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ARTICLE

Nitzschia transtagensis sp. nov. (Bacillariophyceae) from a spring in Southern Portugal

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ABSTRACT
A new species of *Nitzschia* Hassall is described from a small spring in southern Portugal. *Nitzschia transtagensis* sp. nov. has a unique morphology in the *Lineares* section, resembling *Nitzschia hierosolymitana, N. vitrea* and its variety *salinarum*. The new species is unique in that it has a convex primary (raphe) side with a slightly undulated abvalvar edge of the mantle, and a clearly undulated secondary valve side. This taxon also has a dimorphic open, ligulate cingulum composed of an apically asymmetric valvocopula having one row of roundish pores on the pars interior and attached to the valve secondary side, and two rows of pores (one of roundish and one of elongate pores) attached to the primary side of the valve. All remaining girdle elements have a single row of approximately round pores. The new species is compared to morphologically similar taxa and its ecology is discussed based on available information and literature. Additionally, it was found that a variant of the new species had been reported previously from Sardinia as "*Nitzschia* aff. *ebroicensis*", but the Italian population has coarser stria and puncta densities.

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Bacillariaceae; diatoms; Iberian Peninsula; Europe; taxonomy

Introduction

The genus *Nitzschia* Hassall comprises a large group of morphologically diverse species (Mann 1981). Although certain groups within the genus, such as the *Lanceolatae* section (*sensu* Krammer and Lange-Bertalot 1988), including the widely cited *Nitzschia palea* (Kützing) W. Smith and *Nitzschia inconspicua* Grunow, are regarded as lacking sufficient morphological differences to distinguish them reliably (Denys and Lange-Bertalot 1998; Trobajo et al. 2013; Rovira et al. 2015), the reality is that a taxonomy based on geographically and metapopulation wide surveys is still under development. Together with the study of type material, these metapopulation studies could serve in a better delimitation of taxonomic boundaries (Hlúbiková et al. 2009).

Though molecular studies prove to be useful in the distinction of taxa with similar appearance (e.g. Lobban et al. 2019), the analysis of morphological characters using a combination of light (LM) and scanning electron (SEM) microscopy continues to be a powerful tool to find distinguishing features and producing clear cut separations among populations regarded as single, broadly circumscribed species (e.g. Tudesque et al. 2008; Morales 2015).

The recent efforts in using molecular tools to ease the identification of *Nitzschia* species (e.g. Trobajo et al. 2010; Rimet et al. 2011, 2016) are impaired by a dearth of complete reference databases for diatoms and the difficulty to apply such incomplete information on a broad geographical and taxonomically spread bases (Zimmermann et al. 2014; Khan-Bureau et al. 2017). Therefore, traditional morphological identification, which is still largely used in applied ecological analyses, still represents a viable tool and utilizes considerably less and cheaper resources than molecular studies, even when SEM studies are included.

In fact, new species of *Nitzschia* are constantly being described based on morphology, however, very few of the new species can be considered as morphologically strikingly different (e.g. Xie and Li 1994; Lundholm and Moestrup 2000) and, therefore, the great majority often require a strong literature support and taxonomic argumentation (e.g., Hamilton and Laird 2001; Hamsher et al. 2016), and in some cases even the examination of type material is necessary (Hlúbiková et al. 2009).

While some regions of Europe, e.g. central Europe, have been largely explored for diatoms, but continue to yield new taxa, some other regions are seriously understudied and the reference bibliography is poor and insufficient to represent the local floras. This is a serious shortcoming for the implementation of the Water Framework Directive (Wallin et al. 2003), which, at this point, should already count with a rather strong taxonomic basis for water quality determination. The Iberian Peninsula is one of those regions in urgent need of updated taxonomical works since water quality issues are aggravated by dam construction and climate change

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(Morais 2008; Cardoso et al. 2016; Soares et al. 2017). To date, there are only a few floristic works (e.g. Blanco et al. 2010), but the majority of information comes in the form of articles focusing on specific groups (none on *Nitzschia*), or specific habitats (e.g. Cantoral-Uriza and Aboal Sanjurjo 2008; Novais et al. 2015).

In this manuscript, we describe a new *Nitzschia* species in the *Lineares* section (*sensu* Krammer and Lange-Bertalot 1988) from southern Portugal, collected from a spring. The rather distinctive features of the new species are described based on combined LM and SEM data and contrasted to taxa from the literature.

Material and methods

Diatoms were collected in spring (May 26) and summer (Sept. 21) 2017, and winter (Jan. 16) and spring (June 7) 2018 from the João Dias spring and the stream of the same name formed by it (Guadiana basin, Figure 1, coordinates 37º 51′ 17.75″ N, 7º 18′ 00.52″ W).

