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First report of the genus *Spicaticribra* Johansen, Kociolek and Lowe in a Colombian reservoir and revision of the infrageneric taxa present in South America

Primer registro del género Spicaticribra Johansen, Kociolek y Lowe en un embalse colombiano y revisión de los taxones infragenéricos presentes en América del Sur

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Abstract

The genus *Spicaticribra* Johansen, Kociolek, and Lowe is reported for the first time in Colombia, in surface sediment samples collected at Embalse La Fe. Materials were examined with light and scanning electron microscopies and their main morphologic and morphometric characters were compared to those of the remaining species of the genus. Based on these results we assigned them to *Spicaticribra kingstonii* and propose *S. kodaikanaliana* Kartrick and Kocioleck as a synonym. We conclude that the genus is represented in South America by only two species, *S. patagonica* Maidana restricted to the south region of the continent and *S. kingstonii* distributed across a broad latitudinal range.

Key words: Colombia, diatoms, La Fe reservoir, *Spicaticribra kingstonii*, surface sediments, Thalassiosirales

Resumen

El género *Spicaticribra* Johansen, Kociolek y Lowe fue hallado por primera vez en Colombia, en muestras de sedimento superficial, recolectadas en el Embalse La Fe. En este trabajo el material recolectado se estudió bajo microscopios óptico y electrónico de barrido y se compararon los principales caracteres morfológicos y morfométricos con los de las otras especies del género. A partir de estos resultados fue posible establecer que los mismos corresponden a *Spicaticribra kingstonii* y se propone como sinónimo de esta especie a *S. kodaikanaliana* Kartrick y Kocioleck. Concluimos que en América del Sur, el género está representado por sólo dos especies, *S. patagonica* Maidana restringida al sur de continente y *S. kingstonii* distribuida en un amplio rango latitudinal.

Palabras claves: Colombia, diatomeas, embalse La Fe, sedimentos superficiales, *Spicaticribra kingstonii*, Thalassiosirales

INTRODUCTION

The genus *Thalassiosira* Cleve comprises about 300 species (Stachura-Suchoples and Williams 2009), most of them reported from marine environments. It has been recently splited in several genera. Some freshwater species have been allocated in the new genera *Spicaticribra* Johansen et al. (2008) and *Conticribra* Stachura-Suchoples and Williams (2009), based mainly on their different type of areolae. More recently, Khursevich and Kociolek (2012) considered that all species possessing loculate areolae with continuous internal cribra should be transferred from *Conticribra* to *Spicaticribra*.

The genus *Spicaticribra*, Johansen et al. (2008), was redefined by Khursevich and Kociolek (2012) as having both flat or plicate valve surfaces, loculate areolae with internal semi- to continuous criba and external foraminae, one to several rimoportulae located on the valve mantle (with or without external tubulus), marginal fultoportulae with 2-4 satellite pores, with or whithout external tubes arranged in 1-3 rings and absence of valve face fultoportulae (rarely present).

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The type species *Spicaticribra kingstonii* was first collected from Fontana Reservoir, USA, a man-made lake with acid water (pH: 4.7-5.0). In the same issue where the genus was described, Ludwig et al. (2008) described *Thalassiosira rudis* Tremarin et al., a species occurring in lentic and lotic water-bodies in northeastern and southern regions of Brazil, in rather warm waters (23.0-27.5 °C), with neutral pH (6.9-7.0), low conductivity (39.9-40.5 μScm^{-1}), and with total phosphorus and total nitrogen concentrations of 38-60 μgl^{-1} and 156-1428 μgl^{-1}, respectively. This species was first transferred to the genus *Spicaticribra* (Tuji et al. 2012) and then considered a synonym of *S. kingstonii* by Rivera and Cruces (2013).

Karthick and Kociolek (2011) described a similar taxon *S. kodaikanaliana* collected at southern India (Lake Kodaikanal, 10° 14' N, 77° 29' E) and occurring in slightly acidic water (pH: 6.25 ± 0.12), low dissolved oxygen (3.95 ± 0.55 mgl^{-1}) and phosphate and nitrate concentrations of 0.06 ± 0.02 mg l^{-1} and 0.55 ± 0.13 mg l^{-1}, respectively.

