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A new diatom species from a transitional environment (Arade River Estuary, Portugal): *Tetramphora witkowskii* sp. nov.

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With 17 figures and 2 tables

Abstract: Due to the environmental complexity created by physical and chemical gradients, transitional biomes host a large biological diversity. There has been some effort to better understand the diversity of diatoms living in these transitional environments, although many diatom species remain undescribed from these habitats. Gathering this knowledge will significantly improve our ability to halt biodiversity loss due to anthropogenic activities and assess individual water bodies' ecological status. More detailed studies on diatoms from transitional environments will also further enhance the research that uses them as proxies to reconstruct past environmental changes. The current research documents a new diatom species from the genus *Tetramphora,* found on the saltmarshes of the Arade River Estuary (Southern Portugal), and its autecology. As with the other twelve recognised species of the genus, *Tetramphora witkowskii* sp. nov. presents an asymmetrical valve outline, a biarcuate raphe system, slit like areolae, axial costae, a central hyaline area, and a dorsal central thickening. *Tetramphora witkowskii* sp. nov. is characterised by having finely silicified valves with a nearly flat face, 28.0–33.0 µm in length and 6.0–6.5 µm in width. The new species has a stria density of 24–27 in 10 µm with narrow, longitudinally oriented areola openings. It presents proximal raphe endings covered by a projected dorsal-side flap and a developed sternum with thickened costae, weakly formed on the dorsal side of the central area. *Tetramphora witkowskii* sp. nov. was found as a benthic epipelic diatom with a preference for brackish to salt water with circumneutral pH. Further research is required to better understand this species' colony and frustule

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shape, the number, morphology, and position of the chloroplasts, genetic signature, geographical distribution, and environmental tolerance.

Keywords: *Tetramophora*; Saltmarsh; Brackish water; Salt water

Introduction

Transitional environments border terrestrial and marine biomes, including estuaries, deltas, coastal lagoons, and beaches. They are characterised by strong physical and chemical gradients from mixing freshwater flowing into seawaters, making them complex and highly productive (Crossland et al. 2005, Werner & Blanton 2019). The complexity of these environments creates a great diversity of habitats that consequently host a large biological diversity (Gomes & Camacho 2017), including diatoms (Denys & Wolf 1999). Although some efforts have been made to increase knowledge about diatom taxon diversity in transitional environments (e.g. Muylaert & Sabbe 1996, Sabbe et al. 1999, Fernandes & Souza-Mosimann 2001, Massé et al. 2001, Chen et al. 2017, Saber et al. 2020, Li et al. 2021, Yılmaz et al. 2023), there are still many taxa to be discovered and described in these dynamic environments. Knowledge about diatom diversity in these valuable ecosystems is essential to tackle biodiversity loss, following the 2030 Objectives for the Sustainable Development of the United Nations (UN, 2015). Moreover, this knowledge is essential to accurately and precisely assess the ecological status of the water bodies, reconstruct past environmental changes, and predict future ones.

Gomes (2013) studied the modern diatom assemblages found in two estuaries on the southern Portuguese coast where a new diatom genus (Gomes et al. 2013) and additional species were found (Witkowski et al. 2015). In addition to the two new recorded taxa, the analysed samples showed potential to contain others not yet described, including a new species of the genus *Tetramphora* Mereschkowsky emend. Stepanek & Kociolek (2016: 125).

The twelve recognised species of *Tetramphora* are found in the benthos of marine and inland waters with salinity ranging from brackish to salt water, and a pH ranging from circumneutral to alkaline (Stepanek & Kociolek 2016, Mihalić et al. 2019). The genus is characterised by a valvar and copula asymmetry, leaving the living frustule in a girdle orientation with both valve faces on the same plane. This frustule formation is also observed in other amphoroid genera such as *Amphora* Ehrenberg ex Kützing (1844: 107) and *Halamphora* (Cleve) Levkov (Levkov 2009: 165). Initially, Mereschkowsky (1903a) separated *Tetramphora* from Cleve's (1895) *Amphora* sensu lato (included in the *Oxyamphora* subgenus), based on the number, shape and position of the chloroplasts within the cells, but the genus was often ignored by later diatom taxonomists (Stepanek & Kociolek 2016) and it was only in 2016 that the genus *Tetramphora* was re-established based on molecular phylogeny, indicating that *Tetramphora* taxa are monophyletic and distinct (Stepanek & Kociolek 2016). In addition, *Tetramphora* taxa have moderately to strongly dorsiventral valves; stria are composed of transapically or longitudinally oriented areolar slits; they have a biarcuate raphe with a central hyaline area; valves have axial costae and the dorsal central region is thickened (Stepanek & Kociolek 2016).

The current research aims to describe a new diatom species of the genus *Tetramphora*, found in a transitional environment (Arade River Estuary) in Portugal, based on its morphology observed in light and scanning electron microscopy.

