Diatom Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tdia20

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Version of record first published: 31 Oct 2011.

To cite this article: S. E. Sala, E. A. Sar & M. E. Ferrario (1998): REVIEW OF MATERIALS REPORTED AS CONTAINING AMPHORA COFFEAEFORMIS (AGARDH) KÜTZING IN ARGENTINA, Diatom Research, 13:2, 323-336

To link to this article: http://dx.doi.org/10.1080/0269249X.1998.9705454

Diatom Research (1998) Volume 13 (2), 323–336

REVIEW OF MATERIALS REPORTED AS CONTAINING *AMPHORA COFFEAEFORMIS* (AGARDH) KÜTZING IN ARGENTINA

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Amphora coffeaeformis (Agardh) Kützing has been reported in Argentina from marine and inland environments. Since this species has frequently been misidentified, we carried out a review of materials corresponding to the records of *A. coffeaeformis* and compared them with photographs of the type material. All analyzed materials are similar as regards valve outline and dimensions; however, they differ in valve morphology, mainly in the type of striae and areolae. The presence of *A. coffeaeformis* was corroborated in materials from Buenos Aires (Arroyo Tapalqué), Jujuy, San Luis and La Pampa. The materials from Mar del Plata correspond to *Amphora hybrida* Grunow, a new record for South America, and those found in samples from Lago Nahuel Huapi correspond to *A. veneta* var. *capitata* Haworth. Six other taxa purported to be *A. coffeaeformis* could not be determined and will be the subject of further research. Considering that *A. coffeaeformis* has been reported as a potential domoic acid producer, we compared our materials with those in which the toxin was detected. Our results show that the identity of the toxigenic species is uncertain.

INTRODUCTION

Amphora coffeaeformis (Agardh) Kützing has been reported from various sites in Argentina with a wide suite of environmental characteristics. Ferrario & Sar (1985) recorded it from marine shores, Maidana (1994a) in a saline, Maidana & Romero (1995) in a brackish pond, Frenguelli (1923–24) and Maidana (1994 b and c) in lentic and lotic freshwater bodies and Lacoste *et al.* (1983) in a hot spring. This led to doubts on our part concerning the accuracy of these identifications as the species is commonly believed to be mesohalobous (Lowe 1974).

A review of the literature showed that different concepts of *A. coffeaeformis* abound and that various taxa, similar in valve outline and morphometric data, have been misidentified as this species. Archibald & Schoeman (1984) have provided a detailed description of the species based on the study of type material with light and electron microscopy and elucidated numerous taxonomic problems.

Amphora coffeaeformis has been mentioned as a producer of the neurotoxin domoic acid by Shimizu *et al.* (1989) and Maranda *et al.* (1990). These authors isolated a diatom which they identified as *A. coffeaeformis* at the time of an outbreak of amnesic shellfish poisoning, which occurred in 1987 in Prince Edward Island, Canada (clone BPT 11). This report of domoic acid

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production was checked by Bates *et al.* (1989) in two strains of *A. coffeaeformis* from the Provasoli-Guillard Center for Culture of Marine Phytoplankton, isolated from other geographic areas, with negative results.

Taking into account the diversity of environments from which the species was recorded and the confusion regarding the identity of *A. coffeaeformis* in the literature, we undertook a critical review of the materials corresponding to records of the species in Argentina. We also attempted to examine the material in which domoic acid was reported (clone BPT 11).

MATERIAL AND METHODS

The materials used to carry out this research correspond to all records known to us of *Amphora coffeaeformis* in Argentina. We list below the localities where these materials were collected (see Fig. 1):

1. Laguna Pozuelos, Provincia de Jujuy. Sediments from a hypersaline pond. Maidana (pers. com.).
2. Salinas del Bebedero, Provincia de San Luis. Lacustrine sediments (9070–11600 years BP). Maidana (1994a).
3. Laguna La Amarga, Provincia de La Pampa. Hypersaline lake; epiphytic on *Chara halina* García. Maidana & Romero (1995).
4. Arroyo Tapalqué, Provincia de Buenos Aires. Stream with medium conductivity (114–146 μS cm^{-1}). Maidana (1994b).
5. Mar del Plata, Provincia de Buenos Aires, Colección del Departamento Científico Ficología (LPC 3121). Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Intertidal rocky shore; epiphytic on macroalgae. Ferrario & Sar (1985).
6. Lago Nahuel Huapi, Provincia de Río Negro. Quaternary sediments from an oligotrophic lake. Maidana (1994c).
8. Chorrillo Uaquen, Provincia de Tierra del Fuego, Colección Frenguelli, slide no. 234, Departamento Científico Ficología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. Stream. Frenguelli (1923–1924).

The materials from Termas del Domuyo, (a hot-spring and location 7 in Fig. 1) Provincia de Neuquén were not available for study; therefore, only the description and illustration in Lacoste *et al.* (1983) could be evaluated. In the case of clone BPT 11, in which domoic acid was reported, we could only analyze a micrograph because there is no longer any material available (Maranda & Shimizu, pers. com.).

Raw samples were obtained and, except in the case of Frenguelli's materials, cleaning was done as described by Hasle & Fryxell (1970) and Schrader (1974). Preparations for light microscopy (LM) and scanning electron microscopy (SEM) were made following Ferrario *et al.* (1995). The observations were carried out with Wild M 20 phase contrast and Nikon Microphot–FX light microscopes and a Jeol JSM T 100 scanning electron microscope.

The terminology used is that suggested by Ross *et al.* (1979), Schoeman & Archibald (1979) and Cox & Ross (1980).

