

New records of amphoroid diatoms (Bacillariophyceae) from Cachoeira River, Northeast Brazil

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Abstract

Amphoroid taxa have been revised in recent decades. Many species formerly assigned to *Amphora* have been transferred to other recently proposed genera, as *Seminavis* (Naviculaceae) and *Halamphora* (Catenulaceae). In Brazil, there are few studies focused on amphoroid taxonomy. This study presents a taxonomic investigation of five uncommon amphoroid taxa from Brazilian diatom flora: *Seminavis pusilla*, *S. strigosa*, *Amphora ectorii*, *Halamphora ghanensis* and *Halamphora* sp. *Seminavis strigosa* is identical in valve morphology and morphometrical data to *Amphora twenteana*, and its synonymy is proposed. *Seminavis pusilla*, poorly found in Brazilian waters, has expanded its distribution. *Halamphora ghanensis* is a new record to American continent while *Amphora ectorii* are new to Brazilian aquatic systems. *Halamphora* sp. has distinct ultrastructural features in relation to similar species and is probably new for science.

Keywords: *Amphora*, Bahia state, coastal river, *Halamphora*, *Seminavis*.

Novas ocorrências de diatomáceas anforóides (Bacillariophyceae) no Rio Cachoeira, nordeste do Brasil

Resumo

Táxons anforóides foram revisados nas últimas décadas. Várias espécies, previamente atribuídas a *Amphora*, foram transferidas para outros gêneros recentemente propostos, tais como *Seminavis* (Naviculaceae) e *Halamphora* (Catenulaceae). No Brasil, há poucos estudos com foco na taxonomia das diatomáceas anforóides. Este estudo apresenta uma investigação taxonômica de cinco táxons do grupo, incomuns na diatomoflora brasileira: *Seminavis pusilla*, *S. strigosa*, *Amphora ectorii*, *Halamphora ghanensis* e *Halamphora* sp. *Seminavis strigosa* é idêntica em morfologia e métrica da valva à *Amphora twenteana*, e a sinonimização destas espécies é proposta. *Seminavis pusilla*, raramente encontrada em águas brasileiras, tem a sua distribuição ampliada. *Halamphora ghanensis* é um novo registro para o continente Americano, enquanto *Amphora ectorii* é uma novidade para sistemas aquáticos brasileiros. *Halamphora* sp. possui características ultraestruturais distintas em relação a espécies similares e provavelmente seja uma nova espécie para a ciência.

Palavras-chave: *Amphora*, Bahia, rio costeiro, *Halamphora*, *Seminavis*.

1. Introduction

Dorsiventrality was considered a relevant character throughout the taxonomic history of the diatoms. However, it is known that this feature occurs in at least seven diatom orders and evolved apart many times along diatom evolution (Round et al., 1990). Amphoroid taxa are an artificial group characterized by strongly dorsiventral valve outline, that in the past were considered within the genus *Amphora* Ehrenberg (Levkov, 2009). The two valves of a frustule are not parallel in relation to apical plane, due to the girdle bands wider in dorsal side than in ventral one (Round et al., 1990).

The genus *Amphora* was historically seen as a heterogeneous grouping by several authors (Van Heurck, 1880-1885; Cleve, 1895; Hustedt, 1930; Krammer,

1980; Round et al., 1990). Cleve (1895), recognizing the artificiality of *Amphora*, proposed to split it in nine subgenera, widely used by post researches, but probably each related to different raphid orders (Danielidis and Mann, 2002).

In this context, Mann in Round et al. (1990) proposed the genus *Seminavis* Mann, based on old *Amphora* subgenus *Cymbamphora* Cleve, which was characterized by uniseriate striae, lineolate areolae, two plastids in unequal size and raphe structure similar to *Navicula* Bory stricto sensu. *Seminavis* was included in Naviculales, because of raphe and areolae structures more similar to members of this order, rather than valve strongly dorsiventral shared with *Amphora*. Thereafter, a number of papers have shown the

morphology of former *Amphora* species, with transfers and new propositions to the genus *Seminavis* (Garcia-Baptista, 1993; Danielidis and Mann, 2002; 2003; Danielidis et al., 2006; Garcia, 2007; Wachnicka and Gaiser, 2007).