Materials and methods

Study Area

The Arade is the second largest river that flows into the southern coast of Portugal (Fig. 1A). It originates in the Serra do Caldeirão (max. elevation 589 m) and flows for 75 km in an ENE-WSW direction until it reaches the sea near the city of Portimão (SNIRH, 1995–2013). The Arade River Estuary extends to the town of Silves and is approximately 15 km long. Along its banks, there are urban areas, artificial marshes, salt marshes, salt pans and agricultural zones (Gomes 2013; Fig. 1B).

The Arade River's drainage basin covers an area of 966 km^2 (SNIRH, 1995–2013), including Boina, Odelouca and Falacho streams (Ministério do Ambiente e do Ordenamento do Território 2000) and the region has a Mediterranean climate (Csa in Köppen

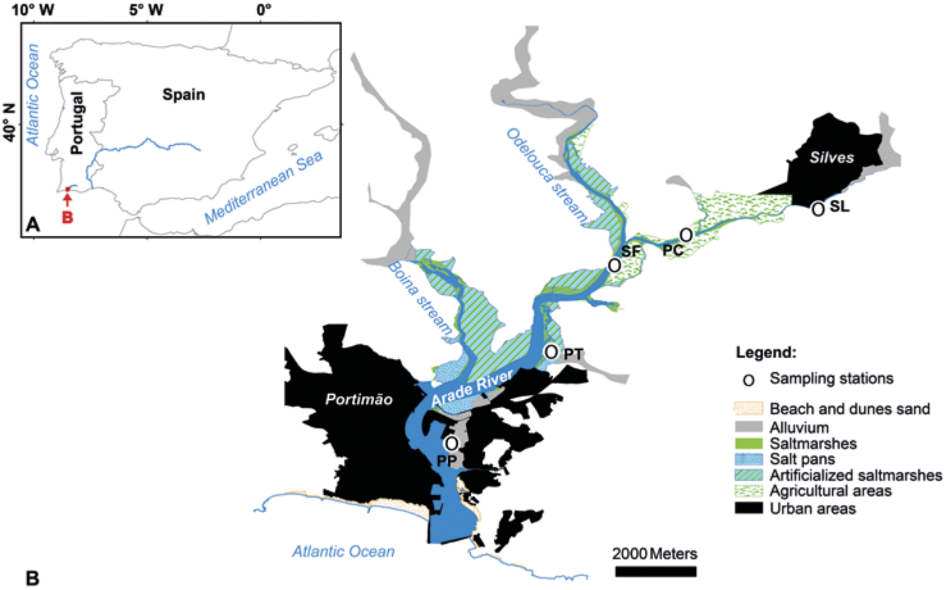


Fig. 1. Study area: **(A)** location in the Iberia Peninsula, **(B)** sampling station's location (PP – Porto do Parchal; PT – Ponte; SF – Sítio das Fontes; PC – Parque de campismo; SL – Silves).

classification; Loureiro & Countinho 1995; IPMA 2024). The average annual rainfall at the river source can reach 2000 mm, and the average annual temperature is 16 °C (Loureiro & Countinho 1995). The minimum temperature is recorded in January (10 °C) and the maximum temperature in August (23 °C) (Loureiro & Coutinho 1995). The average insolation is more than 2800 hours yearly (Loureiro 1983).