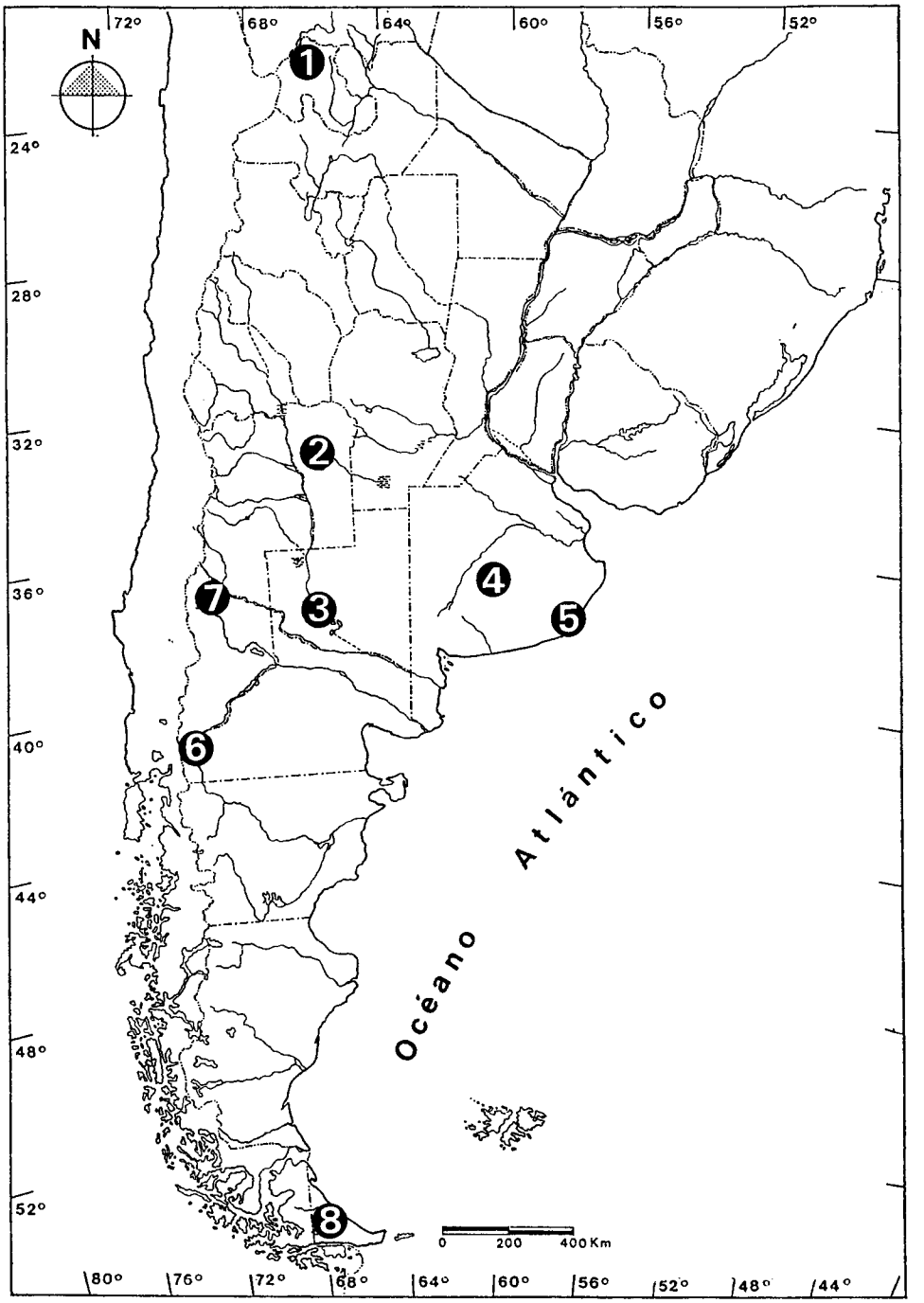


Fig. 1. Map of Argentina showing sites where material examined in the present study was collected: (1) Laguna Pozuelos, Jujuy, (2) Salinas del Bebedero, San Luis, (3) Laguna La Amarga, La Pampa, (4) Arroyo Tapalqué, Buenos Aires, (5) Mar del Plata, Buenos Aires, (6) Lago Nahuel Huapi, Río Negro, (7) Termas del Domuyo, Neuquén and (8) Chorrillo Uaquen, Tierra del Fuego.

OBSERVATIONS

In order to compare our materials with the type material of *Amphora coffeaeformis* we followed Archibald & Schoeman (1984). These authors based their extensive study on the type material of Agardh from Carlsbad (slide LD 4600 from the Agardh Collection), materials belonging to the Kützing Collection (slides BM 18945 and 78009) collected at the same locality, and Kützing and Agardh´s exsiccatae materials. They pointed out that they did not use information from the literature since there was great variability in the concept of this taxon. Taking into account Schoeman & Archibald's (1984) opinion about the difficulties in identifying *A. coffeaeformis,* especially when materials are analyzed only with LM, we studied the samples corresponding to the records of the species in Argentina in the SEM whenever possible.

Circumscription of *Amphora coffeaeformis* based on Archibald & Schoeman (1984)

Frustules elliptical to lanceolate with ends protracted into sub-rostrate to rostrate poles; pervalvar axis strongly curved so that the valvar planes of both valves subtend each other at an obtuse angle. Girdle broad and convex in dorsal view and more or less concave and narrow in ventral view. Girdle bands open at one end and having a thickened axial rib; on either side of the rib there is a single row of linear, oval or roundish pores.