Later, Vyverman et al. (1998) and Williams and Reid (2006) proposed *Eunophora* Vyverman, Sabbe et Mann and *Colliculoamphora* Williams et Reid respectively, both classified into Eunotiales, to which some *Amphora* species were also transferred (Levkov, 2009).

Halamphora (Cleve) Levkov is another genus recently described, by splitting *Amphora* group, based on the presence of a single H-shaped plastid not extending to the dorsal girdle, raphe ledge in the dorsal side only, fused helictoglossa in proximal raphe endings and areolae internally occluded by hymenes and externally opened by simple foramina or complicated with short finger-like processes (Levkov, 2009). Nevertheless, there are still many *Amphora* species that needs further morphological studies to clarify their affiliations.

While there is about 41 species of *Amphora* lato sensu recorded to Brazilian freshwater, brackish and marine environments (Torgan et al., 1999; Procopiak et al., 2006; Tremarin et al., 2009; Silva et al., 2011; Eskinazi-Leça et al., 2012), little taxonomic information about amporoid taxa were published. Most of these records have no sufficiently morphometrical and photographic data to confirm the identifications, except in Sylvestre et al. (2001), Garcia (2007), Garcia and Souza (2008), Garcia and Talgatti (2011), Souza-Mosimann et al. (2011) and Bes et al. (2012).

During a floristic survey performed in Cachoeira river, Northeast Brazil, two taxa belonging to *Seminavis*, one belonging to *Amphora* and two assigned to *Halamphora* were identified. The aim of this study is to describe them, comparing with allied species and documenting new records to Brazil and the Americas.

2. Material and Methods

The Cachoeira river is situated in the Eastern Basin, state of Bahia, Northeast Brazil. This coastal river is around 500 km long and has 4,600 km² of drainage area. Inserted into the Atlantic rainforest, it rises to 800 m elev., cover the major urban centers and flows onto the continental shelf of Ilhéus municipality.

Plankton and periphyton attached to *Eichornia crassipes* (Martius) Solms-Laubach were collected from Cachoeira river, located at Itabuna municipality downtown (14° 47' 14.24" S; 39° 16' 10.12" W), in July 2009, about 25 km away from the coast. Samples were fixed with 4% formalin solution.

Subsamples were cleaned with KMnO₄ and HCl, according to Simonsen' method (1974) modified by Moreira-Filho and Valente-Moreira (1981). Permanent slides were mounted with Naphrax® (R.I.=1.74). Diatoms were observed, measured and photographed at Olympus BX40 light microscope equipped with Differential Interference Contrast and Olympus DP71 digital imaging capture system. For Scanning Electron Microscopy (SEM) analyses, subsamples

of cleaned valves were dried on stubs and covered with gold by sputter Balzers SCD030 and examined with a JEOL JSM 6360 at 15 kV, housed at the Electron Microscopy Center from the Federal University of Paraná, Brazil.

The classification and morphological terminology was based on Round et al. (1990) and Levkov (2009). Sample and slides was stored at the Federal University of Paraná herbarium (UPCB 65979, UPCB 65980).

3. Results and Discussion

Order Naviculales Bessey 1907 emend. D.G. Mann in Round et al. (1990)

Suborder Naviculineae Hendey 1937

Family Naviculaceae Kützing 1844

Genus *Seminavis* D.G. Mann in Round et al. (1990)

Seminavis pusilla (Grunow ex. A. Schmidt) Cox et Reid 2004, Seventeenth International Diatom Symposium 2002, p. 60.

