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# The unexpected diversity in *Amphora* sensu lato (Bacillariophyta) at Sozopol Bay, the western Black Sea

RALITSA ZIDAROVA[1,3*], PLAMEN IVANOV[2,4], OGNYANA HRISTOVA[1,5], BORYANA DZHUROVA[1,6] & ELITSA HINEVA[1,7]

[1]*Institute of Oceanology at the Bulgarian Academy of Sciences, 9000 Varna, Bulgaria*
[2]*Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[3] *zidarova.r@gmail.com, https://orcid.org/0000-0002-6451-0099*
[4] *plamen_new@abv.bg, https://orcid.org/0000-0003-2215-7984*
[5] *o.hristova@io-bas.bg, https://orcid.org/0000-0002-1487-1662*
[6] *dzhurova@abv.bg, https://orcid.org/0000-0003-0849-8184*
[7] *elitsa.hineva@abv.bg, https://orcid.org/0000-0002-9540-5267*
**corresponding author: zidarova.r@gmail.com*

## Abstract

During a survey of benthic diatoms from the Bulgarian Black Sea coast near the historic town of Sozopol, we found a high diversity of *Amphora* sensu lato taxa at a single site and on a single type of artificial substratum. A total of 40 taxa from the current genera *Amphora, Halamphora, Seminavis* and *Tetramphora* were recognized during light microscopy (LM) and scanning electron microscopy (SEM) observations. We were able to identify with a high degree of certainty only 6 taxa based on the literature, whereas for 19 taxa (or almost 50% of all taxa) we could not find even sufficiently similar species, and they remained unidentified to species level. Many of the species had small valve dimensions. The paper presents all taxa with short morphometric data and microphotographs under LM and SEM, where possible. All taxa are discussed and compared with similar known taxa. The illustrative materials in earlier works from the Black Sea and species descriptions, where such were available, were also consulted. Diversity of *Amphora* sensu lato in the Black Sea coastal waters seems to be underestimated, whereas some of the past records are uncertain. A discussion on the current state of knowledge of *Amphora* sensu lato species from the Black Sea is given.

**keywords**: marine benthos, Mediterranean Region

## Introduction

*Amphora* Kützing ex Brébisson sensu lato is a large, heterogenous group of diatom species, currently containing taxa from several distinct genera, including *Amphora* sensu stricto and *Halamphora* (P.T.Cleve) Levkov (Levkov 2009, Stepanek & Kociolek 2018), *Tetramphora* Mereschkowsky (Stepanek & Kociolek 2016) and *Seminavis* D.G.Mann (Round *et al.* 1990). By the 6th of October 2021, a total of 1659 *Amphora* sensu lato species and infraspecific taxa are listed as extant in DiatomBase (Kociolek *et al.* 2021), making it one of the largest diatom genera, even if some of these records include synonymy. Due to the high diversity and numerous misinterpretations of the species over a period of almost 200 years, the genus is also one of the most complicated taxonomically. The studies of the type materials of a number of *Amphora* taxa allowed the recognition of the real identity of certain species (e.g. Archibald & Schoeman 1984, Clavero *et al*. 2000, Danielidis & Mann 2002, Sala *et al.* 2006, Levkov 2009, Ács *et al*. 2011, Desianti *et al.* 2015, etc.), while hundreds of new species were also described from all over the world in the past years (e.g. Danielidis & Mann 2002, Garcia 2007, Levkov 2009, Stepanek & Kociolek 2013, 2018, Van de Vijver *et al*. 2014, López-Fuerte *et al.* 2020, and others). Many of the species thrive in brackish/marine coastal benthic habitats (Levkov 2009, Stepanek & Kociolek 2018).

The Black Sea is a unique ecosystem, characterized by nutrient-rich waters with a lower salinity, strong stratification and anoxic conditions at higher depths (Borysova *et al.* 2005, Murray *et al.* 2007). Diatoms from the Black Sea were first reported by Mereschkowsky in the beginning of the 20th century (Proshkina-Lavrenko 1963 and references therein), but the most comprehensive data for benthic diatoms were collected after the mid 20th century. During the past

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60 years, studies on the northern coasts of the Black Sea have provided large floristic data from a variety of substrates (Proshkina-Lavrenko 1963, Guslyakov *et al.* 1992, Nevrova 2013a, b, 2014a,b, 2015a, 2016, Petrov & Nevrova 2013, Nevrova & Petrov 2019a, Ryabushko 2020, etc.), including artificial/anthropogenic ones (Proshkina-Lavrenko 1963, Milchakova *et al.* 2002, Lokhova 2012, etc.). However, other coasts remained understudied. Particularly, information for benthic diatoms from the Bulgarian (i.e., western) coast is scarce, in only a few publications, all issued almost 30 years ago. The largest data was provided by Petrova *et al.* (1991) and Temniskova-Topalova *et al.* (1994), but without any icongraphic material. Diatoms were also part of several studies of Late Holocene coastal deposits at Sozopol Bay (Ognjanova-Rumenova 1995, Ognjanova-Rumenova & Zaprjanova 1998, Ognjanova-Rumenova *et al.* 1998, 1999), of which two included microphotographs of some of the recorded taxa (i.e. Ognjanova-Rumenova 1995; Ognjanova-Rumenova & Zaprjanova 1998). During the first diatom survey of the Bulgarian Black Sea coast since then, which was focused on diatom colonization rate (ongoing research) and during which we also had to perform community analyses, we found a surprisingly high diversity of *Amphora* sensu lato taxa at only one site and on a single type of artificial substratum. Many of the species had small valve dimensions and we were unable to find them in the literature. Despite the numerous studies from the northern coasts of the sea, currently there is no relevant literature for the identification of *Amphora* sensu lato taxa from the Black Sea. The impossibility to identify the taxa forced us to take a closer look at the species we observed. We found it crucial to at least document and report the observed diversity, while trying to summarize it and to discuss the species we discovered in the light of the currently adopted taxonomy (e.g. Round *et al*. 1990, Levkov 2009, Stepanek & Kociolek 2016, 2018) and the historical data from the Black Sea. Although many taxa remained with uncertain identification, or no identification could be provided at all, we refrained from describing new taxa in this paper, till more observations on different populations from the Black Sea become available. The paper presents the taxa in our material with morphometric data and microphotographs. Comparisons with morphologically similar taxa are given for each taxon, where appropriate or necessary, including with earlier identifications from the Black Sea, where possible. The current state of our knowledge of *Amphora* sensu lato from the Black Sea is also discussed.

## Materials and methods

Samples (a total of 14) were obtained from artificial substrata, placed into the water column at a single site near the yacht port Marina Port Sozopol, Sozopol Bay, the western Black Sea coast (fig. 1). The substrata were roughly hand-sanded plexiglass tiles (25 $cm^2$ each), submerged at two depths (1 m and 3 m) for a period of 58 days in July–October 2020, and sampled consecutively on days 10, 17, 26, 30, 39 and 58. Each sample contained the biofilm present on 3 randomly selected tiles (total area of 75 $cm^2$). The biofilm was collected with a toothbrush into 20 mL vials with distilled water and preserved with 3% formaldehyde *in situ*. During sampling environmental data were also obtained, including Secchi depth, sea water temperature, pH, dissolved and saturated oxygen, conductivity and salinity, at a depth of 2 m. Water chemistry analysis (including TN, $N\text{-}NO_2$, $N\text{-}NO_3$, $N\text{-}NH_4$, TP, IP) and chlorophyll *a* concentrations measurements were done four times during the sampling period (table 1), following standard spectrophotometric methods (Edler 1979, Grasshoff *et al.* 1999).

Diatom samples were prepared for light microscopy (LM) by the method of Hasle & Fryxell (1970), after the material was pretreated with several drops of concentrated HCl in order to dissolve possibly present carbonates in the samples. Diatoms were mounted in Naphrax® and slides were studied using 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 air-drying, coated with a platinum layer of 20 nm and studied using a JEOL-JSM-7100F field emission scanning electron microscope at 1 kV. Stubs are stored at the BR-collection (Meise Botanic Garden, Belgium). For taxa identification and comparisons we consulted the following larger works: Proshkina-Lavrenko (1963), Guslyakov *et al.* (1992), Witkowski *et al.* (2000), Wachnicka & Gaiser (2007), Levkov (2009), Stepanek & Kociolek (2018), a large number of papers published in the last century (particular references given in taxa discussions), and works from the northern coasts of the sea, such as Guslyakov (1987), Nevrova (2013a, b, 2014a, b, 2015 a, b, 2016), Petrov & Nevrova (2013), Nevrova & Petrov (2019a, b), Ryabushko *et al.* (2005, 2019), and others. In addition, all diatom taxon files, present in the digitalized collection of the Academy of Natural Sciences, Philadelphia (ANSP) (Potapova *et al.* 2021), were also examined. Taxa were arranged in plates using Adobe Photoshop®. Measurements of stria density in 10 µm were done starting from the middle of each valve towards the apex.

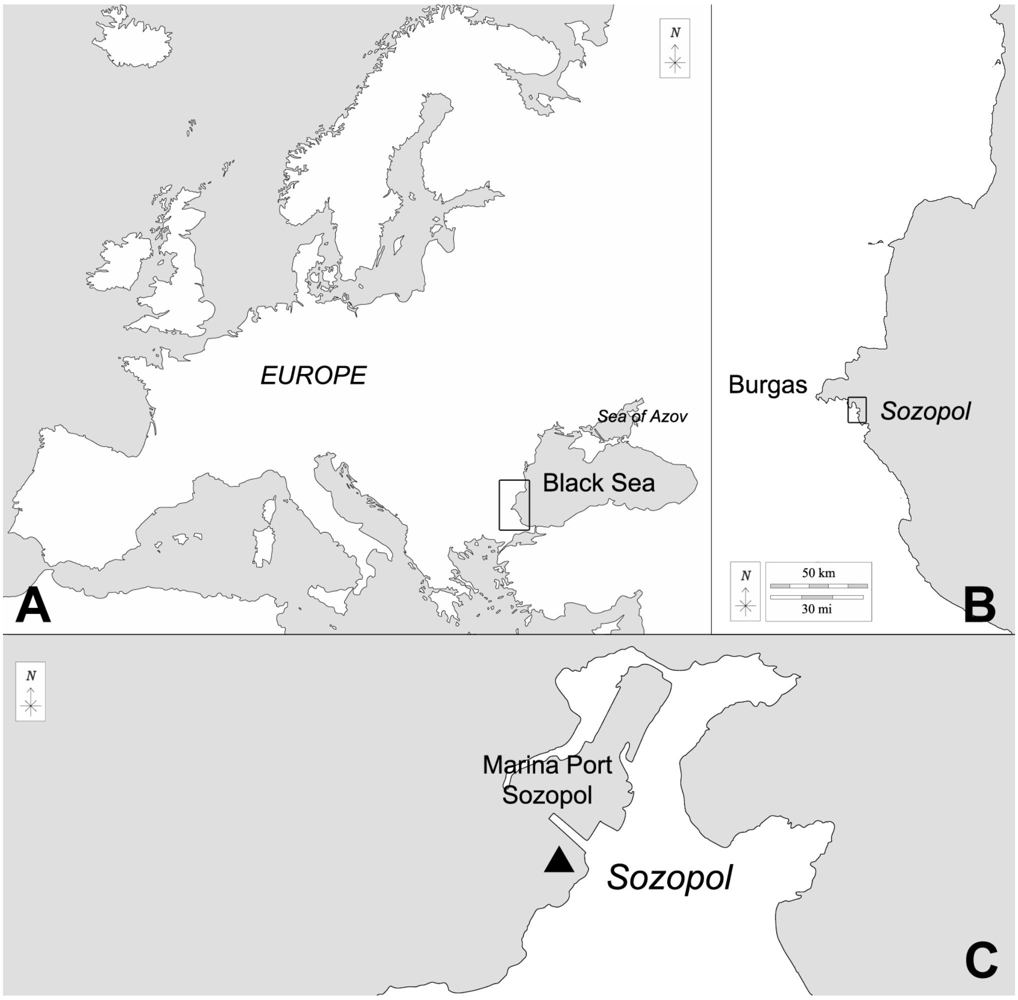

**FIGURE 1.** Map of the region showing the geographic position of the Black Sea (A), Sozopol area (B) and the sampling site (black triangle, C). Maps on figures A & B are based on maps of Europe and Bulgaria at © d-maps.com, whereas the outlines on C are based on © OpenStreetMap contributors. Maps were edited and arranged using Adobe Illustrator © and Adobe Photoshop ©.