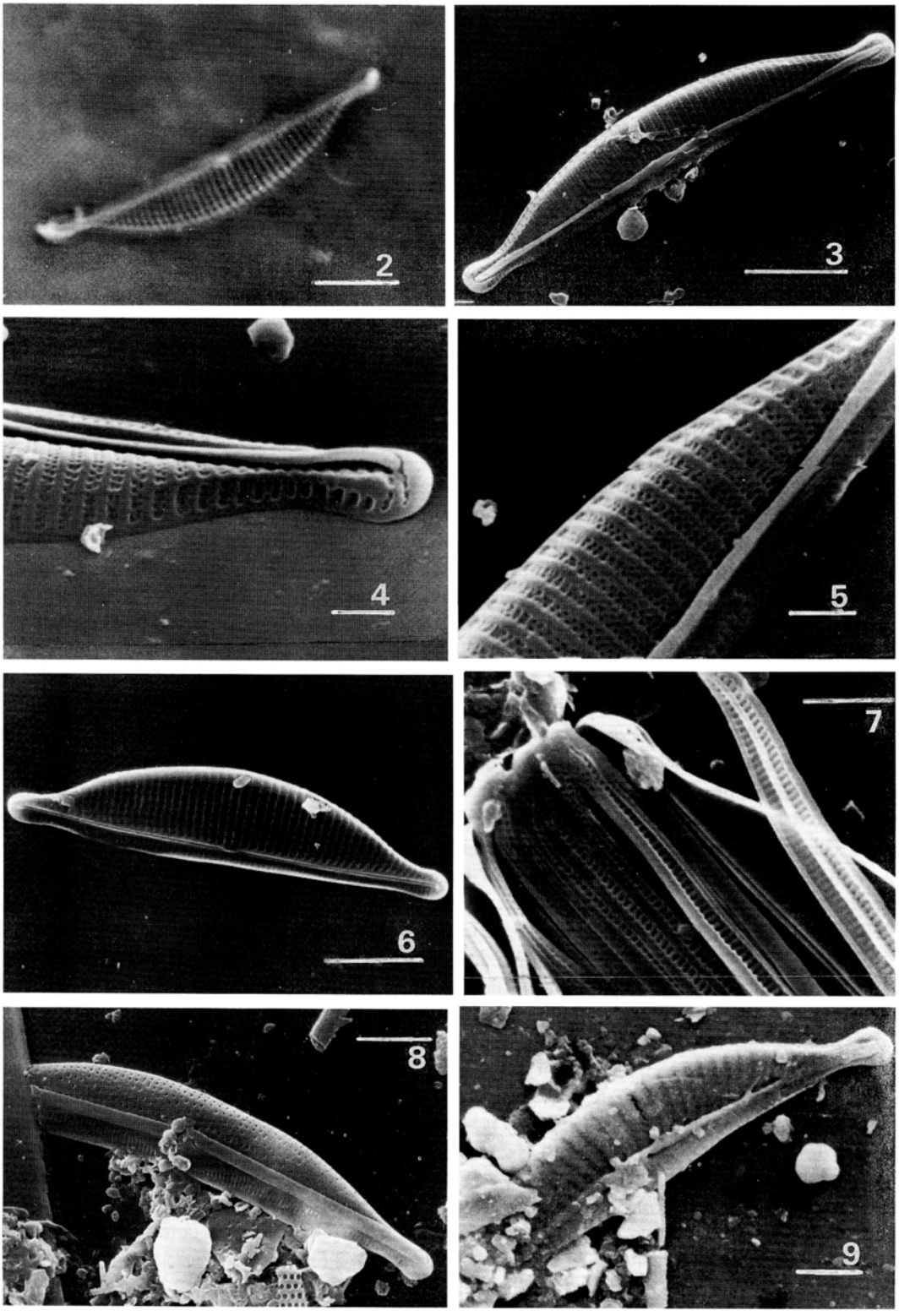
Valves semi-lanceolate; dorsal margin convex, sometimes slightly flattened or indented at the center in larger specimens; ventral margin straight with a slight central inflation or weakly concave. Poles somewhat ventrally deflected, with apices subrostrate, rostrate or capitate. Valve face flat to smoothly curved with a relatively high dorsal mantle lying more or less at right angles to the valve face. Usually a weak longitudinal costa, complete or interrupted, lies between the dorsal mantle and valve face. Dorsal striae biseriate, radiate; ventral striae short, radiate at the center and convergent at the ends of the valve, usually absent beneath the central nodule. Raphe filiform, lying near the ventral margin, within an axial rib. This rib has an external, narrow extension along its dorsal margin (i.e. the conopeum) and internally possesses a tongue-like expansion at the central nodule. Raphe branches slope slightly upwards from the poles to the center. External raphe fissure with proximal ends expanded as small pores and distal ones ending in short, dorsally deflected terminal fissures.

Morphometric data: valve length = 14–55 μm; valve width = 3.5–7.3 μm; dorsal striae = 16–26 in 10 μm at the center and 20–30 in 10 μm at the poles; areolar density within dorsal striae = 51–93 in 10 μm; ventral striae = 21–36 in 10 μm; girdle band pores = 37–45 in 10 μm.

Consideration of Argentinean materials

In the sample from Laguna La Amarga, which corresponds to the publication of Maidana & Romero (1985), two entities similar in valve outline and morphometric data were found which seem to belong to *Amphora coffeaeformis*; however, analysis with SEM reveals morphological differences. Specimens illustrated in Figs 2–7 show various features in common with the type material. Valves

Figs 2–9. *Amphora* taxa, all but Fig. 2 = SEM. Scale bars = 5 μm (Figs 2–6, 8, 9) or 2 μm (Fig.7). **Figs 2–7.** *Amphora coffeaeformis*, Laguna La Amarga. **Fig. 2.** Valve view, LM. **Fig. 3.** External valve view. **Fig. 4.** Detail of external valve end. Note conopeum and short rib at the valve ends. **Fig. 5.** External valve view showing biseriate dorsal striae and single row of ventral areolae. **Fig. 6.** Internal valve view. **Fig. 7.** Dorsal view of the frustule showing detail of girdle bands. **Fig. 8.** *Amphora* sp. 1, Laguna La Amarga. External valve view. Note uniseriate dorsal striae, slit-like nature of ventral striae and straight conopeum. **Fig. 9.** *Amphora* sp. 2, Arroyo Tapalqué. External valve view.



are similar in type and position of the raphe, morphology of the conopeum, biseriate dorsal striae and girdle and band morphology. Nevertheless, we found differences in the striae distribution pattern: the dorsal striae are parallel in the majority of our specimens and radiate in the type material, and the ventral striae are continuous along the valve margin while in the type material they are usually interrupted below the central nodule. Only small morphometric differences were evident and these included the density of ventral striae in 10 μm (42–48 in our material versus 21–36 in type material) and the density of pores in 10 μm in the girdle bands (52 in our material versus 37–45 in type material) (Table 1). On the basis of the morphological analysis, we believe that these specimens correspond to *A. coffeaeformis*, and that the range of variability of the species should be widened to accommodate the observations made here.