Simultaneously with diatom sampling, water chemistry was determined *in situ* using a multi parametric probe TROLL 9500 PROFILER XP, measuring temperature (T, °C), pH, dissolved oxygen (DO, % of O_2 saturation), and electrical conductivity (EC, µS cm^{-1}). In the laboratory, the following parameters were determined following APHA (1995) standard methods, total nitrogen (TN, mg N L^{-1}), nitrates (NO_3, mg NO_3-N L^{-1}), nitrites (NO_2, mg NO_2-N L^{-1}), total phosphorus (TP, mg P L^{-1}) and phosphates (PO_4, mg PO_4-P L^{-1}).

Biological samples collected in the stream were obtained by brushing 5–7 hand-sized rocks with a toothbrush and washing them with ethyl alcohol (96%), while the spring samples were collected from moss. Aliquots from each sample were oxidized in the laboratory with hydrogen peroxide (35%) in a sand bath (210°C) for 36 h after which material was rinsed with distilled water until neutrality (INAG 2008). Permanent slides were mounted in Naphrax® and valves and frustules were identified to species level using a Leica DMLB light microscope equipped with a 100x HCX PL APO oil immersion objective (N.A. 1.40) and a Leica DC 500 camera. A minimum of 400 valves were identified and counted on each slide in order to assess the relative abundance of taxa composing the community (INAG I. P. 2008). The identification was based on reference floras (e.g., Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Hofmann et al. 2011; Lange-Bertalot et al. 2017) as well as recent bibliographic sources, including the series "Diatoms of Europe", "Iconographia Diatomologica", "Bibliotheca Diatomologica" and relevant taxonomic papers, such as Reichardt (1997), Van de Vijver et al. (2011) and Novais et al. (2011).

For SEM analysis, a portion of the oxidized material was rinsed with deionized water over a 3-µm pore glass fiber filter and coated with platinum using a BAL-TEC MED 020 Modular High Vacuum Coating System for 30 s at 100 mA. A Hitachi SU-70 electron microscope operated at 5 kV and 10 mm distance was used for the analysis. All micrographs were digitally manipulated

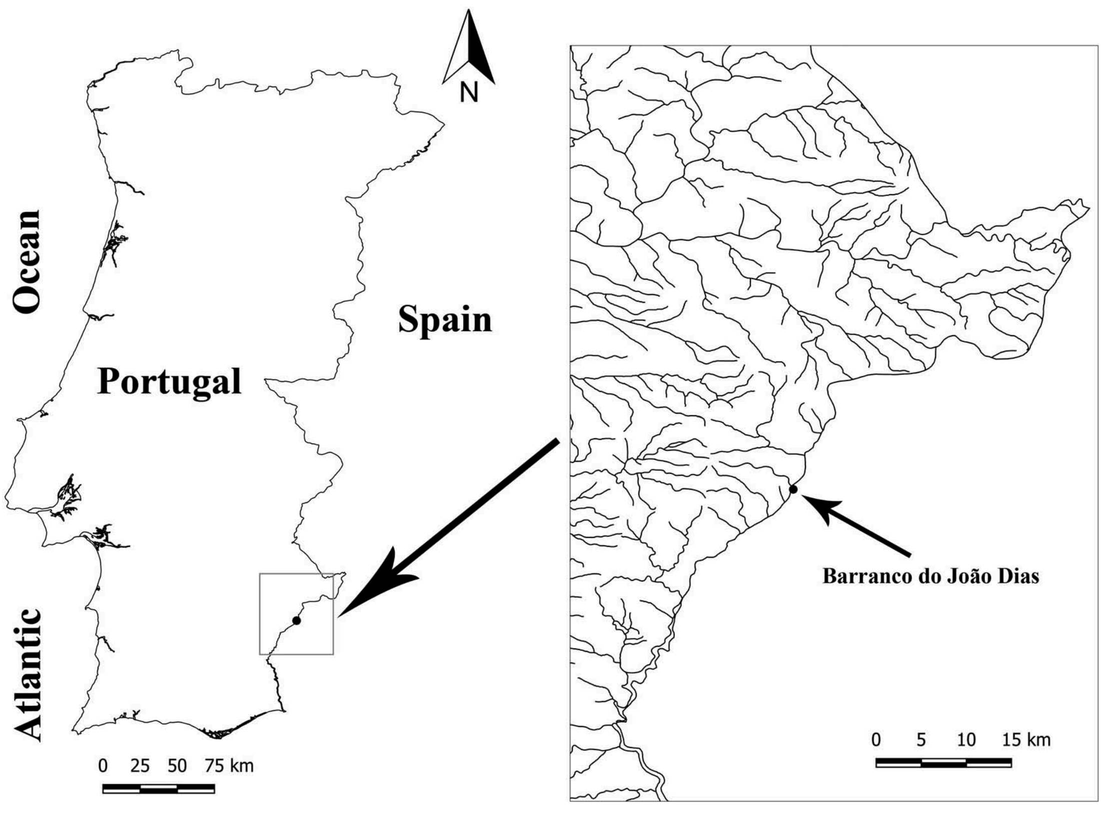

Figure 1. Localization of type locality in southern Portugal.

and plates containing LM and SEM pictures were mounted using CorelDraw X6®.

