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AN EMENDED DESCRIPTION OF *AMPHORA TUMIDA* HUSTEDT (BACILLARIOPHYCEAE)

Eugenia A. Sar[1], **Silvia E. Sala**

Departamento Científico Ficología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900 La Plata, Argentina

Friedel Hinz

Alfred Wegener Institut für Polar und Meeresforschung (AWI), Am Handelshafen 12, D – 27570 Bremerhaven, Germany

Inés Sunesen [2]

Departamento Científico Ficología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900 La Plata, Argentina

Material chosen as the lectotype of *Amphora tumida* Hustedt by Simonsen was examined with light and scanning electron microscopy. Based on this analysis a more comprehensive concept of this taxon was established and the description emended. A comparison with the morphologically similar *A. subacutiuscula* Schoeman and *A. acutiuscula* Kützing sensu Archibald strongly suggests that *A. tumida* is present in South African waters, in addition to its confirmed records from Venezuela and Argentina.

INTRODUCTION

Species of the genus *Amphora* Ehrenberg belonging to the Halamphora group are easily misidentified when analysed with light microscopy due to the similarity in their valve outline and dimensions (Archibald & Schoeman 1984, Sala *et al*. 1998, Clavero *et al*. 2000, Sar *et al*. 2003). The current taxonomic confusion might be reduced if type material of all described species of this group were studied with electron microscopy and their descriptions emended to include additional morphological information. Following this idea we have re-examined material of some species created by Hustedt within the group starting with *A. holsatica* (Sar *et al*. 2003). Another problematic member of the Halamphora, *Amphora tumida*, was described by Hustedt (1956) from material collected in Lago de Maracaibo, Venezuela. Simonsen (1987) examined the three slides marked by Hustedt as containing *A. tumida* and chose Slide 343/95b as the lectotype. Although Simonsen produced excellent light micrographs, they are insufficient to see details of the morphology of the striae, conopeum, raphe and girdle bands that allow differentiation of species belonging to Halamphora. Despite *A. tumida* being described almost 50 years ago, the name has not been reported since that time. VanLandingham (1967) included *A. tumida* as a valid taxonomic entity without a list of

[1] Consejo Nacional de Investigaciones Científicas y Técnicas. E-mail: easar@museo.fcnym.unlp.edu.ar
[2] Comisión de Investigaciones Científicas de la Provincia de Buenos Aires

references, presumably indicating that the species had not been identified in general works up to 1967. Furthermore, there have been apparently no electron microscopical studies, as neither Gaul *et al*. (1993) nor Henderson & Reimer (2003) included this taxon in their catalogues.

In order to provide a more detailed description of *Amphora tumida* and to establish its morphological variation, we examined specimens on the lectotype slide designated by Simonsen (1987). We also compared *A. tumida* with specimens identified by Archibald (1983) as *A. acutiuscula* Kützing and with *A. subacutiuscula* Schoeman.

MATERIAL AND METHODS

The material used to carry out this research corresponds to the lectotype of *Amphora tumida:* Sample Am2036, Slide 343/95b from Station 17, Lago de Maracaibo, Venezuela, collected by F. Gessner in October 1952 and deposited in the Hustedt Collection at the Alfred Wegener Institut für Polar und Meeresforschung in Bremerhaven (BRM, Germany). In order to obtain the morphometric data from a large number of specimens, we considered those photographed by Simonsen (1987) and several other specimens found by us on the lectotype slide and in Sample Am2036. The photomicrographs were obtained with a Axioplan D 7082 light microscope (LM) in brightfield and Nomarski differential interference contrast. Material corresponding to the same sample used by Hustedt for the preparation of the lectotype slide was mounted following the methods of Ferrario *et al*. (1995) and examined with a ISI-DS 130 scanning electron microscope (SEM).

Densities of areolae and striae were measured according to Anonymous (1975) and the terminology used to describe valve morphology is that suggested by Ross *et al*. (1979), Cox & Ross (1980) and Lee & Round (1987). Details of frustule construction in the genus *Amphora* and the morphology of the valves in different positions are ably presented in Schoeman & Archibald (1979).