The material illustrated in Fig. 8 corresponds to the other taxon found in Laguna La Amarga. Although with LM it could be confounded with *A. coffeaeformis*, based on valve dimensions and striae density (Table 1), it possesses morphological features that make it possible to differentiate it. The dorsal striae are uniseriate and consist of a row of pores irregular in size and shape (roundish or transapically elongated). The conopeum is broad and straight. This material is similar to specimens of *A. tenerrima* Aleem & Hustedt illustrated in Bérard-Therriault *et al.* (1986, figs 20–22) but it differs in size, which is greater than that mentioned by these authors (length = 37–39 μm and width = 5.8–6.5 μm in our material versus length = 14–22 μm and width = 3–4 μm in material of Bérard-Therriault *et al.*). The type material of *A. tenerrima* described in Aleem & Hustedt (1951) and illustrated in Simonsen (1987) also differs from our material in size but the micrographs do not permit one to deduce the fine structure of the striae. The specimens of *Amphora holsatica* Hustedt illustrated by Hustedt (1930) and Simonsen (1987) are similar to our material in regard to the uniseriate striae, but the striae and areolar densities are greater than those described by Hustedt (striae density = 22–30 in 10 μm and areolar density = 27–30 in 10 μm in our material versus striae density = 13–15 in 10 μm and areolar density = 13 in 10 μm in type material of *Amphora holsatica*). Taking into account all of the above data we choose to designate our specimens as *Amphora* sp. 1.

Maidana (1994b) recorded *A. coffeaeformis* in Arroyo Tapalqué. When we analyzed these samples we found specimens of *A. coffeaeformis* (Table 1) and a few other valves similar in valve outline, position of the raphe and striae structure (biseriate), but with a much lower density of dorsal striae (7 in 10 μm at the center and 10 in 10 μm at the poles) (Fig. 9). Three species, *Amphora turgida* Gregory, *A. cymbifera* Gregory and *A. granulata* Gregory, match the specimen illustrated in Fig. 9 in terms of the pattern and density of the dorsal striae. Comparison of our material with micrographs related to the type materials of *A. cymbifera* and *A. granulata* in Schoeman & Archibald (1987) and the descriptions and illustrations of Gregory (1857) revealed differences in valve size. *A. cymbifera* is larger (length = 63–114.3 μm and valve width = 11–13 μm in the type material versus length = 41μm and valve width = 8 μm in our material). Furthermore, the dorsal striae are slightly radiate and uniformly distributed in the type material while in our specimens they are strongly radiate and the central ones are more distant. *A. granulata* differs from our materials in size (length: 43–76 μm) and in its valve outline with straight dorsal and ventral margins. The micrographs in Schoeman & Archibald (1987, figs 37 a, b) do not show details of the raphe; however, the conopeum appears to be broad and straight while in our specimens it widens from the center to the poles where it abruptly narrows. *A. turgida* is the taxon that best resembles our material in shape and size (length = 25–50.8 μm), but descriptions and illustrations in Gregory (1857) and Peragallo & Peragallo (1897–1908) are insufficient to attribute them to this species. It would be necessary to study the fine structure of the type material, but there is no material available for SEM work (G. Reid, pers. com.). In view of these uncertainties we have designated our material as *Amphora* sp. 2 (Table 1).

Table 1. Morphometric measurements of *Amphora* taxa collected in Argentina and of the type material of *Amphora coffeaeformis* from Carlsbad (data from Archibald & Schoeman 1984). Sampling localities and illustrations are also listed. Dashes indicate no available data.