**TABLE 1.** Values of the measured environmental parameters, water chemistry data and chl *a* concentrations at Sozopol Bay site during the sampling period.

| **date** | **pH** | **salinity, PSU** | **conductivity, mS/cm** | **O$_2$, %** | **O$_2$, µg.L$^{-1}$** | **water T, °C** | **Secchi depth, m** |
|---|---|---|---|---|---|---|---|
| 18/7/2020 | 8.31 | 17.24 | 28.03 | 83.6 | 6.75 | 25.80 | |
| 27/7/2020 | 8.37 | 17.83 | 28.92 | 90.1 | 7.17 | 25.68 | 5.40 |
| 05/8/2020 | 8.22 | 17.99 | 29.15 | 69.1 | 5.59 | 25.67 | 4.95 |
| 14/8/2020 | 8.31 | 17.87 | 28.98 | 79.3 | 6.25 | 26.02 | 4.25 |
| 19/8/2020 | 8.35 | 18.06 | 29.26 | 81.6 | 6.50 | 25.64 | 3.20 |
| 25/8/2020 | 8.34 | 17.82 | 28.90 | 86.2 | 6.82 | 25.84 | 4.70 |
| 30/8/2020 | 8.34 | 17.80 | 28.87 | 87.6 | 6.97 | 25.54 | 4.30 |
| 12/9/2020 | 8.39 | 18.04 | 29.21 | 56.0 | 4.68 | 24.83 | 4.80 |
| 30/9/2020 | 8.47 | 18.24 | 29.47 | 91.0 | 6.97 | 22.67 | 4.25 |
| mean | 8.34 | 17.88 | 28.98 | 80.5 | 6.41 | 25.30 | 4.48 |
| | **TN, µg.L$^{-1}$** | **N-NO$_3$, µg.L$^{-1}$** | **N-NO$_2$, µg.L$^{-1}$** | **N-NH$_4$, µg.L$^{-1}$** | **TP, µg.L$^{-1}$** | **IP, µg.L$^{-1}$** | **chl *a*, mg. m$^{-3}$** |
| 27/7/2020 | 273.47 | 0.00 | 5.34 | 144.41 | 7.75 | 4.67 | 0.522 |
| 17/8/2020 | 167.22 | 0.00 | 6.16 | 3.82 | 7.14 | 5.83 | 1.532 |
| 01/9/2020 | 266.06 | 1.27 | 2.05 | 3.72 | 8.05 | 5.54 | 0.646 |
| 07/9/2020 | 364.76 | 3.67 | 2.60 | 4.21 | 10.47 | 4.96 | 3.887 |
| mean | 267.88 | 1.23 | 4.04 | 39.04 | 8.35 | 5.25 | 1.56 |

## Results

The site near the town of Sozopol was characterized by deteriorated oxygen conditions, although excellent in regard to inorganic forms of nitrogen and good in terms of phosphorous concentrations, according to Regulation No H-4 for summer season of our coast. Very high concentrations of ammonium nitrogen were present in July, whereas in September the organic content in water increased, the latter consistent with the maximum measured concentration of chlorophyll *a* (table1).

We were able to distinguish a total of 36 *Amphora* sensu lato taxa under LM. Of these, 13 taxa (33%) were only incidentally observed. Other 4 taxa were only found during the SEM observations, bringing the total number of taxa to 40. Only 6 of all taxa (15%) agreed well with (original) descriptions of known taxa in the available literature. For 14 taxa (35%) the identifications are uncertain at present (either cf. or aff.). Almost half of the taxa (19 out of 40) remained unidentified to species level. All taxa are considered consecutively below by their corresponding figures under LM, whereas the few taxa observed only in SEM are added at the end. This unusual arrangement was done in order to allow for a better visual comparison between the morphologically similar taxa under LM. The following abbreviations are used in the morphometric data: L—valve length, W—valve width, DS—dorsal striae number in 10 µm, VS—ventral striae number in 10 µm, (n=x)—number of measured valves under LM.

### *Amphora* sensu lato taxa encountered in the study with notes on their identities

#### figs 2A–C & 8D: *Amphora* cf. *proteus* W.Gregory

**Morphometric data:—**L: 40.7–52.5 µm; W: 8.5–9.8 µm; DS: 11–13; VS: 10–12 (n=5).

**Notes:—**These valves do not fit entirely *A. proteus* since they usually have a single elongated areola on the ventral side, and not two rows of areolae as in *A. proteus* (Schoeman & Archibald 1986, Levkov 2009), although a very short second row could be present (figs 2B–C), as in some of the valves of *A. proteus* in Levkov (2009, Pl. 248: 6). Valves are also slightly smaller than those of the original *A. proteus* (length > 50 µm, width > 9 µm, Schoeman & Archibald 1986, Levkov 2009). However, the only valve we were able to find under SEM (fig. 8D) showed the presence of dimple-like depressions internally near the elongated central nodule, as in *A. proteus* (Levkov 2009, Pl. 248: 6). Another similar taxon in both dimensions and striation pattern, *A. proteoides* f. *varians* Proshkina-Lavrenko, was described from the Black Sea (Proshkina-Lavrenko 1963). Unfortunately, it is only known from her description and drawings (Proshkina-Lavrenko 1963, Pl. IX: 1–4), presenting insufficient details for species separation nowadays.

#### figs 2D–E: *Amphora* cf. *proteus* f. *ambigua* Proshkina-Lavrenko

**Morphometric data:—**L: 24.3–25.1 µm; W: 4.7–5.3 µm; DS: 14–15; VS: 15 (n=2).

**Notes:—**Proshkina-Lavrenko (1963, Pl. IX: 7) described *A. proteus* f. *ambigua* as having a length of 25–48.5 µm, a width of 5.5–9 µm, 12–15 dorsal and 8–13 ventral striae in 10 µm, interrupted in the middle, and a lanceolate hyaline area on the dorsal side. This is another taxon only known from the single drawing accompanying the description, which makes our identification doubtful. The valves we observed are also similar to the smaller valves of *Amphora allanta* Hohn & Hellerman in Desianti *et al.* (2015, figs 45–50), which dimensions and striation (length 23–56 µm, width 5.1–9.5 µm, and 13–14 striae in 10 µm) overlap with those of *A. proteus* f. *ambigua*.

#### fig. 2F: *Amphora* sp. S3

**Morphometric data:—**L: 30.5 µm; W: 5.5 µm; DS: 15, punctate; VS: 15, punctate (n=1).

**Notes:—**At present we are unable to provide identification of this species, neither we could place it within other taxa in the study. From *Amphora* aff. *pusio* P.T.Cleve (see below and figs 2G–H) it differs by the larger dimensions and punctate striae, straighter raphe and absence of hyaline area on the dorsal side. *Amphora allanta* has distinctly more arched raphe and its proximal raphe endings are bent towards the dorsal side (Desianti *et al.* 2015, figs 38–52).

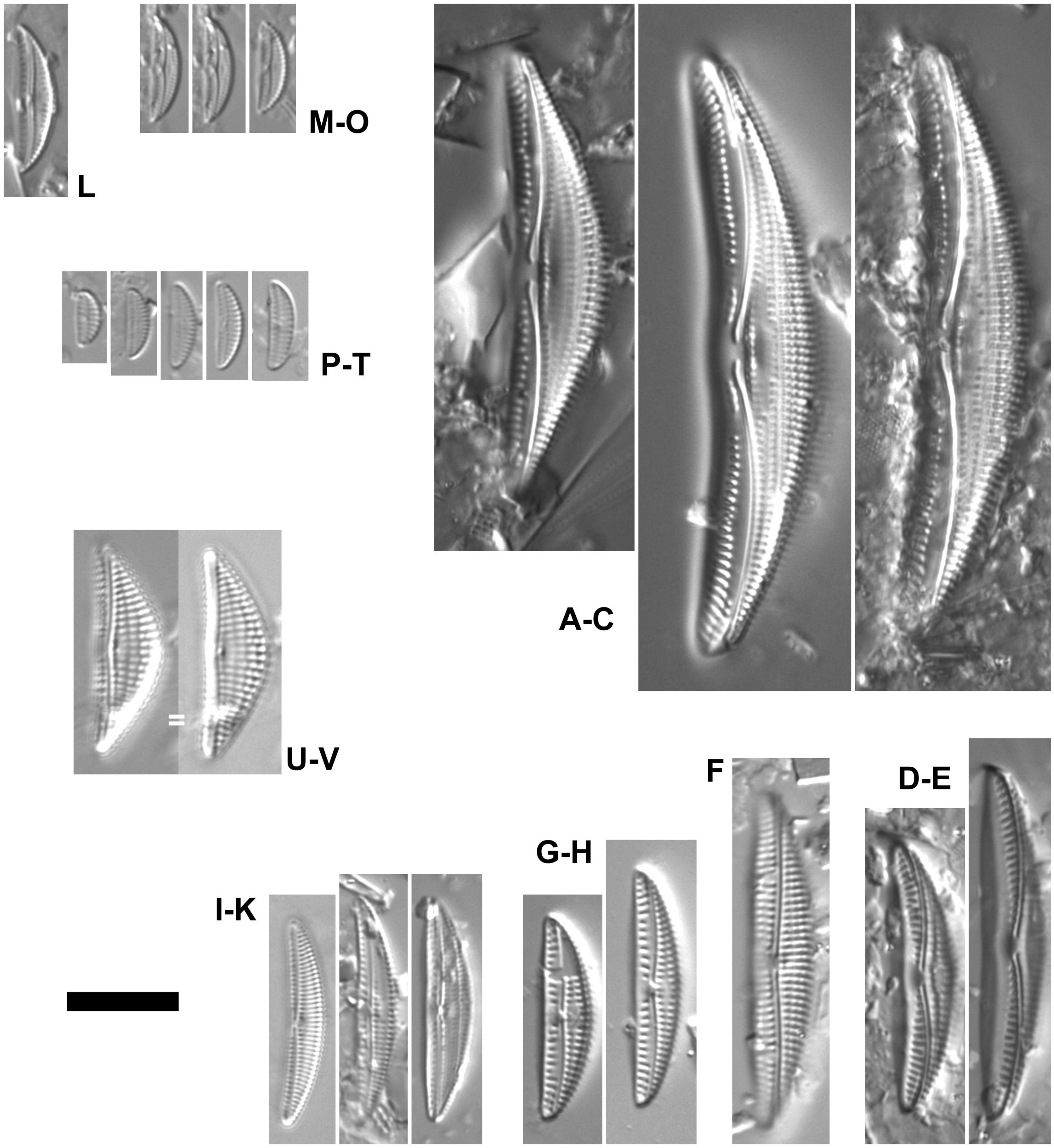

**FIGURE 2.** *Amphora* sensu lato taxa from Sozopol Bay under LM. A–C. *Amphora* cf. *proteus*. D–E. *Amphora* cf. *proteus* f. *ambigua*. F. *Amphora* sp. S3. G–H. *Amphora* aff. *pusio*. I–K. *Amphora* sp. S7. L. *Amphora* cf. *helenensis*. M–O. *Amphora* sp. aff. *A. helenensis*. P–T. *Amphora* sp. S11. U–V. *Amphora* sp. S26. Scale bar = 10 µm.