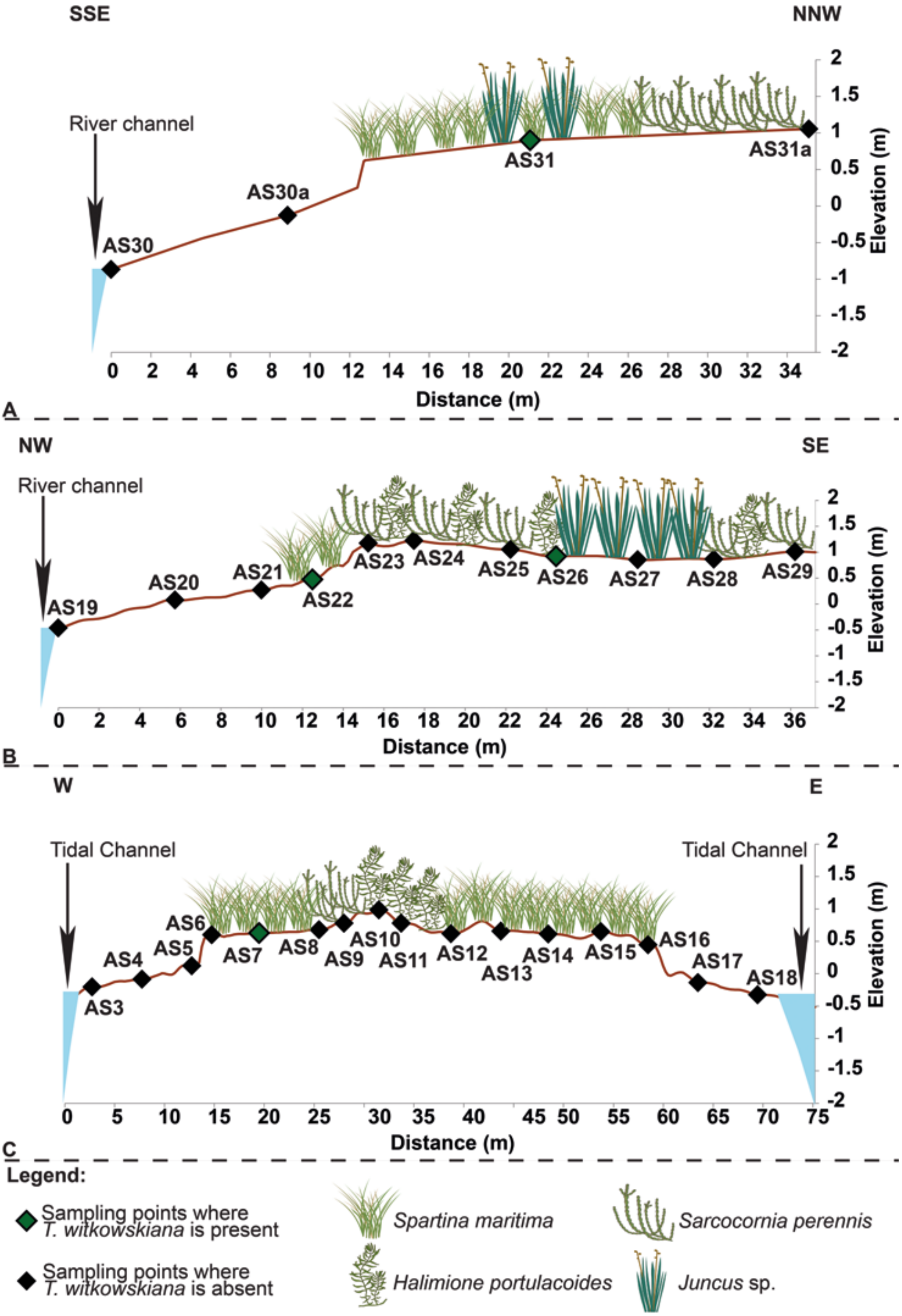
As dams strongly regulate the Arade River, its freshwater flow downstream is, on average, 10 m^3 s^{-1} throughout the year (Portela & Duarte 2016). However, there are exceptions, such as during local intense rainfall events (Correia et al. 2020). Thus, freshwater outflows have limited to no expression in the estuary (Santos-Ferreira 2006). The Arade Estuary experiences a semi-diurnal and mesotidal regime, with an average tidal amplitude of 2 m, presenting moderate tidal currents, varying between 0.25 m s^{-1} in flood and 0.35 m s^{-1} in ebb conditions (Santos-Ferreira 2006).

Part of the Arade River basin is an area classified under Rede Natura 2000 (ICNF 2017–2023). The main identified environmental threats are due to high human pressure (Correia 2020). These are the freshwater retention by the dams, diffused agricultural pollution, discharges of urban wastewater treatment plants and pig farms, cutting of riparian vegetation, dredging, and nautic sports activities (ICNF 2017–2023; Correia 2020).

Sampling for diatom analysis

Surface sediment samples, up to a depth of 0.5 cm, were collected for diatom analysis at thirty-seven sampling points, distributed across five sampling stations along the Arade Estuary, Southern Portugal (Fig. 1B). The stations were located at different distances from the river mouth and were spaced equidistantly (considering accessibility to the river) to ensure they covered areas with varying marine influences. At each station, sampling points were defined in 5- or 10-meter intervals from the river water line during the low tide of the spring tides along transects perpendicular to the river channel. Sampling points were selected based on changes in the transect slope gradient, sediment texture and vegetation along the intertidal zone to capture the most significant spectrum of environmental variation and, consequently, the greatest diversity of diatom taxon (Fig. 2). Samples were collected on May 28th, 2010, and stored in plastic vials at 4 °C.

Fig. 2. Schematic representation of the sampling transects where *Tetramphora witkowskii* sp. nov. was present (elevation relative to the mean sea-level): **A** – Parque de campismo sampling station, **B** – Sítio das Fontes sampling station, and **C** – Ponte sampling station (Fig. 1B). Illustrations of *Spartina maritima* (Curtis) Fernald (1916: 180) by Saxby (2010), *Sarcocornia perennis* (Miller) A. J. Scott (1977: 367) & *Halimione portulacoides* (L.) Aellen (1938: 126) by Tracey (2010), and *Juncus* L. (1753: 325) by Tracey (1999).


SSE
NNW
River channel
AS30
AS30a
AS31
AS31a
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Elevation (m)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Distance (m)
A
NW
SE
River channel
AS19
AS20
AS21
AS22
AS23
AS24
AS25
AS26
AS27
AS28
AS29
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Elevation (m)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Distance (m)
B
W
E
Tidal Channel
Tidal Channel
AS3
AS4
AS5
AS6
AS7
AS8
AS9
AS10
AS11
AS12
AS13
AS14
AS15
AS16
AS17
AS18
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
Elevation (m)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
Distance (m)
C
Legend:
Sampling points where T. witkowskiana is present
Sampling points where T. witkowskiana is absent
Spartina maritima
Halimione portulacoides
Sarcocornia perennis
Juncus sp.