Besides the mentioned taxa, six species, formerly allocated in the genera *Thalassiosira* and *Conticribra*, have been transferred to the genus *Spicaticribra* by Khursevich and Kociołek (2012): *Thalassiosira kilarskii* Kaczmarska, *T. inlandica* Hayashi, *T. nevadica* Khursevich and Van Landingham, *T. patagonica* Maidana; *T. kamszatica* (Lupikina) Lupikina and Khursevich, and *Conticribra tricircularis* Stachura-Suchoples and D. M. Williams. They also proposed that other several species of *Thalassiosira* should be considered for transferring to the genus *Spicaticribra*: *T. lacustris* (Grunow) Hasle, *T. australiensis* (Grunow) Hasle, *T. gessneri* Hustedt, *T. guillardii* Hasle, *T. weissflogii* (Grunow) Fryxell and Hasle, and *T. pseudonana* Hasle and Heimdal, because they have radial, zig-zag paths of continuous cribra on the internal surface.

In South America were mentioned three species of *Spicaticribra*: *S. patagonica* restricted until now to a volcanic lake in Santa Cruz, Argentina (Maidana 1999); *S. kingstonii* restricted to a small lake from Central Chile (Rivera and Cruces 2013) and *S. rudis* from Brazil.

A study of diatoms from the recent sediments of La Fe reservoir Antioquia (Colombia) revealed that one of the most abundant species was a taxon belonging to the genus *Spicaticribra* (Gallo 2014). With the purpose of establishing its identity, this material was examined with light and scanning electron microscopy and its valve morphology was compared to the rest of the species of this genus, with special reference to materials from South America.

MATERIALS AND METHODS

The samples examined were collected at La Fe reservoir (06° 06' 50'' N, 75° 30' 15'' W), state of Antioquia, Colombia. This reservoir was built in two stages, known as north and south basins, filled in 1973 and 1987, respectively. The reservoir is located at 2255 m. a. s. l., occupies an area of 1.39 km^2 and it is used for drinking water supply and recreational activities. The main water quality variables and their respective mean values during the sampling period were: water temperature 13.0-26.0 °C, pH 5.6-9.9, conductivity 42.0-104.0 μScm^{-1} and dissolved oxygen 7-14 mgl^{-1} (Palacio et al. 2013).

Samples of surface sediments were taken during 2010 in 70 sites within the water-body by means of an Ekman dredge sampler.

Organic matter was eliminated with strong acids combining the methodologies proposed by Simonsen (1974) and Taylor et al. (2007). For light microscopy (**LM**) study, cleaned materials were mounted in permanent slides with Naphrax® and observed with a Leica DM 2500 microscope equipped with phase contrast optics. Digital images were taken with a Leica DFC420 digital camera. Scanning electron microscopy (**SEM**) was held on cleaned material air-dried onto cover glasses, attached to bronze stubs, sputter-coated with gold-palladium and examined with JEOL JSM-6360LV and JEOL JSM6490LV electron microscopes.

Subsamples of materials from La Fe reservoir were incorporated to the herbarium of the División Ficología (**LPC**) of El Museo de la Plata (Argentina).

Terminology used is that proposed by Anonymous (1975) and Johansen et al. (2008).

Results

Frustules of the examined specimens are solitary, with flat valve face, without central fultoportulae (figure 1) and mantle with a gentle slope to the thickened valve margin (figure 2 A-B). Valves are 9-24 µm in diameter. Valve face in external view is distinctly different from the internal view. Externally, striae are uniseriate (figure 2 A-B), 20-25 in 10 µm, areolae have round to ovoid external foramina, 20-22 in 10 µm; central and marginal areolae are larger (up to 0.6 and 0.7 µm diameter, respectively) than those located in the middle portion of the valve face (0.1- 0.25 µm