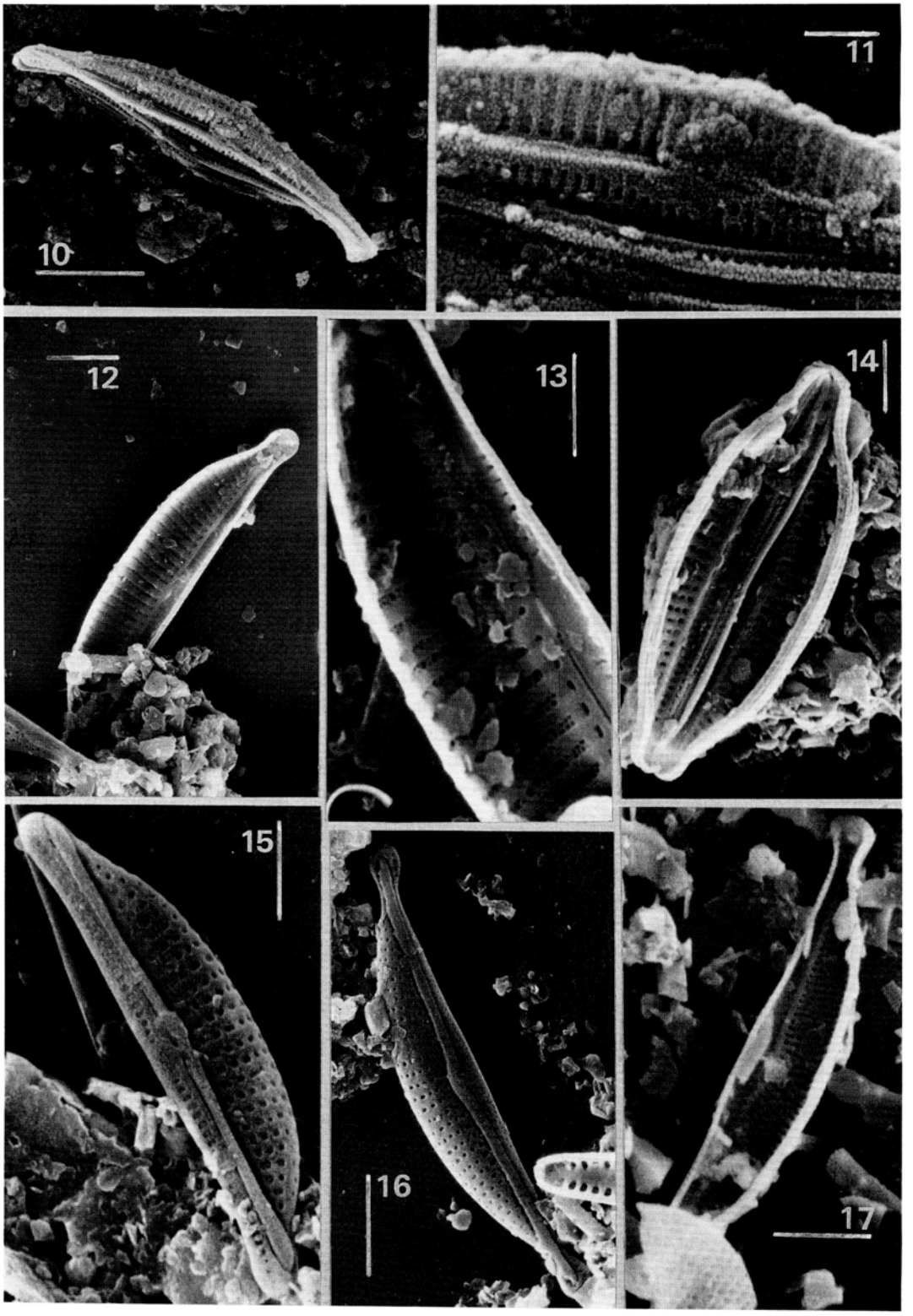
Taxon	Sampling site	Illustrations in this study	Valve dimensions		Dorsal striae in 10 µm		Ventral striae in 10 µm	Areolar density in 10 µm (dorsal striae)	Pores in 10 µm of the bands
			length (µm)	width (µm)	at center	at poles			
Amphora coffeaeformis	Carlsbad	–	14–55	3.5–7.3	16–26	20–30	21–36	51–93	37–45
	Laguna La Amarga	Figs 2–7	21–35.6	3.6–5.6	22–26	26–28	42–48	70	52
	Arroyo Tapalqué	–	19	3.5	22	–	40	–	–
	Laguna Pozuelos	–	15–19	3	22	–	40	–	–
	Salina del Bebedero	–	21–23	3.4–4.2	22–23	24	–	70–80	35
Amphora sp. 1	Laguna La Amarga	Fig. 8	37–39	5.8–6.5	22–23	30	32–35	27–30	–
Amphora sp. 2	Arroyo Tapalqué	Fig. 9	41	8	7	10	18–20	–	–
Amphora sp. 3	Laguna Pozuelos	Figs 10, 11	34	2.7	28	–	42	80–84	–
Amphora sp. 4	Salina del Bebedero	Figs 12, 13	21–28	5–6	13–16	20	30	22–26	–
A. cf. *tenerrima*	Salina del Bebedero	Figs 14, 15	13–14.4	2.6–2.8	25	32	40	40	42
Amphora sp. 5	Salina del Bebedero	Fig. 16	24–35.5	4–5.7	15–19	20–24	26–39	20–25	–
A. veneta var. *capitata*	Lago Nahuel Huapi	Figs 17, 18	24	5	25	–	32	15	–
A. hybrida	Mar del Plata	Figs 19–22	21–31	3.5–6.5	18–24	20–26	30–35	6	30–32
Amphora sp. 6	Chorrillo Uaquen	Fig. 23	22–35	4–6	20	–	–	–	–
A. coffeaeformis ?	Chorrillo Uaquen	Fig. 24	49–51	6–9	14	–	–	–	–

In the samples from Laguna Pozuelos two taxa similar in valve outline were found; one corresponds to *A. coffeaeformis* (Table 1). The valve of the other taxon (Figs 10, 11) coincides with *A. coffeaeformis* in morphometric data but it differs in the SEM as the former possesses longitudinal costae crossing the dorsal striae and a conopeum scarcely developed only at the ends of the valve. Our material resembles *Amphora hybrida* Grunow [= *A. castellata* Giffen] (Schoeman & Archibald 1984, Archibald & Schoeman 1985), but differs in striae structure and in the development of the conopeum. *A. hybrida* has uniseriate striae composed of 2–5 transapically elongated areolae while our specimens have biseriate striae with small roundish pores (Fig. 11). We have not been able to find a taxon that fits the morphological pattern of our material so we conclude it is probably a new species. However, considering that we studied only a few specimens, we prefer for the moment to designate it as *Amphora* sp. 3.

Maidana (1994a) recorded *Amphora acutiuscula* Kützing and *A. coffeaeformis* in Salinas del Bebedero. *A. acutiuscula* and *A. coffeaeformis* are two species that might be confused in LM but the analysis of the sample with SEM allowed us to confirm both records. However, we found three other taxa in the Salinas del Bebedero material similar to *A. coffeaeformis* in valve outline and dimensions but which differ in valve morphology. Figs 12, 13 show a specimen with biseriate striae, which are less dense than those of *A. coffeaeformis* (Table 1), and a longitudinal costa, parallel to and close to the raphe which interrupts the dorsal striae. We observed a similar longitudinal costa in *Amphora eunotia* var. *gigantea* (Grunow) Cleve illustrated in Bérard-Therriault *et al.* (1986, figs 39, 44); however, the valves of this taxon are much larger (length = 74–90 μm, width = 10–14 μm) and it has uniseriate striae. A taxon that coincides in striae density, dimensions and valve outline is *Amphora sabiniana* Reimer in Patrick & Reimer (1975). Reimer described the species based solely on LM observations and pointed out that it has a longitudinal line immediately dorsal to the raphe that could be equivalent to the costa found in our material. Nevertheless, in the protologue a transapical thickening at the center of the valve face was described that was not observed in the material from Salinas del Bebedero. It would be necessary to study the type material of *A. sabiniana* with SEM to determine whether the morphological pattern of this species coincides with that of our material. Taking into account these doubts we have called this taxon *Amphora* sp. 4.