Measurement of environmental parameters

To determine diatom ecological preferences, subsamples of the collected sediment samples were analyzed for grain size and organic content. Grain-size analysis was carried out using a *Malvern Mastersizer* particle size analyzer for fractions between 0.3–300 µm and by dry sieving for coarser fractions (> 300 µm). The results obtained from both methods were merged and analyzed using GRADISTAT software, version 8.0 (Blott & Pye 2001). Sediment organic content was determined by elemental gas chromatography using a Carlo Erba analyzer (model EA1108). At each sampling point, the salinity and pH of the sediment interstitial water were measured using a YSI 556 MPS handheld multiparameter probe and a portable pH meter from EUTECH (model spear) between May 2010 and January 2011. Salinity, pH, dissolved oxygen and temperature were also measured in the river water column next to each sampling station at both high and low tides.

Sample preparation

For diatom analysis using light (LM) and scanning electron microscope (SEM), sediment samples were treated with hydrogen peroxide (30%) and hydrochloric acid (10%) following the method described in Swift (1967). To obtain permanent microscopic slides, cleaned material was mounted in Naphrax®. The slides were analysed using a Nikon Ci light microscope at 1000× magnification with a 100× Plan Apochromat oil immersion objective (NA = 1.4) in the Diatom Laboratory at Kütahya Dumlupınar University. The ultrastructure morphological observations were done using SEM. For that purpose, part of the cleaned material was filtered through a polycarbonate membrane filter with a pore diameter of 5 µm. These membrane filters were fixed on aluminum stubs after air–drying. Stubs were sputter-coated with a gold layer reaching a thickness of ~ 20 nm and studied using a ZEISS Ultra field emission scanning electron microscope at the Eskişehir Technical University in Türkiye.

Taxon description

The new species was described by the measurements carried out in LM and SEM analysis (length, width and number of areolae in 10 µm) and using the terminology presented in Barber & Haworth (1981) and Round et al. (1990). For typification, article 8.2 of the International Code for Botanical Nomenclature (Turland et al. 2018) was followed, indicating the entire microscopic slide as the holotype. Type materials and the holotype slide are deposited in the Canadian Museum of Nature (Ottawa, Ontario, Canada), and the isotype slides are at Kütahya Dumlupınar University (Turkey) and at the University of Algarve (Portugal).

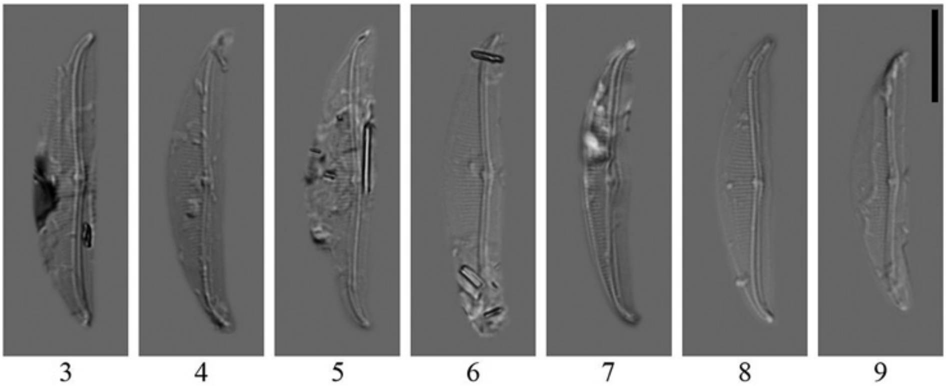
Results

Tetramphora witkowskii **P.B.Hamilton, A.I.Gomes & C.N.Solak sp. nov. (Figs. 3–17)**

