

MORPHOLOGY AND TAXONOMY OF *AMPHORA ATACAMAE* FRENGUELLI (BACILLARIOPHYCEAE)

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Materials containing *Amphora atacamae* Frenguelli from the type locality in Chile and two localities in Argentina have been examined with LM and SEM. This diatom has narrowly lanceolate to elliptic-lanceolate frustules, semi-lanceolate to slightly triangular valves with a convex dorsal margin and concave ventral margin. The raphe is filiform and slightly curved; dorsal and ventral striae are uniseriate, composed of conspicuous, transapically elongated areolae. The girdle is composed of numerous copulae. Type material of *Amphora boliviana* Patrick was also studied with LM and SEM, and we conclude this species should be considered a synonym of *A. atacamae*. The diagnosis of *A. atacamae* has been emended based on our observations, and a lectotype has been selected as Frenguelli did not designate a holotype slide from his collection.

INTRODUCTION

The genus *Amphora* Ehrenberg has a worldwide distribution in both marine and continental environments with a high diversity of species in saline lakes (e.g. Frenguelli 1928, Baudrimont 1973, Czarnecki *et al.* 1981, Aboal 1988, Noël 1984, Maidana 1994, Maidana & Romero 1995). This genus is comprised of approximately 300 species (VanLandingham 1967), but little is known about the morphology of most of these species. The difficulty of identifying species without the aid of the electron microscope was demonstrated by several studies (Archibald & Schoeman 1984, Sala *et al.* 1998, Clavero *et al.* 2000). The problems are mainly due to the overall similarity in valve outline and dimensions of many species and to the incomplete knowledge regarding the fine structure of the valves and cingulum in type material.

Frenguelli (1928) described *Amphora atacamae* based on a single valve found in sediments from the Salar de Atacama, Chile. When analysing sediments from saline lakes from La Rioja and San Luis Provinces (NW Argentina) we found several specimens that were similar in valve outline and morphometric data to the protologue and illustration given by Frenguelli (1928). With the objective of establishing a more comprehensive concept of the species, we carried out a detailed SEM analysis of all these materials. This analysis also allowed us to compare *A. atacamae* with a similar species, *Amphora boliviana* Patrick, collected in Laguna Colorado, Bolivia (Patrick 1961).

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MATERIAL AND METHODS

Specimens of *Amphora atacamae* from the following samples were studied.

- 1) Caravajal, Salar de Atacama, Chile, Colección Frenguelli, Series 5 (sediment sample and Slides 1–4). Hole dug 500 m from the Salar margin (23°42' S, 68°11' W), 2,200 m above sea level.
- 2) La Rioja Province, Argentina (Slide LPC 3490). Superficial sediments collected in 1997 near the shore of a secondary water body on NNW side of Laguna Brava, Departamento de Vinchina (28° 27'–28° 42' S, 68° 27'–69° 00' W) > 4,000 m above sea level.
- 3) San Luis Province, Argentina (Slide LPC 3491). Sediments from a 10 m core (Core IV) collected in 1998 in the middle of Salinas del Bebedero Lake (33° 32' 34'' S, 66° 39' 15'' W). 380 m above sea level.

Material of *Amphora boliviana* Patrick was obtained in the form of its type slide (A.–GC #26098a labeled Bolivia, Puna de Atacama, Laguna Colorado, 4,400 m above sea level, 1957, Holotypus; Herbarium of The Academy of Natural Sciences of Philadelphia) and as cleaned material of the same sample (#10291).

Samples from La Rioja and San Luis were heated with hydrogen peroxide to oxidize all organic material (Battarbee 1986) and permanent slides were mounted in Naphrax and deposited in the Museo de La Plata Herbarium (Slides LPC 3490, 3491). Diatoms were examined with a Carl Zeiss 54974 photomicroscope provided with a Leica Quantimet 520 image analyzer and a Sanyo VC 2512 camera and a Jeol T 100 scanning electron microscope. Areolae and striae density were measured according to Anonymous (1975) and the terminology followed is that suggested by Ross *et al.* (1979) and Cox & Ross (1980).