The Specific Pollution Sensitivity Index (SPI) was calculated from diatom abundances (Coste in Cemagref 1982), using the OMNIDIA v. 5.5 software (Lecointe et al. 1993).

Morphological terminology follows Barber and Haworth (1981) for valve shape and stria pattern, Cox and Ross (1981) and Cox (2012) for lateral extensions (virgae) and crossbars (vimines), and Round et al. (1990) for areolar substructures, raphe and girdle bands.

Results

Nitzschia transtagensis E.Morales, Novais, C.E.Wetzel, Morais & Ector sp. nov. (Figure 2–26)

Description

Frustules with nitzschioid symmetry, lanceolate with a convex raphe side and clearly undulated opposite side of the valve (Figure 2–12, 20). Valves with rostrate to capitate ends, apically asymmetrical (Figure 2–11), sometimes slightly heteropolar (Figure 3, 4, 10) with convex primary side, but sometimes with undulated abvalvar edge of mantle (Figure 12, 23), and conspicuously undulated secondary side (Figure 2–12, 20). Length 30.5–50.4 µm, width 4.5–6.0 µm, fibula density 6–10 in 10 µm, stria density 30–35 in 10 µm (n = 15). Raphe eccentric, situated along an unornamented hyaline area, distinctly elevated on a keel with respect to the valve face and mantle (Figure 12–16). Raphe slit continuous at central nodule (Figure 12, 14), curved toward mantle at both distal extremes (Figure 15, 16) and internally ending in raised helictoglossa (Figure 19, 20). Fibulae round to square, irregularly spaced at central nodule, with valve face base extending onto 2–4 virgae (Figure 2–11, 17–22). Fibula at central nodule wider. Striae barely visible in LM, composed of round to transapically elongated areolae (Figure 12–22), continuous from valve face to the secondary side of mantle, but interrupted by the hyaline area of the keel on the primary side. Areolae unevenly spaced due to variability in vimen length (Figure 12–16, 18–22), and internally occluded by hymens (Figure 18–22). Girdle bands open, ligulate and with a recessed pars interior to accommodate the valve mantle or the previous girdle element (Figure 23–26). Copula narrower, bearing a single row of poroids located on the transition between pars interior and exterior (Figure 23–26). Valvocopula with one row of ca. round poroids on pars interior located on the valve secondary side, and two rows, one of elongated and another of ca. round pores, positioned on both sides of the transition between pars interior and exterior on primary (raphe) side of the valve (Figure 24).

Holotype

Slide BR-4564, Botanic Garden Meise, Belgium (BR). The holotype is represented by Figure 2 herein.

Isotypes. ZU11/27 and ZU11/28, Hustedt Collection, Bremerhaven (ZU), not illustrated herein.

Type locality

The new species was found in a spring feeding the small creek Barranco do João Dias, Alentejo region, Portugal (Figure 1), coordinates 37° 51′ 17.75” N, 7° 18′ 00.52” W, Collected by E.A. Morales on 16 January 2018.

Etymology

The species epithet makes reference to the Portuguese southern region of Alentejo, which name etymologically translates as “beyond the Tagus River”.

Associated diatom species

Nitzschia transtagensis was found growing on mosses in a spring feeding a small watercourse called Barranco de João Dias. The relative abundance of *N. transtagensis* was 0.6%, the taxa richness was 33, and the value of the Shannon diversity index was 3.45. The associated diatoms co–occurring with this taxon were *Achnanthidium minutissimum* (Kützing) Czarnecki (33.1%), unidentified *Gomphonema* (16.1%) and *Pinnularia* (10.6%) species, *Nitzschia* cf. *inconspicua* Grunow (6.8%), *Nitzschia paleacea* (Grunow) Grunow (6.1%), *Humidophila contenta* (Grunow) R.L.Lowe et al. (4.2%), *Nitzschia amphibia* Grunow (4.2%) and *Nitzschia inconspicua* Grunow (3.8%). From these, there is no information yet about the ecological requirements of the unidentified *Gomphonema* and *Pinnularia* species, *N.* cf. *inconspicua* and *Humidophila contenta*. From the remaining taxa, only *A. minutissimum* has high sensibility value (5.0), is circumneutral, nitrogen autotrophic tolerating elevated concentrations of organically bound nitrogen, β-mesosaprobous and requires continuously high dissolved oxygen concentrations. Whilst *N. paleacea, N. amphibia* and *N. inconspicua* present lower sensibility values (2.5; 2.0, and 2.8, respectively), are considered alkaliphilous (pH>7), are facultatively or obligately nitrogen-heterotrophic taxa, needing periodically or continuously elevated concentrations of organically bound nitrogen, require moderate dissolved oxygen concentrations (>50% sat. O_2) and are α-mesosaprobous (Van Dam et al. 1994) retrieved from the 2013 OMNIDIA v. 5.5 (Omnis Software, Inc.) database (Lecointe et al. 1993). The presence of these taxa more tolerant to organic contamination leads to an SPI index value of 14.2.

