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Abstract

During a survey of the diatom flora present on an artificial substratum (plexiglass) submerged off the southern Bulgarian Black Sea coast, a small-celled diatom taxon, reported previously from the Black Sea as *Amphora* (*Halamphora*) sp. S21, was observed. Detailed light and scanning electron microscope observations showed that the original taxon actually included two different taxa: an unknown *Halamphora* species described here as *H. valdeminutissima sp. nov.,* and a second taxon, which, based on SEM observations, could be identified as *Amphora micrometra* Giffen. The morphology of both taxa is described in detail and a comparison with similar taxa is provided. Basic environmental data are added.

Key words: Bacillariophyta, marine benthos, Mediterranean region, taxonomy, morphology

Introduction

The genus *Amphora* Ehrenberg ex Kützing (1844: 107) is one of the largest diatom (Bacillariophyta) genera in terms of number of taxa. Originally, the genus encompassed all species that are currently placed in the genera *Amphora*, *Halamphora* (Cleve 1895: 17) Levkov (2009: 165), *Colliculoamphora* D.M.Williams & G.Reid (2006: 153) and *Tetramphora* Mereschkowsky (1903) but since the revision in Levkov (2009), the genus name *Amphora* is again restricted to species with strictly uniseriate striae (*Halamphora* species often have biseriate striae) and a maximum of three plain copulae (versus numerous perforated copulae in *Halamphora*) (Levkov 2009). Among them, *Amphora* and *Halamphora* are highly diverse and inhabit various freshwater, brackish and marine aquatic environments (Levkov 2009, Stepanek & Kociolek 2018). All recent major works dealing with *Amphora* and *Halamphora* describe a large number of new species (e.g. Wachnicka & Gaiser 2007, Levkov 2009, Stepanek & Kociolek 2018 and references therein, etc.), but also leaving a number of still unknown taxa (Wachnicka & Gaiser 2007, Stepanek & Kociolek 2018).

Recently, in a single site off the Bulgarian Black Sea coast near the historic town of Sozopol Zidarova *et al*. (2022) observed diversity much higher than the one that could be expected based on older, regional literature data. Among the 40 *Amphora*, *Halamphora* and *Tetramphora* taxa recorded on artificial substratum, Zidarova *et al.* (2022, p. 117, figures 5AA–AG) showed and discussed a very small-celled species that could not be identified using the currently available literature. The species was reported as *Amphora* (*Halamphora*) sp. S21. Zidarova *et al.* (2022) commented that some of the valves of the latter taxon on their figures 5AA–AG (*i.e*., figures 5AD and 5AF) differed from the rest of the valves in the population in having a more semi-elliptic valve outline and a slightly more ventrally positioned raphe. However, due to insufficient scanning electron microscope (SEM) observations at that time, they considered that all valves on their figure 5AA–AG belonged to

one taxon. This provisionally single and unknown taxon was also illustrated on their figure 8H in SEM. Further SEM observations on the samples and comparisons with the results from the light microscopy, however, revealed that *Amphora* (*Halamphora*) sp. S21 in Zidarova *et al.* (2022) actually comprised two different taxa: an unknown taxon that could not be identified and that subsequently is described here as a new taxon: *Halamphora valdeminutissima sp. nov.*, and a second taxon that, based on SEM observations and comparison with published descriptions and images in historic literature, was finally identified as *Amphora micrometra* Giffen (1967: 253). The new species is identical with the taxon shown on figure 8H in Zidarova *et al.* (2022). The morphology of both species is discussed and illustrated and compared with similar species worldwide. Brief notes on their observed ecology are added.

Materials and Methods

The Black Sea is located between Europe and Asia covering an area of more than 430 000 sq. km and having a maximum depth of ca 2200 m. Due to the freshwater input from several large rivers (Danube, Dnipro and Don) and the narrow connection with the Mediterranean Sea via the Sea of Marmara and Bosporus and Dardanelles Straits (Fig. 1), it is characterized by low salinity and nutrient-rich waters with a strong stratification, together with anoxic conditions at larger depths (Borysova *et al.* 2005, Murray *et al.* 2007).

Diatom samples were obtained from artificial substrata (roughly hand-sanded plexiglass tiles) placed into the water column at two sites off the western Black Sea coast: near Marina Port Sozopol and off Maslen nos Cape (Figs 1B–D), both located in Bulgaria. Substrata were submerged for a period of 58 days in July–October 2020. Two samples were selected in this study: sample C3.1 collected on 30 August 2020 from Sozopol Bay (42°25'05.3"N, 27°41'20.6"E), and sample M4.3 collected on 28 August 2020 off Maslen nos

Cape (42°18'23.3"N, 27°47'23.4"E) (Figs 1C, D). Samples were collected after 28 (sample C3.1) and 43 (sample M4.3) days of substrate exposure. Each sample was composed of the biofilms present on 3 randomly selected tiles (with a final total sampled area of 75 cm^2). The biofilm was were collected using a toothbrush, placed in 20 mL vials with distilled water and preserved immediately with 3% formaldehyde. Basic environmental data, including sea water temperature, pH, salinity and conductivity at a depth of 2 m taken during sampling in July–August 2020 are summarized in Table 1.

