-like portion opening as slits to the valve interior and as a single row of poroids to the exterior, are also unique characters in the new taxon. Based on a literature review, a comparison of the newly proposed species with morphologically similar taxa was made. Also, published information shows the potential of girdle bands to distinguish groups of species and species themselves within *Frustulia*. Likewise, remarks on the ecological and distribution aspects of *Frustulia* in the Bolivian Altiplano are included, focusing on taxonomic quality, geographic coverage and sampling, and potential to represent the genus in the high Bolivian Andean plateau.

**Keywords:** Bolivian Andean Altiplano; Bacillariophyta; biogeography; bogs; cingulum; girdle bands; high altitude wetlands

## 1. Introduction

Despite a considerable number of studies, the diatoms from the Andean mountains remain poorly known [1]. Those studies have concentrated mainly on material collected from accessible, anthropogenically affected ecosystems or on paleo-material, often with a rather imprecise taxonomic accuracy [2]. The number of studies for the Bolivian Andean Altiplano (25% of the country's territorial extension) are even more scarce, with those done by Frenguelli [3], Servant-Vildary [4–6], Metzeltin et al. [7], and Rumrich et al. [8] being the most representative.

Thus, additional sampling in the Altiplano is prone to yield new taxa (e.g., [1,2,9–15]), as in fact is the case of the material collected for the present study, coming from a high altitude peatland that has been subjected to human pressure only in the last decades. A new species of *Frustulia* Rabenhorst is described here from this material, a taxon that has unique features setting it readily apart from other taxa with similar valve outline and structure.

Though commonly reported from South America, the taxonomy of species in the genus *Frustulia* has not been revised in depth for the continent. The species frequently included in lists are those that also appear in samples from North America and Europe (e.g., *Frustulia rhomboides* (Ehrenberg) De Toni (an invalid name since the type does not correspond to a *Frustulia* species, but to a *Navicula* [16]) and *F. vulgaris* (Thwaites) De Toni). Whether this is the result of a cosmopolitan habit of those taxa, the effect of taxonomic drift, or force fitting into European identification keys [2] will remain subject to revision.

In terms of new taxa, the books of Metzeltin & Lange-Bertalot [7,17], Rumrich et al. [8], and Metzeltin et al. [18] presented 20 new species, three subspecies, and one variety described from South

America, out of the total of 26 species they reported for the continent. In addition, 25 morphospecies designated with “sp.” or “cf.” appeared illustrated in these references and probably constitute additional species, new to science.

There are other publications presenting new *Frustulia* taxa for South American waters. For example, [19–21] list 12 taxa not reported in the above books (six of them being new to science and 3 morphospecies designated as “sp.”). However, all these references are insufficient to show the variation in the reported taxa at the regional or continental levels, since the numbers of samples are insufficient to represent the type of habitats studied or the total diversity of habitats in the continent (own observation of the cited literature, but also see a more general discussion in [7]). This is a major drawback for applied studies including biogeography [22]. On the other hand, those articles solely containing taxa lists, but lacking cross-referencing or illustrations, impede an in-depth historical analysis of the genus in South America.

In all *Frustulia* species, the sternum internally presents thickenings, called longitudinal ribs, on both sides of the raphe slit [23,24]. Externally, the raphe proximal ends are either straight or T-shaped, while internally they are straight and opposite to each other. The distal ends are straight or T-shaped externally, while internally they end in helictoglossa of varying sizes. These variations are species specific and constitute reliable diagnostic features [24].

The longitudinal ribs fuse with the central nodule, producing a conspicuously thickly silicified central region [25], which degree of silicification, size and shape can also be used to distinguish species. In the great majority of *Frustulia* species, the longitudinal ribs distally fuse with the helictoglossa forming a massive polar nodule [25], the silicification, size, and shape of which can also vary from a general pattern resembling a “pencil point” [26] or “porte-crayon” [23] structure that also provides useful taxonomic information.

Analysis of these latter features (raphe and its interaction with the longitudinal ribs) in published size diminution series under light (LM) and scanning electron microscopy (SEM) shows that they vary little with valve size reduction and are trustable characters for species recognition. See for example the material and discussions presented in [20,24,27]. Moreover, the stability of the raphe system and the central sternum seems to be related to other invariable structures such as the hymens internally covering the areolae [28].

The structure of the raphe and flanking longitudinal ribs in *Frustulia* are apparently sufficient to separate its species from other genera in the Family Amphipleuraceae, i.e., *Amphipleura* Kützinger (with raphe branches restricted to the valve ends) [23], *Frickea* Heiden (in which the helictoglossa never fuse with the ribs, and the latter are separated from the raphe slit by at least two rows of areole) [23], and *Pseudofrustulia* Sawai & Nagumo (in which the longitudinal ribs are not fused with the central nodule only with the helictoglossae at the polar nodules) [29].

Very little is known regarding the structure of the cingulum in *Frustulia* and the available information is scattered through the literature with very little taxonomic consideration. Only some references contain SEM data on girdle bands (e.g., [23,27]), showing a variable morphological complexity that it is perhaps worth exploring taxonomically. The analysis done to date, though concentrated on only a few species, shows promising results to separate recognized genera (i.e., *Frustulia*, *Frickea* and *Pseudofrustulia* [29]), but patterns within *Frustulia* have not been explored to date.

The new *Frustulia* species is described in the context of what is known regarding the morphology of other species currently assigned to this genus. The ultrastructure of its cingulum is compared to other species for which there is published information, in an attempt to find patterns within *Frustulia* that would help in species recognition. Additionally, remarks are made on the biogeography and ecology of *Frustulia* in the Bolivian Andes, indicating which South American published records belong to this region.

## 2. Materials and Methods

### 2.1. Study Area

The Bolivian Andean region is an area of 142,815.53 km<sup>2</sup> located on the western flank of the country. Once in Bolivian territory, the Andes range splits into the Western Cordillera with a highest peak of 6542 m a.s.l. (Sajama Volcano) and acts as a natural border with Peru and Chile, and the Eastern Cordillera with a highest peak of 4438 m a.s.l. (The Illimani). The Bolivian Altiplano itself is a plateau sandwiched between the two Andean branches with an average altitude of 3800 m a.s.l. and an extension of 307,000 km<sup>2</sup>. Politically, the Altiplano belongs to the Departments of La Paz (recognized here as the northern Altiplano), Cochabamba and Oruro (central Altiplano), Chuquisaca, Potosí and Tarija (central and southern Altiplano) [30].

The sample used in this study was collected from a peatland in the Eastern Andean Cordillera, from a section locally known as the Tunari Cordillera, on March, 2014 (end of the rainy season). The specific collection site was located near the town of Uyuni (not to be confused with the Uyuni Salt Flat located in the Department of Potosí, Bolivia), Quillacollo Province, Department of Cochabamba (coordinates given below under Type material) (Figure 1).