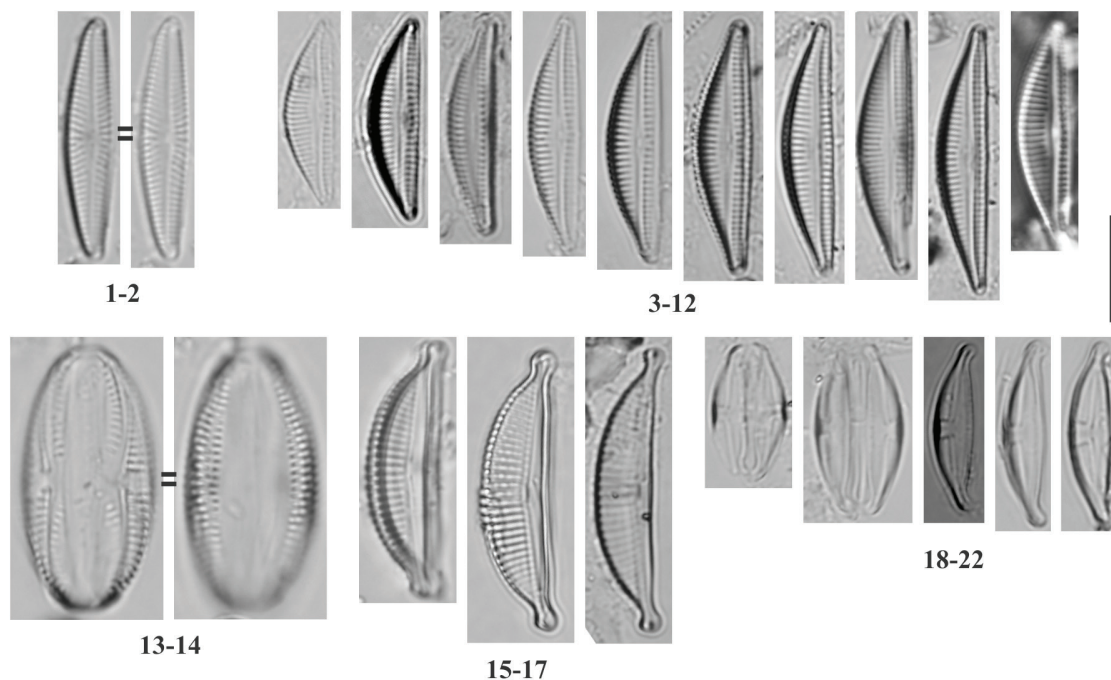
Basionym: *Cymbella pusilla* Grunow in A. Schmidt 1875, Issue 3, pl. 9, figs 36-37 (Figures 1, 2).

Valve moderately dorsiventral, semi-lanceolate, with convex dorsal margin and straight ventral margin, slightly convex in central part; apices rounded, slightly ventrally curved; axial area narrow, central area asymmetrical, more expanded for the dorsal side; striae radiate, some shortened in the central area; areolae inconspicuous; raphe straight, proximal endings slightly expanded, distal endings inconspicuous. Length 22.7 µm, width 4.6 µm, 16 striae in 10 µm.

Taxonomic Remarks: historically, the taxonomy of this species has been confused. *Cymbella pusilla* Grunow is the original proposition. Krammer (1997) transferred it to the cymbelloid monospecific genus *Navicella* Krammer (a later homonym), after renamed *Navicymbula* Krammer (Krammer, 2003). This genus was characterized by a combination of dorsiventrality (related to cymbelloid taxa), areolae and raphe structures typical of *Navicula* sensu stricto, and ecological data. It was the single "*Cymbella*" that could occur in high salinity environments (Krammer, 2003). However, Krammer (2003) provided SEM images that already make clear that this taxon is assigned to *Seminavis*, which has priority. Finally, Cox and Reid (2004) transferred this species to the latter genus, based on cladistical analysis into Naviculineae.

Distribution: it is an unmistakably species, cosmopolitan and common in brackish and freshwater waters (Cleve, 1895; Krammer, 2003). In Brazil, *Seminavis pusilla* is rarely found and has been only recorded to São Paulo State (Tundisi and Hino, 1981; Ludwig, 1996). This is the first citation of species to northeast Brazilian system.

Seminavis strigosa (Hustedt) Danielidis et Economou-Amilli in Danielidis & Mann 2003, Diatom Research, p. 30, figs 23-32.



Figures 1-22. Amphoroid diatoms from Cachoeira river, NE Brazil, LM. Figures 1, 2. *Seminavis pusilla*. Figures 3-12. *Seminavis strigosa*. Figures 13, 14. *Amphora ectorii*. Figures 15-17. *Halamphora ghanensis*. Figures 18-22. *Halamphora* sp. Scale bar = 10 µm.

Basionym: *Amphora strigosa* Hustedt 1949, p. 44, figs 30-33.

Synonym: *Amphora twenteana* Krammer 2003, p. 150, figs 138: 25-29 (Figures 3-12, 23-28).

