Hantzschia subandina Frenguelli (Bacillariophyceae): Morphology, status and typification, as well as the description of a new species of *Nitzschia*

Hantzschia subandina Frenguelli (Bacillariophyceae): Morfología, status y tipificación, asi como la descripción de una nueva especie de *Nitzschia*

John P. Kociolek[1]* & Amelia A. Vouilloud[2]

1. Museum of Natural History and Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA.
2. División Ficología, Facultad de Ciencias Naturales y Museo. Universidad Nacional de La Plata, (1900) La Plata, Argentina

*Patrick.Kociolek@colorado.edu

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Summary

Background and aims: *Hantzschia* Grunow is a genus characterized by having valves asymmetric to the apical axis, markedly dorsiventral, with an eccentric raphe on the ventral side supported by fibulae and 'hantzschioid' symmetry of the frustules. In 1942 J. Frenguelli published the flora of diatoms of Neuquén province (Argentina), work in which he erected *Hantzschia subandina* as a new species for science. This work investigates the identity and taxonomic status of this taxon.

M&M: Original material of *Hantzschia subandina* of the Frenguelli Collection was analysed with light and scanning electron microscopy.

Results and Conclusions: Observations on the material suggested two different species were included in the concept of this species assigned originally to the genus *Hantzschia*. Furthermore, neither of the two species present are assignable to the genus *Hantzschia*. *Nitzschia subandina* (Frenguelli) comb. nov. has a large central nodule and a slight asymmetry about the apical axis; it also produces frustules with nitzschioid and hantzschioid symmetry. We designate a lectotype for this taxon. In addition, another diatom in the same material with slight asymmetry about the apical axis has distinctive ornamentation on the mantle of the valve and produces nitzschioid and hantzschioid frustules. For this taxon we tentatively assign it to the non-monophyletic genus *Nitzschia*, describing it as *N. araucana* sp. nov. We discuss the possible phylogenetic position of this new taxon, and suggest that the genus *Nitzschia* is "the next *Navicula*" ready to be further subdivided into distinct genera.

Key words

Bacillariales, diatoms, *Hantzschia, Nitzschia,* SEM, systematics, valve morphology

Resumen

Introducción y objetivos: *Hantzschia* Grunow es un género de diatomeas caracterizado por la asimetría dorsiventral de sus valvas, rafe soportado por fíbulas sobre el margen ventral, y simetría hatzschioide de los frústulos. En 1942 J. Frenguelli publicó la flora de diatomeas de la provincia de Neuquén (Argentina), obra en la que erigió a *Hantzschia subandina* como una nueva especie para la ciencia. El objetivo de este trabajo es analizar la identidad de este taxón y su status taxonómico.

M&M: El material original de *Hantzschia subandina,* presente en la Colección Frenguelli depositada en el Herbario de la División Ficología del Museo de La Plata, fue analizado con microscopías óptica y electrónica de barrido.

Resultados y Conclusiones: la observación del material original de *Hantzschia subandina* sugiere que dos entidades distintas fueron incluídas en el concepto de esta especie. Ambas producen frústulos tanto con simetría nitzschiode como hantzschioide, por lo que ninguna de ellas pertenece al género *Hantzschia*. La primera presenta un nódulo central conspicuo y leve asimetría según el eje apical; consideramos que estos ejemplares corresponden a *Nitzschia subandina* (Frenguelli) comb. nov., y realizamos la enmienda y lectotipificación del taxón. La segunda entidad presente en el mismo material, posee una leve asimetría según el eje apical, y una distintiva ornamentación en el manto valvar; hemos asignado este taxón al género *Nitzschia*, describiendo *N. araucana* sp. nov. Discutimos la posible posición filogenética de este nuevo taxón dentro de *Nitzschia*, género polifilético que requiere de una profunda revisión y subdivisión en géneros, tal como ocurrió con el género *Navicula*.

Palabras clave

Bacillariales, diatomeas, *Hantzschia, Nitzschia,* MEB, sistemática, morfología valvar

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Introduction

The Bacillariales Ehrenberg is a large order of diatoms, containing over 3500 taxa (Kociolek *et al.,* 2020). In terms of number of species, the order is of approximately the same size as Mammalia (ca. 6000 taxa; Burgin *et al.,* 2018). The Bacillariales are characterized by having cells linear or slightly sigmoid, usually isopolar, with a fibulate raphe in a keel that is usually marginal (Cox, 2015). The species and subspecific entities of the Bacillariales are accommodated in less than 30 genera (Kociolek *et al.*, 2020).

Nitzschia Hassall 1845 is the most diverse genus of this order and contains more than half of the taxa described within the group. According to Round *et al.* (1990) *Nitzschia* is characterized by valves symmetrical in outline with respect to the apical plane, but strongly asymmetric in structure; eccentric raphe supported by fibulae and 'nitzschioid' symmetry of the frustules (the position of the raphe on one valve is on one margin, and on the opposite margin on the other valve of that frustule) or 'hantzschioid' symmetry (the position of the raphe on one valve is on one margin, and on the same margin on the other valve of that frustule). There is a high degree of variability across the species that are included in the genus, and these have been accommodated into infrageneric groups and reorganized several times (Mann, 1986). The type species, *N. sigmoidea* (Nitzsch) W. Smith (1853), is characterized by the presence of a conopeum, which is an external covering of silica over a portion of the valve face.

Hantzschia Grunow 1877, second but distant in terms of species diversity within the Bacillariales (Kociolek *et al.*, 2020), is characterized by having valves asymmetric to the apical axis, markedly dorsiventral, with an eccentric raphe on the ventral side supported by fibulae and 'hantzschioid' symmetry of the frustules (Round *et al.*, 1990). The genus, as typified by *H. amphioxys* (Ehrenberg) Grunow (lectotypified by Boyer 1927), has uniseriate or biseriate striae formed by small, rounded areolae occluded by hymens (Jahn *et al.*, 2014). There is a complex structure of the cingulum, that may contain open or closed elements and with a variable number of porelli (Round *et al.*, 1990). A recently-described species assigned to the genus was the first to include a conopeum as part of the valve ultrastructure (Blanco *et al.*, 2019).

The present study was developed in the framework of a project aimed to revise the type materials of diatom taxa described by Joaquín Frenguelli. The diatom collection of Frenguelli is housed at División Ficología del Museo de La Plata (Argentina). This work is focused in *Hantzschia subandina* Frenguelli, published by the author in a study of the diatom flora of Neuquen province, Argentina (Frenguelli, 1942). *Hantzschia subandina* has not been cited *a posteriori*. Ambiguities between the original description by Frenguelli and illustrations provided by him suggest that lectotypification for *H. subandina* is required. In addition, another species included in Frenguelli's original concept of *H. subandina* appears to be new to science, and we provide a formal description of that taxon.