For light microscopy (LM), samples were treated following the method described in Hasle & Fryxell (1970), after pretreatment with a few drops of concentrated HCl in order to dissolve possibly present carbonates in the samples. Diatoms were mounted in Naphrax® and slides were studied using an Olympus BX51 light microscope at 1000x magnification (N.A. 1.30), equipped with Differential Interference Contrast (DIC) optics and Olympus digital imaging system. For scanning electron microscopy (SEM), part of the suspension was filtered through 5-µm Isopore™ polycarbonate membrane filters (Merck Millipore), pieces of which were fixed on aluminium stubs after airdrying, coated with a platinum layer of 20 nm and studied using a JEOL-JSM-7100F field emission scanning electron microscope at 2 kV at Meise Botanic Garden, Belgium. Cleaned sub-samples and stubs are stored at BR Collection, Meise Botanic Garden, Belgium.

Terminology for species descriptions follows Round *et al.* (1990), Levkov (2009) and Stepanek & Kociolek (2018).

For typification of the species, the entire slide was chosen as the type, following article 8.2 of the International Code for Botanical Nomenclature (Turland *et al.* 2018). Diatoms show a broad variability along their cell cycle making the choice for the entire population on the slide more obvious, but because of admixtures, one valve was indicated to illustrate the taxon best

(see Figures). All novelties are registered proactively according to Art. 42.3 (Turland *et al.* 2018).

Results

Division **Bacillariophyta**

Class **Bacillariophyceae**

Subclass **Bacillariophycidae**

Order **Naviculales**

Family **Amphipleuraceae** (according to Algaebase, Guiry & Guiry 2023)

Genus ***Halamphora***

***Halamphora valdeminutissima* Zidarova, Pottiez, P.Ivanov, M.de Haan & Van de Vijver**, *sp.nov.*

(Figs 2–11, 16–19)

Description

LM observations (Figs 2–10):—Valves semi-lanceolate with distinctly convex dorsal margin (Figs 2–10) and straight (Fig. 2) to slightly convex (Figs 7, 9) ventral margin, occasionally slightly concave in the middle (Fig. 3). Valve apices small, protracted, subrostrate, bent toward the ventral side. Valve dimensions (n=14): length 6.0–13.5 µm, width 1.5–3.0 µm. Raphe slightly arcuate, located almost centrally along the vertical axis of the valve, slightly closer to the ventral side. Other characteristic features difficult to discern in LM. Valves from a second small population (sample M4.3) had the same characteristics (Fig. 11).

SEM observations (Figs 16–19):—Externally, valve face flat with a small marginal dorsal ridge (indicated on Fig. 17, white arrow) interrupting the striae at the valve face/mantle junction (Figs 17, 18). Dorsal striae ca 45 in 10 µm (n=7), parallel to weakly radiate towards

the apices, usually composed of a single elongated areola on the valve face and a single areola on the mantle (Fig. 17). Areolae possessing recessed foramina between relatively thick virgae. Occasionally, irregularities in this striation pattern were observed (Fig. 16). Ventral striae 55–65 in 10 µm (n=7), composed of a single areola with recessed foramina, parallel to slightly radiate, distinctly shorter near the valve middle, where slightly arched in the center, and leaving a small semi-elliptic central area on the ventral side (Fig. 17, black arrow). Distinct raphe ledge present along the entire length of the valve on the dorsal side (indicated on Fig. 18, white arrow), slightly widening at the valve middle, then running straight, weakly widening once again towards the apices, and finally gradually narrowing at the apices (see Figs 16–18). Small thickening present on the ventral side near the central raphe endings (Figs 16, 18, black arrow). Raphe slightly arcuate, central raphe endings close one to another, drop-like enlarged and shortly bent towards the dorsal side (Figs 16–18). Distal raphe fissures weakly dorsally deflected (Figs 16–18). Internally (Fig. 19), striae located between slightly raised virgae. Central raphe endings terminating onto fused helictoglossae (Fig. 19, white arrow), and terminal raphe endings finishing onto small helictoglossae (Fig. 19, black arrow). Finer areolar structure externally and internally not resolved.

Type—BULGARIA. The Black Sea: Sozopol Bay, south of Marina Port Sozopol, 42°25'05.3"N, 27°41'20.6"E. Sample C3.1, *Leg. R. Zidarova & P. Ivanov*, *30 August 2020* (holotype: stub BR-4804! in BR Collection, Meise Botanic Garden Meise, Belgium. Fig. 18 represents the holotype specimen. Additionally, cleaned material of sample C3.1 is stored at BR Collection, Meise Botanic Garden, Belgium).