Nitzschia transtagensis was also identified from two other streams in southern Portugal: Lampreia stream (Guadiana basin), on 29 March 2017 and in Valverde stream (Sado basin) on 30 March 2017. However, in these sites, it was only identified in the qualitative survey and did not appear along counting transects.

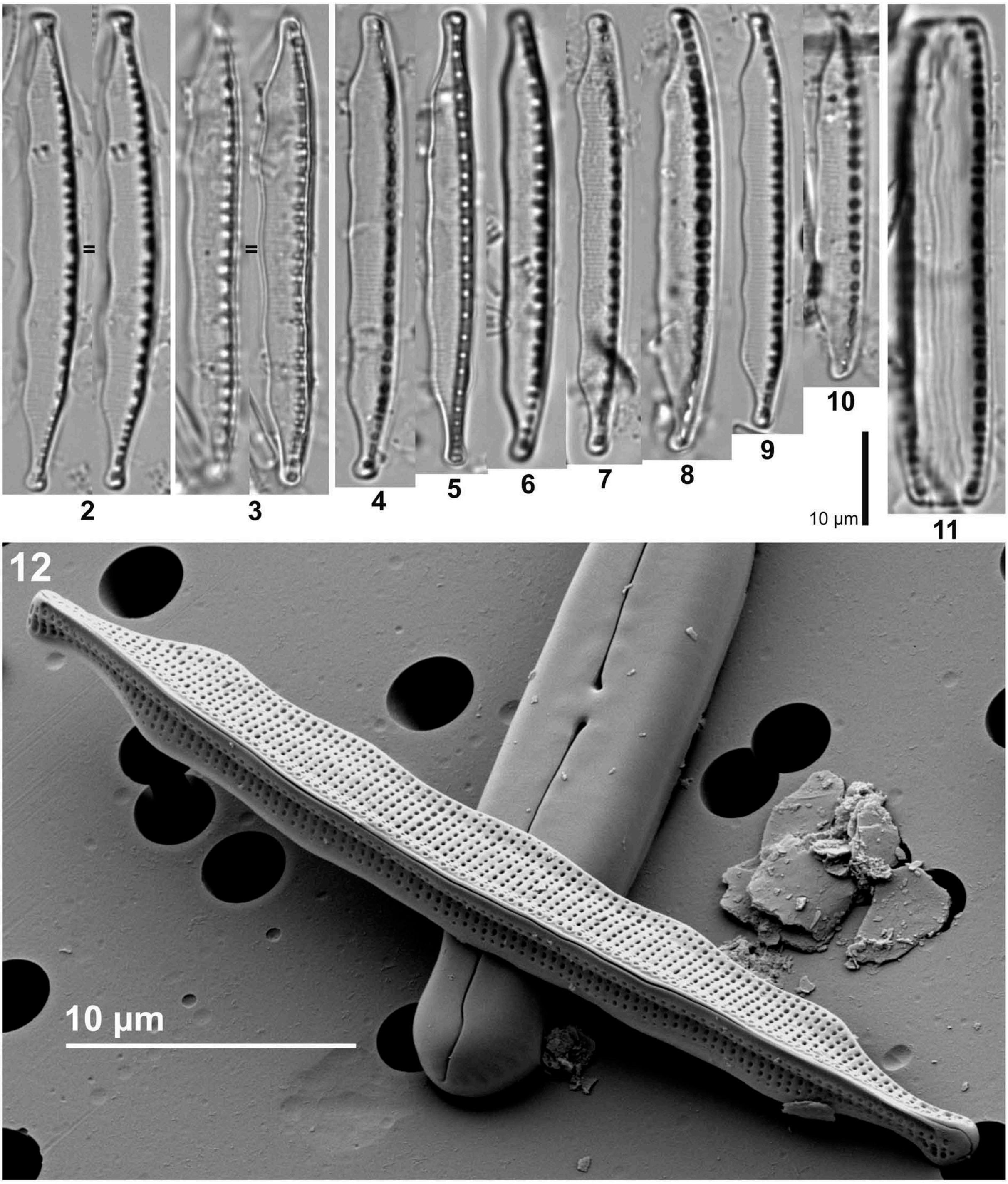

Figure 2-12. *Nitzschia transtagensis*. LM and SEM from type material. Figure 2–10. LM photographs from slide BR-4564. Valve views showing convex primary side and undulated secondary side. Figure 11. LM. Girdle view showing wavy girdle bands. Figure 12. SEM. Tilted and external view of a valve showing continuous raphe running along a suspended hyaline area, and pattern of areolation.