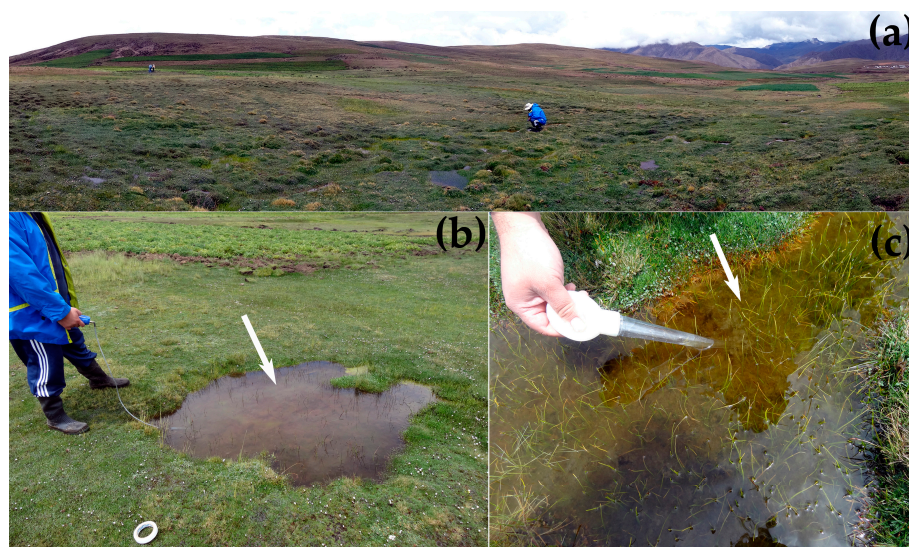
**Figure 1.** Geographic location of the type locality of *F. tunariensis* sp. nov., depicted by a white dot in the right lower part, northeast of the town of Uyuni.

The peatland rests on deposits of organic material which depth has not been determined. On top of such deposits there is vegetation growing (see below) and rivulets and shallow pools spread throughout the entire area. The wetland freezes at night due to temperatures reaching values below  $-4^{\circ}\text{C}$ . Ice from shallow pools and surfaces melt due to high solar radiation at around 9–11 a.m.

The peatland has recently been subjected to human activity pressure, mainly livestock raising (llamas, sheep, and cows) and agriculture (mainly potatoes). Many of the local rivers draining melting and ground water in the area were diverted toward the dam, so much of the water feeding the peatlands was drastically reduced. Water was thus transported through pipes and channels to artificially maintain the peatlands and to supply water for the human population and livestock that were moved from



the dam flooding area to nearby grounds. The town of Uyuni was created for a small group of that human population, a town located in a southwest direction from the type locality sampled for this study (Figure 1). Without proper technical support from the local and national governments, by 2015, a year after collection of the material studied here, roughly half of the peatland seen in Figure 2 had been plowed for agriculture.



**Figure 2.** Photographs taken during sample collection. (a) Peatland area with small rivulets and shallow pools located on undecomposed organic material, several meters thick. (b) Shallow pool corresponding to the type locality, arrow shows cloudy growths in the water. (c) Sample collection using a turkey baster, absorbing the cloudy growths depicted by arrow.

Prior to plowing, the peatland, as seen in Figure 2a, was dominated by mounds of *Distichia muscoides* Nees & Meyen (Juncaceae) and flat growths of *Plantago tubulosa* Decaisne (Plantaginaceae) (dark green patches in Figure 2b). Associated to this, in non-flooded areas, there were growths of *Deyeuxia rigescens* (J.Presl) Türpe, *D. curvula* Weddell and *D. eminens* J.Presl (Poaceae), *Gentiana sedifolia* Kunth, *Gentianella primuloides* Gilg, *G. larecajensis* (Gilg) T.N.Ho & S.W.Liu (Gentianaceae), *Werneria heterolba* Weddell (Asteraceae) and *Castilleja plumila* (Bentham) Weddell (Orobanchaceae). In zones affected by human activity, *Phylloscirus deserticola* (Philippi) Dhooze & Goetghebeur (Cyperaceae) and *Lachemilla diplophylla* (Diels) Rothmaler (Rosaceae) are also present. In the water, there were growths of *Lilaeopsis macloviana* (Gandoger) A.W.Hill (Apiaceae) (Figure 2c) and *Eleocharis* sp. (Cyperaceae).

## 2.2. Sample Collection, Treatment and Analysis

Samples of cloudy growths were collected from a 40-cm-deep shallow pool after water surface melting (ca. 10 a.m.) with a turkey baster (Figure 2b,c). Samples were stored in Ziploc bags and fixed with 10 drops of a 40% formalin solution. Water chemistry variables (temperature, pH, electric conductivity, and total dissolved solids) were measured in situ using a portable meter calibrated prior to sampling (HI 99300, Hanna Instruments S.L., Eibar, Spain).

In the laboratory, wet mounts of samples were analyzed under the microscope at magnifications from  $\times 125$  to  $\times 500$  and soft algae (excluding diatoms) were identified to genus level based on [31].

From each sample, 20 mL portions were oxidized with hot nitric acid. After repeated rinsing and decantation with distilled water, air-dried aliquots were mounted on permanent glass slides using the resin Naphrax (R.I. = 1.74). At least 50 valves of the new species were measured in LM at a magnification of  $\times 1250$  using a Zeiss Universal microscope equipped with Nomarski interference contrast and a Plan  $\times 100$  (1.25 NA) immersion objective. Images of live and oxidized material were taken using a Jenoptik CF color digital camera and ProGres CapturePro v. 2.8 software.



In the absence of taxonomic keys and floras for the region, diatoms were identified to genus and species based on regional floras such as [7,8,17,18]. Taxonomic articles, such as [3,5,32–34], and the annotated checklist by Hohn [35], were also utilized. In limited cases, some European references were also used [24,36–44].

A count of 400 valves [45] was performed along a random transect on the type permanent slide. The transect was randomly positioned and its width was determined by the diameter of the field of view. All valves appearing along the transect were counted, even pieces that could be identified.

For SEM analysis, aliquots of cleaned slurries were dried onto aluminium stubs at room temperature before being coated with gold and examined using a Carl Zeiss SUPRA 40 (at 15 kV) at the Advanced Microcopy Center, University of Buenos Aires, Argentina. SEM images were used to confirm LM identifications and also to support the description of the new species.

Morphological terminology used in the description and comparison of the new taxon follows [23] for valve features and girdle bands. For the latter, [27,46,47] were additionally used.

In order to list the species of the genus *Frustulia* that occur in the Bolivian Altiplano, a search for literature was done and, in every article and book, records of species within the genus were annotated. The summarized information was tabulated, and localities and coordinates were included to express distribution.

### 3. Results

#### 3.1. Accompanying Flora and Diatom Count

The cloudy growths found in the sampled shallow pool were slimy to the touch. Under LM, the growths were composed mainly by filamentous cyanobacteria (*Anabaena* A. Jussieu, *Calothrix* C. Agardh, *Geitlerinema* (Anagnostidis & Komárek) Anagnostidis, *Nostoc* Vaucher ex Bornet & Flahault, and *Oscillatoria* Vaucher ex Gomont), green algae (*Bulbochaete* C. Agardh, *Oedogonium* Link and *Ulothrix* (Kützinger) De Toni, and *Zygnema* C. Agardh). Embedded in the mucilaginous matrix were various coccoid cyanobacteria, coccoid green algae, desmid, and diatom species (including the new species presented here, Table 1), together with bacteria of different shapes and sizes.