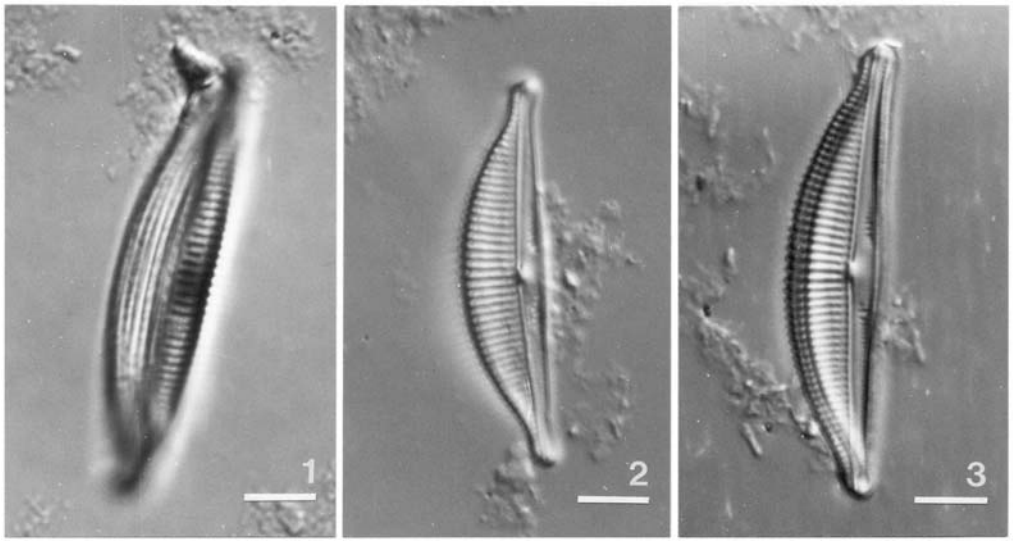
RESULTS

In examining Sample Am2036 used to prepare lectotype Slide BRM 343/95b, we found several specimens that conformed in valve outline and morphometric parameters to Hustedt's (1956) *Amphora tumida*. SEM analysis of this material allowed us to establish that all these specimens belong to the same taxonomic entity. In Table 1 we present morphometric data taken from the protologue of Hustedt (1956), photomicrographs of Simonsen (1987, pl. 654, figs 12–19), and eighteen specimens we examined in LM (lectotype slide) and SEM (Sample Am2036).

Amphora tumida Hustedt emend. Sar, Sala, Hinz & Sunesen

Light microscopy

Frustule with valvar planes of both valves making acute angle. Girdle narrow in dorsal view with several punctate girdle bands (Fig. 1). Valves semi-lanceolate with convex dorsal margin and ventral margin more or less straight, slightly tumid at central region (Figs 2, 3). Poles ventrally deflected, protracted into rostrate to sub-capitate apices. Dorsal striae slightly radiate throughout the valve, almost parallel at centre (Figs 2, 3); ventral striae short, more densely arranged, interrupted at mid-valve (Fig. 3). Raphe branches almost straight, somewhat distant from ventral margin, with proximal ends gently curved dorsally (Figs 2, 3). Central nodule conspicuous.