Physical and chemical characteristics of the spring water

The stream fed by the spring presented, at the time of collection of the type material, 21.3 ºC, pH moderately alkaline (8.4), high DO saturation (80.9%), low EC (237 µS cm^{-1}), low TN (2.04 mg N L^{-1}), NO_3 (0.89 mg NO_3 L^{-1}) and NO_2 (0.003 mg NO_2 L^{-1}) concentrations; low PO_4 concentration (0.4 mg PO_4 L^{-1}). However, the TP concentration (1.44 mg P L^{-1}) is above the limit (≤0.13 mg P L^{-1}) for the "good" ecological status classification for small streams in southern Portugal (type S1 <100 km^2), according to the Water Framework Directive.

Additional sampling of the spring, carried out on 15 November 2018, yielded a neutral pH (7.2), medium DO saturation (58.7%), EC of 337 µS cm^{-1}, low concentrations of TN (2.42 mg N L^{-1}), NO_3 (6.99 mg NO_3 L^{-1}) and NO_2 (0.003 mg NO_2 L^{-1}), low PO_4 concentration (0.1 mg PO_4 L^{-1}), but high TP concentration (0.92 mg P L^{-1}). These fall measurements

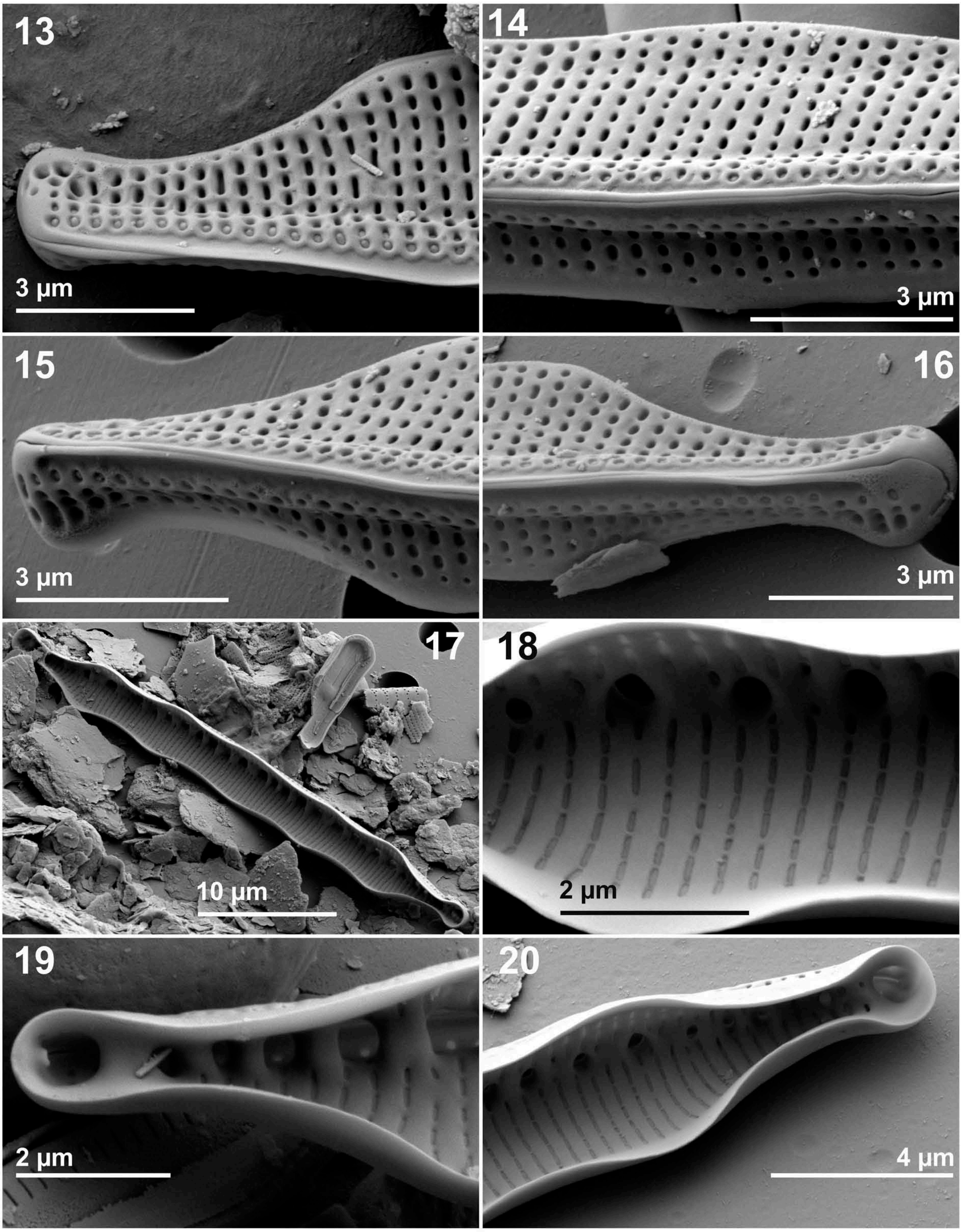

Figure 13-20. SEM images of *Nitzschia transtagensis* from type material. Figure 13, 15, 16. External views of distal ends showing features of the raphe, areolae, hyaline suspended area and valve face and mantle. Figure 14. External view of the central area showing continuous raphe. Internal views of whole (17) and distal ends of valves. Notice position of hymens occluding the areolae, fibulae, raphe canal and the helictoglossa.