The most speciose genera in the diatom community present in the sample were *Pinnularia* Ehrenberg (12 spp.), *Encyonema* Kützinger (six), and *Nitzschia* Hassall (five) (Table 1). These species represented ca. 41% of the 56 taxa encountered under LM. Using the cited literature, 22 corresponded to morphospecies identified under “sp.” or “cf.”, representing ca. 39% of the total number of taxa. In terms of relative abundance, the diatom community was dominated by *Nitzschia* cf. *perminuta* Grunow (26.5%), *N. cf. acidoclinata* Lange-Bertalot (17.5%), *Encyonopsis* cf. *thumensis* (Hustedt) Krammer (11.25%), *Frustulia tunariensis* sp. nov. (7.5%), *Encyonema neogratile* Krammer (6.5%), *Kobayasiella pseudosubtilissima* (Manguin) Lange-Bertalot & E.Reichardt (5.75%), *Fragilaria tenera* (W. Smith) Lange-Bertalot (5.5%), *Pinnularia subcapitata* var. *elongata* Krammer, and *Tabellaria flocculosa* (Roth) Kützinger, each reaching 1.25% of the total count.

The new taxon is one of two species of *Frustulia* found in the analyzed sample. The other species is *Frustulia saxonica* Rabenhorst, present in much lower abundance (Table 1, Figure 3b,c’).

**Table 1.** List of species identified from the type preparation (BR-4598), and count and percentage of taxa appearing along a random transect in a count of 400 valves.

Taxon Name	Valve Count	Percentage
<i>Achnantheidium</i> cf. <i>nanum</i> (F. Meister) Novais & I. Jüttner		
<i>Achnantheidium minutissimum</i> (Kützinger) Czarnecki	2	0.5
<i>Chammaepinnularia</i> sp. 1		
<i>Cymbopleura naviculiformis</i> (Auerswald) Krammer		

Table 1. Cont.

Taxon Name	Valve Count	Percentage
<i>Diploneis kahlui</i> Lange-Bertalot & Rumrich		
<i>Encyonema aueri</i> (Krasske) Krammer	2	0.5
<i>Encyonema dubium</i> Krammer		
<i>Encyonema minutiforme</i> Krammer		
<i>Encyonema neogracile</i> Krammer	26	6.5
<i>Encyonema rumrichae</i> Krammer	4	1
<i>Encyonema silesiacum</i> (Bleisch) D.G. Mann	2	0.5
<i>Encyonopsis</i> cf. <i>ruttneri</i> (Hustedt) Krammer	15	3.75
<i>Encyonopsis</i> cf. <i>thumensis</i> Krammer	45	11.25
<i>Encyonopsis recta</i> Krammer		
<i>Encyonopsis</i> sp. 1	10	2.5
<i>Eunotia</i> cf. <i>biggiba</i> Kützing		
<i>Eunotia</i> cf. <i>major</i> (W. Smith) Rabenhorst		
<i>Eunotia parapectinalis</i> Lange-Bertalot & U. Rumrich		
<i>Fragilaria rumpens</i> (Kützing) Carlson		
<i>Fragilaria tenera</i> (W. Smith) Lange-Bertalot	22	5.5
<i>Frustulia saxonica</i> Rabenhorst	1	0.25
<i>Frustulia tunariensis</i> sp. nov.	30	7.5
<i>Gomphonema</i> cf. <i>gracile</i> Ehrenberg		
<i>Gomphonema</i> cf. <i>parvulum</i> (Kützing) Kützing		
<i>Gomphonema</i> cf. <i>subclavatum</i> (Grunow) Grunow		
<i>Kobayasiella pseudosubtilissima</i> (Manguin) Lange-Bertalot & E.Reichardt	23	5.75
<i>Krasskella</i> sp. 1		
<i>Neidium</i> cf. <i>bisulcatum</i> (Lagerstedt) Cleve		
<i>Nitzschia</i> cf. <i>acidoclinata</i> Lange-Bertalot	70	17.5
<i>Nitzschia</i> cf. <i>perminuta</i> Grunow	106	26.5
<i>Nitzschia</i> cf. <i>subacicularis</i> Hustedt	2	0.5
<i>Nitzschia</i> cf. <i>strelnikovae</i> Lange-Bertalot, Genkal & Vekhov		
<i>Nitzschia neotropica</i> Lange-Bertalot & U. Rumrich	2	0.5
<i>Pinnularia borealis</i> Ehrenberg		
<i>Pinnularia borealis</i> var. <i>islandica</i> Krammer	2	0.5
<i>Pinnularia</i> cf. <i>ampulliformis</i> Manguin	7	1.75
<i>Pinnularia divergentissima</i> Grunow		
<i>Pinnularia microstauron</i> (Ehrenberg) Cleve	14	3.5
<i>Pinnularia pseudogibba</i> Krammer		
<i>Pinnularia rabenhorstii</i> var. <i>franconica</i> Krammer		
<i>Pinnularia similiformis</i> Krammer		
<i>Pinnularia spinosissima</i> Lange-Bertalot, Rumrich & Krammer	2	0.5
<i>Pinnularia subcapitata</i> var. <i>elongata</i> Krammer	5	1.25
<i>Pinnularia subgibba</i> Krammer		

Table 1. Cont.

Taxon Name	Valve Count	Percentage
<i>Pinnularia tsoneka</i> Lange-Bertalot & Metzeltin		
<i>Pseudostaurosira</i> sp. 1		
<i>Rhopalodia</i> cf. <i>operculata</i> (C.Agardh) Håkanasson		
<i>Sellaphora fusticulus</i> (Østrup) Lange-Bertalot	2	0.5
<i>Stauroneis acidoclinata</i> Lange-Bertalot & Werum		
<i>Stauroneis</i> cf. <i>frauenfeldianum</i> (Grunow) Heiden		
<i>Stauroneis subgracilis</i> Lange-Bertalot & Krammer		
<i>Stenopterobia</i> cf. <i>delicatissima</i> (F.W.Lewis) Brébisson ex Van Heurck		
<i>Stenopterobia densistriata</i> (Hustedt) Krammer		
<i>Tabellaria flocculosa</i> (Roth) Kützing	5	1.25
<i>Ulnaria acus</i> (Kützing) Aboal	1	0.25
<i>Ulnaria</i> sp. 1		
TOTAL	400	100

### 3.2. New Species Description

Division Bacillariophyta.

Sub-division Bacillariophytina Medlin & Kaczmarska 2004.

Class Bacillariophyceae Haeckel 1878 (emend. Medlin & Kaczmarska 2004).

Subclass Bacillariophycidae D.G. Mann in Round et al. 1990.