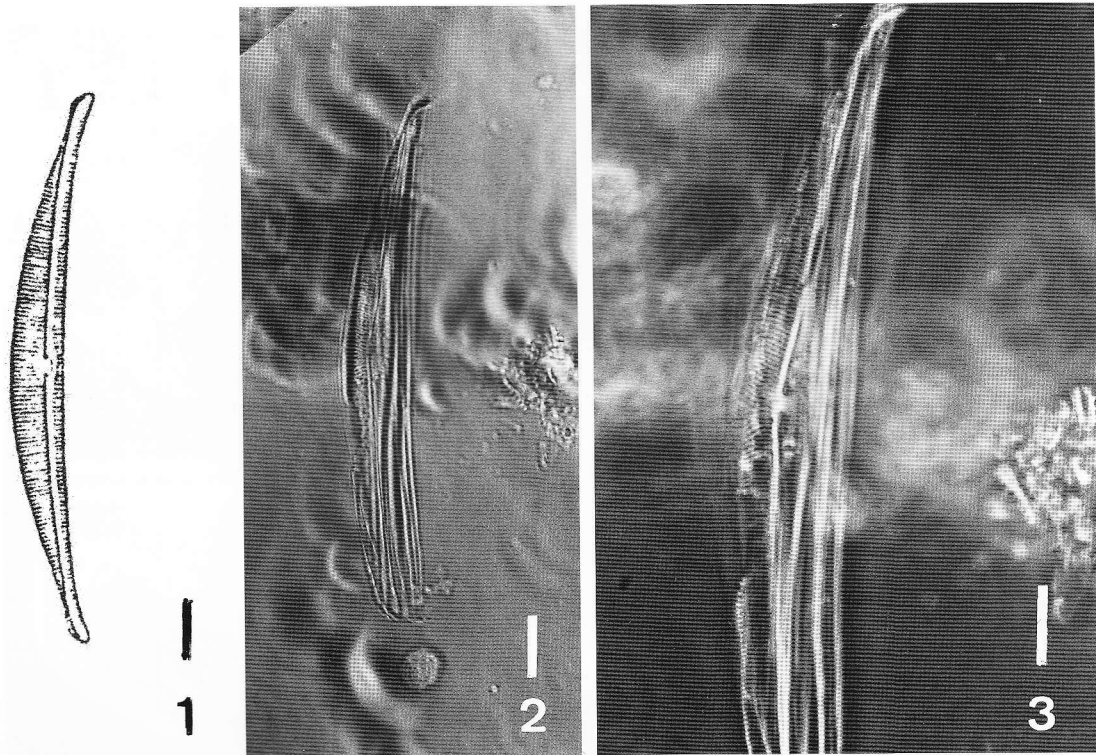
RESULTS AND DISCUSSION

Frenguelli's material of *Amphora atacamae*

In slides of the type material (Slides 1–4, Series 5, Colección Frenguelli), no valve that corresponded exactly to the single specimen of *Amphora atacamae* described and illustrated by Frenguelli (1928, pl. 10, fig. 5 = our Fig. 1) was found. Nevertheless, on Slide 4 we found a single specimen (Figs 2, 3) slightly smaller (length = 86 μm) than the valve figured by Frenguelli which generally agreed with his protologue. In agreement with Frenguelli's drawing the observed specimen is a semi-lanceolate valve with a convex dorsal margin, concave ventral margin, and subcapitate ends which are slightly deflected ventrally. The raphe is filiform and slightly curved with conspicuous central pores. The axial area, not visible on the dorsal side, is conspicuous on the ventral side. The central area is orbicular and more developed on the ventral side. The dorsal striae are radiate throughout, whereas the ventral striae are short and parallel. Individual areolae indistinct in Frenguelli's drawing were visible in the specimen found in slide 4. Although in his protologue Frenguelli stated that the striae density was 12 in 10 μm , we measured 16 dorsal striae in 10 μm and 14 ventral striae in 10 μm on his drawing and 18 dorsal striae in 10 μm in the observed specimen.

