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## ***Tryblionella persuadens* comb. nov. (Bacillariaceae, Diatomeae): new observations on frustule morphology of a seldom recorded diatom**

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### **ABSTRACT**

The species originally described from brackish waters of the Venetian Lagoon as *Nitzschia persuadens* is a diatom rarely cited in the literature since its proposition and it is here recorded for the first time in a freshwater environment in South America. Morphological features of this species, such valve slightly panduriform, with a longitudinal straight fold of the valve face, poroidal areolae, and strongly eccentric raphe system clearly assign this species to *Tryblionella*, and the transfer was made. Here we present new observations on the frustule morphology and comparisons with related species. Light and scanning electron microscopy data of *Tryblionella persuadens* comb. nov. from Cachoeira River, Northeastern Brazil are documented.

**Key words:** Bacillariophyceae, Brazil, coastal river, *Nitzschia persuadens*.

### **INTRODUCTION**

*Tryblionella* W. Smith is a widespread epipelagic genus, occurring in marine, brackish or high conductivity freshwaters (Round et al. 1990). This taxon was erected to generic status by Round et al. (1990), grouping the species included in four old sections from *Nitzschia* Hassal: *Tryblionellae* (W. Smith) Grunow, *Circumsutae* Grunow, *Apiculatae* Grunow and *Pseudotryblionella* Grunow. This proposition have not been adopted by all diatomists, who considered unclear the selected discriminating criteria established (Witkowski et al. 2004). However, molecular studies performed on Bacillariaceae members have showed that *Nitzschia sensu lato* is not a monophyletic group, and probably should be split into several genera

(Lundholm et al. 2002, Rimet et al. 2011). Rimet et al. (2011) advocated the taxonomic separation of *Tryblionella* and *Psammodictyon* D. G. Mann in Round, Crawford et Mann. (specially the later) from *Nitzschia sensu stricto*. Nevertheless, there are no sufficient representatives of *Tryblionella* included in those phylogenetic analyses for a reliable understanding about the relationships among nitzschioid genera. Whereas classification must be consistent with the phylogenetic relationships among groups, molecular tools combined with morphological features based on frustule, life form and plastids position (see Mann 1978) should clarify the genera consistency and their affiliations.

The species assigned to the genus *Tryblionella* were well documented, described and discussed in publications of the nineteenth (eg. Grunow

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1862, Van Heurck 1880-1885, 1896, Peragallo and Peragallo 1897-1908) and twentieth centuries (Frenguelli 1923, 1924, 1942, Hustedt 1930, Mann 1978). All of them, except for Mann (1978), were illustrated by drawings only. There is no recent monograph dealing with all or large number of *Tryblionella* species. Witkowski et al. (2004) is the only recent study exclusively about *Tryblionella* representatives, redefining *T. parvula* (W. Smith) Ohtsuka et Y. Fujita complex and describing four new species based on light and electron microscopy observations.

In Brazil, 23 *Tryblionella* taxa have been recorded; most of them are marine coastal taxa from the Southern region (Torgan et al. 1999, Procopiak et al. 2006, Bes and Torgan 2008, Tremarin et al. 2009, Silva et al. 2011). Little is known about coastal watershed diatom floras, therefore, the same occurs about possible interactions between continental and marine/estuarine communities.

A recent floristic survey of samples collected from a coastal river in Northeast Brazil revealed a species identified as *Nitzschia persuadens* Cholnoky. This taxon is rarely found in the literature, and its transfer is necessary based on its affinities with other *Tryblionella* taxa. This species is described here, based on light and electron microscopy, representing new observations on the frustule structure and the first record to South America.

#### MATERIALS 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 km<sup>2</sup> of drainage area. Inserted into the Atlantic rainforest, it rises to 800 m above sea level, covers major urban centers and flows onto the continental shelf off Ilhéus municipality (Torres et al. 2001).

Plankton and periphyton attached to *Eichornia crassipes* (Martius) Solms-Laubach samples were

collected from Cachoeira River, located at downtown Itabuna (14°47'14.24"S; 39°16'10.12"O), in July 2009, about 25 km away from the coast. Samples were fixed with 4% formalin solution.

Subsamples were cleaned with KMNO<sub>4</sub> and HCl, according to the method proposed by Simonsen (1974), modified by Moreira-Filho and Valente-Moreira (1981). Permanent slides were mounted with Naphrax<sup>®</sup> (R.I. = 1.74) and were stored at the herbarium of the Universidade Federal do Paraná (UPCB 65979, UPCB 65980).