Valves strongly dorsiventral, semi-lanceolate, with convex dorsal margin and straight ventral margin, sometimes slightly convex in central part; apices acuminate; axial area narrow, central area asymmetrical, more expanded to the dorsal side; striae straight, slightly radiate toward the apices; areolae inconspicuous; raphe straight, proximal endings slightly expanded, distal endings inconspicuous. In SEM, striae uniseriate, continuous with the valve mantle (Figures 23 e 24); dorsal axial area wider than the ventral one (Figures 24 e 25); external proximal endings slightly expanded (Figure 24); external distal endings hook-like, dorsally deflected (Figure 25); areolae apically elongate, externally opened by narrow slits (Figures 23-25) and internally occluded by hymens (Figure 28); internally, striae inserted between salient transapical ribs; ventral side expanded over the dorsal side so that the raphe fissure lies towards the dorsal side in the most of its length; small helictoglossa (Figures 26 - 28). Length 17.7-25.3 µm, width 4.4-5.6 µm, 16-22 striae in 10 µm; 60 lineolae in 10 µm.

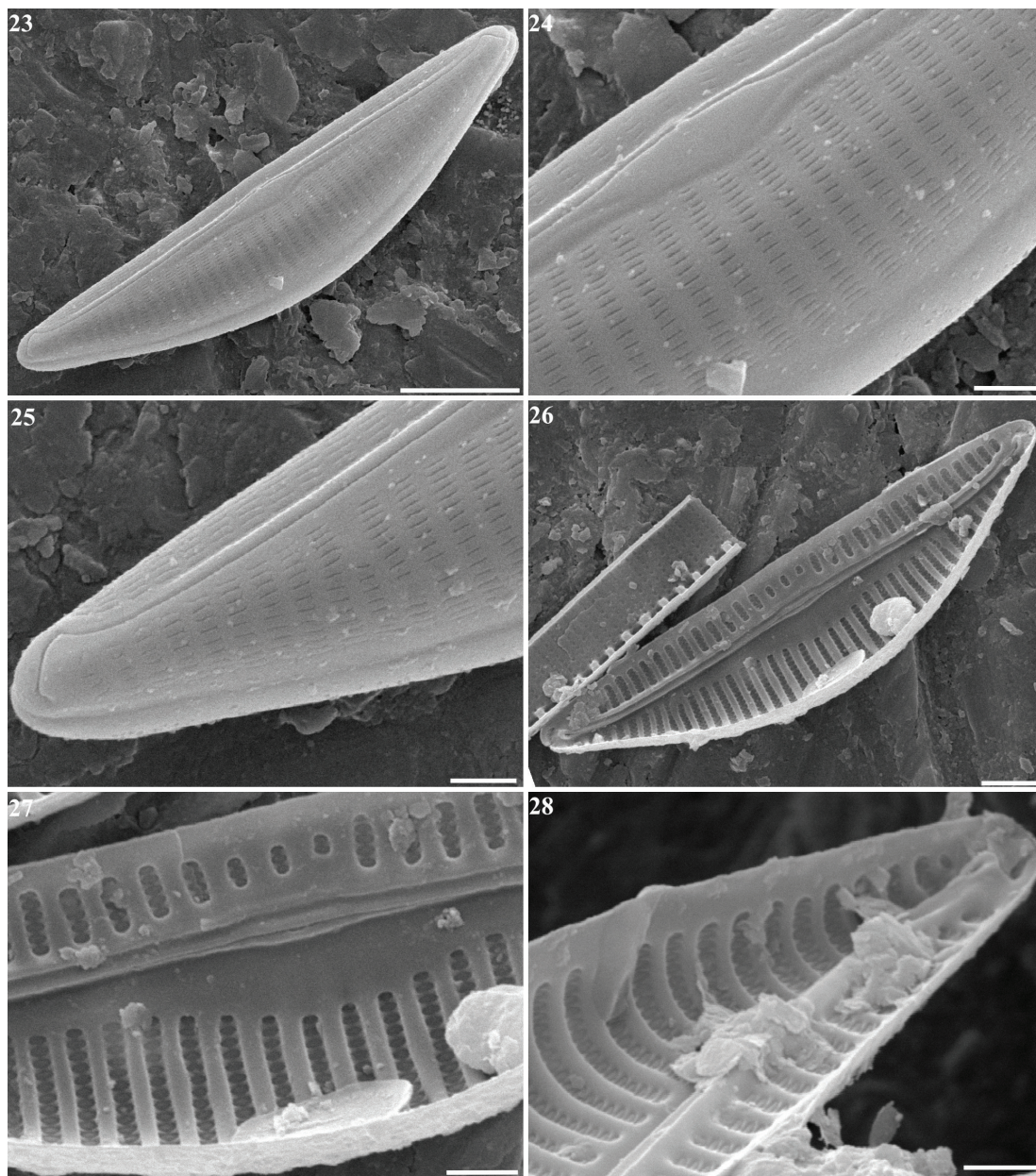
Taxonomic Remarks: *Seminavis strigosa* and *Amphora twenteana* are two identical taxonomic entities that differ only in ecological aspects. *Seminavis strigosa* is characteristically from brackish environments while *A. twenteana* was described from freshwater (see distribution). In both species' protologues, there are no morphological features that allow a clear distinction between the two taxa. We