Order Naviculales Bessey 1907 (emend. D.G. Mann in Round et al. 1990).

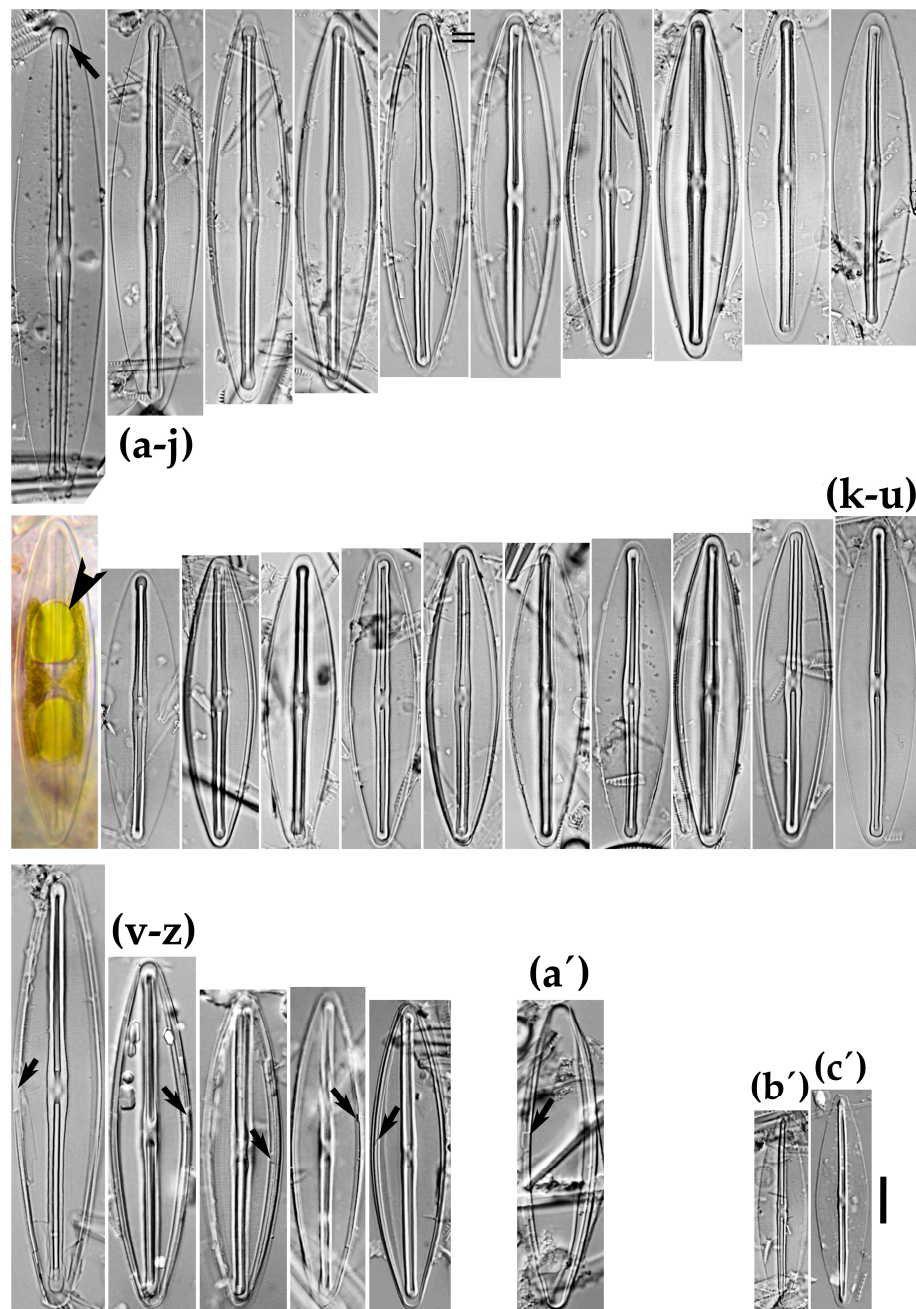
Family Amphipleuraceae Grunow 1862.

Genus *Frustulia* Rabenhorst 1853.

*Frustulia tunariensis* E.Morales sp. nov. (Figure 3 LM, Figures 4 and 5 SEM)

Frustules solitary, rectangular in girdle view. Chromoplasts with granular appearance, H-shaped, golden greenish in color (Figure 3j). Some cells containing large oil droplets (Figure 3j). Valves elliptico-lanceolate with convex margins, with obtusely rounded apices (Figures 3a–z and 4a). Valves 60–110 µm long, 17–18 µm wide. Stria density 39–50 in 10 µm, areolae 40–45 in 10 µm within transapical stria. Striae not clearly resolved under LM, uniseriate, parallel in valve center to convergent toward apices, extending around the latter in circumradiate fashion (Figures 4b–f and 5b). Club-shaped area at apex devoid of ornamentation in external view marks polar nodule where longitudinal ribs and helictoglossa fuse internally (Figures 3a and 4d). Externally, areolar openings from round to elliptic in central area to apically elongated or round toward the valve apices (Figure 4b,c). Internally, areolar openings larger, roundish (Figure 4d–f). Each areola occluded by fine hymen located closer to internal areolar opening (Figure 4f). Raphe filiform with external proximal and distal ends “T”-shaped with bent arms (Figure 4b,c). Proximal and distal raphe ends straight in internal view (Figure 4d,e). Raphe sternum with thick ribs, ca. 2.5 µm wide each (measured transapically), on each side of raphe (Figure 4d,e, externally seen as whitish shadows in Figure 4a). More or less widened structure formed by fusion of ribs with central nodule, constricted on its outer margin (Figure 4e). Distally, ribs fuse with helictoglossa forming globose apical nodules; no evident porte-crayon formation can be seen (Figure 4d). Copulae open, with single row of poroids (Figure 5a). Valvocopula folded, tube-like, lacking internal verrucae (Figure 5c,d). Poroids on valvocopula open as round perforations inside tube chamber (not shown), as single row of round poroids towards valve exterior (Figure 5b), as slits towards valve interior (Figure 5c). Siliceous membrane present on valvocopula as attachment to valve interior (Figure 5a).





**Figure 3.** LM photographs of *Frustulia tunariensis* sp. nov. (a–j,k–u) Size diminution series of part of the population present in type material (BR-4598). Notice thick longitudinal ribs, constricted central nodule and absence of the porte-crayon structure at the valve apices (black arrow in a). (a) depicts holotype specimen. (k) Live cell from type material before processing, showing H-shaped plastid and two large oil droplets (one of them depicted by black arrow head). (v–z) Micrographs showing the notch on valvocopulae still attached to depicted valves (black arrows). (a') Detached girdle with valvocopula showing the notch (black arrow). (b',c') *Frustulia saxonica* found in the same sample. Scale bar in (c'): 10  $\mu$ m.

Type Material: Bolivia, unnamed peatland northeast of the town of Uyuni (17°7'13.1" S, 66°17'51" W), 4155 m a.s.l., area of influence of the Masicuni Hydroelectrical Dam, province of Quillacollo, department of Cochabamba. Coll. March 11, 2014, E.A. Morales & S.F. Rivera.