Diatoms were observed, measured and photographed with a Olympus BX-40 light microscope equipped with phase contrast and a Olympus DP-71 digital imaging system.

For scanning electron microscopy (SEM) analyses, subsamples of cleaned valves were dried on stubs and coated with gold by sputter Balzers SCD030 and examined with a JEOL JSM 6360 at 15 kV. They are housed at the Electron Microscopy Center from the Universidade Federal do Paraná, Brazil.

#### RESULTS

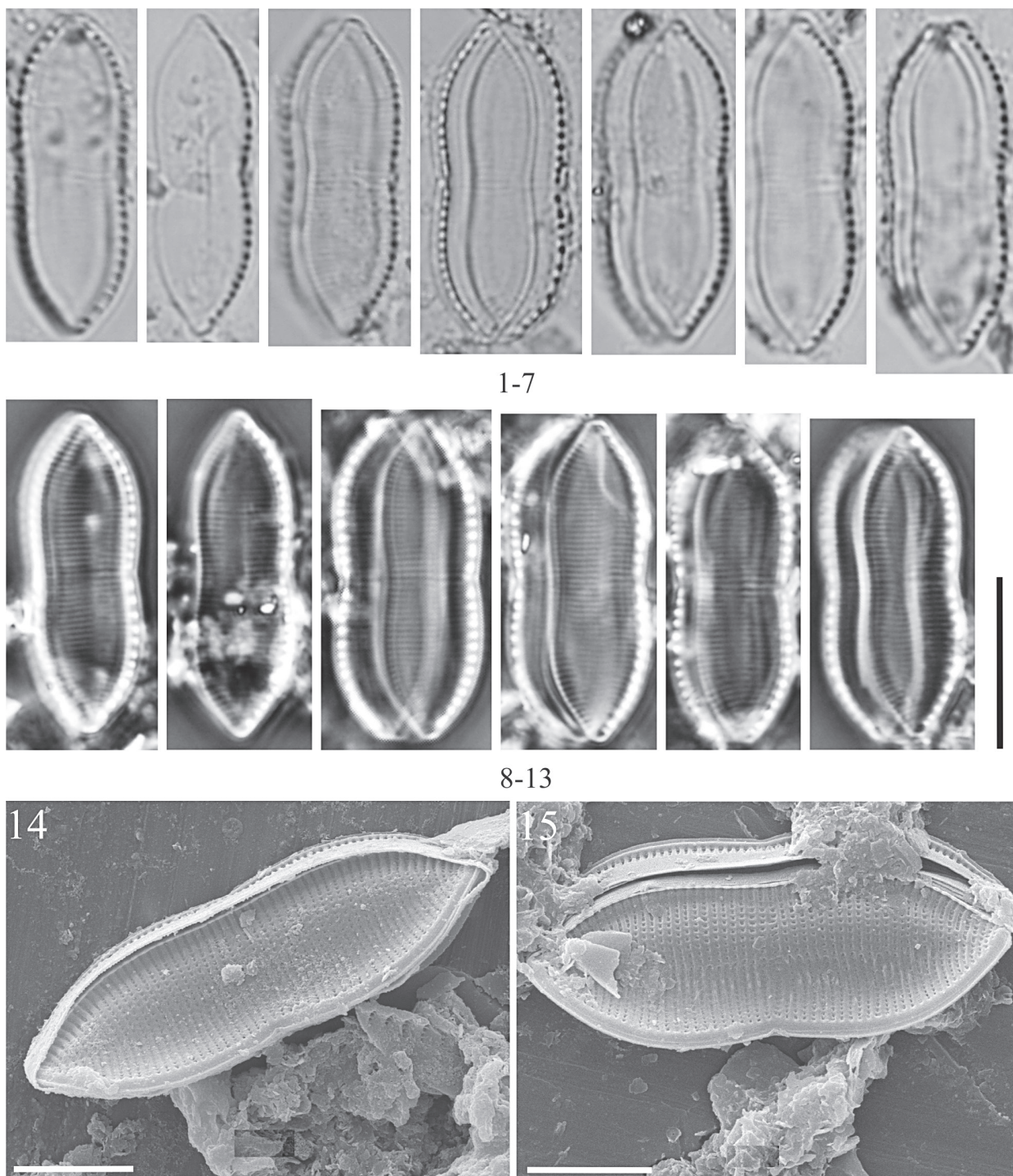
##### *Tryblionella persuadens* (Cholnoky) Cavalcante, Tremarin et T. Ludwig comb. nov.