Materials and Methods

In this study we analyzed series (sample number in the Frenguelli Collection) 329 and 427 of the Frenguelli Collection, corresponding respectively to periphyton samples of the Covunco River and Llimen-Có stream, Neuquén. The series 329 has 5 original slides while series 427 has 5 slides and unmounted material. For light microscopy (LM) analyses, the slides were observed with a Leica DM 500 microscope with phase contrast optics and a Leica DM 2500 with DIC optics and equipped with a photographic camera Leica DFC 420C (both in La Plata, Argentina). Additional observations were made with an Olympus BX-51 light microscope with DIC optics equipped with a BX-71 digital camera (in Boulder, Colorado, USA). Specimens were located on the slides with an England Finder™Graticule. For scanning electron microscopy (SEM) analyses, material from Series 427 was treated with H_2O_2, following the procedure outlined in CEN/TC 230 (2002). Cleaned material was air-dried onto glass stubs that were sputter-coated with gold-palladium. SEM observations were made with a Carl Zeiss NTS SUPRA 40 SEM at the Centro de Microscopías Avanzadas (CMA), Universidad de Buenos Aires and with a JEOL JSM-6360LV at the Servicio de Microscopía Electrónica del Museo de La Plata.

Results

Frenguelli mentioned that *Hantzschia subandina* was found as predominant in series 427 and as rare in series 329. According to this, many specimens were found in the series 427, and only a few in the series 329.

In the protologue, the author described his new species as:

Hantzschia subandina Frenguelli 1942, p. 180, Plate. 8, figs 26-27 (Fig. 1)

"Frustulis e cingulo visis linearibus, rectis, usque ad apices subito obtuso-rotundatis marginibus parallelis, medio subimpressis, 177-248 µ *longis et 11-17 µ latis; valvis 7-8* µ *latis, anguste linearilanceolatis, leniter arcuatis, usque ad apices sensim attenuatis, apicibus valde atenuattis, productis, porrectis, rostrato-capitatis; punctis carinalibus parvis, transverse parum prolixis, 9-10 in 10 µ , duobus mediis inter. se remotis et pseudonodulo distincto separatis; striis transversis tenuissimis, numerosis, circiter 30 et ultra in 10 µ*."

Translated into English, the original description reads: "Frustule and girdle appear linear with parallel margins, straight until suddenly at the apices obtuse-rounded, in the center pinched, 177-248 µm long and 11-17 µm wide; the valve 7-8 µm wide, narrow, linear-lanceolate slightly arched becoming attenuated, strongly attenuate at the apices, produced ends rostrate-capitate. Fibulae small, transversely short, 9-10/ 10 µm. The two central fibulae separated from one another, the central nodule distinct. Striae fine, numerous, about 30 or more in 10 µm."

Initial observations of slides of series 427 show as predominant specimens with outlines similar to Frenguelli's drawings of *Hantzschia subandina*. However, more detailed observations show 95% of the specimens have fibulae more or less regularly-spaced, while only 5 % of the them have the central fibulae widely separated. We never encountered a specimen with both features. Thus we propose that the specimens found in the original Frenguelli material actually correspond to two different entities. As discussed below, both have specimens with hantzschioid and nitzschioid symmetries and therefore cannot belong to the genus *Hantzschia* (Round *et al.*, 1990). We consider the species with separated central fibulae (a condition which is reflected in the specimen illustrated by Frenguelli) (see Fig.1), although less abundant, corresponds to type of *Hantzschia subandina*. This species, however, belongs to the genus *Nitzschia*. Regarding the second taxon, it appears to be new to science and, tentatively, we also assign this species to *Nitzschia*. Both species are compared to other congeners.

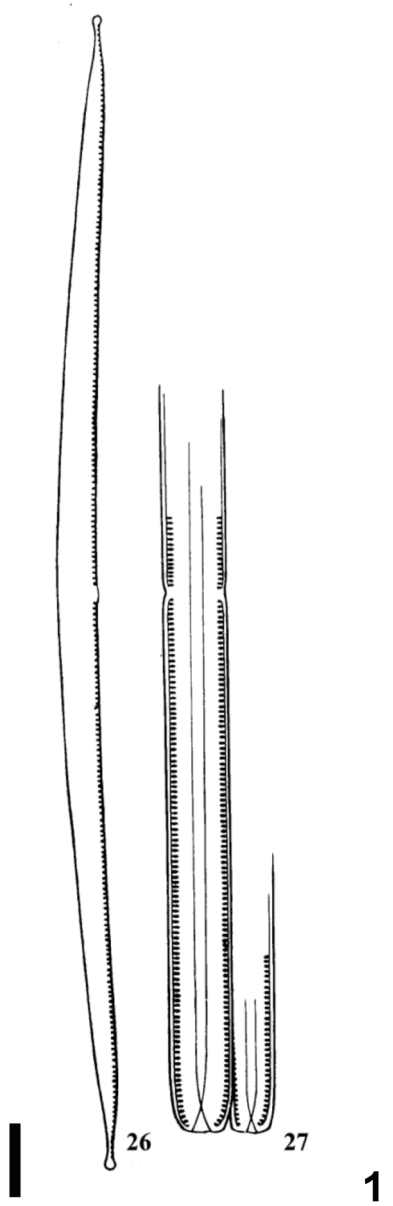

Fig. 1. Drawings of *Hantzschia subandina* from Frenguelli's publication.

Nitzschia subandina (Frenguelli) comb. nov.
(Figs 2-3)

Basionym: *Hantzschia subandina* Frenguelli 1942 (p. 180, Plate, 8, figs 26-27) pro parte.

The metrics herein are based on measurements of 14 specimens in LM and 2 in SEM.

Description

Light Microscope observations (Fig. 2): Valves slightly sigmoid, tapering to the apices which terminate with knob-like endings. Frustules exhibiting both nitzschioid and hantzschioid symmetry (ratio ca. 2:1). Length 100.0-146.5 µm, breadth 6.0-7.4 µm. Fibulae distinct, 11-13/ 10 µm, the two central fibulae more widely spaced forming a distinct central nodule. Striae fine, not easily observable in LM, parallel, indistinctly punctate, 27-30/10 µm. Frustule quadrangular sligthly concave in the middle in girdle view. Raphe elevated off the valve face, positioned to one side.