Registration:—http://phycobank.org/XXXX

Etymology:—The specific epithet *"valdeminutissima"* refers to the very small valve dimensions of this species.

Ecology and distribution:—So far, the species has only been observed with certainty during summer months off the southern Bulgarian Black Sea coast, both near Marina Port Sozopol (sample C3.1) and Maslen nos Cape (sample M4.3). At both sites, and during an entire month of measurements, sea water temperature was on average nearly 26 ^{o}C, mean sea water pH was 8.3, and mean salinity and conductivity values were ca 18 PSU and about 29 mS/cm, respectively, showing very little variation (Table 1). The type locality is characterized by deteriorated oxygen conditions, but excellent condition with regard to inorganic forms of nitrogen and in good state in terms of phosphorous concentrations, according to the local regulations (Zidarova *et al.* 2022). Full record of measured environmental variables at the type locality are given in Zidarova *et al.* (2022, Table 1).

Order **Thalassiophysales**

Family **Catenulaceae**

Genus ***Amphora***

***Amphora micrometra* Giffen (1967: 253) emend. Ács, K.T.Kiss & Levkov in Ács *et al.* (2011: 200)**

(Figs 12–15, 20–21)

References: —Snoeijs & Potapova 1995, p. 26, Ács *et al.* (2011), p. 200, figs 9–11, 30–34

LM observations (Figs 12–15):—Valves narrowly semi-elliptic with moderately convex dorsal side and nearly straight ventral side, and rounded apices. Valve dimensions (n=6): 7.5–11.5 µm, width 1.5–2.0 µm. Raphe straight (Fig. 15) or slightly arcuate (Fig. 12), positioned close to the ventral side of the valve. Other morphological features not discernible in LM.

SEM observations (Figs 20–21):—Due to the rarity of the species in the sample, only three valves were observed in SEM. Externally (Fig. 20), valve face flat with a gradual transition between the valve face and valve mantle. Marginal ridge absent. Axial area very narrow, almost linear (Fig. 20, white arrows). Dorsal striae positioned between raised virgae, parallel to slightly radiate, ca 60 in 10 µm (n=3). Ventral striae parallel to weakly radiate, 70–80 in 10 µm (n=3). Finer areolar structure could not be resolved. Raphe straight, with straight, slightly expanded central raphe endings. Terminal raphe fissures could barely be observed (on Fig. 20 at right upper corner), hooked to the dorsal side. Internally (Fig. 21), central raphe endings straight, terminating onto central fused helictoglossae (Fig. 21, black arrow). Terminal raphe endings straight, terminating onto very weakly developed helictoglossae (Fig. 21, grey arrow). A single portula present at each valve apex near the distal raphe ends (Fig. 21, white arrows).

Ecology and distribution:—Based on literature data listed in Ács *et al.* (2011), *A. micrometra* is a widely distributed species with records from all around the world under variable environmental conditions. However, without a careful morphological check, it is difficult to confirm all these records. In Europe, the species has been recorded with certainty in the Baltic Sea (Snoeijs & Potapova 1995, Witkowski *et al.* 2000). The current observation seems to be the first verified record from the Black Sea. The species, observed at both studied sites (samples C3.1 and M4.3), was more rarely present in the samples compared to *Halamphora valdeminutissima* (Zidarova, pers. obs.).

Discussion

Amphora micrometra is a marine species, originally described from South Africa (Giffen 1967) as a species with small valve dimensions (length of only 7–8 µm, width of 2.5 µm), a convex dorsal margin and slightly protracted rounded apices, a straight or slightly convex ventral margin, straight raphe branches located near the ventral margin, and a fine striation. At

the time of its description, the species was only known from LM observations. As a result of its insufficiently known morphology, valves with similar valve outline and fine/invisible striation have been identified and reported as *Amphora micrometra* from various locations worldwide. These former records are well summarized in Ács *et al.* (2011), who observed a population of a taxon morphologically similar to Giffen's *A. micrometra*, from a brackish and nutrient-rich laguna in the Bolivian Altiplano. Based on LM and SEM observations on the valves from the Bolivian Altiplano and on the type of *A. micrometra* from South Africa, Ács *et al.* (2011) concluded that both populations were conspecific. They emended the description of *A. micrometra*, providing information for the dorsal and ventral striae, raphe structure and girdle bands. However, Ács *et al.* (2011) also addressed the uncertainty regarding the generic position of this taxon. *Amphora micrometra,* unlike *Amphora sensu stricto,* possesses fused central helictoglossae, a feature of the genus *Halamphora* (Levkov et al. 2009). Other differences from the genus *Amphora* observed by Ács *et al.* (2011) included the number of girdle bands and striae structure. But, most strikingly, SEM observations on different populations that could be identified *A. micrometra* (*e.g.*, Snoeijs & Potapova 1995, Ács *et al.* 2011) show the presence of a single portula at each valve apex, as in the taxon we observed (see Fig. 21, arrows), a feature uncommon for both the genera *Amphora* and *Halamphora*, as already discussed by Ács *et al.* (2011). Due to the uncertainties in the generic position of *Amphora micrometra*, Ács *et al.* (2011) left the species in the genus *Amphora,* at least till more information is provided.