**figs 2G–H: *Amphora* aff. *pusio* P.T.Cleve**

**Morphometric data:—**L: 18.1–24.1 µm; W: 4.0–4.2 µm; DS: 15; VS: 16 (n=2).

**Notes:—**By dimensions, striation pattern and the presence of a narrow elongated hyaline area on the dorsal side, these valves fit *A. pusio* under LM, but lack its ventrally bent apices (Levkov 2009).

### figs 2I–K: *Amphora* sp. S7

**Morphometric data:—**L: 18.7–20.4 µm; W: 3.3–4.0 µm; DS: 22–24; VS: 18–22 (n=3).

**Notes:—**The valves of this taxon can be easily separated from the smaller valves of *Amphora* cf. *proteus* (figs 2A–C) and from *Amphora* aff. *pusio* (figs 2G–H) by the smaller width and the distinctly denser striation. Two taxa from the Black Sea are similar in valve outline, *Amphora lydiae* Guslyakov and *A. makarovae* Guslyakov (Guslyakov 1987). The LM micrographs for these two species in Guslyakov *et al.* (1992, Pl. XCIV: 10 & 11 for *A. makarovae* and Pl. XCIV: 16 for *A. lydiae*) do not provide sufficient information for their recognition, but at least *A. makarovae* can be separated with its larger width of 5–7 µm (Guslyakov 1987, Guslyakov *et al.* 1992).

### fig. 2L: *Amphora* cf. *helenensis* Giffen

**Morphometric data:—**L: 13.3 µm, W: 3.1 µm, DS: 21, VS: 18 (n=1).

**Notes:—**Giffen (1973) described the South African *A. helenensis* as a species with a valve length of 10–20 µm, a width of 3–4 µm, a lanceolate area on the dorsal side, 17–20 striae in 10 µm, and interrupted ventral striae in the valve middle. The single valve we observed fits the range of *A. helensis*, although the lanceolate area on the dorsal side is comparatively larger, and we counted 21 striae in 10 µm dorsally. Kaleli *et al.* (2017, fig. 2:1, as *A. helensis*) observed a similar taxon near Sinop, the southern Black Sea coast, although they reported a higher number of striae, 23 in 10 µm. *Amphora* cf. *helensis* from the Mediterranean Sea could also be conspecific, although having a slightly finer striation of 25 dorsal and 21–22 ventral striae in 10 µm (Álvarez-Blanco & Blanco 2014, Pl. 35: 8, 9). The smallest valves of *A. pusio* are also similar in valve outline and dimensions, but the latter has less densely spaced striae, 14–17 in 10 µm, on both the dorsal and ventral sides (Levkov 2009).

### figs 2M–O: *Amphora* sp. aff. *A. helenensis* Giffen

**Morphometric data:—**L: 8.5–10 µm; W: 2.6–3 µm; DS: 25–30; VS: 25–30 (n=2).

**Notes:—**This species is similar to *Amphora helensis* and *A.* cf. *helenensis* (fig. 2L), but the valves are smaller and narrower, and with a much denser striation on both the dorsal and ventral sides (> 25 striae in 10 µm). Another species with a similar valve outline, dimensions (length 6–11 µm, width 2.5 µm) and stria density (ca. 30 striae in 10 µm), *Amphora exilitata* Giffen, was described from South Africa (Giffen 1971). Clearly, Giffen (1971) did not mention any presence of lanceolate hyaline area on the dorsal side, neither the original drawings of the species show such an area on the dorsal side (Giffen 1971, figs 5–7), contrary to the observed species, where it is clearly present (figs 2M–O). Therefore we exclude conspecificity with Giffen's taxon. Some of the valves of *A. proschkiniana* Guslyakov (e.g. fig. 5 in Guslyakov 1987) also closely resemble our taxon in LM. However, *A. proschkiniana* originally shows morphological variability, when figs 10 and 11, or figs 2 and 5 in Gulsyakov (1987) are compared for instance, as well as *A. proschkiniana* in Guslyakov *et al.* (1992, Pl. CIV: 1–10). The identity of the latter taxon is uncertain, since the original materials were never studied.

### figs 2P–T: *Amphora* sp. S11

**Morphometric data:—**L: 5.0–8.8 µm; W: 2.1–2.5 µm; DS: 20–24, punctate; VS: 22–25 (n=11).

**Notes:—**We are unable to place these small valves in the range of any of the observed taxa. *Amphora erezii* Reimer & Lee from the Red Sea is similar in valve outline and dimensions, but it has a finer striation of 28–30 striae (vs 20–24) in 10 µm on the dorsal side, and parallel ventral striae, not interrupted in the middle (Potapova *et al.* 2021). Both *A. indistincta* Levkov and *A. pediculus* has a distinct central area on the dorsal side; moreover, both are freshwater taxa, which excludes conspecificity (Levkov 2009).

### figs 2U–V: *Amphora* sp. S26

**Morphometric data:—**L: 19.1 µm; W: 6.2 µm; DS: 17; VS: 16 (n=1).

**Notes:—**We were unable to find a sufficiently morphologically similar species with areolate striae, which can accommodate the observed single valve.

### fig. 3A–B: *Tetramphora lineolata* (Ehrenberg) Stepanek & Kociolek

**Morphometric data:—**L: 28.8–40.7 µm; W: 6.6–7.6 µm; DS: 19–20, punctate; VS: 21–22 (n=2).

**Notes:—**In LM, these valves agree with the description in Stepanek & Kociolek (2016), although we counted slightly less striae dorsally (19–20 vs 21–23 in 10 µm in Stepanek & Kociolek 2016).

The species was reported from Crimea by Petrov & Nevrova (2013, as *A. lineolata*), but it is possible that some of the numerous records of *Amphora arcus* W.Gregory from the Black Sea (e.g. Nevrova 2013a, b, 2014a, b, 2015a, Nevrova & Petrov 2019a, Ryabushko *et al*. 2019, etc.) represent the same taxon. Guslyakov *et al.* (1992) transferred *A. arcus* to *Cymbella* C.Agardh, as *C. arcus* (W.Gregory) Guslyakov. Some of the valves of *C. arcus* in Guslyakov *et al.* (1992, Pl. LXXXVI: 3) have the same valve outline and raphe, a comparable length of ca. 45 µm and 19 striae in 10 µm (measured by us on their Pl. LXXXVI: 3), as in the valves we observed. However, the reported range of dimensions and stria density in Guslyakov *et al.* (1992) is quite large (length 25–100 µm, 13–20 striae in 10 µm), suggesting that other taxa might have been included within it as well; moreover, Proshkina-Lavrenko (1963) earlier recognized several forms of *Amphora arcus* in the Black Sea samples. These records of *A. arcus* from the Black Sea should be considered doubtful. According to Mann (1994) the original *A. arcus* is a coarsely striated species, with only 10–11.5 striae in 10 µm on the dorsal side, and the depicted valves of *A. arcus* in Levkov (2009, Pl. 275: 1, 2) clearly show a large-celled taxon with only 11 striae in 10 µm as well.

### fig. 3C: *Halamphora pseudohyalina* (Simonsen) Stepanek & Kociolek

**Morphometric data:—**L: 34.5 µm; W: 8.3 µm; DS: 32; VS invisible (n=1).

**Notes:—**The valve matches *Halamphora pseudohyalina* in Stepanek & Kociolek (2018) and in Álvarez-Blanco & Blanco (2014, as *A. pseudohyalina* Simonsen), except that we counted 32, instead of 30 striae in 10 µm. *A. hyalina* Kützing, another species reported from the Black Sea (Ryabushko *et al.* 2005, 2019, Petrov & Nevrova 2013, Nevrova 2014a, 2015a, Nevrova & Petrov 2019a, Ryabushko 2020, etc.), is a larger taxon ( > 50 µm in length) with protracted apices and a lower number of striae in 10 µm (Levkov 2009, Pl. 281: 1, 2, Stepanek & Kociolek 2018, p. 31).

### fig. 3D & (?)6D: *Amphora* sp. aff. *A. pseudograeffeana* Stepanek & Kociolek

**Morphometric data:—**L: 17.7 µm; W: 4.1 µm; DS invisible; VS invisible (n=1).

**Notes:—**The small number of features under LM for this taxon do not allow its proper identification. Although in valve outline the smallest valves of *A. pseudograeffeana* in Stepanek & Kociolek (2018, Pl. 2: 8) resemble the taxon we observed, striae in *A. pseudograffeana* are visible under LM. *A. pseudospectabilis* Levkov and *A. venusta* Østrup are both larger and with a visible striation in LM (Levkov 2009). Under SEM (fig. 6D), we found possibly the same taxon, with an identical outline and arched raphe, and having a fine striation of 35–36 striae in 10 µm dorsally and 46–48 striae in 10 µm ventrally, but larger dimensions (length ca. 36 and width ca. 6.5 µm) and a narrow dorsal stauros internally, a feature we were unable to see under LM.

### figs 3E–F & 6C: *Amphora* sp. aff. *A. ablundens* Simonsen

**Morphometric data:—**L: 19.3–20.5 µm; W: 3.8–4.1 µm; DS invisible; VS invisible (n=2).

**Notes:—**Under LM this species is most similar to *Amphora ablundens* Simonsen and *Amphora* sp. 01 in Stepanek & Kociolek (2018), but the fascia on the dorsal side is narrower and shorter, running about half-way to the dorsal side from the axial area. *Amphora soninkhishigae* Edlund, Shinneman & Levkov, described from hypersaline waters in Mongolia, has narrower valves (2.9–3.8 µm) and a narrow fascia, clearly branching near the dorsal margin (Edlund *et al.* 2009). *Halamphora staurophora* (Juhlin-Danfelt) Álvarez-Blanco & Blanco from the Baltic Sea, a species also reported from the Black Sea (e.g. Nevrova 2014b, Nevrova & Petrov 2019a) and the Mediterranean coasts (Álvarez-Blanco & Blanco 2014), has a broader fascia, widening towards and reaching the dorsal margin (Juhlin-Danfelt 1882, Pl. 1: 9). All *Amphora laevis* var. *minuta* P.T.Cleve, *A. laevissima* var. *perminuta* Grunow in Van Heurck and *A. sublaevis* Hustedt also have a fascia, widening towards the dorsal margin (Edlund *et al*. 2009 and references therein), in contrast to the Black Sea valves.