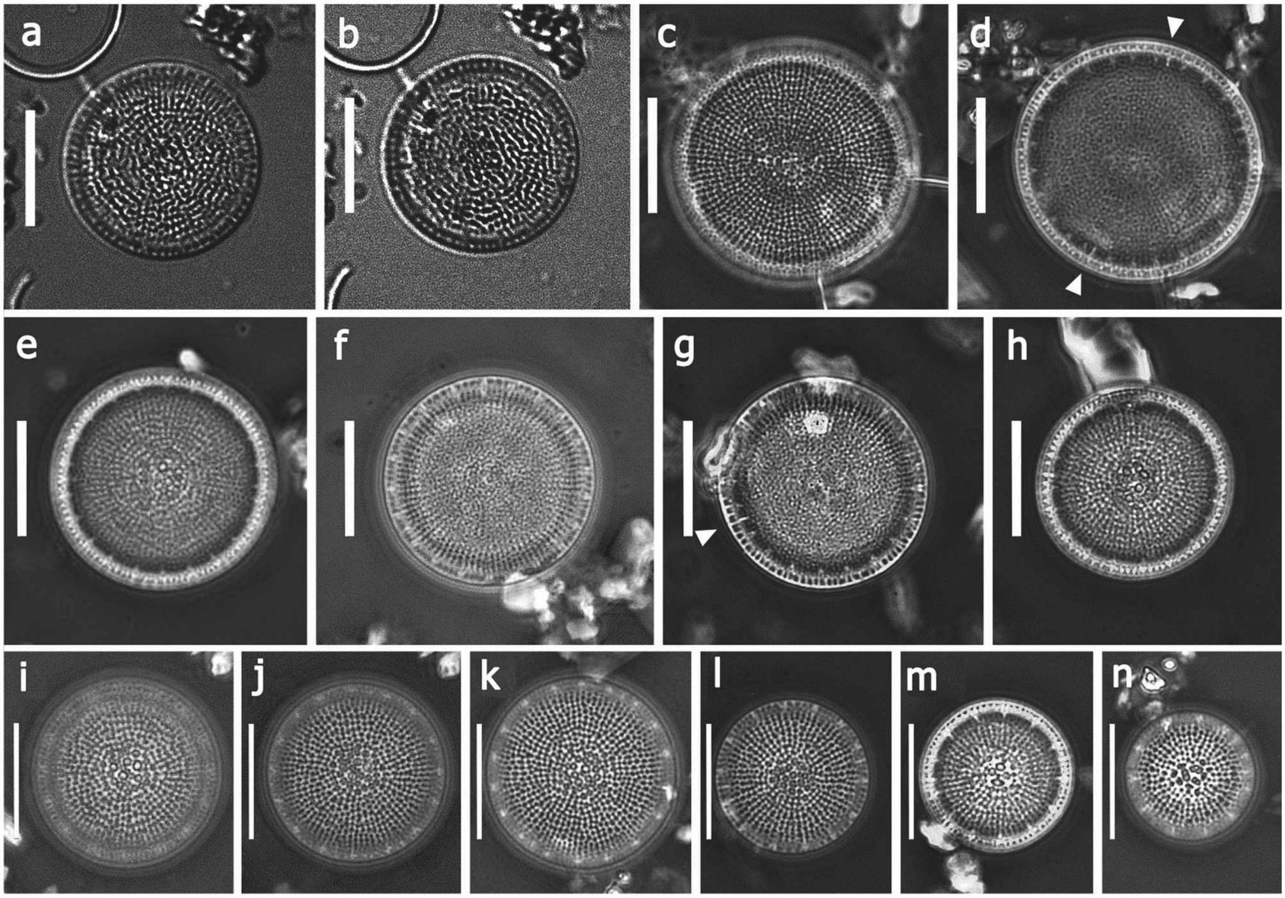

Figure 1. Light micrographs of materials from La Fe reservoir, Colombia. **A-N.** Size reduction series. Arrowheads in **D** and **G** indicate the position of the rimoportulae. **A-D** and **I-J** are de same specimen photographed with different focus (*scale bar* = 10 µm for all figures)

in diameter) (figure 2 A-E). These different sizes are more noticeable in the smallest specimens. Some areolae, especially those located near the valve margin, have a larger external opening and smaller, secondary internal openings (figure 2 D-E).