Based on our observations on the valve outline and dimensions, stria density, raphe structure, and the presence of portulae at the valve apices, the species observed from the Black Sea should be identified as *A. micrometra.*However, some differences exist between the different *A. micrometra* populations observed worldwide, the most obvious being the dorsal stria density, which is slightly lower in the Bolivian Altipano population (44–52 vs usually around

60 striae in 10 µm) compared to the other populations (data summarized in Table 2 in Ács *et al.* 2011, Snoeijs & Potapova 1995). As Giffen (1967) was not able to discern clearly the striation density, he only stated that "striae are fine or scarcely visible" without providing an exact density number. Later, as noted by Ács *et al.* (2011), Giffen (1973) reported only 27 dorsal striae in 10 µm for *A. micrometra*. Given the small number of morphological characteristics discernable in LM for this taxon, Giffen's later observation should be interpreted with some doubt. Ács *et al.* (2011) reported 60 dorsal striae in 10 µm for the type of *A. micrometra.* On the valve from the type material from South Africa, shown on figure 31 in Ács *et al.* (2011) we also measured 58–60 striae in 10 µm, and not 27 as indicated by Giffen (1973). The observed dorsal stria density in the Black Sea populations is comparable to the range of striae given for the type of *A. micrometra* (up to 65 dorsal striae in 10 µm), and to the populations reported from the Baltic Sea (Snoeijs & Potapova 1995, p. 26). A comparable similarity was observed between the Baltic Sea populations and the type of *A. micrometra*, with the number of ventral striae reported for the type being 60 in 10 µm. However, in the Baltic populations a slightly higher ventral stria density, of 68–70 striae in 10 µm, was reported (Snoeijs & Potapova 1995, p. 26). Valves observed in the Black Sea benthos are similar to *Amphora micrometra* from the Baltic Sea depicted by Snoeijs & Potapova (1995, p. 26), although with slightly more densely spaced ventral striae (70–80 vs 68–70 in 10 µm). Another subtle difference between the Bolivian Altiplano population and those from both the Black and Baltic Seas can be observed in the central raphe endings, which appear positioned comparatively closer in both the Baltic Sea (Snoeijs & Potapova 1995, p. 26) and the Black Sea populations (Fig. 20 here) than in those from Bolivia (figures 16–18 in Ács *et al.* 2011,). In addition, both the valves from the Baltic Sea and the Black Sea possessed a very narrow axial area, lacking the slight expansion of the central area, ventrally present in the Bolivian population (see Ács *et al.* 2011 and their figures 16, 18). Whether these small differences

between the European and the Bolivian Altiplano populations are important, is at present not clear and most likely not sufficient to separate the populations as independent species. Recently, a very small-celled taxon was described based on both molecular and morphological data as *Halamphora adumbratoides* Stepanek & Kociolek (2018: 49). Stepanek & Kociolek (2018) considered this taxon very similar to *Amphora adumbrata* Hohn & Hellerman (1966: 119), except in the stria density. In valve outline and striation pattern *Halamphora adumbratoides* is also similar to *Amphora micrometra* (compare figures 1&2 on Pl. 52 in Stepanek & Kociolek 2018 with Figs 20&21 here and figures 30–34 in Ács *et al.* 2011). Its dimensions (length 10.0–18.0 µm and width 1.5–2.5 µm) and stria numbers (56–57 dorsal striae in 10 µm and ca. 70 ventral striae in 10 µm) are also comparable with those of *Amphora micrometra* from both the type, the Baltic Sea and the Black Sea populations (all discussed above). These similarities are also present together with the "nearly absent" raphe ledge in *H. adumbratoides* (Stepanek & Kociolek 2018). Although no portulae were reported or observed for *H. adumbratoides* (Stepanek & Kociolek 2018), the morphological similarity between the two taxa raises the question whether they are conspecific. What the importance of portulae in *Amphora/Halamphora* could be is also yet to be discovered. Therefore, for the moment, we concur with the decision of Ács *et al.* (2011) to keep *Amphora micrometra* as a species within the genus *Amphora*, till more data become available.