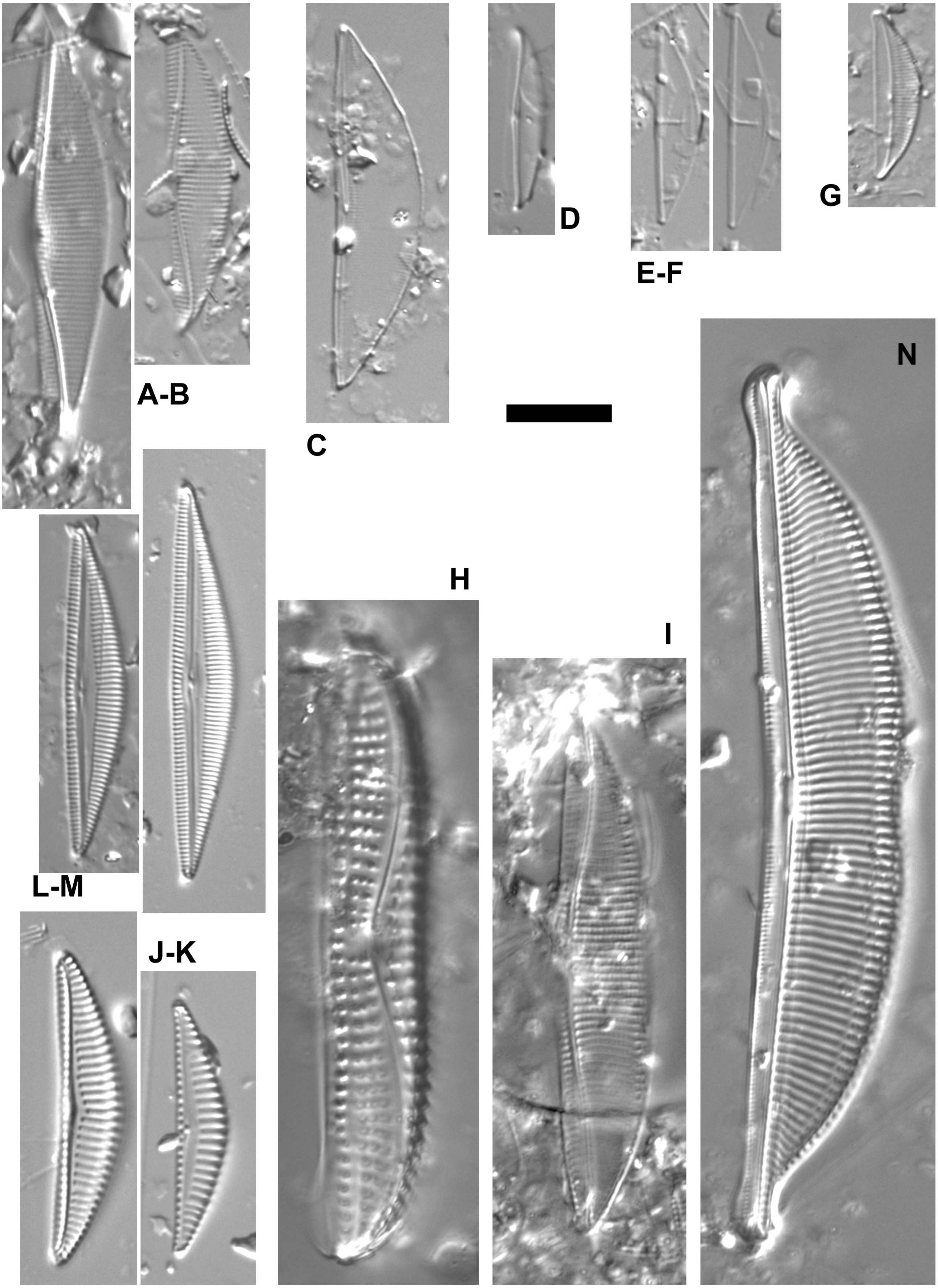

**FIGURE 3.** *Amphora* sensu lato taxa from Sozopol Bay under LM. A–B. *Tetramphora lineolata*. C. *Halamphora pseudohyalina*. D. *Amphora* sp. aff. *A. pseudograeffeana*. E–F. *Amphora* sp. aff. *A. ablundens*. G. *Amphora* sp. S10. H. *Amphora crassa*. I. *Amphora* cf. *praelata*. J–K. *Amphora (Seminavis?)* sp. S4. L–M. *Seminavis* cf. *robusta*. N. *Halamphora* aff. *angularis*. Scale bar = 10 µm.

Under SEM, the only valve that matched this species in outline and dimensions, had elongated areolae near the dorsal margin, a narrow marginal ridge, and central raphe endings first curving ventrally before deflecting towards the dorsal side (fig. 6C). Based on these features the species from the Black Sea is more similar to *Amphora* sp. 01 in Stepanek & Kociolek (2018). However, Stepanek & Kociolek (2018) did not describe *Amphora* sp. 01 as a separate taxon, due to the insufficient information for the variability of *Amphora ablundens*. With regard to stria density, we counted 48 striae in 10 µm dorsally under SEM on the valve from the Black Sea, which number is exactly in between the reported by Stepanek & Kociolek (2018) for *Amphora* sp. 01 (42–43 in 10 µm) and *A. ablundens* (50–53 in 10 µm). Further evaluation is needed. *Amphora insulana* Stepanek & Kociolek has a similar striation pattern, but lacks a fascia (Stepanek & Kociolek 2018).

### fig. 3G: *Amphora* sp. S10

**Morphometric data:—**L: 16.1 µm; W: 4.3 µm; DS: 32; VS invisible (n=1).

**Notes:—**So far we were unable to find a similar taxon, neither this valve could be placed within any of the recorded taxa in the study.

### fig. 3H: *Amphora crassa* W.Gregory

**Morphometric data:—**L: 60 µm; W: 9.3 µm; DS: 6, punctate; VS: 7 (n=1).

**Notes:—**The only valve we observed corresponds to the description in Levkov (2009). *A. crassa* is commonly reported from the Black Sea (e.g. Guslyakov *et al.* 1992, Nevrova 2013a, b, 2014a, b, 2015a, Petrov & Nevrova 2013). The SEM photographs in Guslyakov *et al.* (1992, Pl. CXXXIX) show a taxon with a strongly thickened dorsal marginal ridge, prominent costae on the dorsal valve mantle, large rounded areolae on the valve face dorsally, as described by Levkov (2009, and his Pl. 249: 1–6), with the only subtle difference in the dorsal central area. According to Levkov (2009, Pl. 249: 3) the dorsal central area in *A. crassa* is small, bordered by slightly shortened striae, as also observed by us (fig. 3H), whereas the valves in Guslyakov *et al.* (1992, Pl. CXXXIX) lack a central area dorsally under SEM. We consider this part of the variation in the morphology of the species.

### fig. 3I: *Amphora* cf. *praelata* Hendey

**Morphometric data:—**L: 49.1 µm; W: 9.5 µm; DS: 13–16; VS: 16–17 (n=1).

**Notes:—**This single valve agrees with the species described and illustrated by Hendey (1973, figs 25–30) from the Cornwall coast, UK, except that we measured slightly less striae in 10 µm, compared to the original description (13–16 vs 16–18 striae in 10 µm for the type). Another similar taxon in valve outline, dimensions and striation, *A. alaeziarum* Álvarez-Blanco & Blanco (Álvarez-Blanco & Blanco 2014, Pl. 36: 11–13), was described from the Mediterranean Sea. No comparison with *A. praelata* was made in its description and conspecificity between the two cannot be entirely excluded, since the two taxa overlap in valve outline, dimensions and striation. However, *A. alaeziarum* seems to have slightly more radiate striae and a less arched raphe, compared to *Amphora* cf. *praelata*.

### figs 3J–K: *Amphora (Seminavis?)* sp. S4

**Morphometric data:—**L: 24–37.5 µm; W: 5.2–6.8 µm; DS: 11–12; VS: 12–14 (n=5).

**Notes:—**In valve outline and dimensions this species slightly resembles *A. granulata* var. *costata* Proshkina-Lavrenko (in Proshkina-Lavrenko 1963), but the valves are shorter and slightly wider (length 36–46 µm and width 5–6 µm for *A. granulata* var. *costata*). Compared to the drawing in Proshkina-Lavrenko (1963, Pl. IX: 19), they also have a lanceolate area on the dorsal side, non-interrupted ventral striae in the middle and more closely positioned proximal raphe endings. Two of the valves of *Amphora* (*Seminavis*) *cymbaphora* Cholnoky, shown in Levkov (2009, Pl. 108: 14–15) are also similar; the latter species has slightly more densely spaced striae on the dorsal side (13–14 in 10 µm, Levkov 2009 vs 11–12 in 10 µm).

### figs 3L–M & 6E: *Seminavis* cf. *robusta* Danielidis & D.G.Mann

**Morphometric data:—**L: 25.7–41.8 µm; W: 4.6–6.7 µm; DS: 17–22(26); VS: 16–22 (n=17).

**Notes:—**Occasionally, smaller valves with a finer striation, compared to the description given in Danielidis &

Mann (2002), were observed. With regard to the valve dimensions and stria density, our population has an intermediate position between *S. insignis* Álvarez-Blanco & Blanco (in Álvarez-Blanco & Blanco 2014) and *S. robusta* (in Danielidis & Mann 2002). Futher evaluation is necessary.

From the Black Sea, *Seminavis* sp. 1 in Nevrova & Petrov (2019a, fig. 3: 28) seems conspecific. Valves of a likely identical taxon were also identified and depicted as *Cymbella angusta* (W.Gregory) Guslyakov (var. *angusta,* Guslyakov *et al*. 1992, p. 64, Plate LXXXVII: 2, 4). Earlier, a taxon with similar morphology was reported as *Amphora angusta* W.Gregory by Proshkina-Lavrenko (1963, Pl. VIII: 23) as well. It seems that Gulsyakov *et al*. (1992) followed her identification. However, Guslyakov in Guslyakov *et al.* (1992) noted the differences between the valves they observed and species of the genus *Amphora,* but erroneously concluded that they belong to *A. angusta* and transferred the latter to the genus *Cymbella*. Later, when Danielidis & Mann (2002) studied the materials of Gregory, they considered the Gregory's taxon within *Amphora* sensu stricto, and not within *Seminavis*. *Cymbella angusta var. kujalnitzkensis* Guslyakov & Gerasimyuk (in Guslyakov *et al.* 1992, Pl. LXXXVII: 6, and Pl. LXXXVIII) is another taxon, most likely of *Amphora* sensu stricto.

### fig. 3N: *Halamphora* aff. *angularis* (W.Gregory) Levkov

**Morphometric data:—**L: 83.4 µm; W: 12 µm; DS: 11; VS: 22–23 (n=1).

**Notes:—**Álvarez-Blanco & Blanco (2014, Pl. 34: 14) illustrated a single valve of a very similar taxon from the Mediterranean Sea, which they identified as *Amphora* sp. 1. Based on the valve outline, apices, raphe and striation pattern, and the presence of a longitudinal line, crossing the striae near the dorsal margin, this species is similar to *H. angularis*, but the ventral striae are distinct under LM and the valve length is larger (length < 65 µm for *H. angularis* is reported in Levkov 2009). *Halamphora cymbifera* (Gregory) Levkov (syn.: *A. caroliniana* Giffen, Levkov 2009) is also similar in dimensions and striation, but it has a longitudinal line, crossing the striae near the raphe (Levkov 2009), and not near the dorsal margin.