The taxon illustrated in Figs 14 and 15 is smaller than all other *Amphora* taxa found at Salinas del Bebedero (Table 1) and has uniseriate striae with round or subquadrangular areolae and a straight conopeum, slightly widened at the ends. These materials are similar to *A. tenerrima* in valve outline and dimensions and density of dorsal striae but the description and illustrations in Aleem & Hustedt (1951) and the photographs of the type material in Simonsen (1987) are insufficient to establish a precise concept of this species. Thus, in spite of the similarities pointed out, we designate this population as *Amphora* cf. *tenerrima* because we think it is premature to attribute them to this taxon without analyzing the type material with SEM to establish dorsal striae structure, presence of ventral striae and other morphological features of the valve.

Figs 10–17. *Amphora* taxa, SEM. Scale bars = 5 μm (Figs 10, 12, 16, 17), 2 μm (Figs 13–15) or 1 μm (Fig.11). **Figs 10, 11.** *Amphora* sp. 3, Laguna Pozuelos. **Fig. 10.** External valve view. Note longitudinal ribs crossing dorsal striae. **Fig. 11.** External valve view showing detail of biseriate dorsal striae. **Figs 12, 13.** *Amphora* sp. 4, Salinas del Bebedero. **Fig. 12.** Internal valve view. **Fig. 13.** Detail of valve center showing longitudinal rib parallel to and close to raphe. Note biseriate dorsal striae. **Figs 14, 15.** *Amphora* cf. *tenerrima*, Salinas del Bebedero. **Fig. 14.** Internal view of two valves of a frustule. **Fig. 15.** External valve view showing uniseriate striae and straight conopeum. **Fig. 16.** *Amphora* sp. 5, Salinas del Bebedero. External valve view. Note uniseriate striae, widening of conopeum at center and toward ends of valve, and narrowing of conopeum at poles. **Fig. 17.** *Amphora veneta* var. *capitata*, Lago Nahuel Huapi. Internal valve view.



The taxon from Salinas del Bebedero illustrated in Fig. 16 is larger than *Amphora* cf. *tenerrima* and has fewer striae and areolae in 10 μm. The conopeum is slightly widened at the center and at the ends and it narrows abruptly at the poles. Since this taxon differs from all other *Amphora* species with uniseriate striae described in the literature, we have tentatively designated it as *Amphora* sp. 5. It may well be a new species.

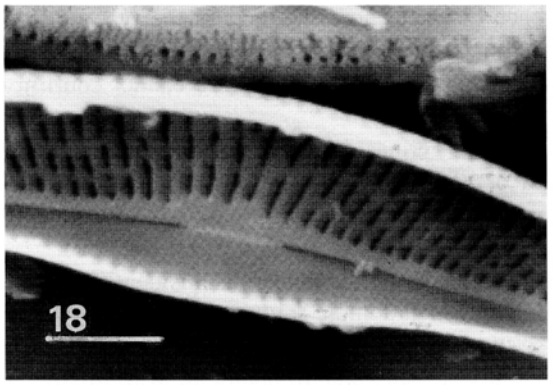
Maidana (1994c) recorded *Amphora coffeaeformis* and *A. veneta* Kützing in Lago Nahuel Huapi. Analyzing these samples with SEM we found only one taxon similar to *A. coffeaeformis* as regards valve outline and morphometric data. However, all these specimens had strongly radiate uniseriate striae with transapically elongated areolae and distant proximal raphe ends (Figs 17, 18). This material corresponds to *Amphora veneta* var. *capitata* Haworth (Haworth 1974, Schoeman & Archibald 1978) (Table 1), a species that has been frequently confused with *A. coffeaeformis* (Archibald & Schoeman 1984).

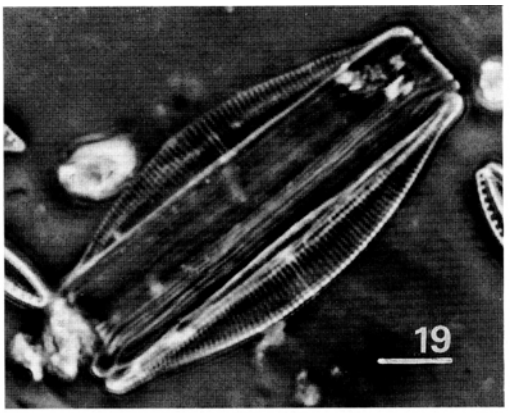
Ferrario & Sar (1985) recorded *A. coffeaeformis* from the rocky shores of Mar del Plata. Although their illustrations and morphometric data suggest that the record is correct, their micrographs show ultrastructural details that correspond to another species. Analysis of the samples with SEM showed the existence of only one taxon. The specimens illustrated in Figs 19–22 have uniseriate dorsal striae with 2 or 3 transapically elongated areolae and two longitudinal costae, one on the valve face and the other at the junction of the valve face and mantle. The structure of the girdle bands coincides with those of *A. coffeaeformis* but in our specimens the pore density is less (Table 1). The species present in our samples is *Amphora hybrida* Grunow, which has been frequently misidentified as *A. coffeaeformis* (Schoeman & Archibald 1984). The confusion is understandable since the image of *A. hybrida* in LM (Fig. 19) bears a very close resemblance with that of *A. coffeaeformis* (Fig. 2), and the valve dimensions and striae densities are similar.