realized that morphometric features known for both species are overlapping (Table 1). Specimens from this study were just slightly smaller than those showed by Hustedt (1949) and Danielidis and Mann (2003) but agree with specimens identified as *A. twenteana* (Krammer, 2003). You et al. (2008) registered longer and wider valves of *A. twenteana*, which overlap with Danielidis and Mann (2003) metrics. In all papers, illustrated individuals have identical valve outline, apices shape, central area and striae pattern. SEM illustrations showed similar morphology to those recorded in Danielidis and Mann (2003) for *S. strigosa*. *Amphora twenteana* have never been pictured in SEM but we believe that, in this case, the optical analysis would be enough to reveal a distinguishable feature.

Ecological differences related to salinity tolerance are not a general rule for all diatoms. Exceptionally, some species are known to occur in both coastal brackish and inland fresh waters, as *Cyclotella meneghiniana* Kützing, *Melosira varians* Agardh, *Pleurosira laevis* (Ehrenberg) Compère, *Fragilariforma subsalina* (Grunow) L. Bukhtiyarova, *Tabularia fasciculata* (Agardh) Williams et Round (Krammer and Lange-Bertalot, 1991) and *Seminavis pusilla* (Krammer, 2003).

We propose, therefore, the recognition of *Amphora twenteana* as later synonym of *Seminavis strigosa*, which has priority based on rules of botanical nomenclature.

Distribution: *Seminavis strigosa* seems to be widely distributed in brackish environments, with records in Wadi Islet and Ayun Musa oasis in the Sinai (Hustedt, 1949), Mesolonghi lagoon, western Greece (Danielidis and Mann,



Figures 23-28. *Seminavis strigosa*, SEM. Figure 23. External valve overview. Figure 24. External central area. Figure 25. External valve apex. Figure 26. Internal valve overview. Figure 27. Internal central area. Figure 28. Internal valve apex. Scale bars = 5 μm (Figure 23), 2 μm (Figure 26) and 1 μm (Figures 24, 25, 27, 28).

Table 1. Comparison between morphometric data of *Seminavis strigosa* and *Amphora twenteana* from this study and the literature. (*) indicate type material informations.

Taxa/ Admeasurements	Source	Length (μm)	Width (μm)	Striae in 10 μm
<i>Seminavis strigosa</i>	This study	17.7-25.3	4.4-5.6	16-22
	Hustedt (1949)*	22-26	\approx 4.0	17
	Danielidis & Mann (2003)	21-38	3.5-6.2	16-24.5
	Silva et al. (2010)	25-30	4-5	16
<i>Amphora twenteana</i>	Krammer (2003)*	18-26	4.7-5.4	16-18 (20)
	You et al. 2008	26-38	5-8	16-20

2003), and Southern Brazil (Silva et al., 2010). *Amphora twenteana* have been reported to freshwater environments, such as Twente Canal, Netherlands (Krammer, 2003) and Xinjiang, Northwestern China (You et al., 2008).

Order Thalassiosiphales D.G. Mann in Round et al. (1990)

Family Catenulaceae Mereschkowsky 1902

Genus *Amphora* Ehrenberg ex. Kützing 1844 emend. Levkov 2009

Amphora ectorii Levkov 2009, p. 58, pl. 66, figs 1-9, pl. 172, figs, 3-6 (Figures 13 and 14).

Valve strongly dorsiventral, semi-lanceolate, with convex dorsal margin and slightly concave ventral margin, margins almost parallel in central part; apices acuminate; axial area narrow, biarcuate; central area broader on ventral side and linearly expanded on dorsal side; striae straight to radiate in dorsal side and radiate to convergent in ventral side; areolae inconspicuous; raphe strongly biarcuate; proximal endings dorsally oriented, distal endings inconspicuous. Length 24.6 μm , valve width 4.6 μm , frustule width 12.3 μm , 14 striae in 10 μm .