Scanning Electron Microscope observations (Fig. 3): Externally, linear-lanceolate valves are nearly straight (Fig. 3A). The raphe is placed on the valve face, towards one side, in an elevated keel (Fig. 3B-E). Flat valve surface forming an angle of 90º with the mantle (Fig. 3 F-H). Uniseriate striae formed by rounded areolae (Fig. 3C-E, I). Areolae are occluded

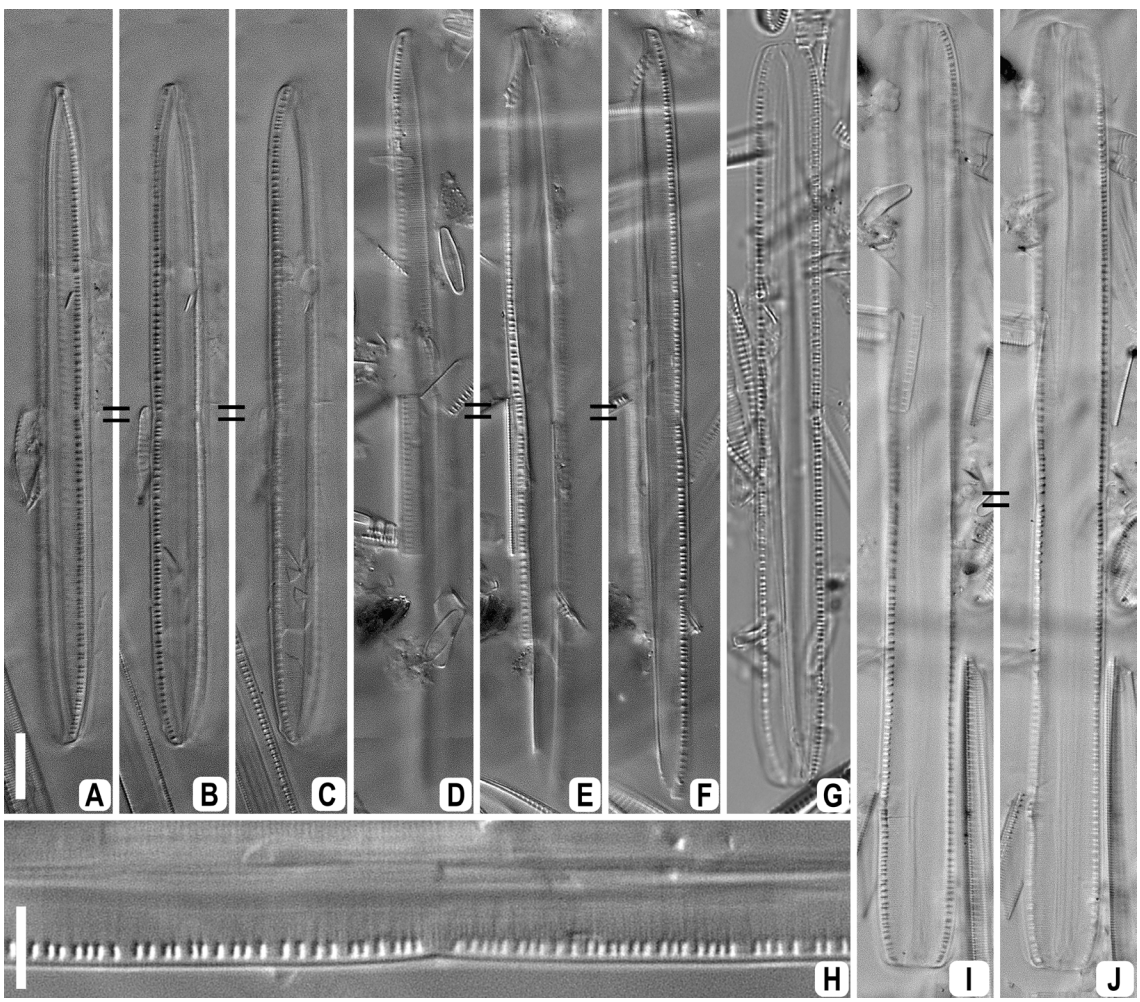

Fig. 2. A-J. *Nitzschia subandina* comb. nov. LM. Specimens of type population. **D-F**. Lectotypus. Scale bars = 10 µm (A-G, I-J); 5 µm (H).

by distinct hymenes (Fig. 3C). Within a stria, areolae density is 30-34/ 10 µm. The valve mantle has rows of areolae similar to the valve surface. The margin of mantle possesses irregular patterns of silica (Fig. 3J-K). The cingulum is complex, formed by several open bands. The valvocopula has one row of poroids occluded by vela; the other cingular elements have one row of poroids occluded and the pars exterior possesses irregular patterns of silica similar to the mantle (Fig. 3I-K).