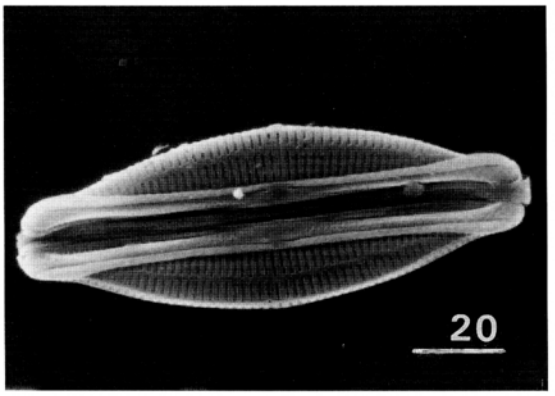
Frenguelli (1923–1924) reported *Amphora acutiuscula*, *A. coffeaeformis* and *A. coffeaeformis* var. *borealis* (Kützing) Cleve from Tierra del Fuego. The materials corresponding to these records (slide no. 234) were studied only with LM since the Frenguelli Collection has no supplementary materials that can be studied with SEM. We only identified two taxa on this slide which differed in valve size and density of dorsal striae. A specimen from the first population is illustrated in Fig. 23. The valves are small (length = 22–35 μm, width = 4–6 μm) and have uniseriate striae (20 in 10 μm) with rounded areolae. This taxon does not seem to correspond to any of the taxa that Frenguelli listed, and is similar to *Amphora tenerrima* in valve outline and type of striae but differs in dimensions and dorsal striae density (length = 8–15 μm; width = 1.5–3 μm; striae = 22–26 in 10 μm, Aleem & Hustedt 1951). As they cannot be accurately determined we have designated them as *Amphora* sp. 6.

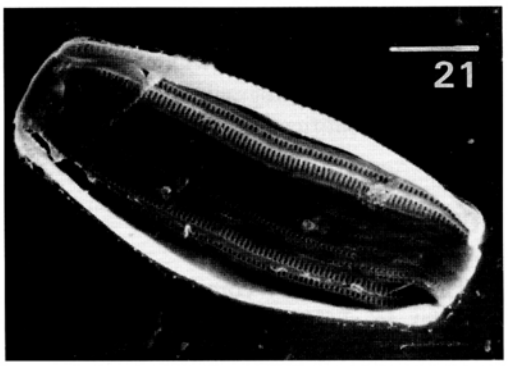
The valves of the second population illustrated in Fig. 24 are larger (length = 49–51 μm, width = 6–9 μm), the dorsal striae are coarser (14 in 10 μm), and the areolae cannot be distinguished with LM. Based on these data this taxon may correspond to what Frenguelli reported as either *A.*

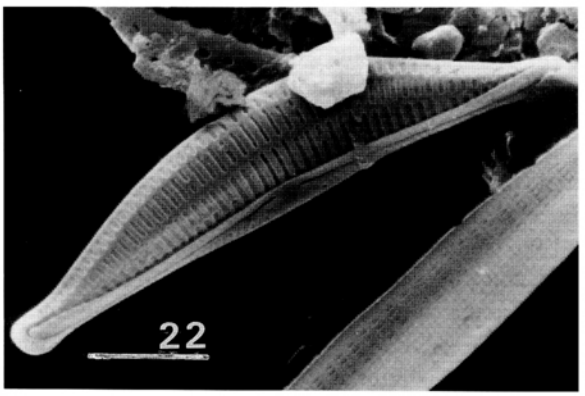
Figs 18–25. *Amphora* taxa. Scale bars = 5 μm (Figs 19–24), 2 μm (Fig. 18) or 1 μm (Fig. 25). **Fig. 18.** *Amphora veneta* var. *capitata*, Lago Nahuel Huapi, SEM. Detail of valve center showing uniseriate, radiate dorsal striae with transapically elongated areolae and distant proximal raphe ends. **Figs 19–22.** *Amphora hybrida*, Mar del Plata. **Fig. 19.** Frustule in ventral girdle view, LM. **Fig. 20.** Frustule in ventral girdle view, SEM. **Fig. 21.** Frustule in dorsal girdle view showing details of girdle bands, SEM. **Fig. 22.** External valve view. Note uniseriate dorsal striae and longitudinal costae, SEM. **Figs 23, 24.** Frenguelli Collection, slide no. 234, Chorrillo Uaquen, LM. **Fig. 23.** *Amphora* sp. 6. Frustule in ventral girdle view. **Fig. 24.** *Amphora coffeaeformis*? Valve view. **Fig. 25.** Clone BPT 11. External valve view, SEM.