indicate that water chemistry is variable at the type locality. Since the highest number of valves of the new taxon was found in January, winter conditions are probably closer to the optima it requires.

Discussion

Nitzschia transtagensis belongs to the *Lineares* section *sensu* Krammer and Lange-Bertalot (1988). The characteristics that the new taxon shares with members of

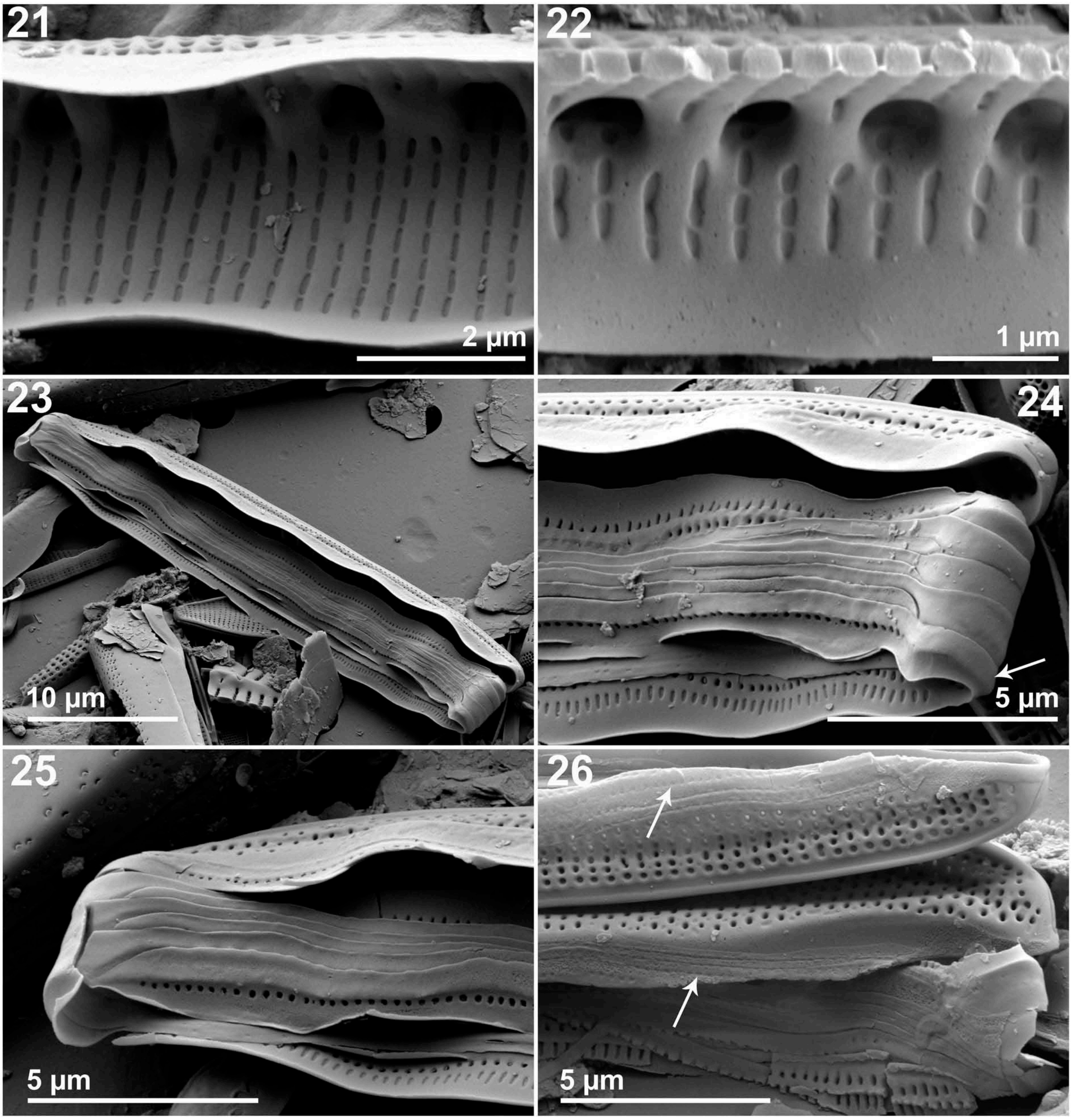

Figure 21–26. SEM images of *Nitzschia transtagensis* from type material. Figure 21, 22. Close internal views of hymens and fibulae. Notice thick structure of the valve in the broken portion of Figure 22. Figure 23–26. Views of the girdle (Figure 24 and 25 are close-ups of 23). Notice apically asymmetric copulae (arrow in Figure 24) and copulae bearing a single row or pores (arrows in Figure 26). Figure 24 also shows close contact of valves of two continuous cells.