### figs 4A & 8B–C: *Halamphora* cf. *acutiuscula* (Kützing) Levkov

**Morphometric data:—**L: 31.0–47.0 µm; W: 5.5–7.5 µm; DS: 13–16, punctate, areolae ca. 20 in 10 µm; VS: 23–25 (n=9).

**Notes:—***H. acutiuscula* (Kützing) Levkov has a slightly finer striation on the dorsal side of 15–18 striae in 10 µm and less densely spaced striae on the ventral side, 19–22 in 10 µm (Levkov 2009). SEM observations on valves from the Black Sea showed the presence of biseriate striae, becoming uniseriate towards the dorsal margin (figs 8B–C). However, in *H. acutiuscula* in Levkov (2009, Pl. 234: 1, 3) striae are biseriate only very close to the raphe ledge, whereas in our valves the biseriate striae reach almost the middle of the dorsal side of the valves (figs 8B–C). Due to these differences, as well as those in stria density, we hesitate in the identification of the observed valves as *H. acutiuscula.* Another similar taxon under LM and SEM is *H. tumida* (Hustedt) Levkov, but it has a slightly denser striation on the dorsal side, of 16–18 and up to 24 striae in 10 µm at the apices (Sar *et al.* 2004), and striae are almost indistinctly punctate (Levkov 2009).

Under LM *H.* cf. *acutiuscula* resembles a number of *Halamphora* taxa with convex dorsal margin, capitate/subcapitate protracted apices and punctate striae: *H. holsatica* (Hustedt) Levkov has wider valves (7–9 µm vs 5.5–7.5 µm) with coarsely punctate dorsal striae, and only 16–18 (vs 23–25) ventral striae in 10 µm (Levkov 2009). *H. subholsatica* (Krammer) Levkov has interrupted in the valve middle ventral striae and less convex dorsal margin, almost parallel in the middle (Levkov 2009), in contrast to the distinctly convex dorsal margin in the Black Sea valves, and it also has clearly more distantly spaced proximal raphe endings (Levkov *et al.* 2009, Pl. 100: 15–24). *H. arcus* Stepanek & Kociolek has broader valves (> 7 µm) and a much finer striation on the ventral side, of 30–34 striae in 10 µm (Stepanek & Kociolek 2018). *Amphora archibaldii* Wachnicka & Gaiser is a much larger species, with a length of above 50 µm and a width of 10–13 µm (Wachnicka & Gaiser 2007). *H. coffeaeformis* (Kützing) Levkov and *H. turgida* (Gregory) Levkov both lack at least punctate striae in LM (Levkov 2009). Finally, the voucher of *Amphora coffeaeformis* var. *tenuissima* Proshkina-Lavrenko in the ANSP collection (Potapova *et al.* 2021) shows a species with a similar valve outline to the Black Sea specimens, punctate dorsal striae and visible ventral striae, but the originally reported number of striae on both the dorsal and ventral sides of the valves is higher (18–22 and 30 in 10 µm, respectively).

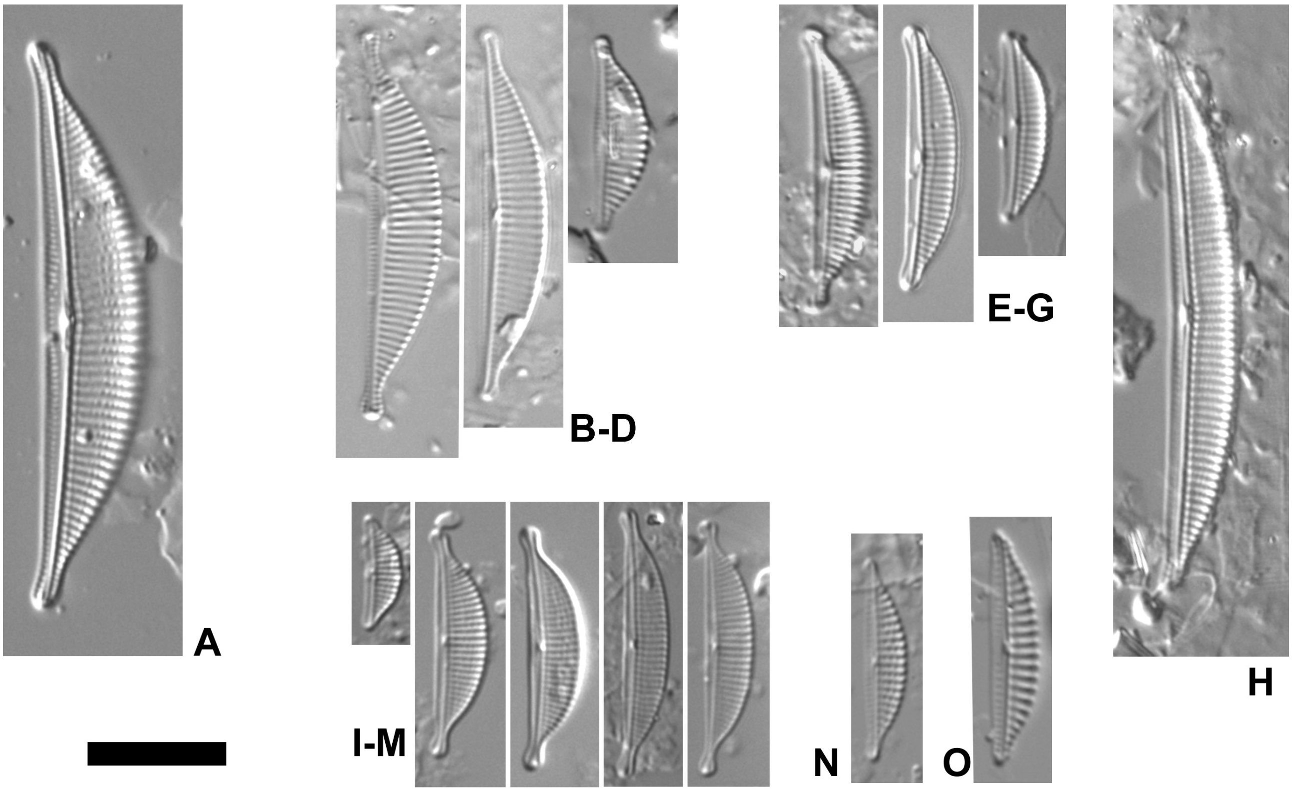

**FIGURE 4.** *Amphora* sensu lato taxa from Sozopol Bay under LM. A. *Halamphora* cf. *acutiuscula*. B–D. *Halamphora coffeaeformis*. E–G. *Halamphora* sp. aff. *H. nagumoi*. H. *Halamphora* sp. aff. *H. cymbifera*. I–M. *Halamphora* sp. S4. N. *Halamphora* sp. aff. *H. pseudoholsatica*. O. *Halamphora* sp. S10. Scale bar = 10 µm.

**figs 4B–D & 8E–F: *Halamphora coffeaeformis* (Kützing) Levkov**

**Morphometric data:—**L: 14.5–31.4 µm; W: 4.0–5.0 µm; DS: 16–20; VS: 25–27 (n=5).

**Notes:—**According to Levkov (2009), *H. coffeaeformis* has indistinct ventral striae under LM and a slightly finer striation of 19–22 striae in 10 µm on the dorsal side. Stepanek & Kociolek (2018) reported for *H. coffeaeformis* less densely spaced dorsal striae, 16–20 in 10 µm (as in our populations), and were also able to distinguish the ventral striae, which they found to be 30–32 in 10 µm. Archibald & Schoeman (1984) gave a wider range, however. The valves we observed had slightly smaller dimensions and a lower number of striae in 10 µm on the ventral side, compared to the range in Stepanek & Kociolek (2018). Under SEM, the valves sometimes had a distinct marginal dorsal ridge (fig. 8E), similarly to *H. isumiensis* Stepanek, Mayama & Kociolek, but unlike the latter they possessed uninterrupted in the middle and much more distantly spaced ventral striae (25–27 vs 36 in 10 µm in *H. isumiensis*, Stepanek & Kociolek 2018). The valves fit *H. coffeaeformis* in Archibald & Schoeman (1984), although showing often a prominent dorsal ridge. Under LM, other similar taxa include *H. tumida* (Hustedt) Levkov, but it has interrupted ventral striae at the valve middle (Levkov 2009, Pl. 236: 1, 4), whereas *H. luciae* (Cholnoky) Levkov has indistinct ventral striae in LM, finely punctate dorsal striae and a longitudinal line, crossing the striae near the raphe on the dorsal side, and both these species have an entirely different structure of the areolae under SEM (details given in Levkov 2009).

**Figs 4E–G & 8G: *Halamphora* sp. aff. *H. nagumoi* Stepanek, Mayama & Kociolek**

**Morphometric data:—**L: 11.5–20.1 µm; W: 3.3–3.9 µm; DS: 18–20; VS invisible, ca. 40 under SEM (n=7).

**Notes:—**The species resembles *H. nagumoi* only under LM. *H. nagumoi* has visible ventral striae and straight proximal raphe endings; under SEM *H. nagumoi* has a very distinct marginal ridge on the dorsal side, strongly thickened external costae and very closely positioned central raphe endings (Stepanek & Kociolek 2018, Pl. 55: 1), not present in the species we observed (fig. 8G). *H. abuensis* (Foged) Levkov from Abu River in Ghana is similar in valve outline under LM, but has more widely spaced, 15–18 in 10 µm, uniseriate (and not biseriate) striae with elongated areolae

(Levkov 2009). *H. cejudoae* Álvarez-Blanco & Blanco from the Mediterranean Sea has comparable valve outline and dimensions, but ventral striae are visible in LM, 20–22 in 10 µm, and striae are composed of transapically elongated areolae with recessed biseriate poroids (Álvarez-Blanco & Blanco 2014, Pl. 97: 2, 4). The valves we observed are also similar to the smallest valves of *H. tumida*, but the later has more distantly spaced striae dorsally (16–18 in 10 µm), distinct ventral striae under LM, and typically larger dimensions (width of 4–7 µm), as well as strongly thickened external costae under SEM (Levkov 2009). From *H. coffeaeformis* (see above and figs 4B–D) the species can be separated by the smaller and narrower (<4 µm in width) valves with a less convex dorsal margin, the clear longitudinal line, crossing the striae on the dorsal side near the raphe, and with the biseriate striae on the valve face, becoming usually uniseriate on the mantle, where composed of mostly 2 enlarged areolae (fig. 8G).