Basionym: *Nitzschia persuadens* Cholnoky. Hydrobiologia, vol. 17, n. 4, p. 319-320, fig. 74, 1961.

##### LM DESCRIPTION:

(Figs 1-13)

Valves linear-lanceolate, slightly panduriform, constricted in the median portion with cuneate-subrostrate apices, 18.1-20.0 µm long, 4.3-5.7 µm wide (n = 20). Valve face with a shallow longitudinal fold, sternum absent. Fibulae 12-16 in 10 µm, regularly distributed along the valve, the median two farther apart. Striae 28-30 in 10 µm, delicate, straight in most of the valve and curved toward the apices. Median stria sometimes wider than the others. Poroids inconspicuous.



**Figs 1-15** - *Tryblionella persuadens* comb. nov. **1-7**. Valves in bright field (LM). **8-13**. Valves in phase-contrast (LM). **14-15**. Whole frustules in SEM. Scale Bars: Figs **1-13** = 10  $\mu$ m, **14-15** = 5  $\mu$ m.

SEM DESCRIPTION:

Valve face with a shallow longitudinal fold, externally depressed in the region near the raphe and

elevated near the opposite margin (Figs 14-16). Margins bounded on one side by raphe strongly eccentric and on the other side by a narrow



marginal ridge, joining the valve face with a shallow mantle (Figs 14, 15). Striae uniseriate. Median striae sometimes biseriate (Fig. 15), seeming to be wider than the others under LM. Poroids round to rectangular, conspicuous only in electron microscopy, 37-40 in 10 µm, occluded by hymenes (Fig. 17). Mantle striated near the raphe, 28-30 striae in 10 µm, with granulated surface (Fig. 18). Girdle bands narrow, delicate, granulated (Fig. 16-18); valvocopula with one row of poroids (Fig. 16). Internally, fibulae are robust (Fig. 19). Poroids are formed by tiny round apertures, which ornate valve face as fibulae wall; there are no longitudinal sternum (Fig. 19-21).

#### DISCUSSION

The species described here is clearly assigned to *Tryblionella*. According to Round et al. (1990), it is a genus difficult to circumscribe. The distinction among *Tryblionella*, *Psammodictyon* and *Nitzschia sensu stricto* is given by a combination of diagnostic features, based on valve, raphe and fibula structures. However, very eccentric raphe system accompanied by a longitudinal straight fold of the valve face is useful to *Tryblionella* affiliation (Mann 1978). In the species protologue, Cholnoky (1961) have already noted that *T. persuadens* should be included in this group (former Section *Tryblionellae*).

Related species are *Tryblionella aerophila* (Hustedt) D.G. Mann in Round, Crawford et Mann, *T. bathurstensis* (Giffen) D.G. Mann in Round, Crawford et Mann, *T. sibula* (Giffen) D.G. Mann in Round, Crawford et Mann, *Nitzschia buschbeckii* Witkowski, Lange-Bertalot et Ruppel, and *N. ligowskii* Witkowski, Lange-Bertalot, Kociolek et Brzezińska. However, all of them present transapical striae interrupted along the apical axis by a longitudinal sternum, while *T. persuadens* does not. Moreover, *T. persuadens* has smaller length and width, fine structure, evidenced by denser fibulae and striae and median stria wider

than the others. Main morfometric, ecologic and distributional distinctive features among these taxa are showed in Table I.

Based on type material analysis made by Krammer and Lange-Bertalot (1988) and by type illustration designated by Simonsen (1987), *T. aerophila* shows wider valves, broader fold, and coarser fibulae. Note that Figure 15 (Plate 51) from Krammer and Lange-Bertalot (1988), named '*N. aff. aerophila*', is similar, if not identical to *T. persuadens*, and should not be confused with *T. aerophila*.