SEM observations of Frenguelli's material resulted in the finding of only two valves and a broken frustule (Figs 4–9). These specimens are smaller and have denser striae than the specimen described by Frenguelli (1928) (see Table 1). The valve is slightly curved and the mantle is scarcely developed with only a single row of areolae (Fig. 6). The raphe is filiform and slightly curved with its internal proximal ends covered by a narrow flange-like expansion (Fig. 7). Internally, the raphe slit terminates distally as rudimentary helictoglossae (Fig. 9). The raphe sternum has an internal costa parallel to the raphe on the

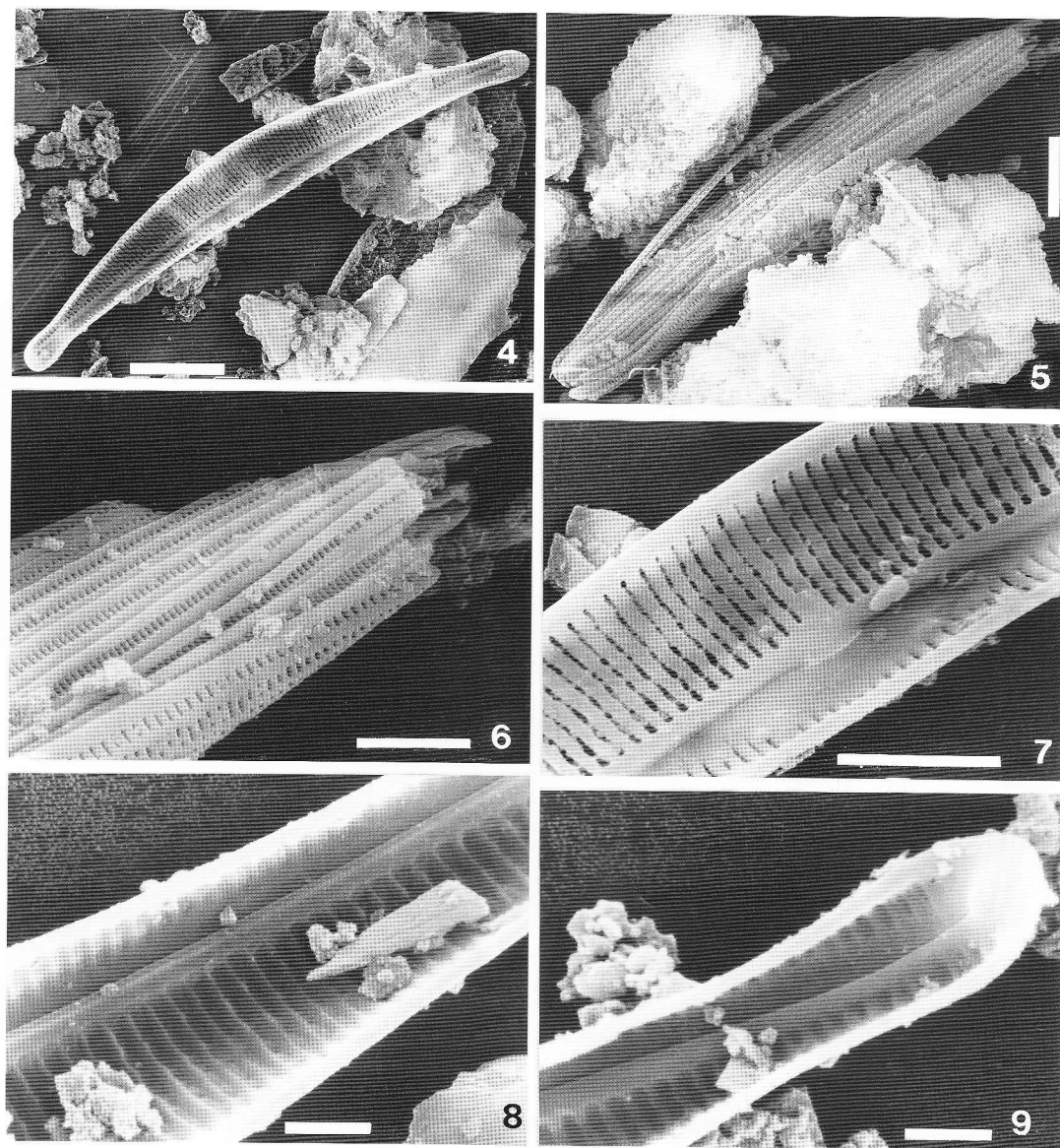
dorsal side (Fig. 8). The axial area is narrow, wider on the ventral than the dorsal side (Figs 4, 7). The central area is orbicular and well developed on the ventral side; on the dorsal side, it is reduced to the foreshortening of the central stria (Figs 4, 7). Dorsal and ventral striae are uniseriate with the areolae transapically elongated and internally occluded (Figs 8, 9). A dorsal view of the frustule reveals that the girdle is composed of numerous copulae with transapically elongated areolae (Fig. 6).