### fig. 4H: *Halamphora* sp. aff. *H. cymbifera* (W.Gregory) Levkov

**Morphometric data:—**L: 39.1 µm; W: 5.0 µm; DS: 16–17, punctate, areolae ca. 27–30 in 10 µm; VS: 21 (n=1).

**Morphometric data:—**We could not place this valve into the range of another species in this study. *H.* cf. *acutiuscula* (this study) has more convex dorsal side, uninterrupted in the middle ventral striae and coarser areolae (fig. 4A, see also above), whereas *H. acutiuscula* has more radiate striae and ventral striae are not interrupted in the middle (Levkov 2009). *Halamphora* sp. aff. *H. nagumoi* (figs 4E–G) has much smaller dimensions, non-punctate dorsal striae, ventral striae are not visible under LM and are much denser (40 vs 21 in 10 µm), and the proximal raphe endings are also distinctly dorsally bent. The species resembles *Halamphora cymbifera* (syn.: *Amphora caroliniana* Giffen, Levkov 2009) in valve outline, but the latter has both longer (> 50 µm) and wider (> 8.5 µm) valves with only 9–11 non-punctate striae in 10 µm (Levkov 2009).

### figs 4I–M: *Halamphora* sp. S4

**Morphometric data:—**L: 8.5–19.8 µm; W: 2.9–3.7 µm; DS: 22–27, punctate; VS not discernible (n=10).

**Notes:—**All *H. bistriata* Stepanek & Kociolek*, H. banzuensis* Stepanek, Mayama & Kociolek, *H. subtropica* (Wachnicka & Gaiser) Stepanek & Kociolek, *H. incelebrata* Stepanek & Kociolek, *H. tenucostata* Stepanek & Kociolek and *H. scatebra* Stepanek & Kociolek are morphologically similar under LM, but they all have more distantly spaced striae, with the number of dorsal striae not exceeding 23 in 10 µm (Stepanek & Kociolek 2018). For comparison with *Halamphora* sp. aff. *H. borealis* (Kützing) Levkov see figs 5A–E and further below.

### figs 4N & 7C–E: *Halamphora* sp. aff. *H. pseudoholsatica* (Nagumo & Kobayasi) Stepanek & Kociolek

**Morphometric data:—**L: 14.8 µm; W: 3.2 µm; DS: 18, punctate; VS: 26 (n=1).

**Notes:—**We depicted only one valve under LM, resembling both *H. pseudoholsatica* and *H. parvipunctata* Stepanek & Kociolek, which both have wider valves (>4 µm) with uniseriate striae (Stepanek & Kociolek 2018). Under SEM we found 3 valves (figs 7C–E), being 18–21 µm in length, 3.3–4.5 µm in width, with 18–20 biseriate dorsal striae of relatively coarse areolae and ca. 30 ventral striae in 10 µm, which in our opinion belong to the same species. One of the valves had somewhat unusual arrangement of striae and areolae on the dorsal side (fig. 7D): areolae have deeply recessed foramina and are located in two rows in between, but on the sides of prominent costae, the latter configuration also well visible internally (see fig. 7E). Externally, areolae are sunken between narrow and raised vimines, extending to the top of the costae, where they fuse with vimines of areolae from the neighboring stria, this way giving the entire dorsal side an appearance of a coarsely knitted fabric. Areolae near the apices are distinctly larger (figs 7C, D), as well as the areolae on the valve mantle, where they fuse to form a single large areola. We were unable to find a similar taxon in the literature. Valves that do not present raised vimines (e.g. fig. 7C) slightly resemble *H. aponina* (Kützing) Levkov, but the latter has larger valves (length > 23µm) with a slightly higher number of dorsal striae in 10 µm (20–22), composed of comparatively smaller poroids (Levkov 2009, Pl. 233: 1, 6).

### figs 4O & 8A: *Halamphora* sp. S10

**Morphometric data:—**L: 17 µm; W: 3.5 µm; DS: 14; VS: 22 (n=1).

**Notes:—**Under SEM (fig. 8A) we observed a single valve internally, having comparable dimensions (length ca. 15 µm, width ca. 3.5 µm) and the same striation pattern of almost parallel in the middle, becoming radiate towards the apices dorsal striae, 14 in 10 µm, and with 23 ventral striae in 10 µm. Striae on the dorsal side are clearly biseriate, composed of rounded areolae, sunken between raised costae internally; the costae almost as wide as the striae. Raphe is almost straight. No other observations were so far possible. Under LM (fig. 4O) this species slightly resembles *Amphora exigua* W.Gregory in Witkowski *et al.* (2000). The latter has many different (mis)interpretations in the past (Levkov 2009 and references therein, as *Halamphora exigua* (W.Gregory) Levkov). From the Black Sea, the valves reported as *A. exigua* by Guslyakov *et al.* (1992), although larger (23–40 µm in length, 4–6 µm in width) have a similar valve outline to the species we observed, and a similar striation pattern of widely spaced, 11–14 biseriate striae in 10 µm dorsally (Guslyakov *et al*. 1992, Pl. CXI: 3, 8). The real *Amphora exigua* is a larger taxon (> 38 µm in length, > 7 µm in width, Levkov 2009).

**figs 5A–E & 7F: *Halamphora* sp. aff. *H. borealis* (Kützing) Levkov**

**Morphometric data:—**L: 7.6–15.7 µm; W: 2.3–3.0 µm; DS: 27–30(33), finely punctate; VS invisible (n=12).

**Notes:—**Under LM the valves slightly resemble those of *H. borealis* (Kützing) Levkov, but are smaller and with a denser striation (27–30 vs 22–24 dorsal striae in *H. borealis,* Levkov 2009). The species is actually most similar to *Halamphora* sp. S4 (figs. 4I–M), but the valves are slightly narrower, with a less convex dorsal margin, and a denser striation of finely punctate striae. Under SEM (fig. 7F) we observed a valve with the same outline and dimensions (length ca. 12 µm, width ca. 3 µm), with 27–30 uniseriate striae in 10 µm on the dorsal side, composed of relatively coarse rectangular areolae, and densely striated ventral side, with ca. 50–55 striae in 10 µm, and an elongated central area, which in valve structure and raphe resembles another species, *H. salinicola* Levkov & Diaz (Levkov 2009, Pl. 207: 5). The later was described from Atacama, Chile, but in our opinion conspecificity between the latter and our taxon is to be excluded, due to the less distantly spaced striae in *H. salinicola*, 21–26 in 10 µm (Levkov 2009).

**figs 5F–H: *Halamphora* sp. S14**

**Morphometric data:—**L: 7.7–13.4 µm; W: 2.5–3.2 µm; DS: 26–30, barely visible; VS invisible (n=5).

**Notes:—**We observed a few valves that do not seem to fit *Halamphora* sp. aff. *H. borealis* (above), since no punctation could be seen in the striae under LM and the dorsal side is more convex. With regard to the fine and parallel striae in the middle, these valves slightly resemble *H. tenuis* Stepanek & Kociolek, but the latter has slender, more elongated valves with a slight constriction in the middle of the dorsal side (Stepanek & Kociolek 2018, Pl. 47: 5–8). *H. pelliculla* Stepanek & Kociolek (Stepanek & Kociolek 2018) and *H.* aff. *pellicula* (see below and figs. 5I–L) both have indistinct dorsal striae in LM.

**figs 5I–L: *Halamphora* aff. *pellicula* Stepanek & Kociolek**

**Morphometric data:—**L: 8.5–12.2 µm; W: 2.5–2.8 µm; DS and VS invisible (n=8).

**Notes:—**The invisible structure of this taxon in LM does not allow its identification at present. Valves from the Black Sea have a less convex ventral margin and the raphe is less arched, compared to the valves of the species in Stepanek & Kociolek (2018, Pl. 50: 9–12). *Amphora pseudotenuissima* Wachnicka & Gaiser has a similar valve outline and fine striation, but a convex ventral side and an arched raphe (Wachnicka & Gaiser 2007, figs 39–40).

**figs 5M–O,** (?)**P: *Amphora (Halamphora)* sp. S8**

**Morphometric data:—**L: 8.5–12.1 (17.9) µm; W: 2.4–2.6 µm; DS > 30, difficult to resolve; VS invisible (n=8).

**Notes:—**This species slightly resembles the smaller valves of *H. sardiniensis* Lange-Bertalot & Levkov. The latter is known from only a few freshwater localities in Sardinia and has wider valves (width > 3 µm) with protracted apices (Levkov 2009). *H. pellicula* have similar outline and dimensions, but weakly silicified valves with > 40 striae in 10 µm, comparatively more protracted apices, and more arched raphe (Stepanek & Kociolek 2018, Pl. 50: 9–16). From *H.* aff. *pellicula* (see above and figs 5I–L) it differs by the less convex dorsal side and visible structure under LM, even if striae are difficult to count. Whether or not the depicted longer valve (fig. 5P, compare the striation of fig. 5O and fig. 5P) belongs to the same taxon could be clarified with further observations on a larger number of valves, and SEM.

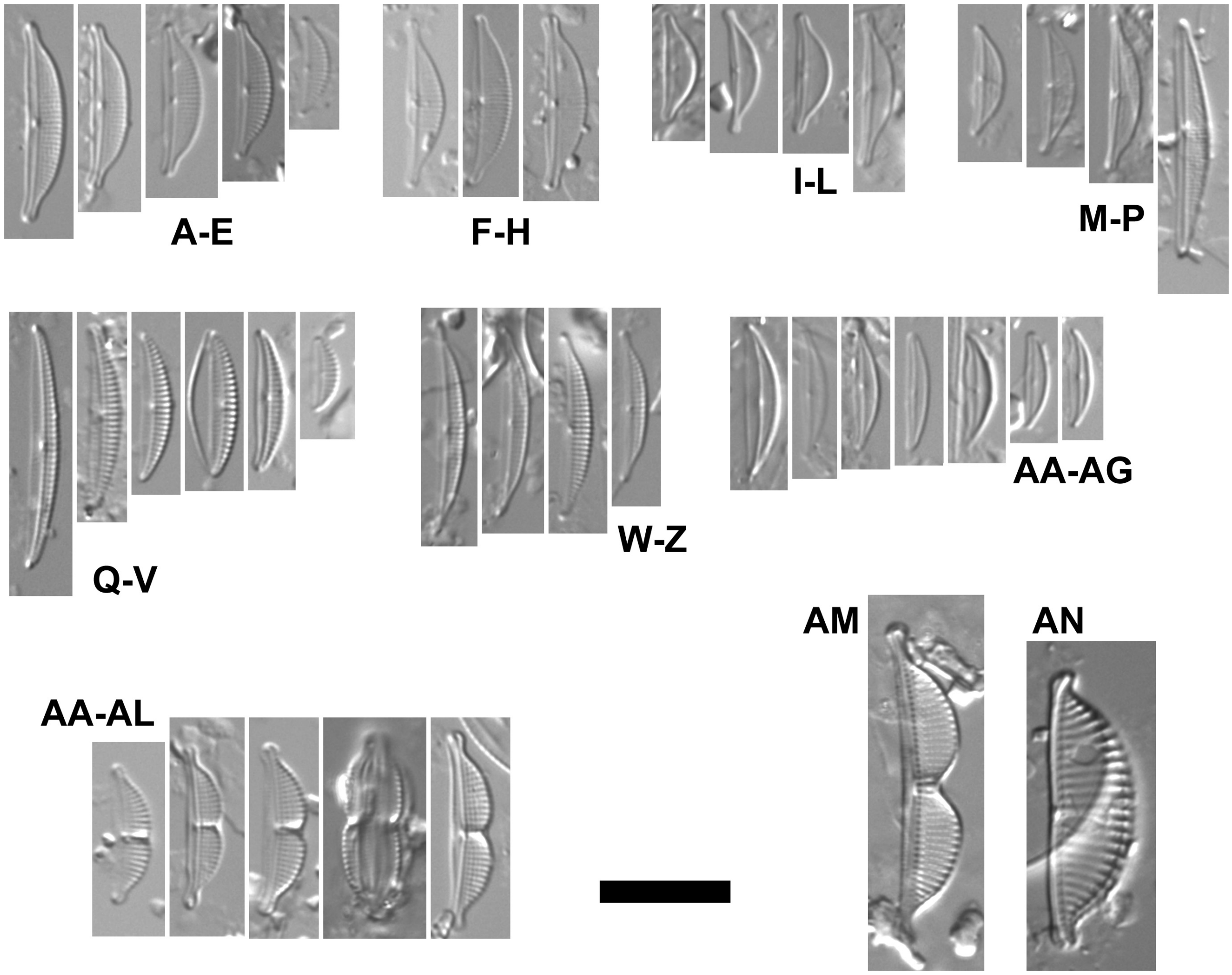

**FIGURE 5.** *Amphora* sensu lato taxa from Sozopol Bay under LM. A–E. *Halamphora* sp. aff. *H. borealis*. F–H. *Halamphora* sp. S14. I–L. *Halamphora* aff. *pellicula*. M–P. *Amphora (Halamphora)* sp. S8. Q–V. *Amphora (Halamphora)* sp. S18. W–Z. *Halamphora* aff. *tenerrima*. AA–AG. *Amphora (Halamphora)* sp. S21. AH–AL. *Halamphora kolbei*. AM. *Halamphora capitata*. AN. *Halamphora* aff. *turgida*. Scale bar = 10 µm.