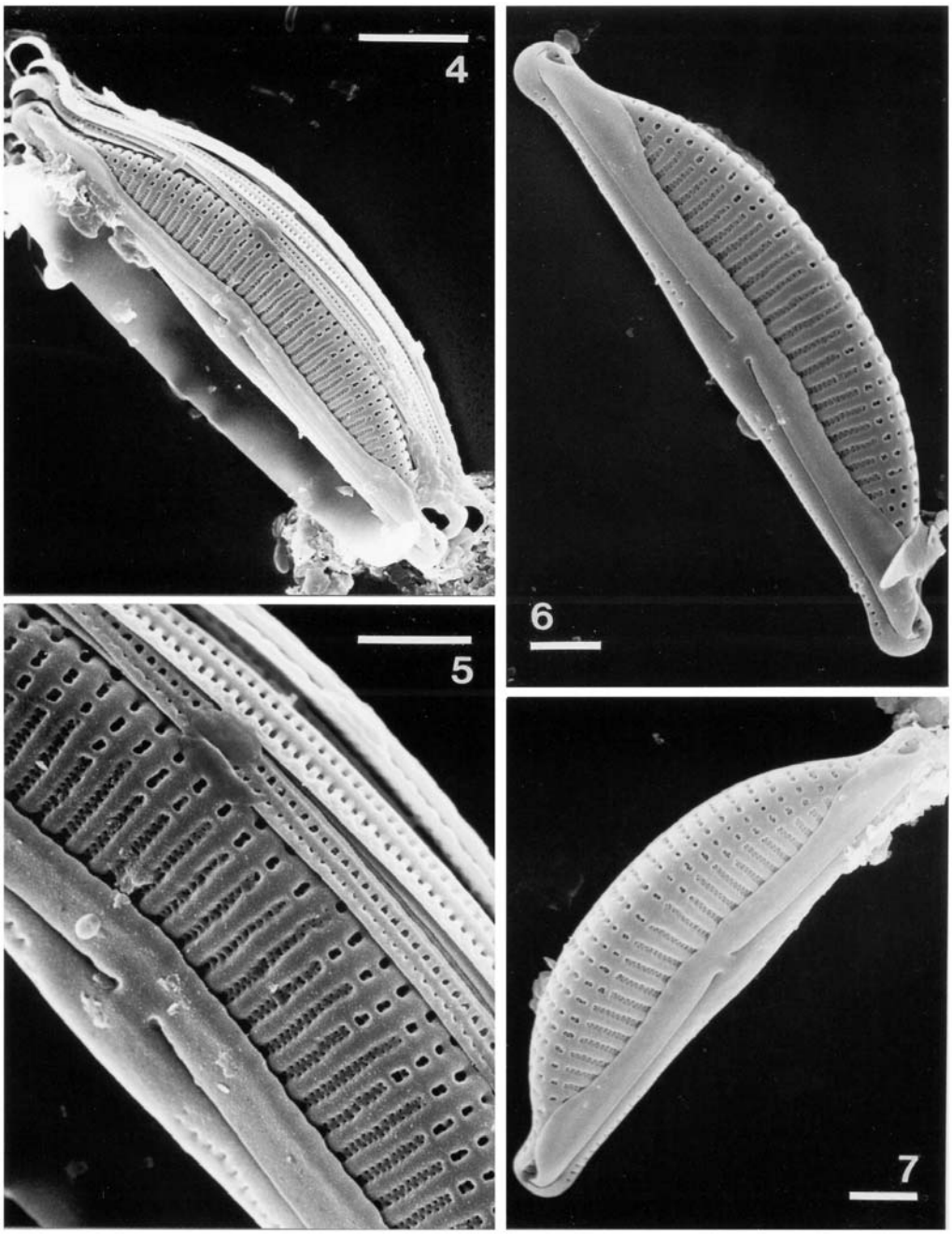
Figs 1–3. *Amphora tumida*, lectotype material BRM 343/95b, LM. Scale bars = 5 µm. **Fig. 1**. Frustule showing dorsal aspect of girdle bands. **Figs 2, 3**. Valves showing dorsal and ventral striae, raphe branches and conspicuous central nodule.