Figs 1–3. *Amphora atacamae*, LM. Scale bars = 10 μm . **Fig. 1.** Reproduction of Frenguelli (1928, pl. 10, fig. 5). **Figs 2, 3.** Lectotype specimen at two different magnifications, slide 4, series 5, Frenguelli Collection.

Materials from Argentina

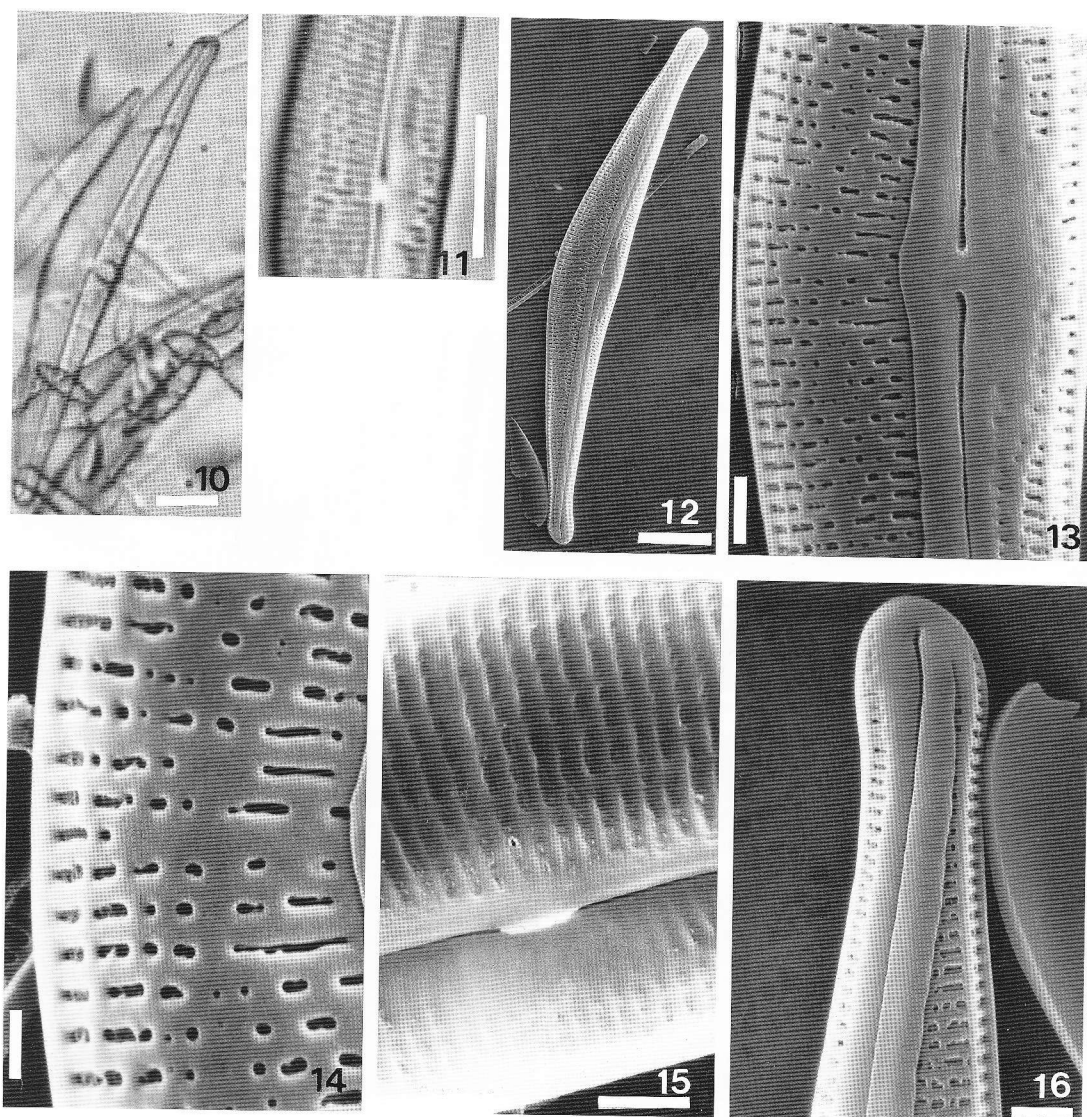
Amphora atacamae was abundant in samples from La Rioja and scarce in samples from San Luis. LM observations revealed that the frustules are narrowly elliptical–lanceolate with poorly differentiated ends. Valves are semilanceolate and slightly triangular. The dorsal margin is convex and the ventral margin slightly concave, with subcapitate ends, which are slightly recurved ventrally (Fig. 10). The axial area is narrow, broader on the ventral than the dorsal side (Fig. 11). The central area is orbicular on the ventral side of the valve and inconspicuous on the dorsal side (Fig. 11). The striae are coarse, uniseriate, with transapically elongated areolae of irregular length (Fig. 11). Dorsal striae are radiate throughout and ventral striae are parallel to slightly radiate at the valve centre and radiate to convergent at the ends.