### figs 5Q–V: *Amphora (Halamphora)* sp. S18

**Morphometric data:—**L: 6.0–19.0 µm; W: 1.8–2.7 µm; DS: 22–24; VS: 28–30, not always visible (n=20).

**Notes:—**We were unable to find a sufficiently similar species in the literature under LM. The largest valves of this taxon only slightly resemble *H. borealis* in Stepanek & Kociolek (2018, Pl. 47: 21–22) in valve outline and striation. The Back Sea valves are smaller (< 19 µm in length and < 3 µm in width), apices are not protracted capitate, and ventral striae could be discerned, in contrast to *H. borealis* (Levkov 2009, Stepanek & Kociolek 2018).

### figs 5W–Z: *Halamphora* aff. *tenerrima* (Aleem & Husted) Levkov

**Morphometric data:—**L: 11.7–16.7 µm; W: 2.1–2.5 µm; DS: 27–30, punctate; VS invisible (n=11).

**Notes:—**Dorsal striae in *H. tenerrima* lack punctation in LM (Levkov 2009); further, it can only be differentiated with certainty under SEM (Clavero *et al*. 2000), and since no SEM observations are available at the moment for this species, we cannot confirm the identification. *H. borealis* has punctate striae, but its valves are larger, 19–40 µm in length and 3–4 µm in width, with more widely spaced striae, 20–24 in 10 µm (Levkov 2009). *H. salinicola* Levkov & Diaz from Chile is again larger with less densely spaced striae (21–26 striae in 10 µm dorsally, Levkov 2009). *H. subsalina* Levkov (Levkov 2009) and *H. tenuis* Stepanek & Kociolek (Stepanek & Kociolek 2018) both have a denser striation on the dorsal side (> 30 striae in 10 µm).

### figs 5AA–AG & 8H: *Amphora (Halamphora)* sp. S21

**Morphometric data:—**L: 7.2–12.0 µm; W: 1.6–2.6 µm; DS and VS invisible (n=11).

**Notes:—**Although some of the depicted valves show more semi-elliptic valve outline and a slightly more ventrally positioned raphe (e.g. figs 5AD and 5AF), at present we consider all these valves as part of the range of a single taxon. Further SEM observations, on a larger number of valves, would be needed to confirm that the differences are important, and not a result of the valve position on the slide, for instance. In valve outline these small valves with invisible structure in LM slightly resemble the freshwater *Halamphora obscura* (Krasske) Levkov, but the latter has larger valve dimensions (15–25 µm in length and 2.4–6 µm in width) and striae are resolvable (Levkov 2009). *Amphora delicatissima* Krasske, according to both Witkowski *et al.* (2000) and Wachnicka & Gaiser (2007) is also larger, with a valve width > 3 µm, and 36–38 striae in 10 µm. The valve that we believe to represent the same taxon under SEM (fig. 8H) has a narrow, but distinct raphe ledge, expanded central raphe endings bent towards the dorsal side, and ca. 45 dorsal striae in 10 µm, composed of a single areola. We could not find other similar taxa in the literature so far.

### figs 5AH–AL & 8I: *Halamphora kolbei* (Aleem) Álvarez-Blanco & S.Blanco

**Morphometric data:—**L: 10.5–15.5 µm; W: 2.3–2.4 µm; DS: 24–27; VS invisible (n=8).

**Notes:—**In our opinion this taxon is identitical to the species, reported and illustrated by Álvarez-Blanco & Blanco (2014) as *H. kolbei*, although they hesitated that the valve they observed under SEM belongs to the latter, due to the presence of ventral striae (Álvarez-Blanco & Blanco 2014, Pl. 81: 2, as *Amphora* sp. 4). However, ventral striae are present in the Black Sea population (fig. 8I) and could be seen under LM occasionally, even if faintly (e.g. figs 5AC, 5AL), while depending on the valve position they may not be visible at all (fig. 5AB). Both the valves from our material and the Mediterranean Sea (Álvarez-Blanco & Blanco 2014) have a slightly denser striation, compared to the original *Amphora kolbei* Aleem (24–27 vs 21–24, or up to 25, see Wachnicka & Gaiser 2007). However, Kaleli *et al.* (2017), who also recorded *H. kolbei* from the Black Sea, reported a lower number of striae, 22 in 10 µm (as in the type). A similar taxon with deeply constricted dorsal margin was described from Loch Fine, under the name *A. lyrata* W.Gregory (Gregory, 1857, Pl. XIII: 82). We were unable to find further information for this species.

### fig. 5AM: *Halamphora capitata* (Hagelstein) Álvarez-Blanco & S.Blanco

**Morphometric data:—**L: 24.8 µm; W: 3 µm; DS: 20, punctate; VS invisible (n=1).

**Notes:—**This single valve agrees with *H. capitata* in Álvarez-Blanco & Blanco (2014, Pl. 36: 9) from the Mediterranean Sea. All valves, recently reported from the Black Sea (Kaleli *et al.* 2017, and this study) and from the Mediterranean Sea (Álvarez-Blanco & Blanco 2014) have more distantly spaced dorsal striae, 20–22 instead of 24–25 striae in 10 µm, as originally given in Hagelstein (1939, as *Amphora bigibba* var. *capitata* Hagelstein, cit. in Álvarez-Blanco & Blanco 2014 and Wachnicka & Gaiser 2007). A taxon with comparable dimensions, capitate apices, deeply constricted dorsal margin, and having 15–20 (usually 16–19, Proshkina-Lavrenko 1963) striae in 10 µm, has been illustrated from the Black Sea as *A. bigibba* Grunow (e.g. Proshkina-Lavrenko 1963, Pl. VIII: 20, Guslyakov *et al.* 1992, Pl. CVIII: 1), and the repeated reports of *A. bigibba* from the Black Sea (e.g. Nevrova 2013a, 2014b, 2015a, Ryabushko *et al*. 2005, Ryabushko 2020, etc.) most likely refer to the same taxon. Further evaluation is needed, since according to the voucher of Hagelstein's *Amphora bigibba* var. *capitata* in the ANSP diatom file collection (Potapova *et al.* 2021), the latter variety differs from *A. bigibba* only with the strongly capitate apices and denser striation. The valves of *H. kolbei* (figs 5AH–AL) are smaller, and at least those from the Black Sea have a denser striation, compared to *H. capitata.*

### fig. 5AN: *Halamphora* aff. *turgida* (W.Gregory) Levkov

**Morphometric data:—**L: 21 µm; W: 6.4 µm; DS: 11; VS invisible (n=1).

**Notes:—**This species seems identical to the species, reported as *Amphora turgida* from the Mediterranean Sea in Álvarez-Blanco & Blanco (2014, Pl. 36: 10), although the number of the dorsal striae is lower (11 vs 14 striae in 10 µm in Álvarez-Blanco & Blanco 2014). We were unable to resolve the ventral striae. According to Levkov (2009), *A. turgida* has larger valves with a length of 32–48 µm and a width of 7.5–9.5 µm, and although Witkowski *et al.* (2000) reported smaller valve lengths for this taxon (17–36 µm), still the valve width of *A. turgida* they reported was to be at least 7 µm. The single valves observed at Sozopol Bay and from the Mediterranean coasts have a smaller width of 6–6.4 µm (see Álvarez-Blanco & Blanco 2014). According to Levkov (2009), *A. turgida* also presents a biarcuate raphe.

The raphe was not observed by us, and it is also difficult to resolve on the figure in Álvarez-Blanco & Blanco (2014, Pl. 36: 10). At present the identification of these valves is uncertain, and considering their smaller dimensions, it is possible that they do not represent the latter taxon, or at least they do not fit *H. turgida* in Levkov (2009), and neither fit entirely *Amphora turgida* in Witkowski *et al.* (2000). *Amphora* sp. 2, observed and depicted by Guslyakov *et al*. (1992, Pl. CXVII: 9) from the Black Sea might be conspecific. Guslyakov *et al.* (1992) did not report valve dimensions, but their *Amphora* sp. 2 has 8–10 striae in 10 µm, composed of rounded, almost fused one with another areolae.

In addition to these taxa, found during the LM observations, a few other taxa were incidentally discovered during the SEM studies:

**fig. 6A: *Tetramphora* aff. *ostrearia*** (Brébisson ex Kutzing) Stepanek & Kociolek

**Morphometric data:—**L: ca.51 µm; W: ca. 11 µm; DS: 14; VS: 15.

**Notes:—**The species has prominent virgae internally and the internal areolar openings are slit-like. In dimensions and stria density it fits *Tetramphora ostrearia* in Stepanek & Kociolek (2016 and references therein), but it has a dorsal (semi) stauros internally, not mentioned for this taxon by Stepanek & Kociolek (2016), neither shown as present on their figures 6–7. It is likely that some of the records of *T. ostrearia* (incl. as *Amphora ostrearia* and its varieties) from the Black Sea (e.g. Guslyakov *et al.* 1992, Petrov & Nevrova 2013, Nevrova 2014a, b, 2015a, etc.) refer to the same taxon we observed.

**fig. 6B: *Amphora* cf. *graeffeana* Hendey**

**Morphometric data:—**L: ca. 24 µm; W: ca. 5 µm; DS: 24–25; VS: underdeveloped, ca. 11.

**Notes:—**A similar taxon in valve outline and dimensions, and with reduced ventral striae, was identified as *A. graeffeana* by Stepanek & Kociolek (2018, Pl. 4: 2). Both our valve and those from Florida, USA, have smaller dimensions, compared to the type of the species (see Stepanek & Kociolek 2018).