Taxonomic Remarks: our specimen is slightly less wide than those designated in the protologue (5.5-8 μm) (Levkov, 2009). *Amphora affinis* Kützing, *A. alpestris* Levkov, *A. copulata* (Kützing) Schoeman et Archibald and *A. subconstricta* Levkov are similar species. However, *A. affinis* and *A. copulata* have less biarcuate raphe, conspicuous elongated areolae and trapezoidal central area on ventral side; *A. alpestris* is longer, has coarser areolae and wider central area on dorsal side; and *A. subconstricta* is tumid in central valve on ventral side, presents parallel striae, less biarcuate raphe and shorter central area (Levkov, 2009).

Distribution: it is reported to brackish/marine environments. The species is common in the type locality, Maracaibo Lagoon, Venezuela, attached to *Potamogeton* L. (Levkov, 2009). This is the first record in Brazilian waters.

Genus *Halamphora* (Cleve) Levkov 2009

Halamphora ghanensis Levkov 2009, p. 194, pl. 105, figs 12-19, pl. 235, figs 1-7 (Figures 15-17).

Valves strongly dorsiventral, semi-lanceolate, with convex dorsal margin and straight ventral margin; apices capitate, ventrally curved; axial area narrow, straight; central area absent; dorsal striae finely punctuate, radiate, ventral striae inconspicuous; raphe branches arched; proximal endings slightly expanded, dorsally oriented; distal endings inconspicuous. Length 23.8-27.2 μm , valve width 5.3-6.4 μm , 13-14 striae in 10 μm .

Taxonomic Remarks: our population agreed with the type material, except to valves wider (5-5.6 μm in the protologue) and slightly less dense striae (14-16 in 10 μm) (Levkov, 2009). Levkov (2009) points out that a peculiar feature of *Halamphora ghanensis* is its narrow range of

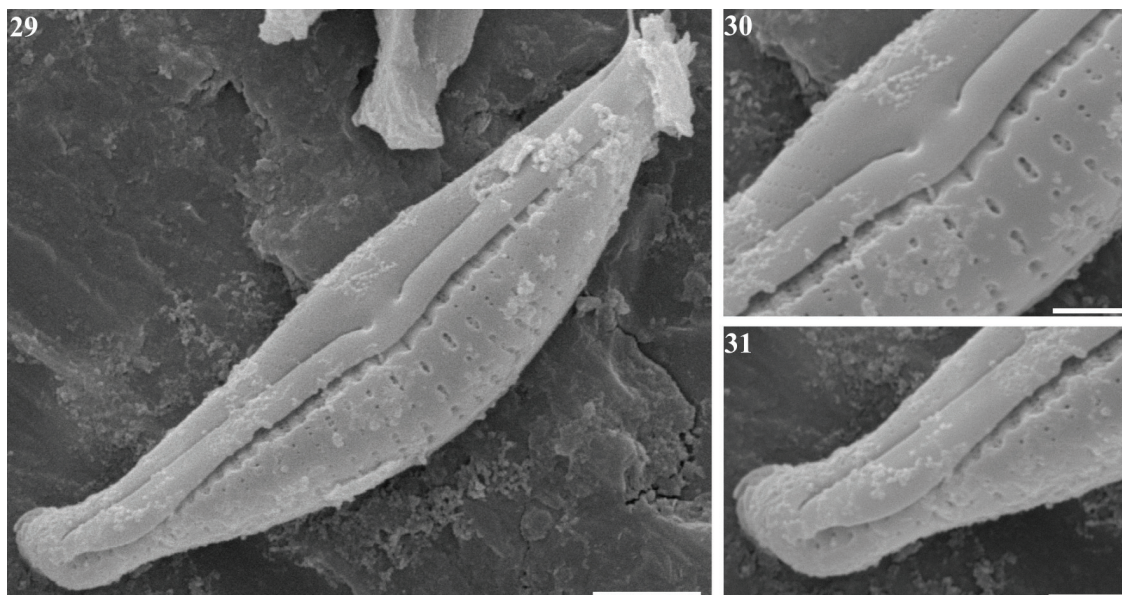
length measurements, varying only in 3 μm (24-27 μm) in type population. This feature was also observed here. Similar species are *H. acutiuscula* (Kützing) Levkov, *H. coffeaformis* (Agardh) Levkov, *H. sabiniana* (Reimer) Levkov, *H. subholsatica* (Krammer) Levkov, *H. tumida* (Hustedt) Levkov and *H. turgida* (Gregory) Levkov. But *H. acutiuscula* has wide axial and central area, absent in *H. ghanensis* (Levkov, 2009); *H. coffeaformis* has higher striae density (16-26 in 10 μm) (Archibald and Schoeman, 1984); *H. sabiniana* has central striae more spaced and broad ventral fascia (Levkov, 2009); *H. subholsatica* has distinct punctuate areolae and tumid ventral margin (Levkov, 2009); *H. tumida* has ventral striae recognizable, slightly tumid ventral margin and apices less ventrally bent than *H. ghanensis* (Sar et al., 2004); finally, *H. turgida* is broader (valve width 7.5-9.5 μm) and lower striae density (10-12 in 10 μm) (Levkov, 2009).