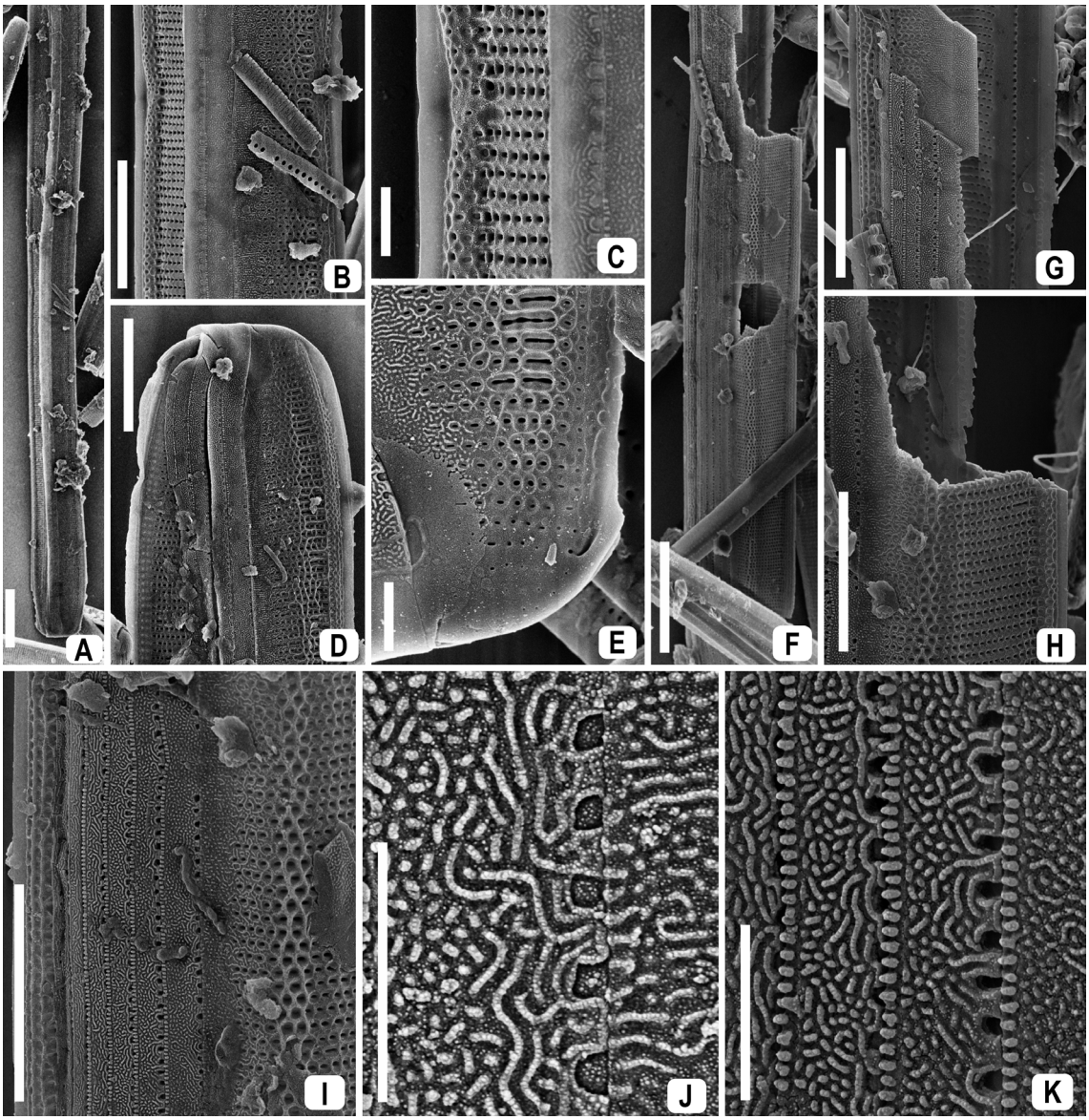

Fig. 3. *Nitzschia subandina*, comb. nov. SEM. External views. Specimens of type population. **A**. Entire frustule, girdle view. **B-C**. Center of the valve, showing distinct central nodule. **D-E**. Valve apices; striae range from round to ellipsoid in shape; External distal raphe end is evident in **E**. **F-H**. Broken valve, showing the valve surface, forming an angle of 90º with the deep valve mantle and cingulum elements. **I-K**. Girdle view, with cingula with a row of round poroids and siliceous markings. **J**. Detail of junction mantle (right) and valvocopula (left). **K**. Detail of cingular bands. Scale bars = 10 µm (A, F); 5 µm (B, D, G-I); 1 µm (C, E, J-K).

Lectotypus (designated here): Frenguelli Collection, Slide 427(1), Finder M34/2.

Type Locality: ARGENTINA, Neuquén Province, *Llimen-Có stream*, submerged plants. January 21st 1941. J. Frenguelli 427 (Frenguelli Collection).

Since Frenguelli did not designate a holotype, and indicated two samples in which his species was found, we have chosen a lectotype for this taxon that best reflects his interpretation of the species in series 427 as indicated in his illustrations of his new taxon (Fig.1).

Nitzschia subandina can be compared to other, similarly-sized and -shaped members of *Hantzschia* and *Nitzschia* (Table 1). Frenguelli (1942, p. 180) compared *H. subandina* with *H. elongata* (Hantzsch) Grunow and *Nitzschia vermicularis* (Kützing) Grunow. In his paper Frenguelli pointed out that *H. elongata* has valves less lanceolate and more symmetric outline with flat valve surface and lower stria and fibula densities; the fine structure reveals more differences in the type and density of areolae, a raphe externally interrupted in the center with a longitudinal groove parallel to the raphe and the mantle margin mantle is smooth. Frenguelli also suggested that *H. subandina* can be confused with *Nitzschia vermicularis*, particularly in bigger exemplars; however, this species differs in its lower fibula density. On the other hand, *N. vermicularis* has a sigmoid outline in girdle view and always shows a nitzschioid symmetry.

The species is also compared here with *Nitzschia linearis* W. Smith, *Hantzschia spectabilis* (Ehrenberg) Hustedt and *H. vivacior* Lange-Bertalot. *N. linearis* is very similar to *N. subandina*, but has differences in shape of apices and higher striae density (35-38/ 10 µm versus 27-30/ 10 µm; Kociolek, 2011).

H. spectabilis resembles *Nitzschia subandina* when observed with LM, but it differs in its greater size and by having lower stria and fibula densities. Another taxon with similar appearance when viewed with LM is *Hantzschia vivacior*; but this species has lower stria and fibula densities; its fine structure also shows differences in characteristics of the areolae and valve mantle; the valve surface is flat; internally it is noted that the fibulae have different shape and size which can be associated with 1-3 virgae.

Nitzschia araucana Vouilloud & Kociolek, sp. nov. (Figs 4-7)

The metrics herein are based on measurements of 50 specimens in LM and 17 specimens in SEM.

Description

Light Microscope observations (Figs 4): Valves straight to arched in valve view, linear-lanceolate

Table 1. Comparison of *Nitzschia subandina* with allied taxa. *measured from of illustrations of the publication. "nd' = not determined.

Taxon	Reference	Apical axis (µm)	Transapical axis (µm)	Fibulae/ 10 µm	Striae/ 10 µm	Areolae/ 10 µm
Nitzschia subandina (Frenguelli) comb. nov.	This study	100-146.5	6-7.4	11-13	27-30	30-34
Hantzschia subandina Frenguelli	Frenguelli 1942	177-248	7-8	10	30 aprox.	nd
Nitzschia linearis W. Smith	Kociolek 2011	60-150	4-6	11-14	35-38	nd
Nitzschia vermicularis (Kütz.) Hantzsch	Krammer & Lange-Bertalot 1988	75-250	5	5-7	30-32	nd
Hantzschia elongata (Hantzsch) Grunow	Lange Bertalot 1993	140-220	14-16	4.5-5.5	13-15	31-35*
Hantzschia spectabilis (Ehrenberg) Hustedt	Krammer & Lange-Bertalot 1988	150-500	10-15	4-6	9-12	nd
Hantzschia vivacior Lange-Bertalot	Lange Bertalot 1993	(60) 90-150	(6.5) 8-11	6-8	13-20	36-42*

in outline, tapering nearly the entire length of the valves, with knob-like apices, appearing asymmetrical to the apical axis, length 113.5-150.0 µm, breadth 5.0-6.4 µm. Frustule quadrangular to arched to slightly twisted about the apical axis in girdle view. Frustules exhibiting both hantzschioid or nitzschioid symmetry. When seen in pairs (sibling cells), frustules exhibited hantzschioid + hantzschioid symmetry or hantzschioid + nitzschioid symmetry, but never nitzschioid + nitzschioid symmetry. Raphe elevated off the valve face, positioned to one side, but extending onto the valve center at the apices. Fibulae distinct, 11-14/ 10 µm, small and equidistant along the length of the valve. Striae uniseriate, parallel, indistinctly punctate, 24-27/ 10 µm.