Scanning electron microscopy

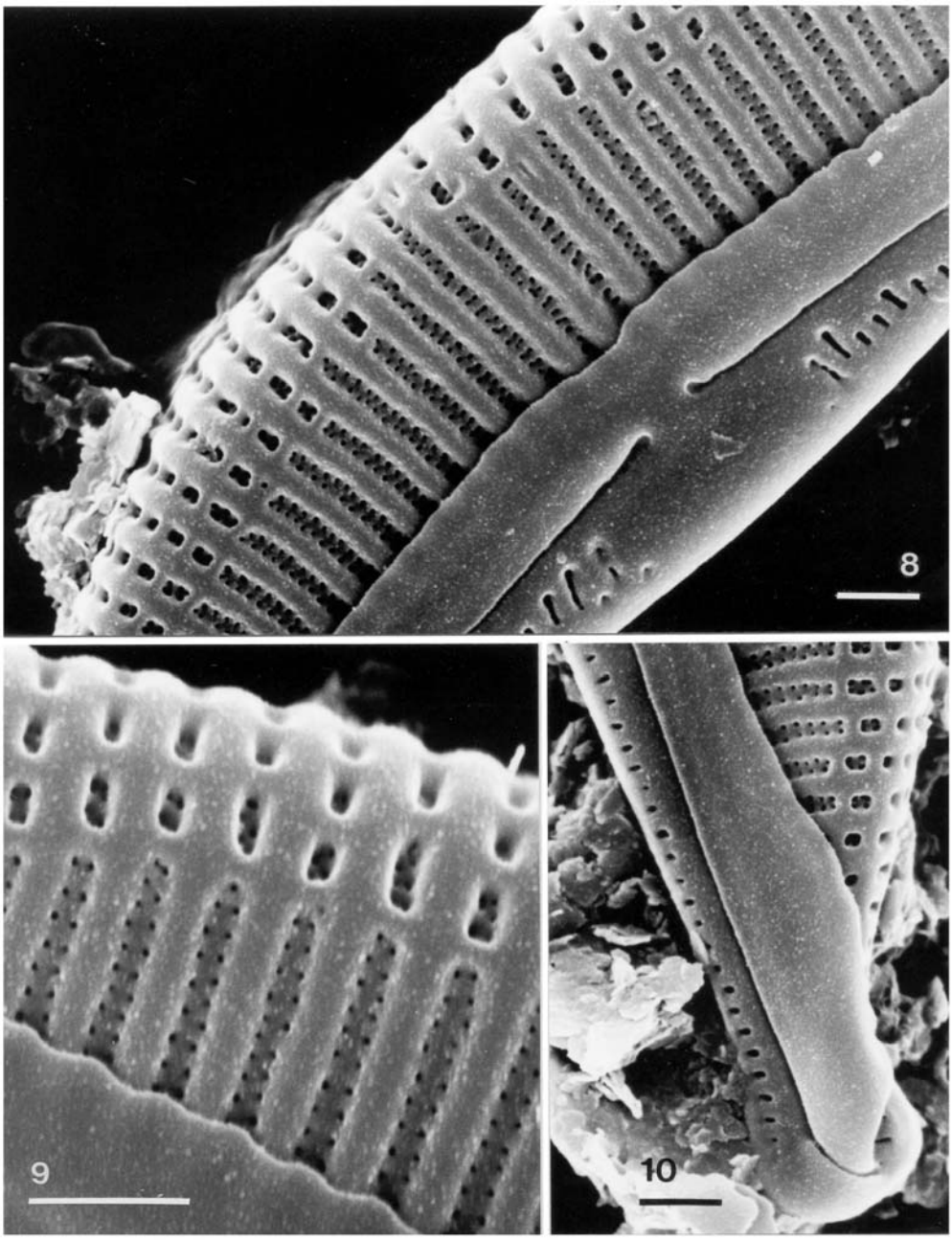
Valve face gently curved into a high, oblique, dorsal mantle (Figs 4–7, 13). Ventral mantle shallow and at a right angle to valve face (Fig. 13). Dorsal striae biseriate, sunken in siliceous layer, interrupted externally by longitudinal, wavy, fragmented costae on dorsal side of valve (Figs 7–10). Areolae of dorsal striae arranged opposite one another or in quincunx (Figs 8–10, 12). Ventral striae slender, more densely arranged than dorsal striae, convergent at valve ends, radiate near valve centre, interrupted at mid-valve (Figs 8, 10–12). Raphe branches nearly parallel to ventral margin for most of their length (Figs 6, 11). Internally, raphe-sternum distinct, narrower on dorsal side (Figs 11, 12, 14). Raphe enclosed by an axial rib (Figs 11, 12, 14). Internal proximal raphe ends ventrally bent, terminating under a tongue-like expansion; terminal ones ventrally deflected as poorly developed helictoglossae (Figs 11–13). Internally a longitudinal rib present, parallel to raphe-sternum, incomplete at valve centre, interrupting dorsal striae and delimiting a single proximal row of areolae (Figs 11–14). Externally, proximal raphe fissures dorsally bent, ending as dilated central pores (Figs 5–8); terminal fissures dorsally deflected (Figs 6, 7, 10). Conopeum well-developed on dorsal side of the valve, broad, straight at valve centre, widening just before valve poles (Figs 6–8, 10). Girdle bands open at one end, with two rows of ovoid, circular or quadrangular poroids separated by a rib on their dorsal side (Figs 5, 14).