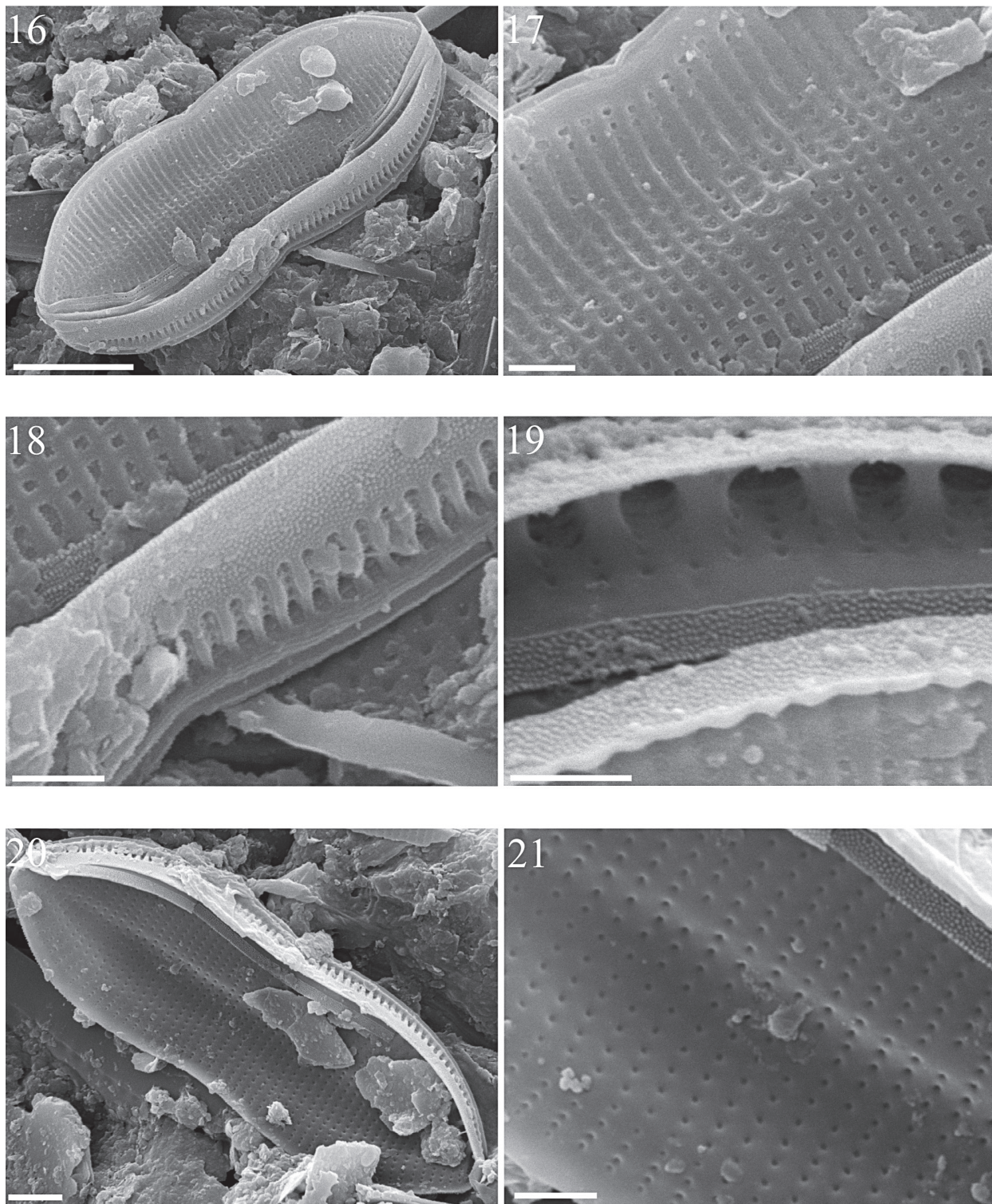
*Tryblionella bathurstensis* distinguishes from *T. persuadens* by having wider valves, less dense striae and faint, though conspicuous, poroids in LM, 28-30 in 10 µm, (Giffen 1970). On the other hand, *T. sibula* is longer, wider, shows more protracted apices, broad sternum and less dense fibulae and striae (Giffen 1973).

*Nitzschia ligowskii*, although a recently described species (Witkowski et al. 2004), used to be known by *N. subconstricta* Grunow, a name never validly published. It differs from *T. persuadens* by being longer and wider with lower striae density, and the marginal poroids are larger (Witkowski et al. 2004). Another similar species, *N. buschbeckii* is longer, wider, possesses a broad sternum, lower striae and fibulae densities and the poroids in the valve margins are coarser (Witkowski et al. 2004). According to our sense on phylogenetic relations among the Bacillariaceae genera, *N. ligowskii* and *N. buschbeckii* should be transferred to *Tryblionella*.