that section are that valves are not sigmoid (Figure 10), no transapical costae are present (Figure 2–11) and thus the fibulae do not extend onto the valve face (Figure 17–22). The new species also lacks a conopeum (Figure 12–16) and the valve apices are rostrate to capitate with the keel fully eccentric. Although there could be a slightly wider separation of fibulae at the valve center in the new taxon (Figure 17, 21), denoting the central nodule, the raphe slit is continuous (Figure 14).

The apical asymmetry with an undulated pattern on the secondary side of *N. transtagensis* has not been reported for any other taxon. In the case of *Nitzschia sinuata* (Thwaites) Grunow both the primary and secondary sides are wavy and in *Nitzschia brevissima* Grunow there is a middle constriction that produces two swollen areas from the center of the valve to its apices on each side of the valves (Krammer and Lange-Bertalot 1997) (Table 1). However, the structure of both of these diatoms is very different from that in the new species. *Nitzschia sinuata* belongs to the *Grunowia* section (Krammer and Lange-Bertalot 1988), which is now recognized as a separate genus from *Nitzschia* (Kociolek 2018), though a type still needs to be chosen for it (Guiry and Guiry 2019). *Nitzschia transtagensis* has a central nodule only visible in internal view (Figures 17 and 21) and denoted by a wide fibula; a central nodule is absent in

N. sinuata. The extended fibulae over the valve face in inner view are absent in *N. transtagensis* and the striae are denser and composed of narrower areolae than in *N. sinuata*.

On the other hand, *Nitzschia brevissima* belongs to the *Obtusae* section (Krammer and Lange-Bertalot 1988). The valve ends are short, generally rostrate, with strong middle valve constriction that results in a wide concavity on the primary side of the valve and a slight to absent concavity on the secondary side. Valves are sigmoid and the central nodule is not only evident in the separation of the fibulae, but also in the discontinuity of the raphe (Krammer and Lange-Bertalot 1997). The multiple undulations on the secondary valve side and the convex primary side of *N. transtagensis* (Figure 2–9) are absent in *N. brevissima*, while the concavity of the primary side present in the latter is absent in *N. transtagensis*. The new species is not sigmoid and, as stated before, the central nodule only shows a wider spacing of the fibulae, but not a discontinuity of the raphe.

The new species (except for the apical asymmetry) resembles species such as *Nitzschia vitrea* G.Norman, *N. vitrea* var. *salinarum* Grunow, and *N. hierosolymitana* D. G.Mann, but none of them has a wavy secondary valve side and a primary abvalvar mantle edge (Mann 1980; Krammer and Lange-Bertalot 1988; Stenger-Kovács and Lengyel 2015) (Table 1). As in the three taxa mentioned above, *N. transtagensis* has square to rectangular fibulae, with square to round interfibular spaces. Also, the three taxa have round to elongated areolae delimited by vimines of varying lengths. Likewise, the three taxa have a raphe that is elevated along a hyaline keel. We have not been able to find high magnification SEM images of the valve interior and central areas of *N. vitrea*, its var. *salinarum* or *N. hierosolymitana*, thus we can not determine if there is also a resemblance with the new species regarding the inner position of hymens and the continuous character of the raphe. Mann (1980) presented two external SEM images of *N. vitrea* in girdle view, partially showing the cingulum. From these images, it can be noticed that the girdle elements are open, ligulated, and that the valvocopula is perforated and larger. Also, it is noticeable that none of the elements in *N. vitrea* are undulated, therefore, the wavy feature of the cingulum of *N. transtagensis* seems to be a unique feature within the group (Table 1).

Regarding the asymmetry in the structure of girdle elements, it is known that copulae and valvocopulae may have different structures in other nitzschioids, as it happens in *Nitzschia bizertensis* Smida, Lundholm, Hlaili & Mabrouk, in which the copulae bear one row of pores, while the valvocopula has two (Smida et al. 2014). Also, *Nitzschia navis-varingica* Lundholm & Moestrup has valvocopulae with 2–3 rows of pores, while the copulae may have 1–2 rows, possess scattered pores or lack them altogether (Lundholm and

Table 1. Comparison of *Nitzschia transtagensis* with close morphological relatives within the *Lineares* section.