Halamphora valdeminutissima sp. nov. is described within the genus *Halamphora* based on the following morphological features: the presence of a well-developed raphe ledge on the dorsal side; striae composed of areolae with recessed foramina; internal central raphe endings terminating onto a fused central helictoglossae in "one solid structure, inwardly elevated from the rest of the valve" (Levkov 2009, see Fig. 19 here). The new species resembles *A. micrometra* in LM when taking only valve outline and dimensions into account, and *A. micrometra* can be easily overlooked if present in small numbers together with *H.*

valdeminutissima. The two species were sympatrically observed in the analyzed samples. Despite their similarity in valve outline and dimensions, and the lack of visible striation in both taxa in LM, it is still possible to separate both using LM observations by: 1. the more semi-elliptic valve outline with smaller and subrostrate protracted apices in *H. valdeminutissima sp. nov.* compared to *Amphora micrometra* (Figs 2–11 and 12–15); 2. the almost centrally positioned raphe in *H. valdeminutissima sp. nov.* compared to the ventrally positioned raphe in *A. micrometra* (Figs 2–11 and 12–15). Not many other *Amphora* and *Halamphora* taxa, similar to *H. valdeminutissima sp. nov*, could be found in the currently available literature. Zidarova *et al.* (2022) commented that based on its valve outline, the new species is slightly similar to *Halamphora obscura* (Krasske 1938: 530) Levkov (2009: 211) and *Amphora delicatissima* Krasske in Hustedt (1930: 346). However, *Halamphora obscura* has more coarsely striated, larger valves (length >15 µm, width >2.4 µm, dorsal striae only 26–30 in 10 µm vs ca 45 in 10 µm); in addition, in SEM striae are uniseriate, composed of a number of elliptical areolae, and the raphe ledge is slightly constricted near the central nodule (Levkov 2009), features absent in *H. valdeminutissima sp. nov*. *Amphora delicatissima* is also much broader (width >3 µm) with more distantly spaced striae, clearly visible in LM (up to 38 striae in 10 µm) (Witkowski *et al.* 2000, Wachnicka & Gaiser 2007).

Under SEM, a similar striation pattern on the dorsal side of the valve as in *H. valdeminutissima sp. nov.* is observed in *H. pellicula* Stepanek & Kociolek (2018: 50), but the latter taxon has broader valves (>2.5 µm) with comparatively more convex dorsal and/or ventral margins and shortly protracted subcapitate apices (vs more protracted and subrostrate apices clearly bent to the ventral side in *H. valdeminutissima sp. nov.*) (compare figures 3–6 on Pl. 52 in Stepanek & Kociolek 2018 and Figs 16–18 here). The raphe in *H. pellicula* is positioned closer to the ventral valve margin, and not near to the valve center as in *H. valdeminutissima sp. nov*. (see figures 9–16 on Pl. 50 in Stepanek & Kociolek 2018 and Figs

2–11 here). The striation on the ventral side of the valves in *H. pellicula* is also coarser (45–52 vs >55 ventral striae in 10 µm in *H. valdeminutissima sp. nov.*). In addition, *Halamphora pellicula* possesses a much broader dorsal raphe ledge, abruptly narrowed to nearly sharply cut at the apices (Pl. 52, figures 3–6 in Stepanek & Kociolek 2018), compared to the thinner and gradually narrowing at the apices raphe ledge in *H. valdeminutissima sp. nov.* (Figs 16–18). Zidarova *et al.* (2022) also illustrated and discussed a finely structured species, *Halamphora* aff. *pellicula,* found in the samples from Sozopol Bay; however, this taxon is impossible to confuse with *H. valdeminutissima sp. nov.,* even during LM observations. *Halamphora* aff. *pellicula* (illustrated on figures 5I–L in Zidarova *et al.* 2022) has much more convex dorsal valve margin and straight ventral margin, straight raphe positioned very close to the ventral margin, and a larger valve width (>2.5 µm, Ziarova *et al.* 2022), contrary to narrower vaves of *H. valdeminutissima sp. nov.* with subrostrate protracted apices and more centrally positioned raphe. Further, SEM observations showed that *Halamphora* aff. *pellicula* is another species that needs attention, since it has uniseriate striae of more or less rounded areolae; therefore, it neither represents *Halamphora valdeminutissima sp. nov.,* nor *H. pellicula* (R.Zidarova & M. Pottiez, unpubl. data), where the dorsal striae are composed of single elongated areola (Figs 16–18 here). The same applies to the related to *Halamphora* aff. *pellicula* taxon illustrated as *Amphora* (*Halamphora*) sp. S8 in Zidarova *et al.* (2022, figures 5M–P). Finally, *Amphora adumbrata,* which is actually another species of *Halamphora* as already noticed by Stepanek & Kociolek (2018), is a taxon having a slender valve outline with more rounded apices, similarly to *Halamphora adumbratoides* and *A. micrometra,* but not to *H. valdeminutissima.* In SEM, *Amphora adumbrata* has comparable structure of the striae, but the striation is coarser (35–40 vs ca 45 striae in 10 µm in *H. valdeminutissima sp.nov.*), and even visible in LM in the valve middle on the dorsal side of the valves. The latter was illustrated on both the drawing in Hohn & Hellerman (1966, Pl. I, figure 20) and on the LM