To a lesser extent, the species mentioned above also differ by habitat. *T. aerophila* seems to be a typically freshwater species. Krammer and Lange-Bertalot (1988) noted that individuals of *T. aerophila* from brackish water bodies recorded in Central and South America and South Africa cannot be safely considered as conspecific; *T. bathurstensis* is from brackish, while *T. sibula*, *N. ligowskii* and *N. buschbeckii* are from marine habitats. *T. persuadens* was found in brackish (original description) and freshwater (this study) environments.

TABLE I  
Morphometric, ecologic and distributional features of *Tryblionella persuadens* and related taxa.

	References	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	Fibulae in 10 $\mu\text{m}$	Striae in 10 $\mu\text{m}$	Poroid structure	Sternum	Habitat	Distribution
<i>T. persuadens</i>	Our data	18.1-21	4.3-6	12-16	28-30	round to rectangular	absent	freshwater	coastal river, NE Brazil
	Cholnoky (1961), protologue	18-25	6-7	12	32	-	absent	brackish	Venetian Lagoon, NE Italy
<i>T. aerophila</i>	Krammer and Lange-Bertalot (1988), type material	23-31	6-8	9-11	26-30	-	present	freshwater, epiphyte in a hepatic bank	Bremen, NW Germany
<i>T. bathurstensis</i>	Giffen (1970), protologue	20-25	8-9	11-13	22-25	-	present	brackish, on sand or mud	Kowie River, Cape Province, South Africa
<i>T. sibula</i>	Giffen (1973), protologue	30-36	7-8	10-12	22-25	-	present	marine	St. Helena Bay, Cape Province, South Africa
<i>Nitzschia</i> <i>buschbeckii</i>	Witkowski et al. (2004), protologue	26-52	6.3-9.3	10-12	21-24	round, variable size	present	marine	Indian Ocean Sector, near to Antarctic continent
<i>Nitzschia</i> <i>ligowskii</i>	Witkowski et al. (2004), protologue	10-57	5-9	12-15	22-27	round, variable size	present	brackish- marine	cosmopolitan



**Figs 16-21** - *Tryblionella persuadens* *comb. nov.* in SEM. **16**. External view of whole valve. **17**. Detail of central valve face, showing poroids structure. **18**. Detail of granulated mantle. **19**. Internal view of fibulae structure. **20**. Valve in internal view. **21**. Detail of valve center in internal view, showing internal poroid aperture. Scale Bars: Figs **16** = 5  $\mu$ m; Figs **17-19**, **21** = 1  $\mu$ m; Fig. **20** = 2  $\mu$ m.



*Tryblionella persuadens* is a poorly recorded taxon. Since its proposition, based on brackish population from Venetian Lagoon (Cholnoky 1961), the only records we have found were in Witkowski et al. (2000, p. 808 and 820), which are doubtful. Figure 7 (p. 808) showed a specimen with the following characteristics: 23.3 µm long, 10.6 µm wide, 11 fibulae and 24 striae in 10 µm, discernible poroids, which are not compatible with the original description of *T. persuadens*. The same occurs with figures 16 (p. 808) and 7 (p. 820), named *N. cf. persuadens*. Those individuals are wider (7.3, 8 µm) and show lower striae (21, 26 in 10 µm) and fibulae (10, 8 in 10 µm) densities. This is therefore the first record of this diatom in continental environments.

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#### RESUMO

*Nitzschia persuadens*, originalmente descrita a partir de águas estuarinas da Laguna de Veneza, é uma diatomácea raramente citada na literatura desde a sua proposição e é registrada aqui pela primeira vez em ambiente epicontinental da América do Sul. Características morfológicas desta espécie, tais quais valva levemente panduriforme, com uma ondulação reta longitudinal na face valvar, areolas poroidais e sistema de rafe fortemente excêntrica, claramente atribui esta ao gênero *Tryblionella*, e a sua transferência foi feita. São apresentadas novas observações sobre a morfologia da frústula e comparações com espécies afins. Análises em microscopia óptica e eletrônica de varredura de *Tryblionella persuadens* comb. nov. do rio Cachoeira, Nordeste do Brasil, foram documentadas.

**Palavras-chave:** Bacillariophyceae, Brasil, rio costeiro, *Nitzschia persuadens*.

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