Some specimens possess small siliceous warts irregularly scattered onto the mantle (figure 2 B-E). In internal valve view, areolae are covered by radial rows of cribra that join towards the valve margin adopting a spike-like, semi-continuous pattern (Figure 3 B, F; 4 C, D).

The marginal fultoportulae, 8-27 per valve, are located at the valve/mantle junction; the external expression is a domed pore (figure 2 D-E) and the internal opening is a long central tube surrounded by three satellite pores with well developed "cowlings" (figure 4 A, D).

The rimoportulae, 1-2 per valve (figure 1 D, E) are located closer to the margin than the fultoportulae (figure 4 B, D). In specimens with two rimoportulae, one of them is positioned near the margin while the other one is near the ring of fultoportulae (figure 3 C; 4 A). In most cases the rimoportulae possess internally a long and bent stalk (figure 4 A-D); the external opening is not discernible from the areolae.

DISCUSSION

The specimens found in the La Fe reservoir were assigned to the genus *Spicaticribra* based on the presence of anastomosing rows of cribra covering internally the areolae of valve face and the lack of valve face fultoportulae. Nevertheless, the attempt to assign these materials to any

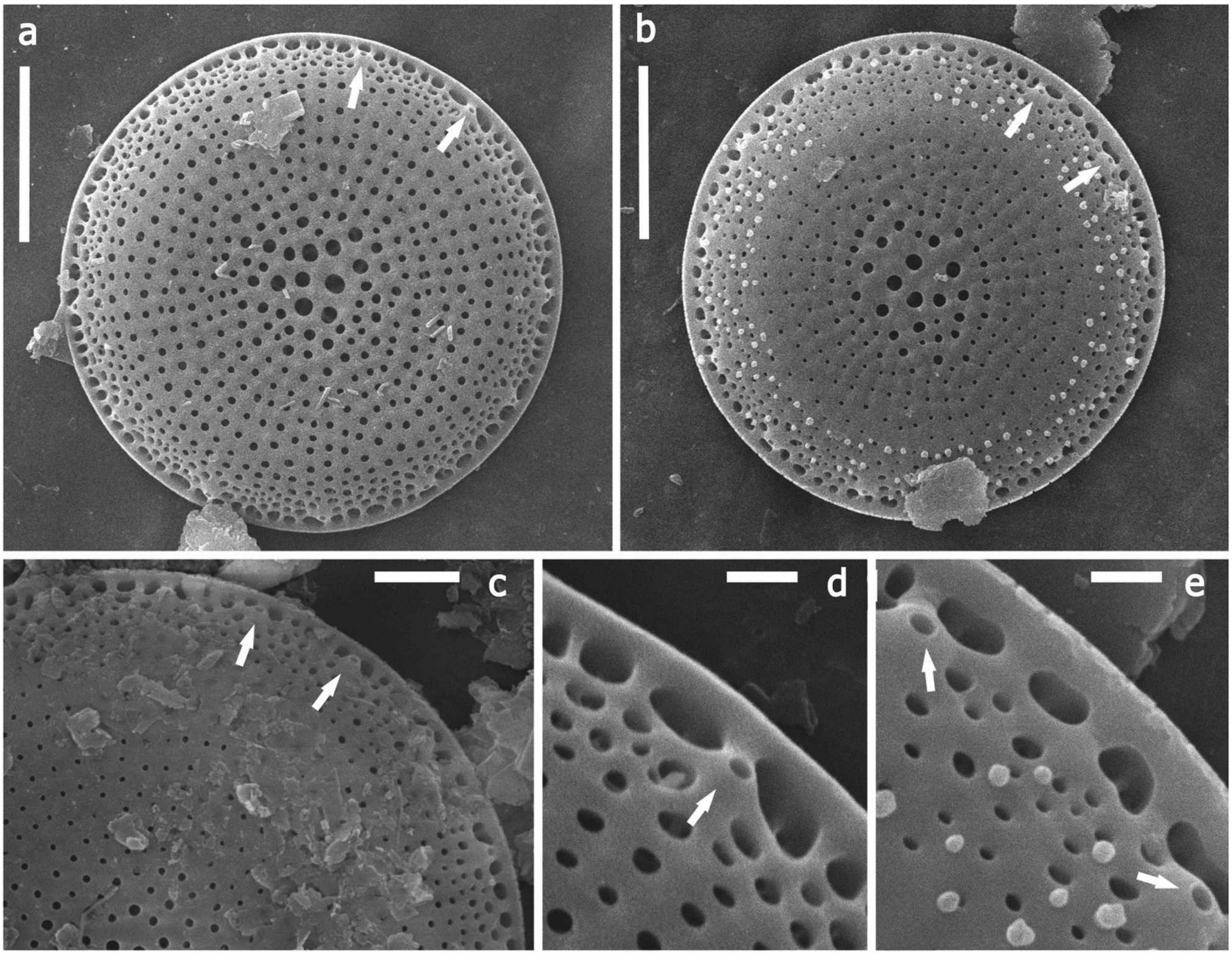

Figure 2. A-E. External view of materials from La Fe reservoir, Colombia. SEM. **A-B.** Valve view. **C-E.** Detail of valve margin. Arrows indicate the raised external opening of the fultoportulae. Note that specimen in B and E has small siliceous warts (*scale bars* = 5 µm in A y B; 2 µm in C; 0.5 µm in D-E)