Holotype: Slide BR-4598 (accession number in process), Meise Botanic Garden, Belgium, Figure 3a herein. Maltwood finder 33/34.

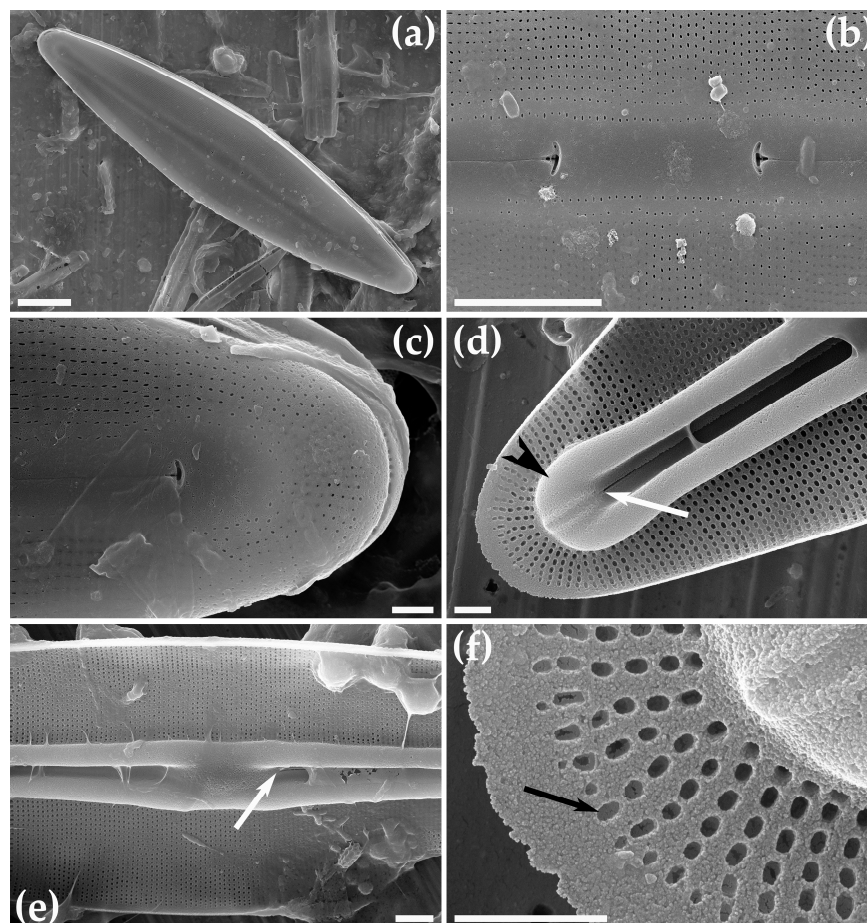
Isotype: Slide DBOL-0821, Diatomothea Boliviensis, Cochabamba, Bolivia.

Habitat: Shallow pool in a high-altitude peatland with cloudy submerged growths in which cells of the new taxon were embedded. Pool freezes overnight at temperatures below  $-4\text{ }^{\circ}\text{C}$  and melts between 9–11 a.m.

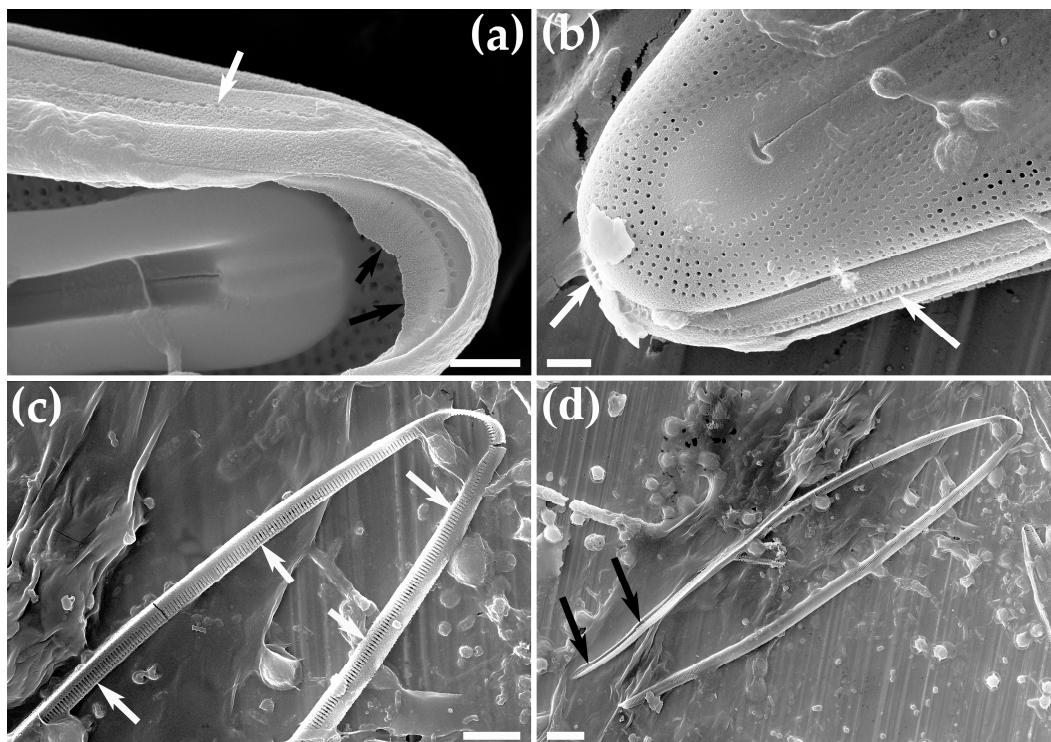
Distribution: Known from type locality only.

Etymology: The epithet “*tunariensis*” refers to the Tunari Cordillera where the type locality is found.

Ecology: The taxon was found in relatively cold ( $T = 18.6\text{ }^{\circ}\text{C}$  at the moment of sampling) and slightly acidic water ( $\text{pH} = 6.3$ ). The water was very clear where the cloudy algal growths were not present. The instrument recorded 0 ppt total dissolved solids and  $0\text{ }\mu\text{S/cm}$  electric conductivity in these clear spots.



**Figure 4.** SEM images of *Frustulia tunariensis* sp. nov. from type material BR-4598. (a) Entire frustule. (b) Central area in external view showing T-shaped proximal raphe ends and round to transapically elliptic areolae, forming parallel striae. (c) Valve apex (external) showing club-shaped shadow area (polar nodule) with T-shaped distal raphe end, circumpolar convergent striae formed by apically elongated areolae below the polar nodule and round at the very apex. (d) Valve apical region (internal) showing polar nodule with fusion of longitudinal ribs and helictoglossa (open arrow), of which faint labia can still be seen, but no porte-crayon structure. Notice large internal areolar openings and the striae going from parallel to convergent toward the apex. White arrow depicts straight distal raphe end. (e) Central nodule with fused longitudinal ribs, parallel striae. White arrow shows straight proximal raphe end. (f) Zoom into apical area showing the areolar occlusions located toward the internal areolar border (black arrow). Scale bars: (a) 10  $\mu\text{m}$ , (b) 5  $\mu\text{m}$ , (c–f) 1  $\mu\text{m}$ .