Scanning Electron Microscope observations (Figs 5-7): Valves linear-lanceolate nearly straight, arched, or twisted (Fig. 5°-C). Externally, raphe placed in a elevated keel on the valve face, towards one side but not eccentric, and curves towards the center of the valve face at the apices (Fig. 5B, D-J). The valve tapers strongly on one side, the other side is extended then tapers quickly. Uniseriate striae formed by rounded areolae which are individually or in groups bordered by an elevated rim or extensions oriented perpendicular to the raphe. These extensions appear to buttress the elevated keel, and help to give the valve a rugose appearance (Fig. 5D-E, K-L). Areolae are occluded by distinct hymenes (Fig. 5 D, K-L). Within a stria, areolae number 30-34/10 µm. The valve mantle has rows of areolae similar to the valve surface (Figs 5L; 7C-D). The margin of the mantle possesses irregular patterns of silica (Fig. 5L). The cingulum is complex, formed by several open bands (Fig. 7C-D). The valvocopula has one or two rows of poroids occluded by vela and pars exterior with a fimbriate margin; the other cingular elements have several rows of occluded poroids (Figs 5J, 7).

Internally each fibula also serves as an interstria (Fig. 6A-D, F), leaving one interstria without a connection between two fibulae. Portules of the canal raphe are nearly round (Fig. 6F-G). The areolae also appear round and are without occlusions (Fig. 6D, F). The raphe is continuous along the length of the valve, terminating at the apices as small helictoglossae positioned on the mantle (Fig. 6C, E).

Type Locality: ARGENTINA, Neuquén Province, *Llimen-Có stream*, submerged plants. January 21st 1941. J. Frenguelli 427 (Frenguelli Collection).

Typification: Holotype (designated here), Frenguelli Collection Slide 427(2), Finder Q36/2.

Etymology. The specific epithet refers to the "Araucanos", an indigenous community of the Patagonian Andean Region.

Nitzschia araucana can be compared with others similar taxa such as *N. heufleriana*, *N. linearis* and *N. tenuis* (Table 2)

It is possible that this taxon might be confused with *Nitzschia heufleriana* Grunow. Fortunately, the type of *N. heufleriana* was illustrated by Lange-Bertalot (1976). The two species are quite dissimilar from one another in terms of outline, with *N. heufleriana* having parallel sides and then tapering close to the apices (see Lange-Bertalot 1976, Fig. 20, which is from Grunow's type material). *Nitzschia araucana* sp. nov. does not have parallel sides; it tapers from mid-valve to the apices.

Nitzschia araucana differs from *N. linearis* in outline, striae and areolae densities, and central nodule present (Kociolek, 2011).

Nitzschia araucana can be also compared to *N. tenuis* W.Smith, since the type material of this taxon was studied in Kobayasi & Kobori (1988). *N. tenuis* has the same kind of elevated raphe system, but it differs from the new species in that the central nodule and raphe are interrupted at the center and they also have differently-structured cingular bands.

This second species amongst *Hantzschia subandina* pro parte was one that Frenguelli found to be predominant in series 427 and rare in series 329.

This species exhibited variation in symmetry about the apical axis, including being asymmetrical to the apical axis, nearly straight and twisted about the apical axis. Frenguelli assigned this species to *Hantzschia* based in part on his observations of valves asymmetrical to the apical axis. Our observations support his description, but they also suggest the symmetry features of this diatom are more complex in the types of symmetry present.

In addition, we noted variation in valve organization amongst frustules and sibling frustules, with both hantzschioid and nitzschioid symmetries