previously described infrageneric taxon raised a series of uncertainties about the criteria used to separate some species within the genus. Of the *Spicaticribra* species so far described (Khursevich and Kociolek 2012), *S. kilarskii*, *S. kamszatica,* and *S. patagonica* can be clearly differentiated from our materials mainly by their plicate valve face. Among the remainder species with non-plicate valve face, *S. tricircularis* differs primarily in the marginal fultoportulae arranged in three rings and opening externally through long tubes. The species *S. nevadica* can also be distinguished because the fultoportulae have tubular external extensions and are internally surrounded by only two satellite pores.

The studied material closely resembles *Spicaticribra kingstonii*, *S. rudis*, *S. kodaikanaliana,* and *S. inlandica* in the overall general valve morphology, i.e., non-plicate valve face, sometimes domed or with a slightly depressed centre, and the marginal fultoportulae with three satellite pores, opening externally through a domed, somewhat conical pore. These species also have a similar size range but exhibit slight differences in the other morphometric parameters (table 1). However, *S. inlandica* has been characterized by the tendency of the fultoportulae located adjacent to the rimoportulae to be abnormally expressed (Hayashi et al. 2007). It remains uncertain whether this feature is really abnormal or genetically fixed so as to be considered a diacritical characteristic that allows the taxonomic separation of *S inlandica* from this group of species. Nevertheless, as *S. inlandica* also have a higher number of fultopotulae per valve and a higher ratio number