Distribution: freshwater species of poorly known distribution. Recorded to Ghana, West Africa (Levkov, 2009). This is the first record to American continent.

Halamphora sp. (Figures 18-22, 29-31).

Valves strongly dorsiventral, semi-lanceolate, with convex dorsal margin and slightly convex ventral margin; apices capitate produced, ventrally curved; axial area narrow on dorsal side, semi-lanceolate on ventral side; central area with a semi-stauros extending until the valve margin, on dorsal side; striae inconspicuous in LM; raphe branches arched, proximal endings slightly expanded, dorsally oriented. In SEM, striae uniseriate; dorsally irregular, biseriate (especially near to raphe ledge), sometimes uniseriate, with coarse areolae; ventral striae uniseriate, shortened in central area, with poroidal and delicate areolae (Figures 29-31); external central area absent on dorsal side, indicating that the central thickening observed in LM is internal (Figure 30); raphe ledge narrow, elevated from the valve surface (Figure 30); proximal raphe endings slightly expanded, dorsally deflected (Figure 30). Length 12.4-17.4 μm , valve width 3-3.9 μm , 25 dorsal striae in 10 μm ; 34 ventral striae in 10 μm .

Taxonomic Remarks: in LM, our taxon is similar to *Amphora charrua* Metzeltin, Lange-Bertalot et García-Rodríguez and *Halamphora submontana* (Hustedt) Levkov, being more closely related to *Halamphora montana* (Krasske) Levkov, with respect to measurements, shape of apices and central area (Levkov, 2009). However, by analyzing the SEM images, it is notable that our taxon does not agree with any of those species. *Amphora charrua* has not produced apices nor raphe ledge, and the morphology of areolae and raphe are clearly distinct; *Halamphora montana* has uniseriate striae on dorsal side near to raphe ledge, wide central area on dorsal side and higher striae density (40-45 in 10 μm); *H. submontana* has biseriate striae on dorsal side near to raphe ledge and central area on dorsal side is areolate, although the central striae are more spaced; however the striae density is higher (32-36 in 10 μm) and the areolae morphology on dorsal side is clear.



Figures 29-31. *Halamphora* sp., SEM. Figure 29. External valve overview. Figure 30. Detail of central area. Figure 31. Detail of apex. Scale bars = 2 μ m (Figure 29) and 1 μ m (Figures 30, 31).

These findings indicate that our taxon is probably distinct from the species described in the literature. Unfortunately, we could not find more valves in SEM analysis, including internal views, due its rarity in the samples. In *Halamphora*, different species have also differences in internal structures. Under these conditions, the precise identity of this taxon is subject to further studies of its morphology by SEM.

Distribution: just found in samples from Cachoeira River, northeastern Brazil.

This study presented the descriptions of five uncommon amphoroid taxa from Brazilian diatom flora. *Halamphora ghanensis* had never been recorded to American continent while *Amphora ectorii* is new records to Brazilian aquatic systems. Additionally, *Amphora twentean* is proposed as heterotypic synonym of *Seminavis strigosa*. *Halamphora* sp. has distinct ultrastructural features in relation of similar species and probably can be a new species for science. This data corroborate the need of more floristic diatom studies in Brazil, especially in unexplored environments, in order to reach a closer knowledge of the real diatom diversity that inhabit this extense country.

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