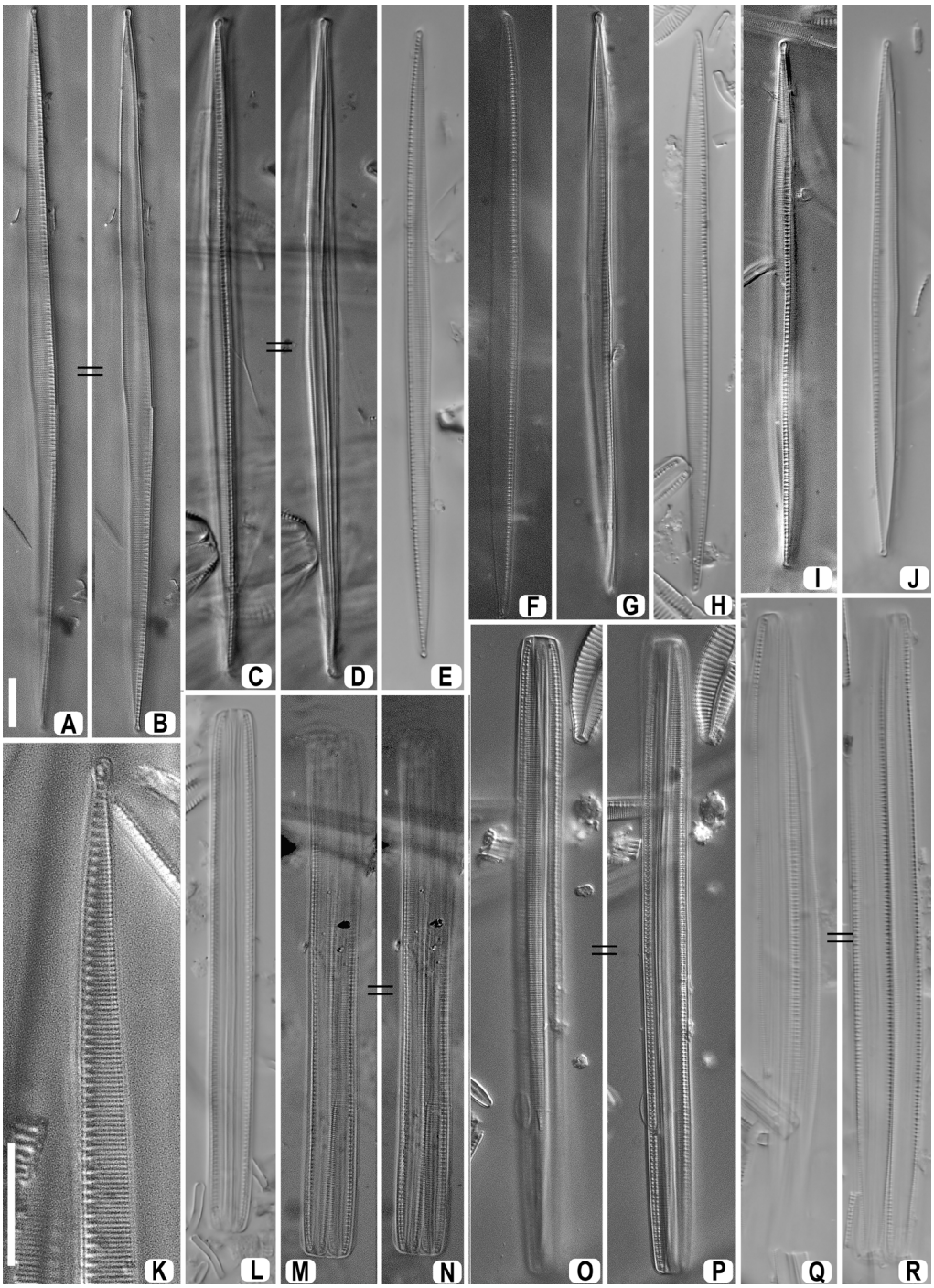

Fig. 4. *Nitzschia araucana*, sp. nov. LM. Specimens of type population. **A-B.** Holotypus. **A-K.** Valve views. **K**. Higher magnification image of the valve apex; fibular and slightly punctate striae are evident. **L-R**. Girdle views showing different frustular symmetries. **M-N**. Two frustules at different levels of focus, one exhibiting hantzschioid symmetry and one with nitzschioid symmetry. **O-P.** Frustule at different focal planes, showing hantzschioid symmetry. **Q-R**. Two frustules at different levels of focus, one exhibiting hantzschioid symmetry and one with nitzschioid symmetry. Scale bars = 10 µm (A-J, L-R); 5 µm (K).

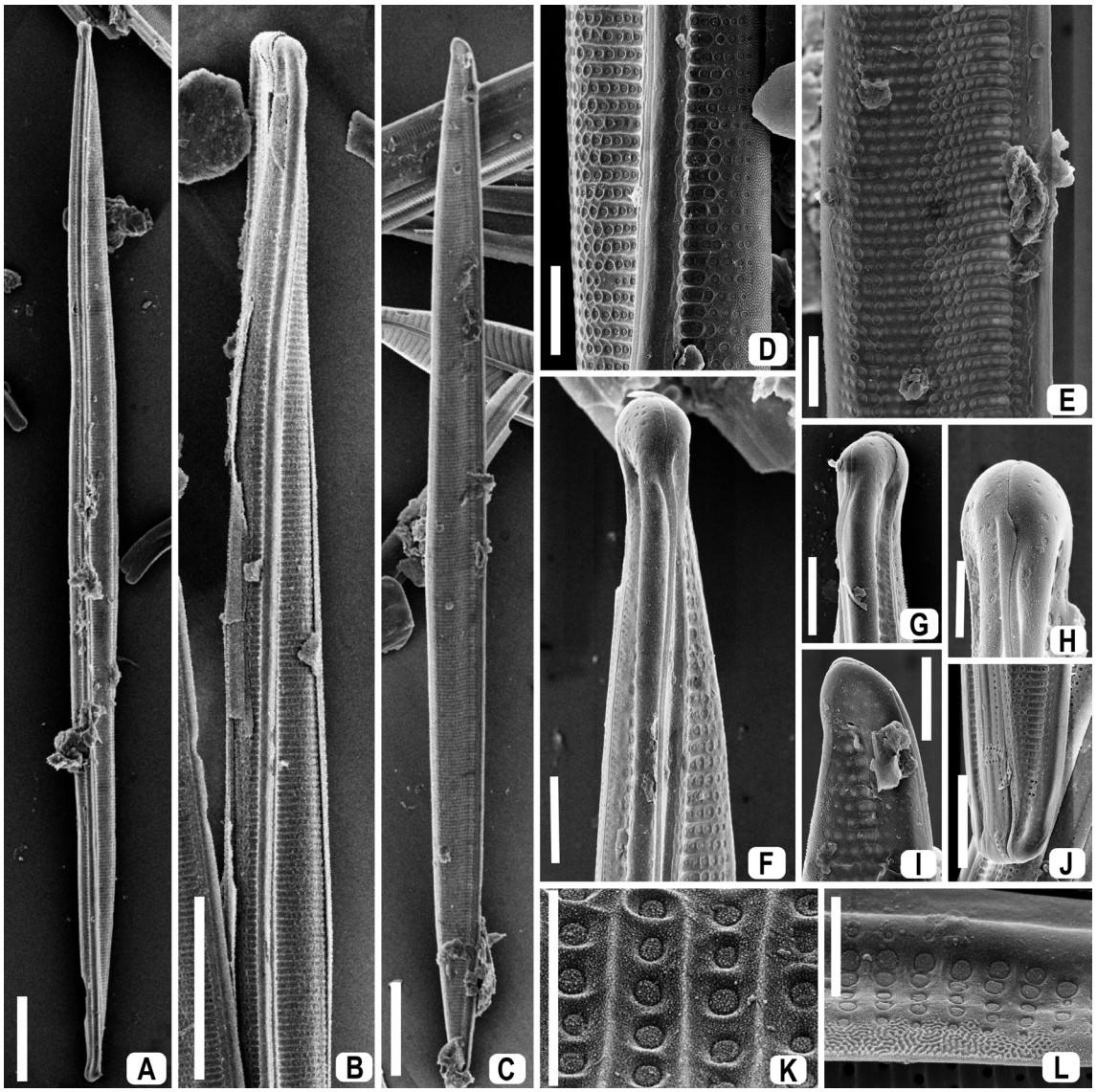

Fig. 5. *Nitzschia araucana*, sp. nov. SEM. External views. **A**. Whole valve, showing linear-elongate shape, raphe in an elevated keel, knob-like apex and curved nature of the raphe. **B**. One end of the valve; curved nature of the raphe system is evident. **C**. Whole frustules in valve view. **D**. Central portion of the valve with elevated raphe and striae in shallow troughs evident. **E**. Central portion of the valve. Undulate nature of the valve is shown. **F-L**. Valve apices showing distal raphe end extending onto the valve mantle. **K**. High magnification of the valve showing external occlusions of areolae. **L**. High magnification of the mantle showing external occlusions of areolae and irregular patterns of silica on the margin. Scale bars = 10 µm (A-C); 5 µm (J); 2 µm (D-G, I); 1 µm (H, K-L).

being present. Summarizing the work of Pickett-Heaps (1983) and Mann (1986), Kociolek & Williams (1987) detailed how differences in the migration paths of nuclei during mitosis relative to the position of keels in the parent valves can produce true-breeding nitzschioid frustules, true-breeding hantzschioid frustules or frustules that can produce both frustules with hantzschioid and nitzschioid symmetry. Thus, based on these observations, we assert that the predominant