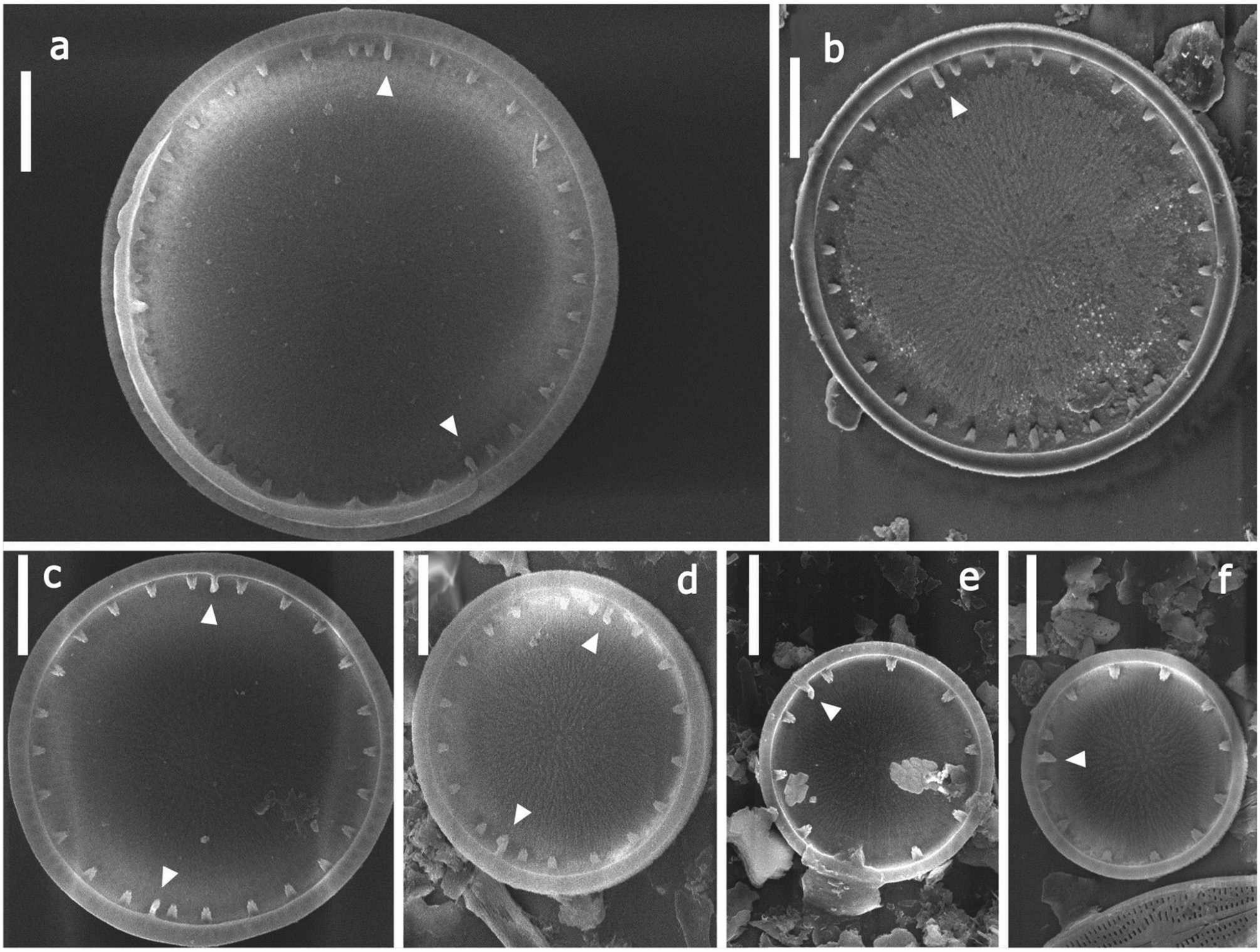

Figure 3. A-F. Internal valve view of different sized specimens from La Fe reservoir, Colombia. SEM. Arrowheads indicate the position of rimoportulae (*scale bar* = 5 µm for all figures)

of fultoportulae/valve diameter (table 1) we agree to consider it a separate taxonomic entity.

On the contrary, our material is hardly distinguished both from *S. kodaikanaliana, S. kingstonii,* and *S. rudis* in morphometric parameters (table 1). Karthick and Kocioleck (2011) pointed out that *S. kodaikanaliana* differs from the other species in the presence of small siliceous warts onto the valve face and mantle and small central areolae. Nevertheless, these characters have proved to be variable in the allied taxa, even within the same population. With regard to siliceous warts we observed that in the same sample from La Fe reservoir there were valves with and without nodules. The presence of siliceous knots might be related to environmental conditions and the occurrence of both types of valves in the same sample might be explained by the fact that a sediment sample integrates the diatoms present in the reservoir in different seasons along the year. In relation to the central areolae, this group of species only exhibits minor differences in their size and shape compared to the rest of the valve face areolae. They are large and irregularly shaped in *S. kingstonii* from USA, Chile, and Japan, larger but almost circular in the material from Colombia and slightly larger in *S. rudis* both from Brazil and Thailand. In view of the variability observed within the group, we consider that these differences are insufficient to differentiate species among populations. For that reason, we think that materials from Colombia should be accommodated within *S. kingstonii* and propose to consider *S. kodaikalaniana* a synonym of that species. Following the same criterion, we agree with Rivera and Cruces (2013) who established the synonymy of *S. rudis* and *S. kingstonii*.