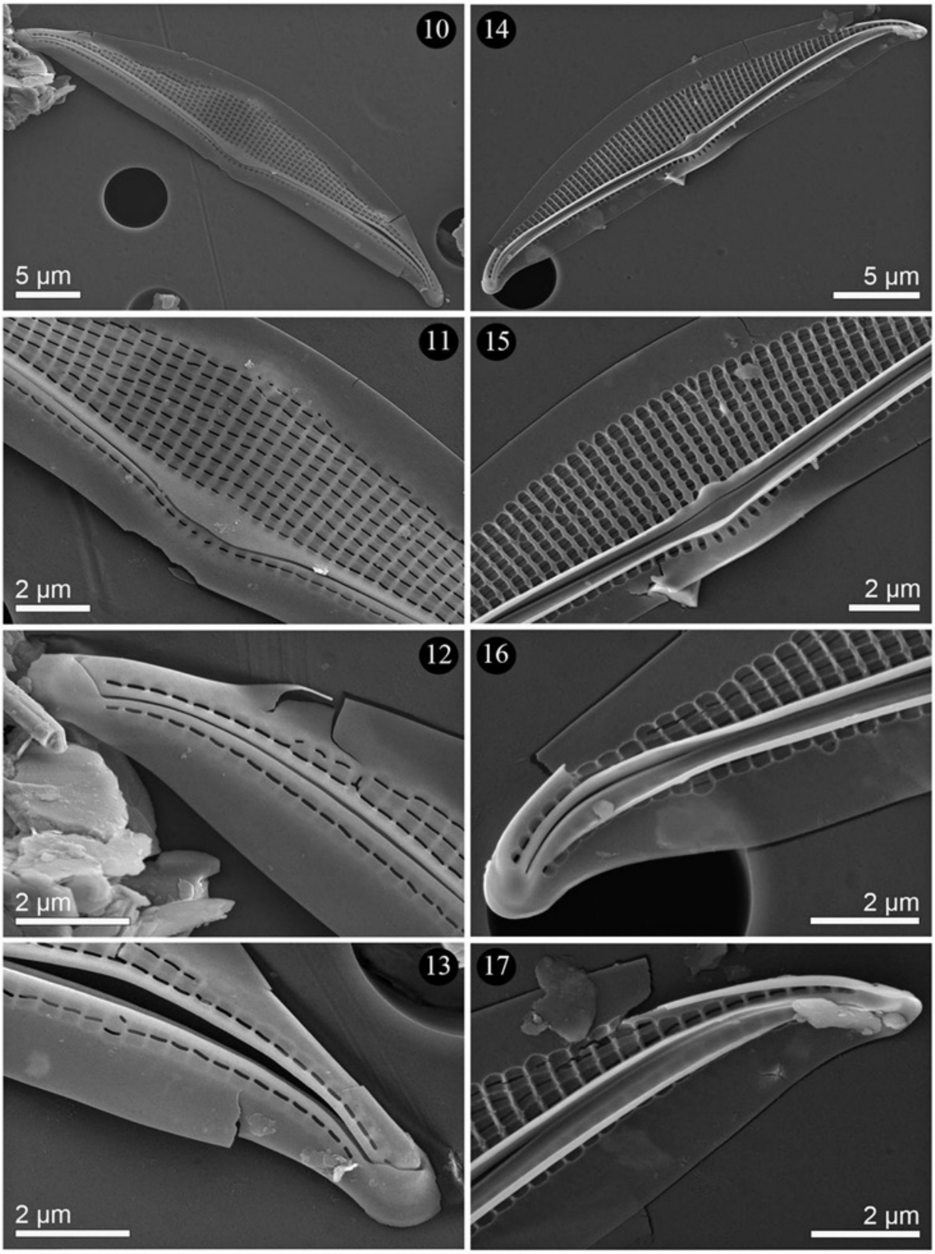
Description

LM (Figs. 3–9): Valves finely silicified, semi-elliptic and strongly dorsiventral. Valve endings narrowly rounded and ventrally deflected. Valve length 28.0–33.0 µm, valve width 6.0–6.5 µm (n = 16). Raphe weakly biarcuate, largely visible in a single focal plane lying near ventral margin. Proximal raphe endings distinct and enlarged (Figs. 3, 4, 7–9). Axial area narrow, with a slight dilation at the central area with thickened sternum. Striae slightly visible in LM, not obviously areolate, parallel at valve centre, becoming weakly radiate near apices. Striae density 24–27 in 10 µm.

SEM (Figs. 10–17): Valve face nearly flat, facilitating raphe observations of raphe. Valve wall thin, externally virgae visable and covered by a fine valve surface plate (Figs. 10 and 11). Valve margins and mantle hyaline. Sternum thickened and slightly elevated from valve face (Figs. 10 and 11). Externally, raphe biarcuate with distal endings strongly deflected dorsally, ending at valve face mantle junction with tear-drop pore (Figs. 10, 12 and 13). Central raphe endings obscured by a projected dorsal-side flap. Areola openings narrow, longitudinally oriented slits (Figs. 10 and 11). Internally, raphe continuing through central area, ending distally onto small helictoglossae on raised thickened end (Figs. 14, 16 and 17). Sternum bordered by thickened axial costae, weakly formed on dorsal side of central area, and with a poorly formed small protuberance (nodule) on dorsal central area (Figs. 14 and 15). Striae separated by thin virgae crossed by weakly developed vimines (Fig. 15).



Figs. 3–9. LM micrographs of *Tetramphora witkowskii* sp. nov. from Arade Estuary, Silves, Portugal. The representative type specimen is presented in Fig. 8. The specimen's valves are displayed showing the variation on the ventral margin shape and size range. Scale bar = 10 µm.



Figs. 10–17. SEM micrographs of *Tetramphora witkowskii* sp. nov. from Arade Estuary, Silves, Portugal. **10.** External view of the whole valve. **11.** Close up to the external view of the center of the valve showing large siliceous flap which covers the continuous raphe. **12, 13.** Close up to the external view of the valve apices showing curved distal raphe endings. **14.** Internal view of the whole valve. **15.** Close up to the internal view of the center of the valve showing the continuous raphe. **16, 17.** Close up to the internal view of the valve apices showing the helictoglossa.