Morphometric data

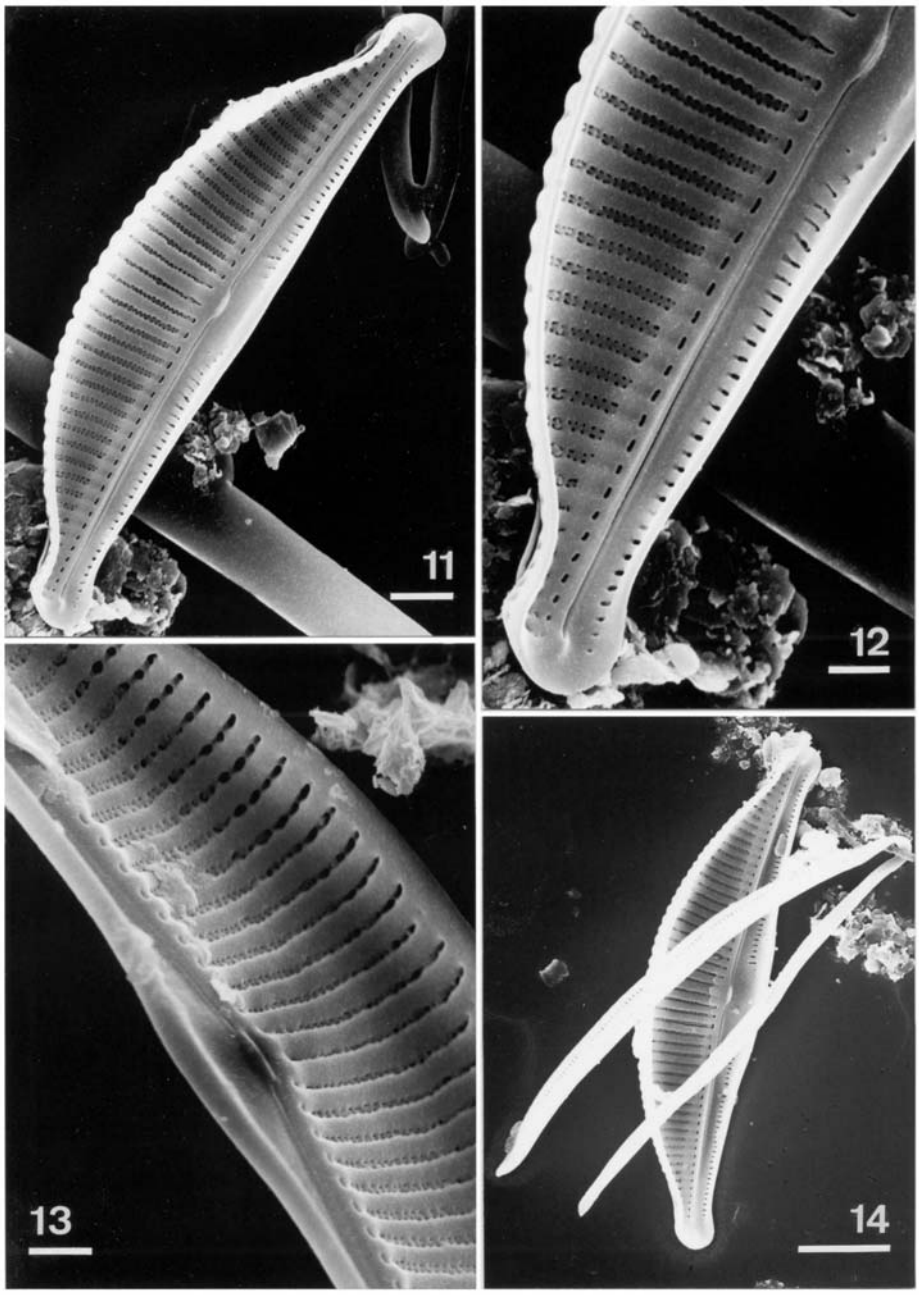
Length, 12–33 µm; valve width, 3–7 µm; dorsal striae in 10 µm at centre, 15–18, at poles, 18–24; ventral striae in 10 µm at centre, 24–32, at poles 32–36; areolae in 10 µm of dorsal striae, 60–80; girdle band areolae in 10 µm, 34–40.