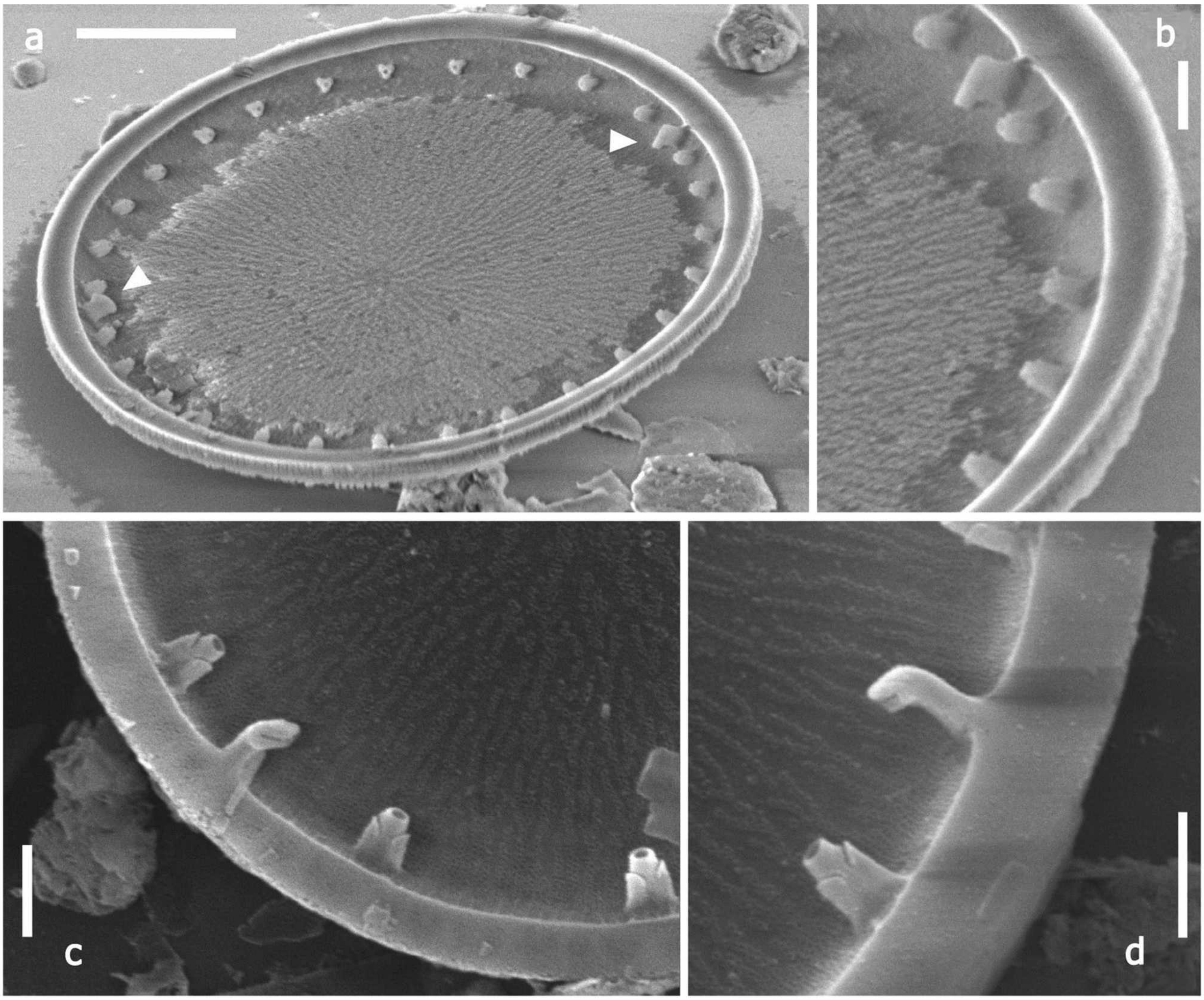

Figure 4. A-D. Internal view of materials from La Fe reservoir, Colombia. SEM. **A.** Valve view of a tilted specimen. Arrowheads indicate the position of rimoportulae. Note internal openings of fultoportulae surrounded by three satellite pores. **B-D.** Detail of valve margin showing different internal openings of the rimoportulae (*scale bars* = 5 µm in A; 1 µm in B-D)