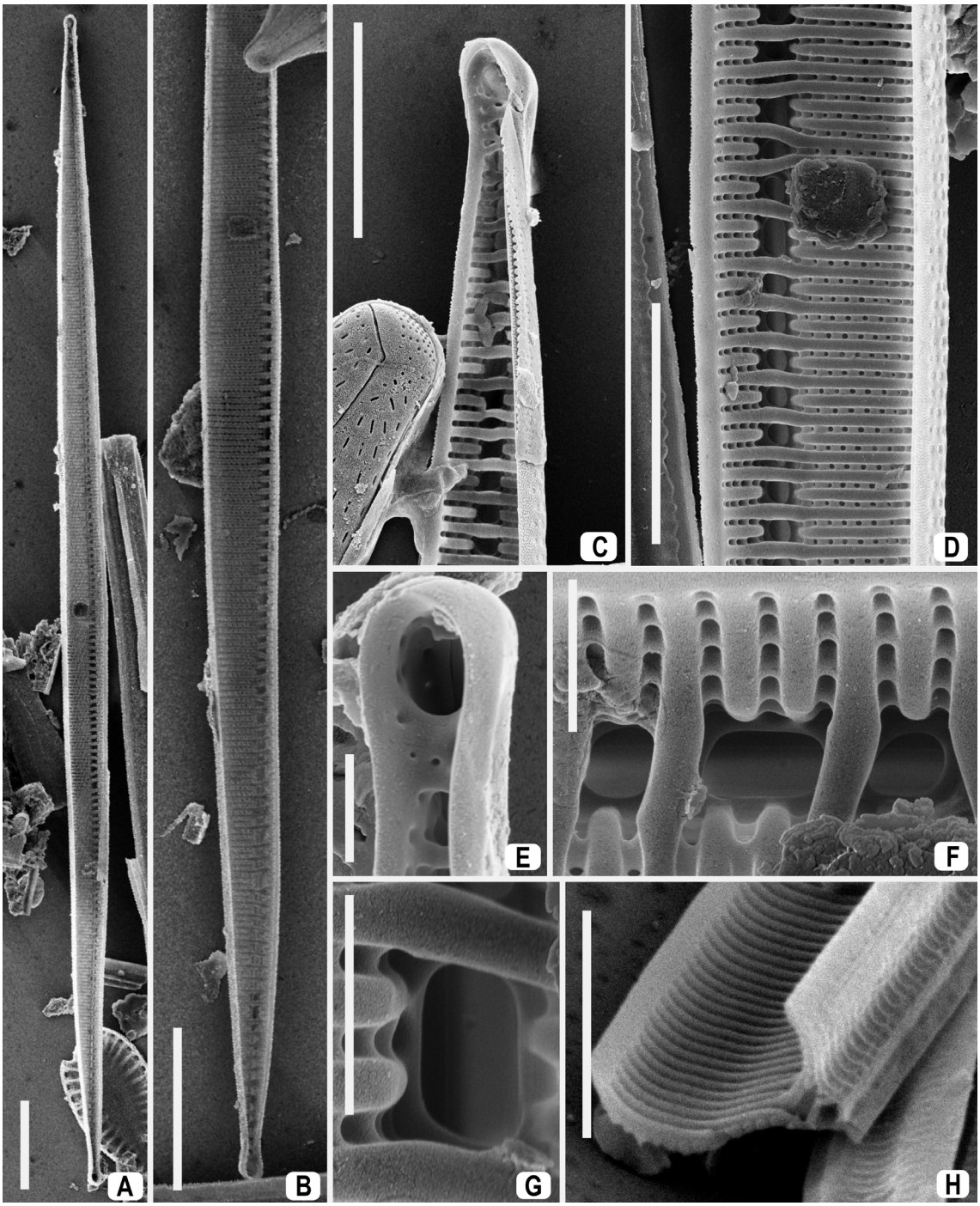

Fig. 6. *Nitzschia araucana*, sp. nov. SEM. Internal views. **A-B**. Valve views, showing evident fibulae and the shift in position of the canal raphe system towards the center of the valve near the apices. Figs **C, E**. Apices of the valve, the helictoglossae is positioned on the mantle. **D, F**. Center of the valve, showing fibulae extending across canal raphe and incomplete, serving as interstriae. **G.** Valve center showing the raphe is continuous. **H.** Transverse section showing the raphe canal. Scale bars = 10 µm (A-B); 5 µm (C-D, H); 1 µm (E-G).

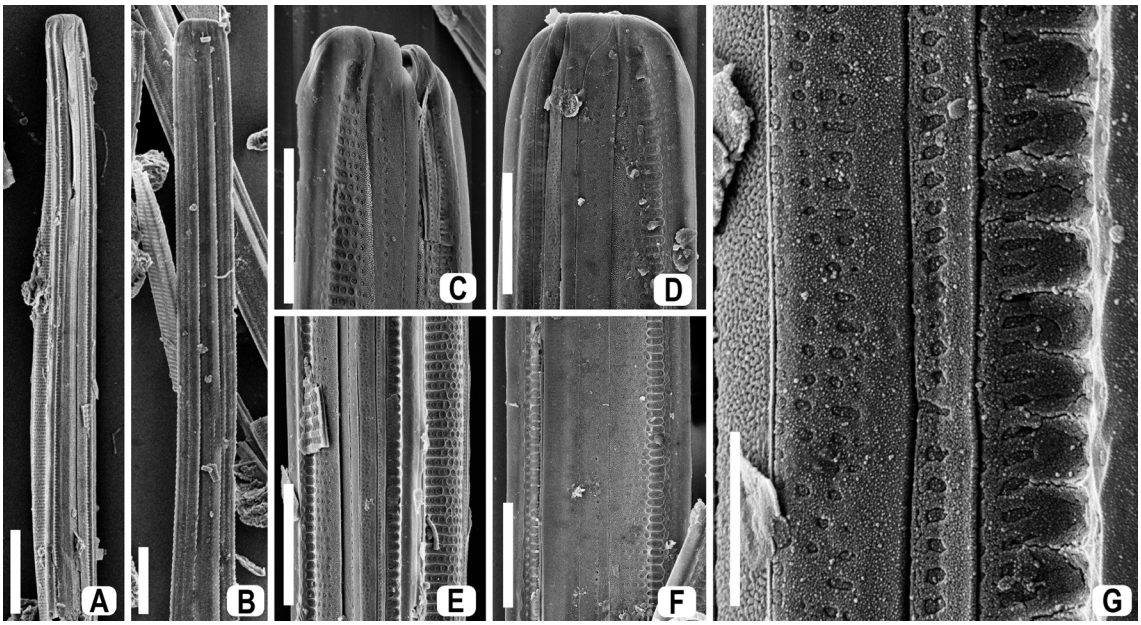

Fig. 7. *Nitzschia araucana*, sp. nov. SEM. Girdle views. **A-B.** Whole frustules. **C.** Apice in oblique view, note the keel elevated and the raphe lateral finished in a fissure. **D.** Apices in girdle view showing the mantle and the open cingular bands. **E-F**. Central portion of the valves. **G**. High magnification view of the girdle region. All figures show the cingulum elements, being of the open type. Most cingula have a row of poroids. Scale bars = 10 µm (A-B); 5 µm (C-F); 1 µm (G).