Holotype: CANA 131923 in the collection at the Canadian Museum of Nature, Ottawa, Canada. Microscope slide designed as a holotype.

Isotypes: PRTG_Arade Estuary_AS31_May 2010 deposed at Kütahya Dumlupınar University Herbarium (DUP), Türkiye, isotype slide no. 15886/ALGU in the ALGU Plant and Algae Collection at the University of Algarve, Portugal.

Registration: http://phycobank.org/105137

Type locality: PORTUGAL, Silves (Parque de campismo sampling station – Figs. 1 and 2), Arade Estuary as a sediment sample (GPS 37°10'42.100"N; 8°28'16.293"W; 1 m a.s.l.), collector: Ana Isabel GOMES, 28th of May 2010.

Etymology: The new species in named in honor of our late colleague Prof. Dr. hab Andrzej Witkowski (1955–2023) for the many scientifically significant contributions to our understanding of salt water environments on a worldwide scale.

Distribution and ecology: Observed from the type locality and at the sampling points AS7, AS22 and AS26 (Figs. 1 and 2). Where *Tetramphora witkowskii* was observed, the sediment organic content varied between 3.31 (AS22) and 3.98 (AS26), and the sediment texture was silty (70.8 to 77.8% of silt, respectively in AS7 and AS26). At the same sampling points, the salinity of the sediment interstitial water ranged from 27.04 ± 3.15 g/kg (AS31) to 34.22 ± 4.63 g/kg (AS22), and the pH was between 6.81 ± 0.24 (AS22) and 7.15 ± 0.18 (AS7) (Table 1). Where the new species was observed (i.e., PT, SF and PC), during low tide, the salinity, pH, dissolved oxygen and temperature varied between 15.08 ± 7.90 (PC) and 25.90 ± 12.83 g/kg (PT), 7.63 ± 0.14 (PC) and 7.89 ± 0.26 (PT), 85.9 ± 22.9 (PC) and 96.1 ± 25.2% (PT), and 17.15 ± 4.74 (PT) and 18.27 ± 4.74 °C (SF), respectively. At high tide, the same parameters fluctuated between 24.29 ± 6.81 (PC) and 32.99 ± 1.11 g/kg (PT), 7.88 ± 0.11 (PC) and 8.09 ± 0.11 (PT), 95.4 ± 28 (PC) and 103.8 ± 32.2% (SF), and 18.01 ± 4.96 (PC) and 18.23 ± 5.11 °C (SF). Detailed information regarding the physicochemical parameters measured at each sampling point is available in Table 1.

Discussion

Based on morphological observations conducted in LM and SEM, a new *Tetramphora* species is proposed: *Tetramphora witkowskii.* Like the other species of the genus, *Tetramphora witkowskii* is characterised by an asymetrical valve outline (specifically semi-elliptic and strongly dorsiventral), the presence of a biarcuate raphe system, slit like areolae, axial costae, a central hyaline area, and a dorsal central thickening (Stepanek & Kociolek 2016). However, it is easily distinguished from the established *Tetramphora* species (Table 2).

Tetramphora witkowskii differs from *T. croatica* Udovič, Mihalić, Stanković & Levkov (Mihalić et al. 2019: 278), *T. fontinalis* J.G.Stepanek & Kociolek (2016: 135), *T. intermedia* (Cleve) J.G.Stepanek & Kociolek (2016: 130), *T. robusta* J.G.Stepanek & Kociolek

Table 1. Physicochemical parameters measured in the sampling points where *Tetramphora witkowskii* sp. nov. was observed.

Sampling stations			**PT**	**SF**		**PC**
Sampling points			**AS7**	**AS22**	**AS26**	**AS31**
Sediment	**Organic content (%)**		3.38	3.31	3.98	3.53
	Texture	**Sand (%)**	11	7.9	4.8	9.8
		Silt (%)	70.8	72.2	77.8	74.5
		Clay (%)	18.2	19.9	17.4	15.7
Sediment interstitial water	**Salinity (g/Kg)**		33.83 ± 3.41	34.22 ± 4.63	33.83 ± 6.72	27.04 ± 3.15
	pH		7.15 ± 0.18	6.81 ± 0.24	7.03 ± 0.19	7.05 ± 0.11
Arade River Estuary water column	**Salinity (g/kg)**	**Low tide**	25.90 ± 12.83	20.58 ± 5.78		15.08 ± 7.90
		High tide	32.99 ± 1.11	32.92 ± 2.30		24.29 ± 6.81
	pH	**Low tide**	7.89 ± 0.26	7.80 ± 0.24		7.63 ± 0.14
		High tide	8.09 ± 0.11	7.94 ± 0.14		7.88 ± 0.11
	Dissolved oxygen (%)	**Low tide**	96.1 ± 25.2	95.1 ± 36.1		85.9 ± 22.9
		High tide	100.8 ± 33.2	103.8 ± 32.2		95.4 ± 28
	Temperature (°C)	**Low tide**	17.15 ± 4.74	18.27 ± 4.74		17.46 ± 4.45
		High tide	18.15 ± 4.66	18.23 ± 5.11		18.01 ± 4.96