Figs 4–7. *Amphora tumida*, BRM sample Am2036, SEM. Scale bars = 5 μm (Fig. 4) or 2 μm (Figs 5–7). **Fig. 4**. Frustule in valvar view showing several girdle bands. **Fig. 5**. Detail of central part of frustule with dorsal girdle bands bearing two rows of poroids separated by a rib. **Figs 6, 7**. External valve views. Note biseriate dorsal striae on valve face, ventral striae, conopeum, raphe branches, and dorsal mantle.



Figs 8–10. *Amphora tumida*, BRM sample Am2036, SEM. Scale bars = 1 μm. **Fig. 8**. Central part of valve in external view. Note differing morphology of striae on dorsal portion of valve, longitudinal costae, ventral striae, broad conopeum and central raphe fissures. **Fig. 9**. Detail of external dorsal valve face and mantle showing striae morphology and scalloped margin of conopeum. **Fig. 10**. Valve pole in external view. Note broadening of conopeum at valve apex, terminal raphe fissure, and single row of ventral areolae.



Figs 11–14. *Amphora tumida*, BRM sample Am2036, SEM. Valves in internal view. Scale bars = 5 μm (Fig. 14), 2 μm (Fig. 11) or 1 μm (Figs 12, 13). **Fig. 11**. Whole valve showing distinct raphe-sternum which is narrower on dorsal side, and raphe enclosed by an axial rib. Note longitudinal rib on dorsal side of raphe-sternum delimiting a single row of apically elongated areolae. **Fig. 12**. Detail of valve apex showing striae morphology, tongue-like expansion of central nodule and small helictoglossa. **Fig. 13**. Oblique view of valve centre showing the vertical, shallow, ventral mantle and evidence of longitudinal costae towards dorsal mantle area. Note that distal portions of dorsal striae appear to be uniseriate only because they are eroded. **Fig. 14**. Detached girdle band open at one end.

DISCUSSION

The specimens of *Amphora tumida* figured in Simonsen (1987) and those photographed by us with LM and SEM show slight differences in valve dimensions and dorsal striae density with respect to the ranges given by Hustedt (1956) in the protologue (Table 1). Despite these differences we feel confident in assigning all specimens we observed to the same taxon and consequently the range of morphometric parameters is enlarged to contain all data obtained in the present study (Table 2).

Table 1. Morphometric data for *Amphora tumida* based on investigations of type material by past and present authors. Abbreviations: C = valve centre; P = valve pole; dash = no data .

Reference	Length (µm)	Valve width (µm)	Dorsal striae in 10 µm		Ventral striae in 10 µm		Areolae in 10 µm	Girdle band areolae in 10 µm
			C	P	C	P		
Hustedt (1956)	12–30	3–6	18–20		_	_	_	35–40
Simonsen (1987)	20–33	5–7	15–16	18–22	24–28	_	_	_
LM (present study)	27–30	6–6.5	16–18	20–22	29–31	35	_	_
SEM (present study)	18–30.5	4.5–6.5	16–18	18–24	28–32	32–36	60–80	34–36

Table 2. Morphometric comparison of *Amphora tumida* with selected species. Abbreviations: C = valve centre; P = valve pole; dash = no data

Species	Reference	Length (µm)	Valve width (µm)	Dorsal striae in 10 µm		Ventral striae in 10 µm		Areolae in 10 µm	Girdle band areolae in 10 µm
				C	P	C	P		
A. tumida	Present study	12–33	3–7	15–18	18–24	24–32	32–36	60–80	34–40
"*A. acutiuscula*"	sensu Archibald (1983)	18–49.5	3.4–6.4	15–20	20–24	22–28	_	_	_
A. subacutiuscula	Schoeman (1972)	13–34.5	3.5–5.5	18–20 (22)	More dense	27–30	Not visible	_	_

SEM analysis of the type material of *Amphora tumida* provided additional morphological information to that contained in Hustedt's (1956) LM description. This new information includes the following: 1) dorsal striae are biseriate, sunken in the siliceous layer and interrupted externally by longitudinal, wavy, fragmented costae on the dorsal side of the valve, 2) conopeum is well-developed on the dorsal side of the valve, broad, straight at valve centre, widening just before valve poles, 3) longitudinal rib parallel to the raphe-sternum is present internally, and 4) girdle bands possess two rows of ovoid, circular or quadrangular poroids separated by a rib on their dorsal side.