**Figure 5.** SEM images of *Frustulia tunariensis* sp. nov. from type material BR-4598. (a) Valvocopula still attached to valve. White arrow depicts single row of round poroids. Black arrows point to siliceous membrane that attaches the valvocopula to the valve. (b) External view of valvocopula showing row of poroids opening externally. (c) Opening slits (white arrows) containing poroids communicating the tubular (partially eroded) valvocopula with the valve interior. (d) Same valvocopula but showing it as an open (split at ends), tubular (folded) structure (black arrows). Scale bars: (a,b) 1 µm, (c) 3 µm, (d) 5 µm.

### 3.3. Distribution of *Frustulia* Species in the Bolivian Altiplano

Only five species have been recorded for the Altiplano region of Bolivia, including the new species described herein (Table 2). Four of these species are rather common in European and North American floras, namely *F. crassinervia* (Brébisson ex Smith) Lange-Bertalot & Krammer, *F. rhomboides*, *F. saxonica* and *F. vulgaris* (Thwaites) De Toni. One, namely *F. franguelli* Manguin, is rarely reported from the Altiplano, in general. Another one, the new species presented here, has been seen only in the type locality.

For the six species in Table 2, two have LM data, three appear named in lists and one (the new species) has been studied in detail using SEM. These species appear reported in northern or central parts of the Bolivian Altiplano, with no records for the southern portion of this region.



**Table 2.** Species of the genus *Frustulia* reported for the Bolivian Altiplano. The new taxon described herein is shaded.

Taxa	Locality and Altitude (m a.s.l.)	Coordinates	General Ecology	Reference & Comments
<i>F. corneliae</i> Lange-Bertalot & U. Rumrich	Small unnamed stream in Sorata, La Paz, 4056	15°42′0.35″ S, 68°36′0.41″ W	Rare, in stream benthos, circumneutral to alkalibiont	[34], as “F. sp. 1”, only LM
<i>F. crassinervia</i> (Brébisson ex Smith) Lange-Bertalot & Krammer	Small unnamed stream in Sorata, La Paz, 4056	15°42′0.35″ S, 68°36′0.41″ W	Rare, in stream benthos, alkalibiont	[34], only LM
<i>F. franguelli</i> Manguin	Ichu Kota Valley, La Paz, 4200–4900	16°12′ S, 69°30′ W	Rare, in high altitude peatland, acidobiont	[4,5], only listed
<i>F. rhomboides</i> (Ehrenberg) De Toni	Ichu Kota Valley, La Paz, 4200–4900	16°12′ S, 69°30′ W	Rare, in high altitude peatland, acidobiont	[4,5], only listed
<i>F. saxonica</i> (Thwaites) De Toni	-Ichu Kota Valley, La Paz, 4200–4900 -Peatland near town of Uyuni, Cochabamba, 4155	-16°12′ S, 69°30′ W 17°7′13.1″ S, 66°17′51″ W	Rare, in high altitude peatlands, acidobiont	[4,5], only listed -This manuscript, only LM (Figure 3a’,b’)
<i>F. tunariensis</i> E. Morales sp. nov.	Peatland near town of Uyuni, Cochabamba, 4155	17°7′13.1″ S, 66°17′51″ W	Common, in high altitude peatland, acidobiont	This manuscript, LM and SEM available
<i>F. vulgaris</i> (Thwaites) De Toni	-Two small unnamed streams in Sorata, La Paz 3993–4056 -Lake Titicaca, La Paz, 3812	-15°42′0.35″ S, 68°36′0.41″ W; 15°51′2.47″ S, 68°38′6.39″ W -15°45′ S, 69°25′ W	Rare, in lake and stream benthos, circumneutral to alkalibiont	[6,34], only listed

## 4. Discussion

### 4.1. Taxonomic Allocation and Distinction of *Frustulia tunariensis* sp. nov. from Similar Species

The morphological analysis presented here places *F. tunariensis* sp. nov. in *Frustulia* mainly due to the structure of the raphe and flanking longitudinal ribs. The fusion of these ribs with the central nodule, and the helictoglossa at the polar nodules is similar to the most common species in the genus, also considered as the most representative species within it (e.g., *F. crassinervia*, *F. rhomboides*, *F. saxonica*, and *F. vulgaris*). The similarity with these taxa is also extended to the features of the raphe (filiform and characteristic terminations at both proximal and distal ends), striae (uniseriate, parallel to convergent), areolae (varying in shape, with larger internal opening) and their occlusions located toward the valve interior), and the general characteristics of the valves, highly determined by the association raphe-valve nodules-longitudinal ribs, that position these species apart from other Amphipleuraceae [23,29].

Table 3 includes species that are similar to *F. tunariensis* sp. nov. The similarity is given in the general elliptico-lanceolate valve outline, pattern of striation and overall features of the longitudinal ribs and their structural association to the polar and central nodules. At the ultrastructural level, however, the taxon has a unique combination of features that sets it apart from the rest of the species included in the mentioned table. Namely, the density of areola (40–45 per 10 µm) and stria (39–50 per 10 µm) are higher than in any of the considered taxa. The longitudinal ribs are also the widest (2.5 µm) recorded for these species and really stand out during LM and SEM analyses (Figures 3a–z and 4d,e). One of the most notorious features of the new species at the LM and SEM levels is the bulbous apical nodule that embeds the helictoglossum (Figures 3a and 4d), not leaving an apical extension in a porte-crayon fashion, a feature not present in any of the taxa included in Table 3.

**Table 3.** Comparative data for *Frustulia* spp. having lanceolate and elliptical shape with evident longitudinal ribs. Taxa with strictly rhomboid shape and taxa with curved raphe are not considered. Distinctive features of *F. tunariensis* sp. nov. (shaded row) are underlined.