Figs 4–9. *Amphora atacamae*, SEM, sediment sample no. 5, Salar de Atacama, Chile, Colección Frenguelli. Scale bars = 10 μm (Figs 4, 5), 5 μm (Figs 6, 7) or 2 μm (Figs 8, 9).

Fig. 4. Internal valve view. **Fig. 5.** Frustule in dorsal view. **Fig. 6.** Detail of dorsal girdle. **Fig. 7.** Internal valve view showing detail of striae, central area and proximal raphe ends. Note foreshortening of central stria. **Fig. 8.** Internal valve view showing detail of striae and axial rib. **Fig. 9.** Internal view of valve end showing rudimentary helictoglossa.

SEM examination of these materials provided an excellent picture of the fine structure of the frustule in contrast to the few, poorly preserved specimens in Frenguelli's material. The valve is slightly curved and the mantle is scarcely developed, with only a single row of areolae (Figs 13, 14). The raphe is filiform and slightly curved; its sternum has an internal costa parallel to the raphe on the dorsal side (Figs 15, 17). The external proximal raphe ends are slightly dilated, one slightly curved

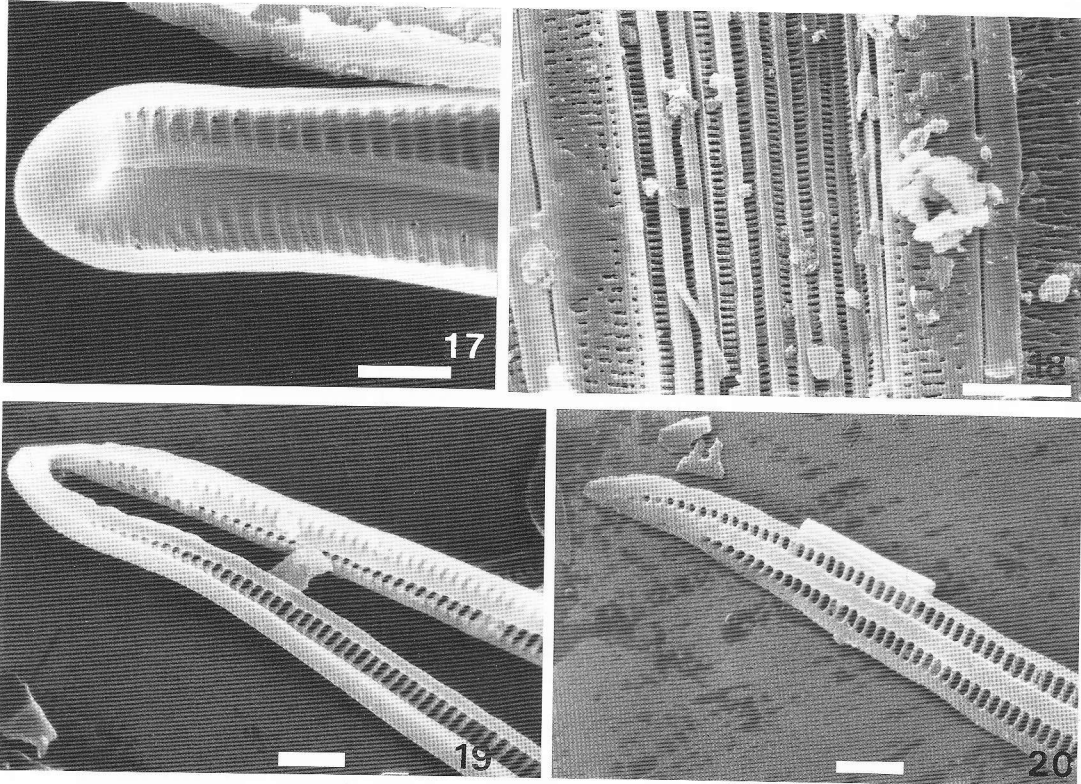


Figs 10–16. *Amphora atacamae*, materials from Argentina.

Scale bars = 10 μm (Figs 10–12), 2 μm (Figs 13, 15, 16) or 1 μm (Fig. 14). Figs 10–11. LM. Figs 12–16. SEM. **Fig. 10.** Single valve. **Fig. 11.** Detail of valve centre. **Fig. 12.** External valve view. **Fig. 13.** Detail of external valve centre showing central area, dorsal conopeum and proximal raphe ends. **Fig. 14.** Detail of external dorsal striae and margin. Note axial costa. **Fig. 15.** Internal valve view showing occlusion of areolae and flange of silica extending ventrally to cover proximal raphe ends. Note central stria is not foreshortened. **Fig. 16.** External view of valve end showing distal striae and conopeum.

towards the dorsal side and the other straight (Fig. 13). Internally, they are covered by a narrow flange-like expansion (Fig. 15). Internal distal raphe endings terminate in small helictoglossae (Fig. 17), whereas the external distal raphe fissures very slightly curved dorsally (Fig. 16). The conopeum is well-developed on the dorsal side of the raphe and is slightly expanded at mid-valve to partially cover the central striae (Figs 13, 16). The axial area is narrow; the central area is orbicular and only