Specimens from the Sundays River in South Africa assigned by Archibald (1983, figs 3, 4, 89–92, 490–492) to *Amphora acutiuscula* Kützing matches the type material of *A. tumida* in valve outline, morphometric data (Table 2), morphology of the dorsal and ventral striae and presence of an internal longitudinal rib parallel to the raphe-sternum. However, considering the statements of Archibald (1983), the name he assigned to these specimens was used in a sense that conflicts with the type material of *A. acutiuscula*. He examined the putative type slide (BM 18173) and commented that "there were few *Amphora* specimens in this material from Genoa … and these comprised three different but rather similar entities. There is thus some uncertainty as to which of the three is the proper *A. acutiuscula*. None of these specimens had very clearly punctate striae …". Archibald also examined Van Heurck's Slide No. 261of *A. acutiuscula* and wrote that " these specimens had a similar valve shape, but the striae were more or less distinctly punctuate …There is thus a significant discrepancy amongst the different materials reputed to contain *A. acutiuscula* …". Finally, he emphasized that in his study "the concept of *A. acutiuscula* is confined to those specimens having more or less distinctly punctate striae" so the Sundays River specimens may not be "the true *A. acutiuscula* when compared with Kützing's material, but they are very similar in striae structure to the examples on Van Heurck's Slide No. 261". Based on these arguments we believe that the name *A. acutiuscula* should not be applied to the Sundays River specimens and they may in fact be conspecific with *A. tumida*.

Maidana (1994) determined material from the Salinas del Bebedero (San Luis, Argentina) as *Amphora acutiuscula* following Archibald (1983). The specimens illustrated in external view (Maidana 1994, fig. 25a,b) correspond to two different taxa. The specimen illustrated in fig. 25b matches the type material of *A. tumida* in its morphometric parameters and the morphology of its dorsal striae and broad conopeum. Specimens from the same sample studied by Maidana (1994) figured in internal view by Sala *et al*. (1998, figs 12, 13) and designated as *Amphora* sp. 4, also coincide with *A. tumida* in their morphometric parameters, morphology of the dorsal striae, and the presence of a longitudinal rib parallel to the raphe-sternum. Thus, we conclude that this population from Salinas del Bebedero should be identified as *A. tumida*.

Amphora subacutiuscula Schoeman is another species that is similar in valve outline and morphometric data to *A. tumida* (Table 2). *A. subacutiuscula* was erected by Schoeman (1972) with a brief description and line drawings as the iconotype. In the protologue the author compared his taxon with *A. tumida* Hustedt, pointing out that its poles are more protracted and the ventral striae, where visible, are longer in the former than in the latter. Subsequently, Archibald (1983, figs 511, 512) analysed the type material of *A. subacutiuscula* with transmission electron microscopy and concluded that more than one taxon might be involved. These micrographs clearly document two taxa that differ in the length of their ventral striae and in the extent of the hyaline central area on the ventral side of the valve. In our opinion, Archibald's fig. 512 is conspecific with *A. tumida* as the specimen illustrated matches the latter in all morphometric parameters and morphology. Based on the fact that the iconotype is not sufficient to fix the application of the name, it would be necessary to study the type material of *A. subacutiuscula* with SEM in order to fully describe its morphology.

The difficulties we found when comparing *Amphora tumida* with similar taxa described in the literature clearly demonstrate that in the Halamphora group of the genus in particular there is a pressing need to examine type material with SEM and TEM to evaluate intraspecific variability and to establish specific limits. The most relevant features to consider are: morphology of dorsal and ventral striae, presence or absence of longitudinal costae, morphology and width of conopeum, path of raphe branches, shape and inclination of proximal and distal raphe fissures, size of helictoglossae and morphology of girdle bands.

This taxon was reported by Hustedt (1956) as present in the Lago de Marcaibo, Venezuela (1.14 psu and 32 ºC). More recently, Maidana (1994) found it as a fossil form in Quaternary sediments from the Salinas del Bebedero, Argentina. The comparisons with *A. subacutiuscula* and *A. acutiuscula* sensu Archibald strongly suggest that *A. tumida* is also present in South African waters.