(2016: 136), *T. securicula* (Peragallo & H.Peragallo) J.G.Stepanek & Kociolek (2016: 130), *T. chilensis* (Hustedt) J.G.Stepanek & Kociolek (2016: 131) and *T. rhombica* (Kitton in Schmidt) J.G.Stepanek & Kociolek (2016: 138) by presenting narrow longitudinally oriented areola openings, while the simailar species have transapically oriented areola openings (Table 2). Additionally, all these species have less stria in 10 µm than *Tetramphora witkowskii*, and all except *T. chilensis* have larger valves (Mihalić et al. 2019, Stepanek & Kociolek 2016). *Tetramphora chilensis* also differs from *T. witkowskii* by the lack of an internal sternum bordered by thickened axial costae, the absence of a residual central nodule on the dorsal side of the raphe and the presence of clear central raphe endings on the internal side of the valve (Stepanek & Kociolek 2016).

Comparing *Tetramphora witkowskii* with the remaining taxon possessing narrow longitudinally oriented areola openings (Table 2), the new species differs from *T. ostrearia* (Brébisson) Mereschkowsky (1903b: 54) and *T. sulcata* (Brébisson) J.G.Stepanek & Kociolek (2016: 128) having smaller valves (28.0–33.0 µm in length and 6.0–6.5 µm in width) with a nearly flat face, more stria (24–27 in 10 µm), and a weakly biarcuate raphe system instead of a highly biarcuate one (Table 2). *Tetramphora lineolatoides* J.G.Stepanek & Kociolek (2016: 133) is similar in size, shape and valve thickness, but differs by the absence of a flap over the external central raphe ends, less stria (18–19 in 10 µm), more areolae on the ventral side of the valve, the presence of a less developed sternum with thickened costae and a clear central nodule on the dorsal side of the continuous internal raphe (Table 2). *Tetramphora lineolata* (Ehrenberg) Mereschkowsky (1903b: 54) is the most similar to *T. witkowskii* in valve shape and size (including having a flat valve face), structural formation of the areolae, and internal sternum bordered by thickened axial costae, weakly formed on the dorsal side of the central area (Table 2). However, *Tetramphora lineolata* differs by its thicker valve, lower stria density, more areolae on the central side of the valve, and the external central raphe ends are not covered with a flap (Table 2; Stepanek & Kociolek 2016).

Living material needs to be cultured to complement further the description of *Tetramphora witkowskii* in terms of colony and frustule shape, as well as the number, shape, and position of the chloroplasts. Diatom cultivation was not initially performed because it was not the focus of Gomes (2013) study during sample collection. As suggested by Stepanek & Kociolek (2016), it is also essential to conduct genetic anlysis, to solve discrepancies observed in the taxon' morphological similarities and the results of molecular phylogenetic analysis, which is planned in future analyses.

Tetramphora witkowskii was found in the Arade River Estuary (Portugal; Fig. 1), located in a temperate climate zone (IPMA 2024). Compared with the global distribution of the remaining taxa of the genus, it appears that *Tetramphora lineolata, T. lineolatoides, T. ostrearia, T. croatica, T. fontinalis* and *T. chilensis* were also recorded in the temperate climate zone (Kaleli & Akçaalan 2021, Mihalić et al. 2019, Sala et al. 2007, Stepanek & Kociolek 2016, Witkowski et al. 2000). *Tetramphora ostrearia, T. sulcata, T. intermedia, T. robusta* and *T. securicula*, in contrast, are found in the subtropical climate zone (Stepanek & Kociolek 2016, Witkowski et al. 2000). Observed in the sediments of Arade Estuary intertidal zone, *Tetramphora witkowskii* is a benthic epipelic diatom (sediments with from 70.8 to 77.8% silt content; Table 1). However, the hypothesis that *Tetramphora witkowskii* can be epiphytic or planktonic or presents both of these life forms, as *T. lineolatoides, T. croatica* and *T. chilensis* (Mihalić et al. 2019, Sala et al. 2007, Stepanek & Kociolek 2016), can not be excluded, since it occurs on saltmarshes that are inundated twice a day (Fig. 2). The new species is observed in a transitional environment where water varies from brackish to salty (27.04 ± 3.15 to 34.22 ± 4.63 g/kg) with circumneutral pH (6.81 ± 0.24 to 7.15 ± 0.18) (Table 1). *Tetramphora lineolata*, *T. lineolatoides, T. ostrearia, T. sulcata, T. intermedia* and *T. robusta* are also benthic species from transitional environments with a preference for brackish to saltwater (Denys 1991/2, Stepanek & Kociolek 2016). Regarding the pH, *Tetramphora ostrearia, T. fontinalis, T. robusta*, *T. securicula* and *T. chilensis* have similar preferences to *T. witkowskii* (Mihalić et al. 2019, Stepanek & Kociolek 2016).