developed on the ventral side with ghost striae (Figs 13, 15). Dorsal and ventral striae are uniseriate (Figs 12–15), externally interrupted at the valve face-mantle junction by a rib (Figs 13, 14, 16). Externally the areolae are transapically elongated, with foramina of variable length and irregular outline (Figs 13, 14, 16). Internally individual areolae are difficult to resolve due to the presence of variably perforated occlusions (Figs 15, 17). The girdle is composed of numerous copulae (Fig. 18), open at one end (Figs 19, 20). On the ventral side of the frustule, each copula has a single row of areolae, a narrow rib on the advalvar side and a larger, thickened rib on the abvalvar side (Fig. 18). Dorsally, each copula possesses two rows of areolae separated by a wide rib and narrow marginal costae (Fig. 19).



Figs 17–20. *Amphora atacamae*, materials from Argentina, SEM. Scale bars = 5 μm (Fig. 18) or 2 μm (Figs 17, 19, 20). **Fig. 17.** Internal view of valve end showing striae and rudimentary helictoglossa. **Fig. 18.** Ventral view of frustule showing ventral striae and numerous copulae. **Fig. 19.** Detail of closed end of one copula showing its 2 rows of areolae. **Fig. 20.** Detail of one end of a copula. Note one areolae row extends further than the other.

Morphometric data for *Amphora atacamae* given by Frenguelli (1928) (excluding striae density) are coincident with dimensions of specimens studied in material from Salar de Atacama, Chile and Argentina (Table 1). In addition, SEM observations revealed the Chile and Argentina materials differ only in the length of the central stria: in the former the central stria is foreshortened whereas in the latter it is not (cf. Figs 7, 15). We believe that this difference is insignificant and that all specimens studied belong to the same species.