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REFERENCES

ANONYMOUS. (1975). Proposals for a standardization of diatom terminology and diagnoses. *Nova Hedwigia*, *Beiheft* **53**, 323–354.

ARCHIBALD, R. E. M. (1983). The diatoms of the Sundays and Great Fish Rivers in the Eastern Cape Province of South Africa. *Bibliotheca Diatomologica*, **1**, 1–362.

ARCHIBALD, R. E. M. & SCHOEMAN, F. R. (1984). *Amphora coffeaeformis* (Agardh) Kützing: a revision of the species under light and electron microscopy. *South African Journal of Botany*, **3**, 83–102.

CLAVERO, E., GRIMALT, J. O. & HERNÁNDEZ-MARINÉ, M. (2000). The fine structure of two small *Amphora* species. *A. tenerrima* Aleem & Hustedt and *A. tenuissima* Hustedt. *Diatom Research*, **15**, 195–208.

COX, E. J. & ROSS, R. (1980). The striae of pennate diatoms. In: *Proceedings of the 6th International Diatom Symposium* (Ross, R., ed.), 267–276. Koeltz, Koenignstein.

FERRARIO, M. E., SAR, E. A. & SALA, S. E. (1995). Metodología básica para el estudio del fitoplancton con especial referencia a las diatomeas. In: *Manual de Métodos Ficológicos* (K. Alveal, M.E. Ferrario, E. Oliveira & E.A. Sar, eds), 1–23. Universidad de Concepción, Concepción.

GAUL, U., GEISSLER, U., HENDERSON, M., MAHONEY, R. & REIMER, C. W. (1993). Bibliography on the fine-structure of diatom frustules (Bacillariophyceae). *Proceedings of the Academy of Natural Sciences of Philadelphia*, **144**, 69–238.

HENDERSON, M. V. & REIMER, C. W. (2003). Bibliography on the fine structure of diatom frustules (Bacillariophyceae). II. (+ Deletions, addenda and corrigenda for Bibliography I). *Diatom Monographs,* **3**, 1–372.

HUSTEDT, F. (1956). Diatomeen aus dem Lago de Maracaibo in Venezuela. *Ergebnisse der deutschen limnologischen Venezuela-Expedition 1952*, **1**, 93–140.

LEE, K. & ROUND, F. E. (1987). Studies on freshwater *Amphora* species. II. *Amphora copulata* (Kütz.) Schoeman & Archibald. *Diatom Research*, **3**, 217–225.

MAIDANA, N. I. (1994). Fossil diatoms from Salinas del Bebedero (San Luis, Argentina). *Diatom Research*, **9**, 99–119.

ROSS, R., COX, E. J., KARAYEVA, N. I., MANN, D. G., PADDOCK, T. B. B., SIMONSEN, R. & SIMS, P. A. (1979). An amended terminology for the siliceous components of the diatom cell. *Nova Hedwigia*, *Beiheft* **64**, 513–533.

SALA, S. E., SAR, E. A. & FERRARIO, M. E. (1998). Review of materials reported as containing *Amphora coffeaeformis* (Agardh) Kützing in Argentina. *Diatom Research*, **13**, 323–336.

SAR, E. A., SALA, S. E., HINZ, F. & SUNESEN, I. (2003). Revision of *Amphora holsatica* (Bacillariophyceae). *European Journal of Phycology*, **38**, 73–81.

SCHOEMAN, F. R. (1972). A further contribution to the diatom flora of sewage enriched waters in southern Africa. *Phycologia*, **11**, 239–245.

SCHOEMAN, F. R. & ARCHIBALD, R. E. M. (1979). *The Diatom Flora of Southern Africa*. No. 5. CSIR Special Report, WAT 50, Pretoria.

SIMONSEN, R. (1987). *Atlas and Catalogue of the Diatom Types of Friedrich Hustedt*. Text (525 pp), Atlas (772 pls.) J. Cramer, Berlin.

VANLANDINGHAM, S. L. (1967). *Catalogue of the Fossil and Recent Genera and Species of Diatom and their Synonyms. Part I. Acanthoceras* through *Bacillaria*, 1–492. J. Cramer, Lehre.