valves in Series 427 of a diatom referred to by Frenguelli as "*H. subandina*" without widely-spaced central fibulae is a separate, unique taxon. Asymmetry about the apical axis of some valves of "*H. subandina*" suggests it could belong to *Hantzschia*, the original placement of the species by Frenguelli. However, its ability to produce frustules both with hantzschioid and nitzschioid symmetry

Table 2. Comparison of *Nitzschia araucana* with allied taxa. *measured from of illustrations of the publication. "nd" = not determined.

Taxon	Reference	Apical axis (µm)	Transapical axis (µm)	Fibulae/ 10 µm	Striae/ 10 µm	Areolae/ 10 µm
Nitzschia araucana sp. nov.	This study	113,5-150	5-6,4	11-14	24-27	30-34
Hantzschia subandina Frenguelli	Frenguelli 1942	177-248	7-8	10	30 aprox	nd
Nitzschia linearis W. Smith	Kociolek 2011	60-150	4-6	11-14	35-38	nd
Nitzschia tenuis W.Smith	Kobayasi & Kobori 1988	108-200	4-6	12-13*	28	35-40*
Nitzschia heufleriana Grunow	Kobayasi & Kobori 1988	83	6.3	11-12	22-24	28-30*
Nitzschia vermicularis (Kütz.) Hantzsch	Krammer & Lange-Bertalot 1988	75-250	5	5-7	30-32	nd

would preclude it from being placed in *Hantzschia*. According to Round *et al.* (1990, p. 610), "All *Hantzschia* spp., however, seem to have a type of division in which cells of hantzschioid symmetry always give rise to two daughter cells both of which have hantzschioid symmetry; this contrasts with the situation in *Nitzschia*." We tentatively include the species within *Nitzschia*, even though the genus, as currently construed, is non-monophyletic (Kim *et al.*, 2019). If one envisions a narrow circumscription of the genus *Nitzschia*, as typified by *N. sigmoidea* (Nitzsch) W. Smith, it would contain those species that have sigmoid valves and a conopeum, features not found in *Nitzschia araucana*.

Discussion

The exemplars that we observed showed some remarkable differences in morphological and morphometric data as compared to the description offered by Frenguelli. In observations on hundreds of valves in Frenguelli's samples, we never encountered valves that were both dorsiventral in shape and possessed a wide spacing of fibulae in the centre of the valves (Fig. 1). Differences, for example, exist in the size ranges between our observations and the description offered by Frenguelli (see Table 1). This mis-match between the reported sizes by Frenguelli and subsequent measurements is unfortunately a characteristic common to Frenguelli's work and has been previously documented in studies of type materials of Frenguelli's taxa (Gorriti *et al.*, 2000; Sala & Maidana, 2003; Sunesen *et al.*, 2017; Wetzel *et al.*, 2017; García *et al.*, 2018; Vouilloud *et al.*, 2018, among others). There does not seem to be some consistent factor that is common to these reports (e.g. that all of Frenguelli's measurements are 2X the size measured subsequently). It is a good reminder of the value of looking directly at specimens in collections, rather than relying on even primary (let alone secondary or tertiary) reports of features that might be incorporated into a table for species comparisons.

We note here that neither of the taxa considered here have been recorded since Frenguelli (1942) described *Hantzschia subandina*. This is a reminder that there have been few taxonomic studies in the region. In addition, due to lack of information in the original publication, we actually know very little about the ecology of these species. It is hoped that studies like the present report will stimulate a review of Frenguelli's taxa, and the diatom flora of Neuquén Province.

Nitzschia, with nearly 3,000 named species and subspecific taxa (Kociolek *et al.*, 2020), might be considered 'the next *Navicula*', given both the morphological diversity that has been forced into this genus (e.g. Krammer & Lange-Bertalot, 1988). Results of phylogenetic studies which suggest that the genus is not only non-monophyletic, but that several other genera are nested within it (*Hantzschia* among them) (Witkowski *et al.*, 2015; Kim *et al.*, 2019) seem to argue for a a more narrow circumscription of *Nitzschia*.

The symmetry of *Nitzschia araucana* sp. nov. and the structure of its fibulae argue for a closer relationship with *Hantzschia*, while variable products of division (hantzschioid + hantzschioid and hantzschioid + nitzschioid symmetry of the sibling cells) suggest this species differs from *Hantzschia*. Similarities between *Nitzschia araucana* sp. nov. and *Hantzschia* in terms of asymmetry might suggest this species occupies a more basal position in the tree of life for the Bacillariales, within the context of the formal analysis presented by Witkowski *et al.* (2015). If so, the possibility exists that *Nitzschia araucana* sp. nov., along with *N. alba* J.C. Lewin & R.A. Lewin (Lauritis *et al.*, 1967), *N. hierosolymitana* D.G. Mann (formerly *H. fenestrata*) and *N. sigmoidea* (Mann, 1980), are transition taxa between the primitive condition of true-breeding *Hantzschia* taxa and the more derived condition of true-breeding *Nitzschia* taxa (Mann's 1980, groups 2 and 3) (Witkowski *et al.*, 2015; Kim *et al.*, 2019). Mann & Trobajo (2014) described species of *Nitzschia* that have conopea and hantzschioid symmetry. The species that are able to produce frustules with both types of symmetry may not necessarily represent a natural group, however, since the morphological features observed in *N. sigmoidea* (Mann, 1986; Knattrup *et al.*, 2007), *N. hierosolymitana* (Mann, 1980) and *N. araucana* reflect a broad diversity of valve features in terms of raphe, fibulae, cingulum organization and structure, presence or absence of a conopeum, among others. Further research is required to detail the phylogenetic relationships of the Bacillariales

and to align the classification of the order with the relationships of evolutionary descent. But it seems clear that the diversity of symmetry, valve features and patterns of reproduction exhibited by taxa currently referred to the genus "*Nitzschia*" argue for a revision of their classification at the level of genus and, perhaps, higher levels of taxonomic hierarchy.

Author contributions

Both authors have participated in the data collection, interpretation and writing of the manuscript.

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