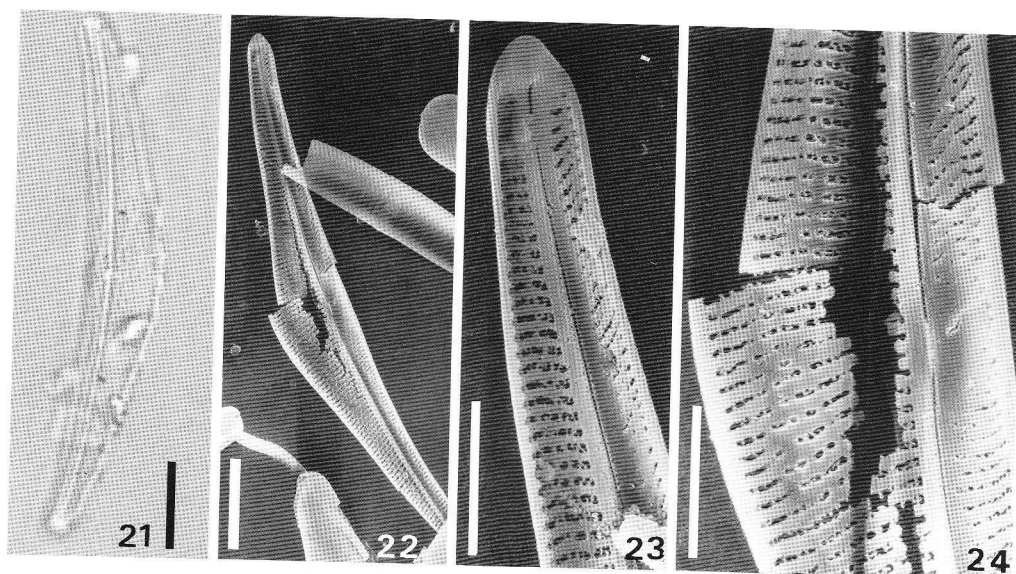
Table 1. Morphometric data for *Amphora atacamae* and *A. boliviana*. A - indicates measurement could not be made.

Species	Reference	Length (μm)	Width (μm)		Valve length/width ratio	Striae in 10 μm			Areolae in 10 μm
			Frustule	Valve		Dorsal	Ventral	Valve	
Chile	Salar de Atacama Frenguelli (1928)	105	-	11	9.5	12 (16?)	12 (14?)	-	-
	Salar de Atacama (this study)	64-86	-	7.5-8	8.5-10.7	17-19	20	-	30-32
Argentina	La Rioja (this study)	62-138	19-34	9-11	7.3-12.5	17-22	19-27	12-21	30-32
	San Luis (this study)	68-100	19-24	9-10	6.9-10.5	21-23	23-24	-	-
	Laguna Colorado Patrick (1961)	57-58	-	7-8	7.2-8.1	18-20	22-23	-	-
<i>A. boliviana</i>	Bolivia	Laguna Colorado (this study)	57-61	-	7.5-8.7	6.5-8.1	18-20	21-24	-

Comparison with *Amphora boliviana* Patrick

Amphora atacamae is similar to *Amphora boliviana*, described by Patrick (1961) from samples collected in Laguna Colorado, Puna de Atacama, Bolivia (4,400 m above sea level). According to Patrick's diagnosis and illustration, *A. boliviana* is slightly smaller (see Table 1) than all studied specimens of *A. atacamae* but the length to width ratio and striae density in both taxa overlap (Table 1). In the material provided by the Academy of Natural Sciences of Philadelphia, we only found one specimen on the type slide (Fig. 21) and a broken, eroded valve in an unmounted sample (Figs 22–24). LM and SEM analysis of these specimens show that there are no relevant morphological or morphometric differences (Table 1) that justify the existence of two separate taxa. As *A. boliviana* was described later, it should be considered a synonym of *A. atacamae*.

Based on LM and SEM observations of material from Chile, Argentina and Bolivia, we have developed a comprehensive concept of the species and emended the diagnosis. Since Frenguelli (1928) published *Amphora atacamae* without designating a holotype, we hereby designate the specimen found on slide N° 4, Series 5 as the lectotype.