illustrations provided by Desianti *et al.* (2015, figures 33, 34). No striae were ever observed in LM on the comparatively shorter but wider valves of *H. valdeminutissima sp. nov.* (see Figs 2–11 here and figures 33–36 in Desianti *et al.* 2015). In addition, as observed by Desianti *et al.* (2015) in SEM, the raphe ledge of *Amphora adumbrata* covers half of the dorsal striae. Such arrangement was also not observed in any of the studied valves of *H. valdeminutissima sp. nov.,* so far.

Another taxon resembling both *A. micrometra* and *H. valdeminutissima sp. nov.,* is *Amphora exilissima* Giffen (1967: 251). Records of the latter species exist also from the Black Sea (Nevrova 2022, as both *A. exilissima* and *A.* cf. *exilissima*), but its identity is still not well known. *Amphora exilissima* was also present in the material that served as type of *A. micrometra* and studied by Ács *et al.* (2011). They were, however, unable to find specimens in SEM or TEM, but based on the original description in Giffen (1967), and their LM observations on the type, *A. exilissima* has a distinct stauros on the dorsal side (figures 13–14 in Ács *et al.* 2011), a feature absent in both *A. micrometra* and *H. valdeminutissima sp. nov.*

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Table 1. Sea water temperature, pH, salinity and conductivity values at the sampling sites per date of measurement, with mean values during July–September 2020 and standard deviation.

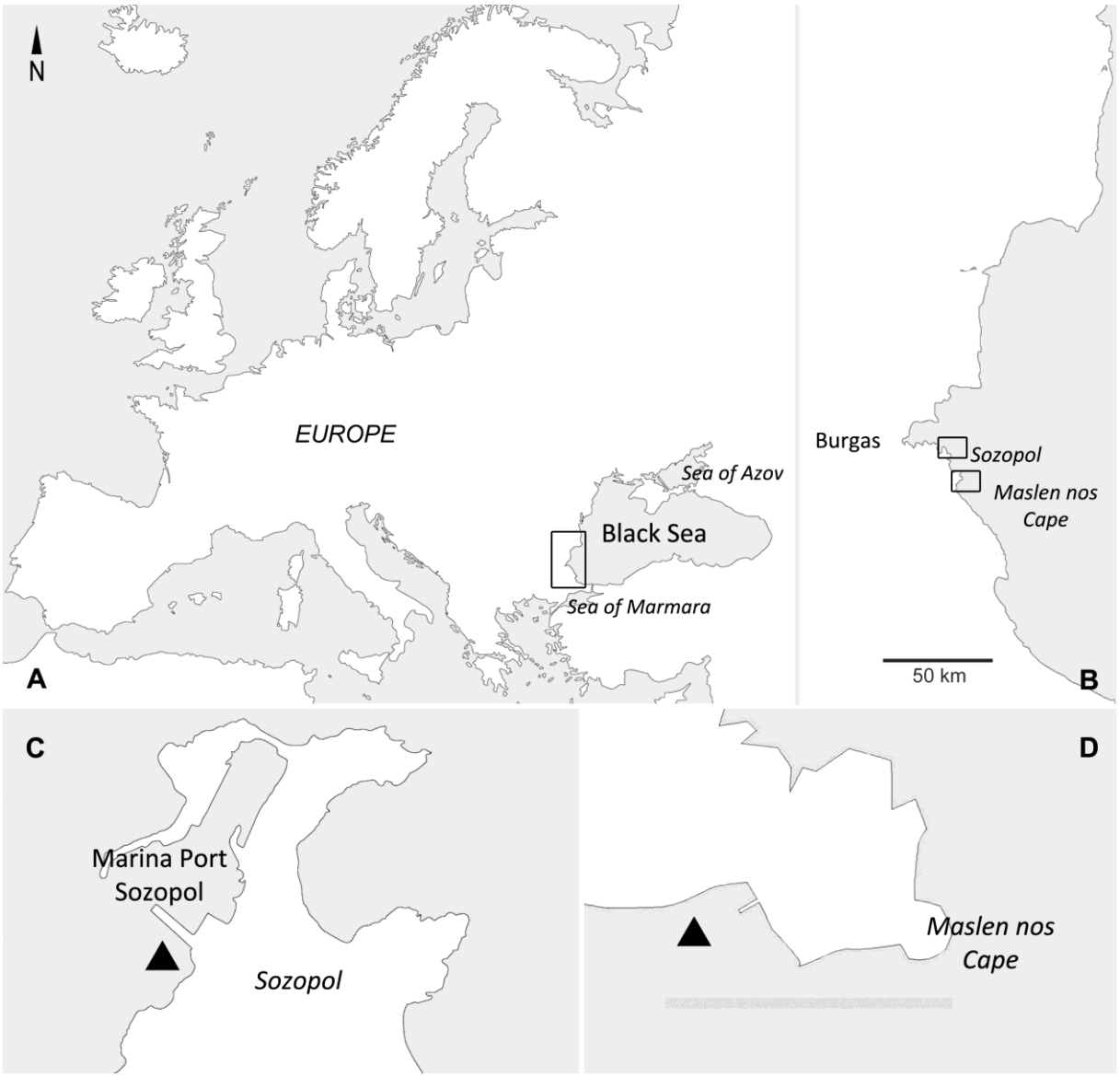
Marina Port Sozopol		**pH**	**salinity, PSU**	**conductivity, mS/cm**	**temperature, °C**
27.07.2020		8.37	17.83	28.92	25.68
05.08.2020		8.22	17.99	29.15	25.67
14.08.2020		8.31	17.87	28.98	26.02
19.08.2020		8.35	18.06	29.26	25.64
25.08.2020		8.34	17.82	28.9	25.84
30.08.2020		8.34	17.80	28.87	25.54
	mean	*8.30 (±0.05)*	*17.89(±0.11)*	*29.01(±0.16)*	*25.70 (±0.17)*
Maslen nos Cape					
28.07.2020		8.31	17.40	28.29	25.74
13.08.2020		8.32	17.47	28.37	25.89
19.08.2020		8.49	17.96	29.14	26.35
28.08.2020		8.27	17.84	28.95	25.83
01.09.2020		8.33	17.92	29.06	26.23
	mean	*8.34 (±0.08)*	*17.72 (±0.26)*	*28.76 (±0.40)*	*26.01 (±0.27)*