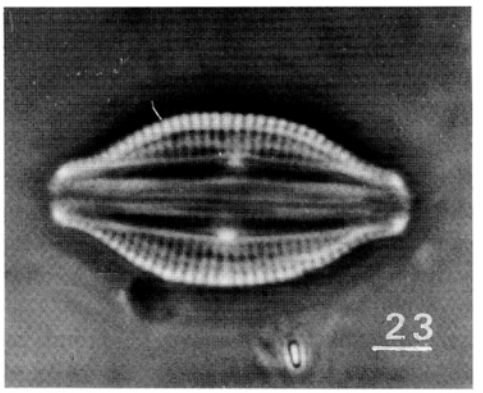


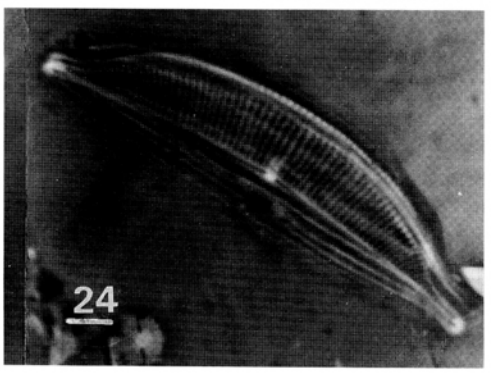


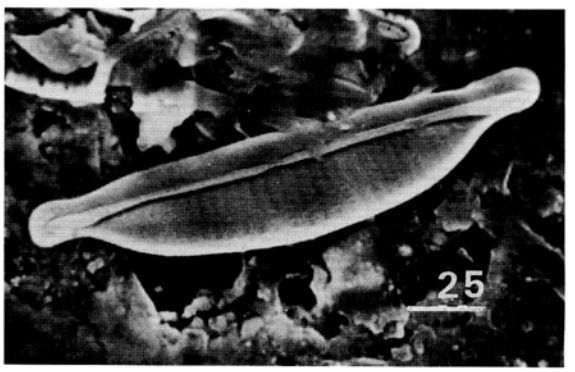












coffeaeformis or *A. acutiuscula*. The main difference between these two taxa is the type of striae: biseriate in *A. coffeaeformis* and uniseriate near the dorsal margin and biseriate near the raphe in *A. acutiuscula* (Archibald 1983, fig. 491). As this feature is not visible in LM we cannot confirm or reject the records of Frenguelli.

Lacoste *et al.* (1983) reported *A. coffeaeformis* from Termas del Domuyo (Neuquén). Since the corresponding samples were not available for LM or SEM study and despite similarities with *A. coffeaeformis* as regards the illustration and dimensions, the available information is insufficient to confirm or reject this record.

After studying the materials from Argentina and considering the numerous misidentifications of *Amphora coffeaeformis*, we wondered if the materials in which domoic acid was detected (clone BPT 11) really correspond to this species. Since no illustrations were published in Shimizu *et al.* (1989) or Maranda *et al.* (1990), we sought relevant material but were only able to obtain an SEM micrograph (Fig. 25) used for its initial identification as no material exists (Maranda, pers. com.). This specimen matches *A. coffeaeformis* in its valve outline and morphology of the conopeum but the valve is smaller (length = 12.3 μm, width = 2.7 μm) and the density of its dorsal striae is greater (30 in 10 μm). Since the photograph does not permit determination of whether the dorsal striae are uni- or biseriate, the identification of clone BPT11 as *A. coffeaeformis* remains uncertain.

DISCUSSION

The present study corroborates the statement by Archibald & Schoeman (1984) about the difficulties in differentiating *Amphora coffeaeformis* from morphometrically similar taxa. The analysis of materials collected in marine and inland waters of Argentina demonstrated that various taxa were misidentified as *A. coffeaeformis*. These misidentifications were due to the similarity of the various non-*A. coffeaeformis* taxa in valve outline, dimensions and striae density. Only *Amphora* sp. 2 has a striae density so low that it can be easily separated from the other taxa.

On the basis of fine structural features we were able to authenticate *A. coffeaeformis* and discriminate 8 other taxa in the Argentinean materials. The most important characters for differentiating these taxa were the type of striae (uni- or biseriate), type of areolae (round, subquadrangular or elongated), presence or absence of longitudinal costae and morphology of the conopeum (more or less expanded, narrowing at the center and/or the ends). As it is not possible to discriminate all these features using LM alone, we consider it inadequate to attempt to separate such taxa without an electron microscope analysis. Taking into account the insufficiency of LM observations mentioned above, the protologues of many species of the subgenus *Halamphora* Cleve are inadequate to provide a precise concept of these taxa. A careful analysis with electron microscopy of the type materials is imperative to avoid even more confusion.

As a result of our study we are able to confirm the presence of *A. coffeaeformis* in the provinces of La Pampa (Laguna La Amarga), Buenos Aires (Arroyo Tapalqué), San Luis (Salinas del Bebedero) and Jujuy (Laguna Pozuelos). On the contrary, we reject the records from Lago Nahuel Huapi and Mar del Plata. In the former case the specimens present in the samples correspond to *A. veneta* var. *capitata* and in the latter to *A. hybrida*, a taxon that is recorded for the first time in South America. We could not corroborate the records from Tierra del Fuego (Chorillo Uaquén) and Neuquén (Termas del Domuyo) because materials for SEM examination were not available. One or more of the other six *Amphora* taxa given numbers here coexist with *A. coffeaeformis* in Laguna La Amarga, Arroyo Tapalqué, Laguna Pozuelos and Salinas del Bebedero. Although we studied their valve morphology in some detail, we considered it premature to assign them to known taxa or

describe them as new species until the type materials of allied taxa are reviewed. This will be the subject of further research.

It is difficult to establish the autoecology of *A. coffeaeformis* because of numerous misidentifications of this species in the literature for reasons mentioned here and in Archibald & Schoeman (1984). Authenticated materials as those studied by Schoeman & Archibald (1984) were collected in inland waters from South Africa while others as those analyzed by Bérard-Therriault *et al.* (1986) were collected in estuarine habitats from Canada. In both cases the authors did not give environmental data. The records reported by Anderson (1975, fig. 1) from deep ocean waters and by Garduño *et al.* (1996, fig. 6) from marine fouling do not correspond to this species but to others with uniseriate striae. In the materials studied by us *A. coffeaeformis* was present only in inland waters and, although three of the sites where it was collected are hypersaline, it was also found in a river with medium electrolytic content (114–146 μS cm^{-1}). The samples analyzed were not accompanied by environmental data or these data were incomplete. On the basis of the available information we think that *A. coffeaeformis* is an inland and estuarine species that seems to tolerate different salinity regimes.

ACKNOWLEDGEMENTS

We are indebted to Dr Nora Maidana who kindly provided part of the materials upon which this paper is based, to Dr L. Maranda who provided a photograph of one specimen from clone BPT 11 and to the Editor, Dr M.J Sullivan, who helped us to improve this paper. We also thank Miss Nilda Malacalza for her technical assistance.

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