There is very little information available about the distribution and ecology of *Tetramphora* taxa, which is crucial for environmental and paleoecological studies. Additionally, species belonging to this genus can be found in both marine and inland water environments (Mihalić et al. 2019, Stepanek & Kociolek 2016; Witkowski et al. 2000). Therefore, it is important to increase research on the geographical distribution and environmental tolerance of *Tetramphora* species, including *Tetramphora witkowskii*.

Table 2. Morphological charcteristics of *Tetramphora witkowskii* sp. nov. compared with other *Tetramphora* taxa described in Stepanek & Kociolek (2016) and Mihalić et al. (2019).

Taxa	Valve outline	Valve face	Valve length (µm)	Valve width (µm)	Raphe	Axial costae	Central area	Stria density (in 10 µm)	Areola openings
Tetramphora witkowskii	semi-elliptic and strongly dorsiventral	nearly flat	28.0–33.0	6.0–6.5	weakly biarcuate	weakly formed on dorsal side of central area	slightly dilated on the dorsal side, with a projected dorsal-side flap; poorly formed nodule on dorsal internal area	24–27	narrow longitudinally oriented
Tetramphora lineolata	semi-elliptic and strongly dorsiventral	flat	25.0–48.0	5.5–8.0	weakly biarcuate	well-developed	slight dilated on the dorsal side with a small projection	21–23	narrow longitudinally oriented
Tetramphora lineolatoides	semi-elliptic and strongly dorsiventral	weakly flexed	28.0–45.0	5.0–8.0	moderately biarcuate	weakly developed	slightly dilated on the dorsal side, without a flap; small protuberance on the internal side	18–19	narrow longitudinally oriented
Tetramphora ostrearia	semi-elliptic and dorsiventral	flexed	58.0–66.0	7.0–8.0	highly biarcuate	dorsally and ventrally raised	small protuberance on the dorsal internal side	13–14	narrow longitudinally oriented
Tetramphora sulcata	semi-elliptic and dorsiventral	flexed	35.0–58.0	5.5–7.5	highly biarcuate	dorsally reduced to a small protuberance and ventrally thickened near the central area	with an unornamented area on the dorsal side, not extending into a flap; small protuberance on the dorsal internal side	18–19	narrow longitudinally oriented
Tetramphora croatica	semi-elliptic and strongly dorsiventral	flat	40.0–78.0	6.5–12.0	highly biarcuate	strongly dorsally and ventrally thickened near the central area	not expressed from both valve sides, with a large unornamented flap	dorsal: 18–22; ventral: 19–24	oblique or transapically oriented

Table 2. cont.

Taxa	Valve outline	Valve face	Valve length (µm)	Valve width (µm)	Raphe	Axial costae	Central area	Stria density (in 10 µm)	Areola openings
Tetramphora fontinalis	narrowly semi-elliptical becoming linear through the central portion of the valve and dorsiventral	flexed	38.0–60.0	6.0–8.0	highly biarcuate	well-developed	with an unornamented area on the dorsal side, well developed into a flap	17–21	small transapically oriented
Tetramphora intermedia	broadly semi-lanceolate and strongly dorsiventral	flexed	80.0–105.0	7.0–19.0	weakly biarcuate	moderately developed	with a large unornamented flap; small protuberance in the internal area	14–17	narrow, transapically oriented
Tetramphora robusta	broadly semi-elliptic and strongly dorsiventral	highly flexed	45.0–65.0	8.0–10.0	weakly biarcuate	well-developed	with an unornamented area, not extending into a flap; protruding central thickening	13–14	small transapically oriented
Tetramphora securicula	semi-elliptic and dorsiventral	flexed	55.0–75.0	8.0–10.0	highly biarcuate	weakly developed	with a large, unornamented flap; small protuberance at the dorsal side	16	small transapically oriented
Tetramphora chilensis	narrowly semi-elliptical and strongly dorsiventral	flat	20.0–42.0	4.5–6.0	nearly straight	weakly developed	with a broad unornamented area on the dorsal side, not extending into a flap; dorsal swelling absent	dorsal: 20–23; ventral: 26–30	small transapically oriented
Tetramphora rhombica	–	–	130–260	21–50	–	present	unornamented dorsal central area	10–12	transapically oriented

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Authors contributions

Ana Gomes was responsible for the fieldwork and article conceptualization, data collection and analysis (sediment samples and measurements of physicochemical parameters), sample preparation for diatom analysis, new taxon identification and description, original draft of the article writing, and funding acquisition. Paul Brian Hamilton was responsible for the new taxon identification and description and the original draft of the article writing. Cüneyt Nadir Solak acquired LM and SEM images, identified and described the new taxon, and wrote the original draft of the article. Tomasz Boski was responsible for the fieldwork conceptualization, funding acquisition, manuscript review, and editing. Delminda Moura was involved in the fieldwork conceptualization, data collection in the field, and manuscript review and editing. Nesil Ertorun was responsible for the acquisition of SEM images. Ferhan Yedidağ prepared samples for LM and SEM analysis.

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