Figure legends

FIGURE 1. Maps showing the geographic position of the Black Sea (A), sampling locations (B) and sampling sites (C, D). Maps based on © d-maps.com (A, B) and © OpenStreetMap contributors (C, D), edited and arranged using Adobe Illustrator © and Adobe Photoshop ©.

FIGURES 2–21. LM and SEM microphotographs of *Halamphora valdeminutissima sp. nov.* and *Amphora micrometra.* 2–10. LM valve views of *Halamphora valdeminutissima sp. nov.* from the type population (sample C3.1, fig. 6 shows an entire frustule). 11. LM valve views of *Halamphora valdeminutissima sp. nov.* from a population near Maslen nos Cape (sample M4.3). 12–15. *Amphora micrometra*, LM views of several valves from the Black Sea, sample M4.3 (Figs 12–14) and sample C3.1 (Fig. 15). 16–18. *Halamphora valdeminutissima sp. nov.*, SEM external views of entire valves, all from the type population (sample C3.1, Fig. 18 represents the holotype specimen). Fig. 16: white arrow indicates the dorsal raphe ledge; black arrow points to the thickening near the central raphe endings. Fig. 17: white arrow indicates the dorsal marginal ridge. Fig. 18: black arrow points the thickening ventrally to the central raphe endings. 19. *Halamphora valdeminutissima sp. nov.*, SEM internal view (type population, sample C3.1). Black arrow indicates the fused central helictoglossae, and white arrow points to the raphe fissures termination onto a weakly developed helictoglossa. 20. *Amphora micrometra*, SEM external view of a single valve from the Black Sea (sample M4.3). White arrow indicates the narrow axial area. 21. *Amphora micrometra*, SEM internal view of a valve from the Black Sea (sample M4.3). White arrows indicate the portulae at each valve apex; grey arrow points the terminal fissures; black arrow shows the fused central helictoglossae

Scale bars: 10 µm (2–15), 1 µm (16–21).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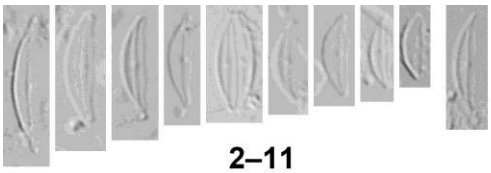
Cape



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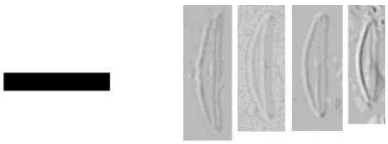

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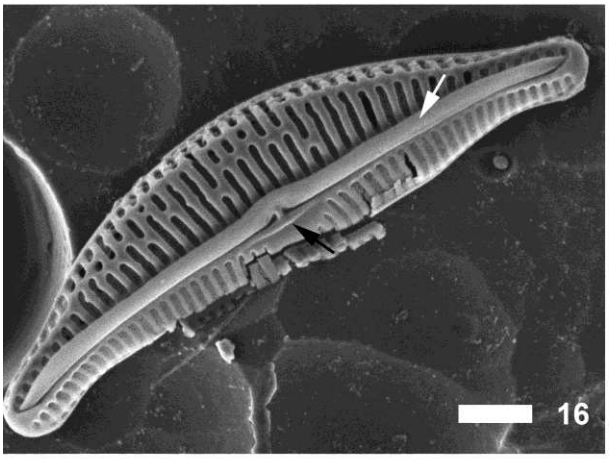