Based on these results and from a biogeographic point of view, the genus *Spicaticribra* is represented in South America by only two species: *S. patagonica*, so far restricted to a maar lake in southern Patagonia, and *S. kingstonii,* widely distributed in temperate to tropical lakes, both natural and man-made. Even though we agree in gathering all these materials under the species *S. kingstonii*, subtle morphological differences found in distant populations, collected in Colombia or either reported as *S. kingstonii* in North America, Japan, and Chile; *S. rudis* in Brazil and Thailand, and *S. kodaikanaliana* in India, raise the question whether *S. kingstonii* is a sub-cosmopolitan species that comprises varieties or races or, in the contrary, it is an ensemble of cryptic taxa with more restricted geographic distribution. The use of ultrastructural characters is insufficient to answer this question, suggesting that it will be necessary to compare populations of different geographical regions with the aid of molecular tools.

In relation to environmental conditions in which the species was found, at the La Fe reservoir since it was fulfilled in 1987. The species dominate the diatom population during approximately six years alternating with *Discostella stelligera:* before and afterwards species richness and equitability raced, prevailing Pennate diatoms (Gallo 2014). During this period pH varied between 7.24-7.45, similar values to those reported in Brazil (pH 6.9-7.0, Ludwig et

Table 1. Morphometric parameters of the studied materials compared to those allied *Spicaticribra* species with non-plicated valve face (***** = measured from the literature; **FP** = fultoportulae; **RP** = rimoportulae)

Taxon	References	Diameter (µm)	FP -. *n per valve* -. *n in 10 µm* -. *n/diameter*	RP	Striae/ 10 µm	Areolae/ 10 µm	Valve face	Siliceous nodules
Materials from Colombia	This study	9-24	-. 8-27 -. 4-6 -. 0.8-1.2	1-2	20-25	20-22	flat	±
S. kingstonii Johansen et al.	Johansen et al. (2008)	9-25	-. 7-27 4* in 10 µm 0.8*	1-3	16-19	18-21	flat	–
	Rivera and Cruces (2013)	13-25	-. 13-14* -. 4-5 in 10 µm -. 0.9-1.1*	1-2	20*	18-19 (21)	flat	+
	Tuji (2012)	11.7-26*	-. 26* -. 3 in 10 µm* -. 1-1.2*	1-3*	–	–	?	–
S. rudis Tremarin et al.	Ludwig et al. (2008)	10.8-23.7	-. 6-26*, -. 2-4 in 10 µm -. 0.75-1.2*	1-3	22-26*	20-30	convex-flat*	±
	Tuji (2012)	12.5-17.5	-. 11* -. 4 in 10 µm* -. 0.7*	2?	–	–	?	±
S. kodaikanaliana Karthick and Kociolek	Karthick and Kociolek (2011)	8-23	-. 8-14 -. 4 in 10 µm -. 0.7*	2-3	16-22	25-32	slightly domed	+
S. inlandica (Hayashi) Khursevich and Kociolek	Hayashi et al. (2007)	9-34	-. up to 45* -. 1.9*	(1) 2-4	14-17 (20)	16*	flat with depressed center	±

al. 2008) but slightly different from those from India (pH: 6.25 ± 0.12, Karthick and Kociolek 2011) and markedly different from those in USA (pH: 4.7-5.0, Johansen et al. 2008) showing a wide range of pH tolerance of the species. Besides, in the study area *S. kingstonii* dominated during a period of nutrient enrichment (Gallo 2014). This coincides with reported high nutrient concentration in the Chilean Lake (Rivera and Cruces 2013) and Brazil waterbodies (Ludwig et al. 2008) but quite different from the lake were the species was collected in India. In relation to conductivity the only data are those from Brazil (39.9-40.5 μScm^{-1}) that also coincide with Colombian reservoir conditions. From this point of view the species appear in similar condition in the Neotropical region.

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