Figs 21–24. *Amphora boliviana*, Laguna Colorado, Puna de Atacama, Bolivia. Scale bars = 10 μm (Figs 21, 22) or 5 μm (Figs 23, 24). Fig. 21. LM. Slide A.–GC #26098a, holotype. Figs 22–24. SEM. Unmounted material (#10291). **Fig. 21.** Single valve. **Fig. 22.** Internal valve view. **Fig. 23.** Internal view of valve end showing striae and rudimentary helictoglossa. **Fig. 24.** Detail of internal valve centre showing central area, proximal raphe ends partially obscured by eroded flange of silica and striae.

Emended diagnosis

Amphora atacamae Frenguelli

Synonym = *Amphora boliviana* Patrick

Light microscopy

Frustules narrowly lanceolate to elliptic–lanceolate with poorly differentiated ends, length 57–138 μm , width 19–34 μm . Valves semi-lanceolate to slightly triangular, dorsal margin convex,

ventral margin slightly concave, width 7–11 μm . Valve ends undifferentiated to subcapitate, slightly deflected ventrally with round to cuneate apices. Axial area narrow. Central area orbicular on the ventral side and variable on the dorsal side, absent or delimited by the foreshortening of the central stria. Raphe filiform, slightly curved. Striae uniseriate. Dorsal striae radiate throughout, 17–23 in 10 μm at valve centre. Ventral striae parallel to slightly radiate at valve centre, radiate to convergent at valve ends, 19–27 in 10 μm . Valve areolae 12–21 in 10 μm , girdle areolae 30–32 in 10 μm .

Scanning electron microscopy

Valve slightly curved, with a slightly developed mantle that has only a single row of areolae. Raphe filiform. Proximal raphe ends slightly dilated, one curved towards the dorsal side and the other straight; internally separated by a flange-like expansion of the raphe sternum. External distal raphe fissures slightly dorsally curved. Internal distal ends terminate in rudimentary helictoglossae and are ventrally bent. Conopeum only developed on dorsal side of valve, straight but slightly expanded at central nodule, partially covering the central dorsal striae. Axial area straight, narrower on dorsal side of valve. Central area orbicular on ventral side of valve, sometimes with ghost striae; on the dorsal side absent or reduced to the foreshortening of the central stria. Dorsal and ventral striae uniseriate, interrupted at valve face-mantle junction by a rib. Externally, areolae are transapically elongated with foramina of variable length and irregular outline. Internally, individual areolae are difficult to resolve due to the presence of variably perforated occlusions. Girdle composed of numerous copulae, open at one end. On ventral side of frustule each copula has a single row of areolae, a narrow rib on the advalvar side and a thick rib on the abvalvar side. On dorsal side of frustule each copula has two rows of areolae separated by a wide rib and narrow marginal costae.

Lectotype: Specimen from Caravajal, Chile, on slide 4, series 5 of the Frenguelli collection, Museo de Ciencias Naturales, La Plata, Argentina (Figs 2, 3).

Type locality: Caravajal, Salar de Atacama, Chile, 2,200 m above sea level, 23°42' S, 68°11' W.

Amphora atacamae has only been recorded from arid or hypersaline environments of South America, most of them located over 2,000 m above sea level. The species was reported by Rumrich *et al.* (2000, pl. 122, figs 1, 2) from Chile Altiplano (brook above Putre, 3,600 m above sea level). These authors also illustrated a specimen (pl. 122, figs 3, 4) from Bolivian Altiplano (Río Grande at 4,000 m above sea level) with a tumid/gibbous dorsal margin. Servant-Vildary *et al.* (2000) recorded the species (as *A. boliviana*) in saline habitats from Ovest-Lipez, SW Bolivian Altiplano.

Environmental information at the sites where *Amphora atacamae* was found is lacking. However, based on the great abundance of this species observed in La Rioja in contrast to its scarcity at all other studied sites, we can infer that conditions here were nearest to optimal for the species. Measured environmental factors were: salinity 3.376 g l^{-1} , pH = 6, total solids 33,376 mg l^{-1} , conductivity 5.28 mS cm^{-1} , hardness 416 mg l^{-1} , Na (+K) 880 mg l^{-1} , bicarbonate 720 mg l^{-1} , chloride 1,372 mg l^{-1} , sulphate 162 mg l^{-1} , nitrite 0.12 mg l^{-1} , fluoride 0.40 mg l^{-1} and arsenic 0.80 mg l^{-1} (Carrizo *et al.* 1997).

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