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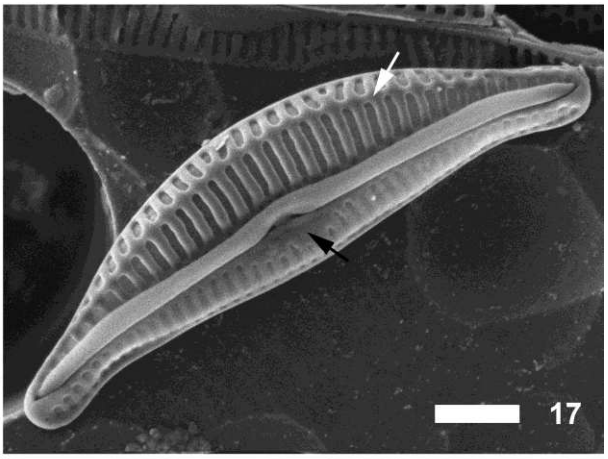

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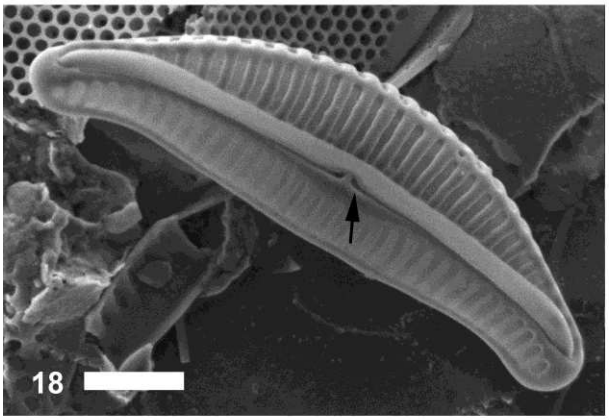

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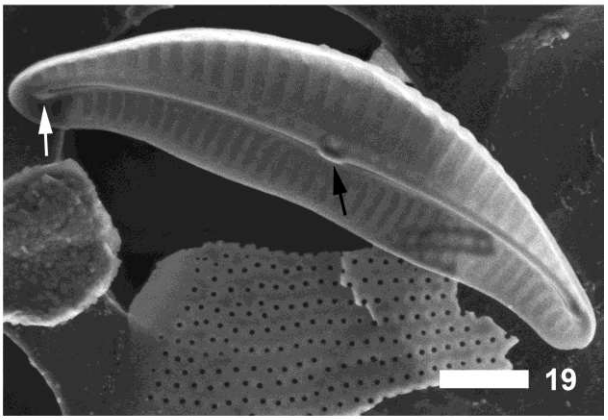

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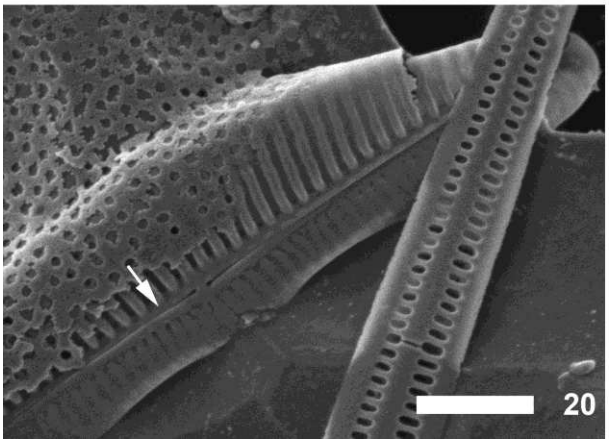

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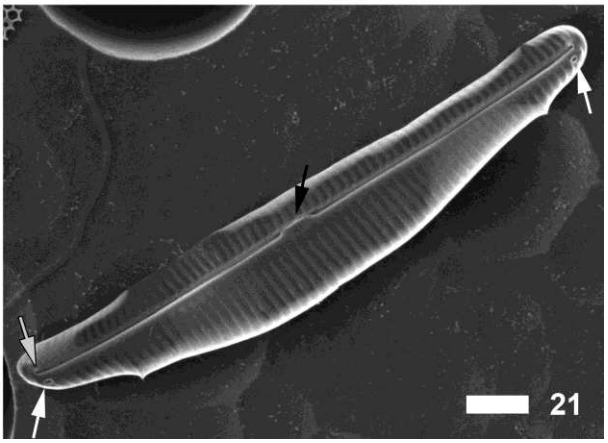

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