Taxon/Features	Valve Shape	Valve Dimensions ( $\mu\text{m}$ )/Areola and Stria Density (in 10 $\mu\text{m}$ ), and Orientation	Raphe Endings	Longitudinal Ribs/Helictoglossa Complex	Comment/Reference
<i>F. altimontana</i> Metzeltin & Lange-Bertalot	Lanceolate with obtusely to broadly rounded apices	Length: 115–145 Width: 22–27 Areolae: 15–19 Striae: 22–24, parallel to convergent; circumradial at apices	Unknown	Ribs ca. 1.3 $\mu\text{m}$ thick. Helictoglossa unknown. Apical nodule non-bulbose, but unclear from LM; apical extension unclear. Central nodule biconvex	Only known from LM [7]
<i>F. bahlsii</i> Edlund & Brant	Lanceolate to rhombic-lanceolate with broadly rounded ends, sometimes rostrate	Length: 98–193 Width: 24–33 Areolae: 16–23 Striae: 20–26, parallel; slightly circumparallel to circumradial at apices	Externally, proximal and distal ends T-shaped. Internally, the distal and proximal terminations straight	Ribs ca. 0.8 $\mu\text{m}$ thick. Helictoglossa inconspicuous and porte-crayon structure very low. Apical nodule notably bulbous without an apical extension. Central nodule constricted	[28,48]
<i>F. chilensis</i> Lange-Bertalot & Rumrich	Elliptico-lanceolate with short, rostrate, widely rounded apices	Length: 94–110 Width: 20–24 Areolae: 26–29 Striae: 20–27, parallel to subparallel; unknown pattern at apices	Unknown	Ribs ca. 1.3 $\mu\text{m}$ thick. Helictoglossa unknown. Polar nodule bulbous with very short apical extension. Central nodule diffuse and unclear	Only known from LM [8]
<i>F. blancheana</i> Maillard	Lanceolate with subrostrate, broadly rounded apices	Length: 70–84 Width: 10–13 Areolae: 25–30 Striae: 28–33, parallel to slightly convergent; circumradiate around apices	Externally, proximal ends slightly deflected in the same direction, distal ends straight. Internally, the distal and proximal terminations straight	Ribs ca. 1.7 $\mu\text{m}$ thick. Helictoglossa a relatively high porte-crayon structure. Apical nodule notably bulbous with long apical extension. Central nodule constricted	[47]
<i>F. crassiareolaeta</i> Metzeltin & Lange-Bertalot	Lanceolate with faintly subrostrate, broadly rounded apices	Length: 77–100 Width: 17.5–21 Areolae: 20–22 Striae: 23–26.5, parallel to subparallel; circumradiate around apices	Unknown	Ribs ca. 1 $\mu\text{m}$ thick. Helictoglossa unknown. Polar nodule non-bulbose with long apical extension. Central nodule irregular, sometimes constricted	Only known from LM [7]
<i>F. crassiareolaetoides</i> Metzeltin & Lange-Bertalot	Lanceolate with broadly rounded apices	Length: 77–115 Width: 17–23 Areolae: 20–22 Striae: 24–26, parallel to subparallel; circumradiate around apices	Unknown	Ribs ca. 1 $\mu\text{m}$ thick. Helictoglossa unknown. Apical nodule bulbous with large apical extension. Central nodule highly constricted	Only known from LM [17]
<i>F. ellipticolanceolata</i> Casa, Mataloni & Van de Vijver	Lanceolate with subrostrate ends	Length: 51–69 Width: 12.5–15.5 Areolae: 25–35 Striae: 30–32, parallel at center, weakly convergent toward ends; circumradiate around apices, sometimes reduced to a single apical areola per apical stria.	Externally, proximal and distal ends T-shaped. Internally, the distal and proximal terminations straight	Ribs up to ca. 0.9 $\mu\text{m}$ thick. Helictoglossa a relatively high porte-crayon structure. Apical nodule bulbous with apical extension. Central nodule constricted	[20]

Table 3. Cont.

Taxon/Features	Valve Shape	Valve Dimensions ( $\mu\text{m}$ )/Areola and Stria Density (in 10 $\mu\text{m}$ ), and Orientation	Raphe Endings	Longitudinal Ribs/Helictoglossa Complex	Comment/Reference
<i>F. fuegiana</i> Casa, Mataloni & Van de Vijver	Lanceolate with faint subrostrate to bluntly cuneate ends.	Length: 44–90 Width: 12–17 Areolae: 24–28 Striae: 26–29, parallel at center becoming weakly convergent toward ends	Externally, proximal and distal ends T-shaped. Internally, the distal and proximal terminations straight	Ribs up to ca. 0.8 $\mu\text{m}$ thick. Helictoglossa a faint porte-crayon structure. Apical nodule faintly bulbous. Central nodule weakly to noticeably constricted	[20]
<i>F. magna</i> Metzeltin & Lange-Bertalot	Lanceolate to rhombic-lanceolate with broadly rounded ends	Length: 130–160 Width: 31–33 Areolae: 18–20 Striae: 20–22, parallel to convergent; circumradiate around apices	Unknown	Ribs ca. 1.7 $\mu\text{m}$ thick. Helictoglossa unknown. Apical nodule non-bulbose with large apical extension. Central nodule slightly biconvex to highly constricted	Only known from LM [7]
<i>F. neofrenguelli</i> Lange-Bertalot & Rumrich	Elliptico-lanceolate, with short subrostrate and obtusely rounded apices	Length: 55–80 Width: 16–18 Areolae: 24–26 Striae: 24–26, parallel; circumradiate around apices	Externally, proximal and distal ends T-shaped. Internally, the distal terminations end in a small punctum; proximal ends unknown	Ribs ca. 1 $\mu\text{m}$ thick. Helictoglossa, a low porte-crayon structure forming a short apical extension on a non-bulbose polar nodule. Central nodule constricted and only known in LM.	SEM information incomplete [8]
<i>F. pangaeopsis</i> Lange-Bertalot	Elliptico-lanceolate with rostrate to subcapitate, widely rounded apices	Length: 75–90 Width: 16–18.5 Areolae: 24–26 Striae: 6–27, parallel to slightly convergent; circumradiate around apices	Externally, proximal and distal ends unknown. Internally, the distal terminations straight; proximal ends unknown	Ribs ca. 0.7 $\mu\text{m}$ thick. Helictoglossa, inconspicuous and porte-crayon structure faint, forming an also faint short apical extension on a slightly bulbous polar nodule. Central nodule constricted and only known in LM.	SEM information incomplete [24]
<i>F. tunariensis</i> E. Morales sp. nov.	Elliptico-lanceolate with obtusely rounded apices	Length: 60–110 Width: 15.5–18 Areolae: 40–45 Striae: 39–50, parallel to convergent, circumradiate around apices	Externally, proximal and distal ends T-shaped. Internally, the distal and proximal terminations straight	Ribs ca. 2.5 $\mu\text{m}$ thick. Helictoglossa inconspicuous and porte-crayon structure faint. Apical nodule notably bulbous without an a Central nodule irregular, sometimes constricted	This manuscript
<i>F. turfosa</i> Metzeltin & Lange-Bertalot	Lanceolate with subrostrate, broadly rounded apices	Length: 100–150 Width: 16.6–19 Areolae: 18–21 Striae: 27, parallel to convergent, circumradiate around apices	Unknown	Ribs ca. 1 $\mu\text{m}$ thick. Helictoglossa unknown. Apical nodule bulbous without an apical extension. Central nodule constricted	Only known from LM [17]



#### 4.2. Ecology of *F. tunariensis* sp. nov.

Table 1 shows that type material is dominated by species of *Pinnularia*, *Encyonema* and *Nitzschia*, found in oligo to mesotrophic, low electrolyte, acidic waters and in peatlands (*Pinnularia*) or with several species living in such places (*Encyonema* and *Nitzschia*) [8,24,41,42]. At the species level, the taxa accompanying the new species are all found in oligo to mesotrophic environments, with pH varying from slightly acidic to circumneutral [49–51], suggesting that the new taxon also falls within this autecological category. The high relative abundance reached by *F. tunariensis* sp. nov. (7.5%), places this taxon within the general definition of *Frustulia* as a conglomerate of acidophilous, oligo to mesotraphentic taxa living in peatlands and acid ponds and rivers with low electrolyte content [20,23,52].