Feature	*N. transtagensis*	*N. ebroicensis*	*N.* aff. *ebroicensis*	*N. hierosolymitana*	*N. vitrea*	*N. vitrea* var. *salinarum*
Length; width (µm)	L: 30.5–50.4; W: 4.5–6.0	L: 32–40; W: 4–5	L: 34–45; W: 4.5–4.8	L: 25–45; W: 5–6	L: 30–200; W: 9.5–14	L: 30–85; W: 5–9
Striae, fibulae and areolae in 10 µm	Str.: 30–35; Fib.: 8–10; Ar.: 35–45	Str.: 28–30; Fib.: 7–8; Ar.: 26	Str.: 26; Fib.: ca. 9; Ar.: 23–25	Str.: 33–35; Fib.: 2.5–5	Str.: 20–22; Fib.: 4–8	Str.: 25–30; Fib.: 5–7
Outline	Convex primary side with slightly undulated abvalvar edge of mantle, undulated secondary side	Convex primary side, convex to straight secondary side	Convex primary side, undulated secondary side	Convex primary side, convex to straight secondary side	Convex primary side, convex to straight secondary side	Convex primary side, convex to straight secondary side
Cingulum	Dimorphic with apically asymmetric valvocopula. All elements open	Unknown	Unknown	Dimorphic, valvocopula closed, not apically asymmetric, bearing a single row of pores and fringed abvalvar edge. All other elements open	Valvocopula larger, but can not be determined to be dimorphic based on available information. All elements open	Unknown
Reference	This study	Lange-Bertalot et al. (2003)	Lange-Bertalot et al. (2003)	Mann (1980, also as *Hantzschia fenestrata* Hustedt)	Krammer and Lange-Bertalot (1988); Lange-Bertalot et al. (2003)	Krammer and Lange-Bertalot (1988)

Moestrup 2000). Mann (1980) showed that *N. hierosolymitana* also has dimorphic girdle bands. The valvocopulae in this species are closed, have a single row of pores and are fringed along their abvalvar perimeter, while the open copulae bear a single row of pores and are not fringed. Mann (1980) also showed that the row of pores in all elements is located on the pars interior so when the girdle is intact they are unnoticed. *Nitzschia transtagensis* in turn, has girdle elements that are open all less silicified, but the pores in all are located in the pars interior as in *N. hierosolymitana* (Figure 23–25).

Nitzschia transtagensis, besides having dimorphic girdle elements, also exhibits an apical asymmetry in the valvocopula with one row of pores on the portion attached to the valve secondary side and two rows present on the portion attached to the primary valve side (Table 1, Figure 23, 24). As far as we know, this feature is also unique to this species and constitutes the first report of an apically asymmetric valvocopula within the Bacillariaceae. However, this uniqueness has to be confirmed by close inspection of the cingulum in the *Dubiae* and *Bilobatae* sections of *Nitzschia* species.

Lange-Bertalot et al. (2003) reported *Nitzschia* (aff.) *ebroicensis* R.Maillard from Sardinian pools. Their Figure 11–13 (pl. 97) coincide with what we are presenting here as *N. transtagensis*. Both populations have a convex primary side and an undulated secondary side. Length and width of the valves (34–45 µm and 4.5–4.8 µm in the aff. *ebroicensis* against 30.5–50.4 µm and 4.5–6.0 µm in *N. transtagensis*, respectively) overlap in both, and the features of the fibulae (9 in aff. *ebroicensis* against 8–10 in the Portuguese taxon) and interfibular spaces are also similar. Regarding the stria density, however, the aff. *ebroicensis* from Sardinia is coarser (26 in 10 µm against 30–35 in 10 µm in *N. transtagensis*). Additionally, the punctae are also coarser in the Sardinian population (23–25 in 10 µm against 35–45 in 10 µm in the Portuguese population) (Table 1).

We agree with Lange-Bertalot et al. (2003) that the Sardinian population is not *N. ebroicensis* for all the arguments they exposed (e.g. difference in valve outline and dimensions) and that what they called *N.* aff. *ebroicensis* should be considered as a new taxon. They did not erect a new species since they considered they lacked sufficient information, but with the information we collected from Portugal, we consider that it is now possible to include the Sardinian population within *N. transtagensis*. However, since there are differences in stria and puncta density, the Italian population could be kept as a separate variety. Since the locality and sample information in Lange-Bertalot et al. (2003) is not clear, the erection of the new variety is postponed until more information is available.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Notes on contributors

Eduardo A. Morales Contribution: Fieldwork, LM analysis, discovery of the new species, paper project designer and promoter, writing and development of the manuscript.

Carlos E. Wetzel Contribution: SEM analysis, plates, discussion of results and development of the manuscript.

Maria Helena Novais Contribution: fieldwork, sample processing and preparation, data analysis and development of the manuscript.

M. Manuela Morais Contribution: research project designer, paper promoter and development of the manuscript.

Luc Ector Contribution: Provision of literature, discussion of results and development of the manuscript.

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