Interestingly, the conductivity and total suspended solids were zero at the time of measurement (after 5 trials and making sure the same instrument worked properly in other sites). This suggests that there is a chemical process, unidentified during this study, which causes a scarcity of charged salts and other solids in the water column. Taking into account the presence of conspicuous healthy algal growth in the sampled shallow pool, it is possible that the nil values might be indicating a strong absorption of nutrients after the waters have melted and the algal growths started active photosynthetic activity.

#### 4.3. Use of Girdle Bands to Characterize *Frustulia* spp.

Another structure that could produce potentially useful features to separate groups and species within *Frustulia* is the cingulum, which has not received sufficient attention thus far but that could yield a series of useful characters. I have found relatively complete information for only five species and one morphospecies, and I compared them to the information gathered for the new species presented herein (Table 4).

**Table 4.** Comparison of girdle elements among *Frustulia* taxa with available published information.

Taxon/Features	Copula	Valvocopula	Openings in Valvocopula	Notch and Verrucae on Valvocopula	Reference
<i>F. blanchiana</i> Maillard	Open, with one row of poroids	Open, without fimbriae	Slits in tube-like portion opening as slits toward the interior and as a single row of poroids toward valve exterior	Present, absent	[47]
<i>F. creuzburgensis</i> (Krasske) Hustedt	Open, with two rows of poroids	Closed, bearing fimbria	With two rows of poroids in tube-like portion, apparently not open to either valve interior or exterior	Present, verrucae alternate with poroids	[46]
<i>F. cf. krammeri</i>	Unknown	Open, lacking fimbriae, but with a flap (pars interior) running on top of the tube-like portion	With one row of slits on the longitudinal flap and one row of slits in the tube-like portion, presumably opening as slits toward the valve interior and not opening towards the valve exterior	Present, verrucae randomly distributed	[27]
<i>F. latita</i> Graeff & Kociolek	Open with one or two rows of poroids	Closed, fimbriae undetermined	Poroids inside tube-like portion, opening toward the valve interior undetermined, opening as single row of slits towards the valve exterior becoming a row of poroids toward the valve apices	Present, verrucae alternate with poroids	[43]
<i>F. rhomboides</i> (Ehrenberg) De Toni	Open with several rows of poroids	Open, lacking fimbriae, but with an unornamented flap (pars interior) running on top of the tube-like portion	Slits in tube-like portion, open as poroids towards the valve interior and as slits towards the exterior	Present, absent	[23,47]
<i>F. tunariensis</i> sp. nov.	Open with one row of poroids	Open, lacking fimbriae but with siliceous membrane on pars interior	Poroids in tube-like portion, open as slits to the interior and as single row of poroids towards the valve exterior	Present, absent	This manuscript

Table 4 provides evidence for the existence of two groups of taxa within *Frustulia*, groups that also differ in the structure of the longitudinal ribs and their association to the valvar nodules and raphe [46,53]. A first group is composed by *F. creuzburgensis* (Krasske) Hustedt and *F. latita* Graeff & Kociolek, taxa that have unilaterally deflected proximal raphe ends and that either lack longitudinal ribs or have them very little-developed. The cingulum of both of these species have closed valvocopulae and open copulae, a feature absent in the other *Frustulia* in Table 4, which have open girdle elements combined with well-developed longitudinal ribs.

At the species level, considering the structure of the cingulum is also useful to separate taxa. Each species in Table 4 has at least one unique feature. *Frustulia tunariensis* sp. nov. differs in at least two regards, the presence of a unique extended siliceous membrane in the pars interior that secures the valvocopula to the valve mantle interior. Other taxa have either finger-like projections (fimbriae, e.g., *F. creuzburgensis*), a flap that runs longitudinally atop the tube-like portion of the valvocopula (e.g., *F. rhomboides*), or they lack these structures altogether (e.g., *F. blanchiana*). The poroids present in the tube-like portion of the valvocopula, opening as slits to the interior of the valve and as single row of poroids towards the valve exterior is also a unique character in the new species.

In agreement with [46,53], there is a need for a revision of the genus *Frustulia*, which I have defined here, (largely based on [23,26]), as a group of species with proximal raphe ends that are straightly opposite to each other and with well-developed longitudinal ribs interacting with the raphe and the valvar nodules. Now, it can be added that all girdle elements of typical *Frustulia* species are open. Information on additional species is required to confirm this.

#### 4.4. Distribution of *Frustulia* spp. from the Bolivian Altiplano

Table 2 shows that knowledge of the genus *Frustulia* in the Bolivian Altiplano is scant and that the literature is centered on the northern and central parts of this geographical region. That is, data are only available for the northern and part of the central Altiplano, with no data for the rest of the central and the southern portions. Even for the areas represented with publications, the latter are insufficient given the extensive areas covered by peatlands in the Andean Altiplano, represented by the biogeographic provinces known as the Peruvian Puna (Lake Titicaca and surroundings) and the Altiplanic Province (down south up to the border with Argentina), both part of the High Andean Hydroecoregion in Bolivia [54,55].

Under-sampling is another issue regarding the knowledge of *Frustulia* in the Bolivian Altiplano, since the combination of all references in Table 2 does not yield more than 60 samples. Given the large number of species reported from other high mountain localities in South America [7,8,17,18], it can be inferred that this under-sampling is seriously biasing the knowledge of *Frustulia* in Bolivian high plateau peatlands.

A third problem denoted in Table 2 is that only *F. tunariensis* sp. nov. has been studied in detail by combination of LM and SEM techniques, while the other taxa have been either listed or studied only under LM. This is definitely a shortcoming for any taxonomic assessment since three taxa included in the mentioned table are only listed and three have only LM and no ultrastructure data. This observation is in agreement with [2], who implied that the taxonomic depth with which Andean taxa are usually treated is rather limited.

In the absence of detailed morphological analyses, it is extremely difficult to assess if the common European species reported for the Bolivian Altiplano (e.g., *F. crassinervia*, *F. rhomboides*, *F. saxonica* and *F. vulgaris*) do occur in Andean ecosystems. Also, with no means to verify the taxonomy of these taxa in *Frustulia*, making deep biogeographic interpretations is rather risky (e.g., [22]) because there is no way to know what is really being represented in lists or whether we are dealing with species complexes, in which case, SEM information is pivotal in solving species identities [1,2].

For the reasons exposed above, it can be inferred that any taxonomical or biogeographic assessment of *Frustulia* in the Andean Altiplano is currently premature. A higher sampling effort displayed on a wider geographic range is required, together with the application of combined LM and SEM, including

molecular techniques if possible, to produce a taxonomically sound database that would then allow for a distributional and ecological characterization of the genus.

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