**fig. 7A: *Halamphora* aff. *cuneata* (P.T.Cleve) Levkov**

**Morphometric data:—**L: ca. 38.5 µm; W: ca. 6.5 µm; DS: 12; VS: not observed.

**Notes:—**This single valve, although eroded, clearly presents a raphe ledge on the dorsal side (fig. 7A, white arrow), as well as a prominent dorsal marginal ridge (fig. 7A, black arrow). In valve outline, with a convex ventral margin in the middle, protracted capitate and ventrally bent apices, as well as in raphe endings and stria density, the species is very similar to *H. cuneata* in Levkov (2009). However, the striae in the valve we observed have an unusual arrangement of the areolae. In *H. cuneata* striae are composed of several rectangular areolae, located between raised costae (Levkov 2009, Pl. 243: 5, 6). In the valve we observed, striae are composed of only two areolae, unlike *H. cuneata*: one row of smaller rounded areolae near the raphe, interrupted at the dorsal central area (as in *H. cuneata*), and a second row near the dorsal ridge, where areolae are transapically elongated, with the few areolae opposite the dorsal central area longer than the rest (as in *H. cuneata*) and extending towards the raphe. Near the apices only a single, transapically elongated areola is present. Areolae of each stria are positioned in shallow transapical depressions, placed between raised costae. This arrangement of areolae forms a large, B-shaped, non-perforated area on the dorsal side of the valve, a feature not observed by Levkov (2009) in *H. cuneata.* The valve is also shorter, compared to the range given in Levkov (2009, length > 47 µm). At present we are uncertain whether these differences are important or are part of the variability of *H. cuneata*. Levkov (2009) considered *Amphora japonica* Meister identical with *H. cuneata*, and the voucher of *A. japonica* in the ANSP diatom files collection (Potapova *et al*. 2021) shows a taxon with the same arrangement of striae as in *H. cuneata.* The species illustrated as *H. cuneata* in Guslyakov *et al*. (1992, Pl. CIV: 11), although with smaller dimensions compared to *H. cuneata* in Levkov (2009), also seems to have striae composed of several areolae. Nevrova (2014b) reported from Balaklava Bay (the northern Black Sea) a species, identified as *A.* cf. *cuneata*, but it was not illustrated and we cannot compare our finding with her observations. Finally, similar arrangement of areolae is present in *Amphora maletractata* Simonsen (=*A. interrupta* Heiden, *Halamphora interrupta* (Heiden) Levkov) and its var. *constricta* (Heiden) Simonsen (Simonsen, 1992, Pl. 67: 5–15). No SEM studies on the original materials of these taxa are available and we hesitate they are conspecific with our taxon, since these taxa were described from the Southern Hemisphere. The taxon illustrated by Park & Koh (2012, figs 3l–m) as *A. maletractata* var. *constricta* from Korea lacks capitate apices, no striae are present opposite the central area dorsally and, clearly, is not identical with the taxon we observed.

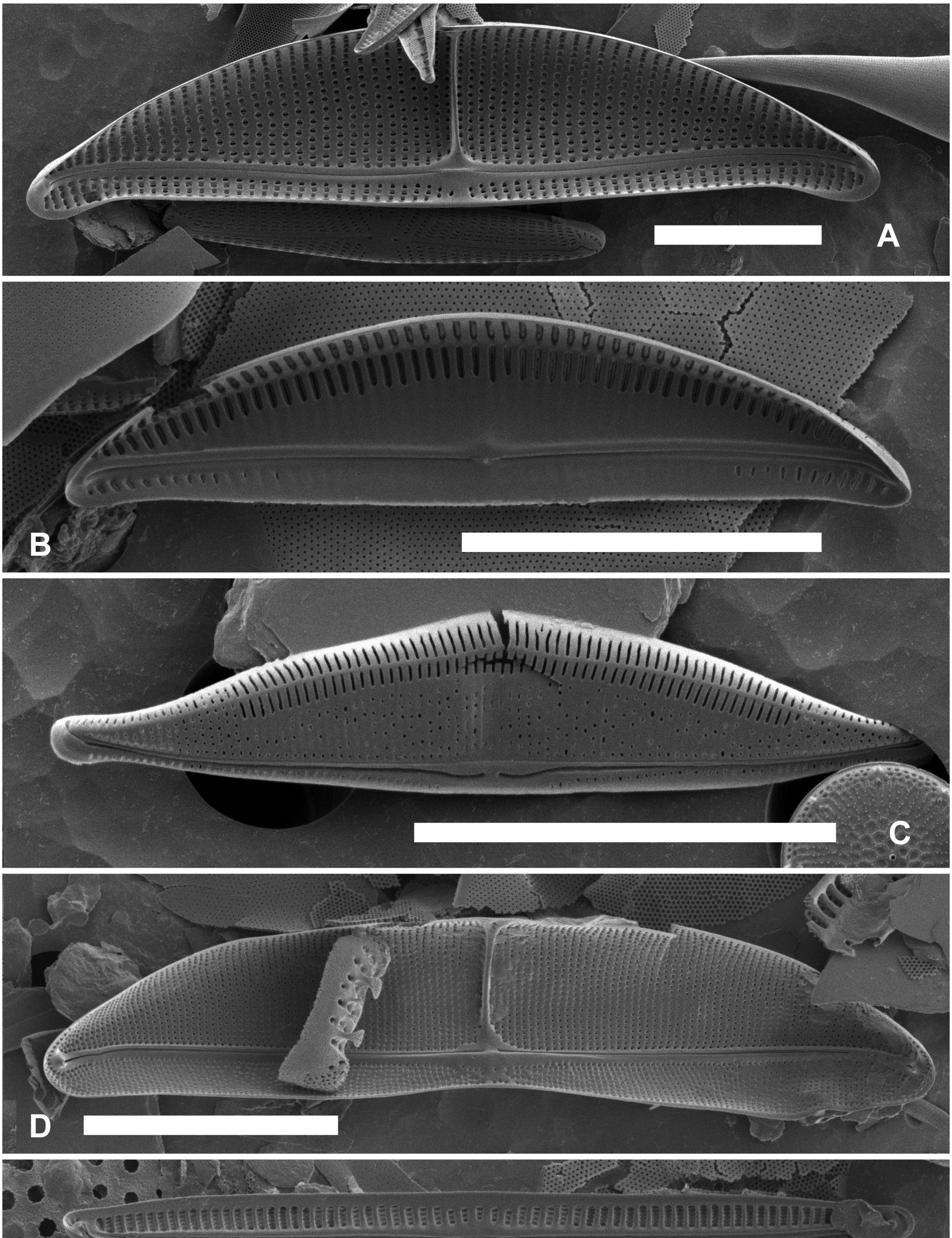

**FIGURE 6.** *Amphora* sensu lato taxa from Sozopol Bay under SEM. A. *Tetramphora* aff. *ostrearia*, internal view. B. *Amphora* cf. *graeffeana,* internal view. C. *Amphora* sp. aff. *A. ablundens,* external view. D. *Amphora* sp. aff. *A. pseudograeffeana,* internal view. E. *Seminavis* cf. *robusta,* internal view. Scale bars = 10 µm.

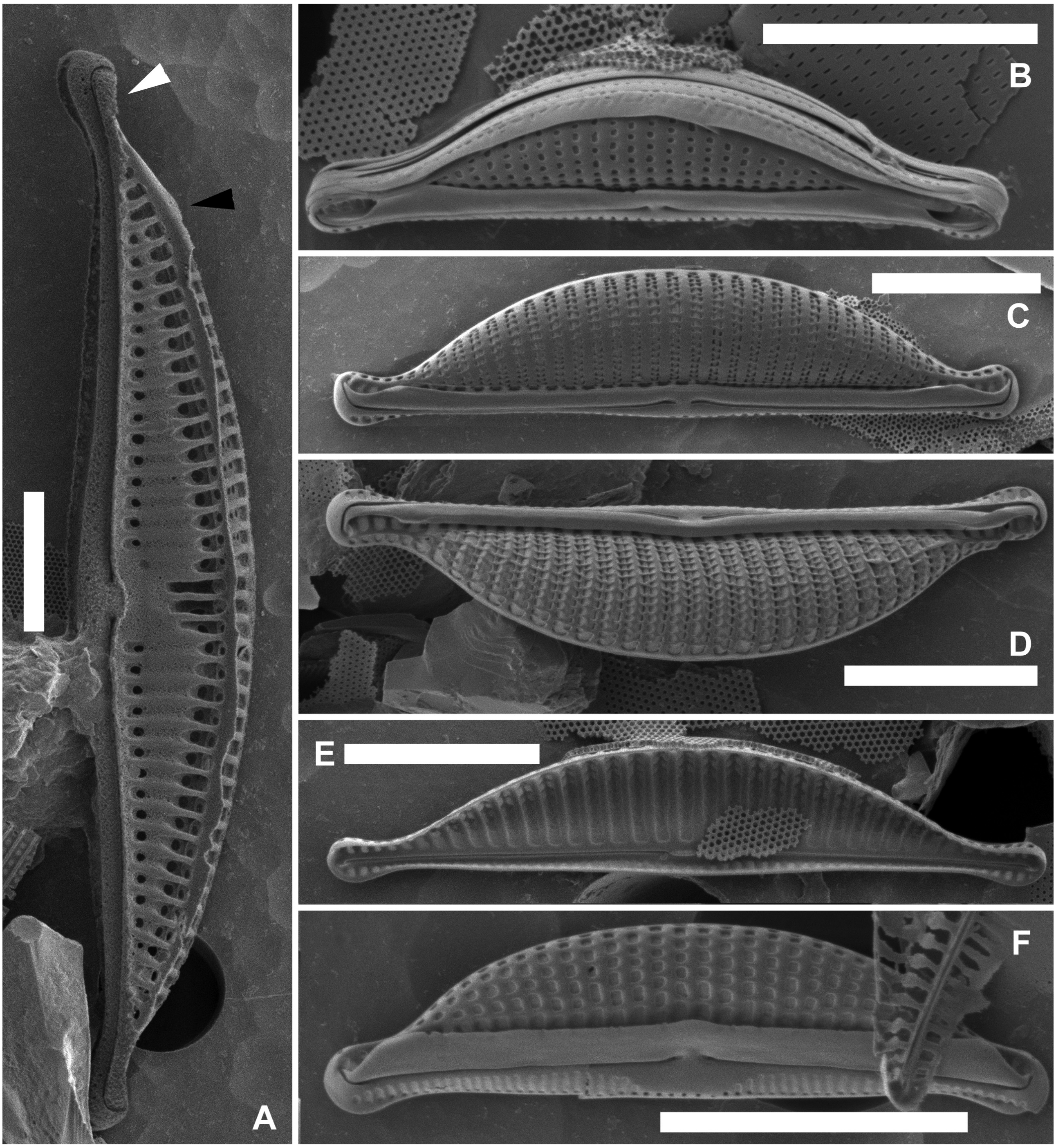

**FIGURE 7**. *Amphora* sensu lato taxa from Sozopol Bay under SEM. A. *Halamphora* aff. *cuneata,* external view, white arrow points to the raphe ledge, black arrow indicates the distinct marginal ridge of the single eroded valve. B. *Halamphora* aff. *pseudotenuissima*, external view. C–E. *Halamphora* sp. aff. *H. pseudoholsatica,* C, D represent external valve view and E is internal valve view. F. *Halamphora* sp. aff. *H. borealis*, external valve view. Scale bars = 5 µm.

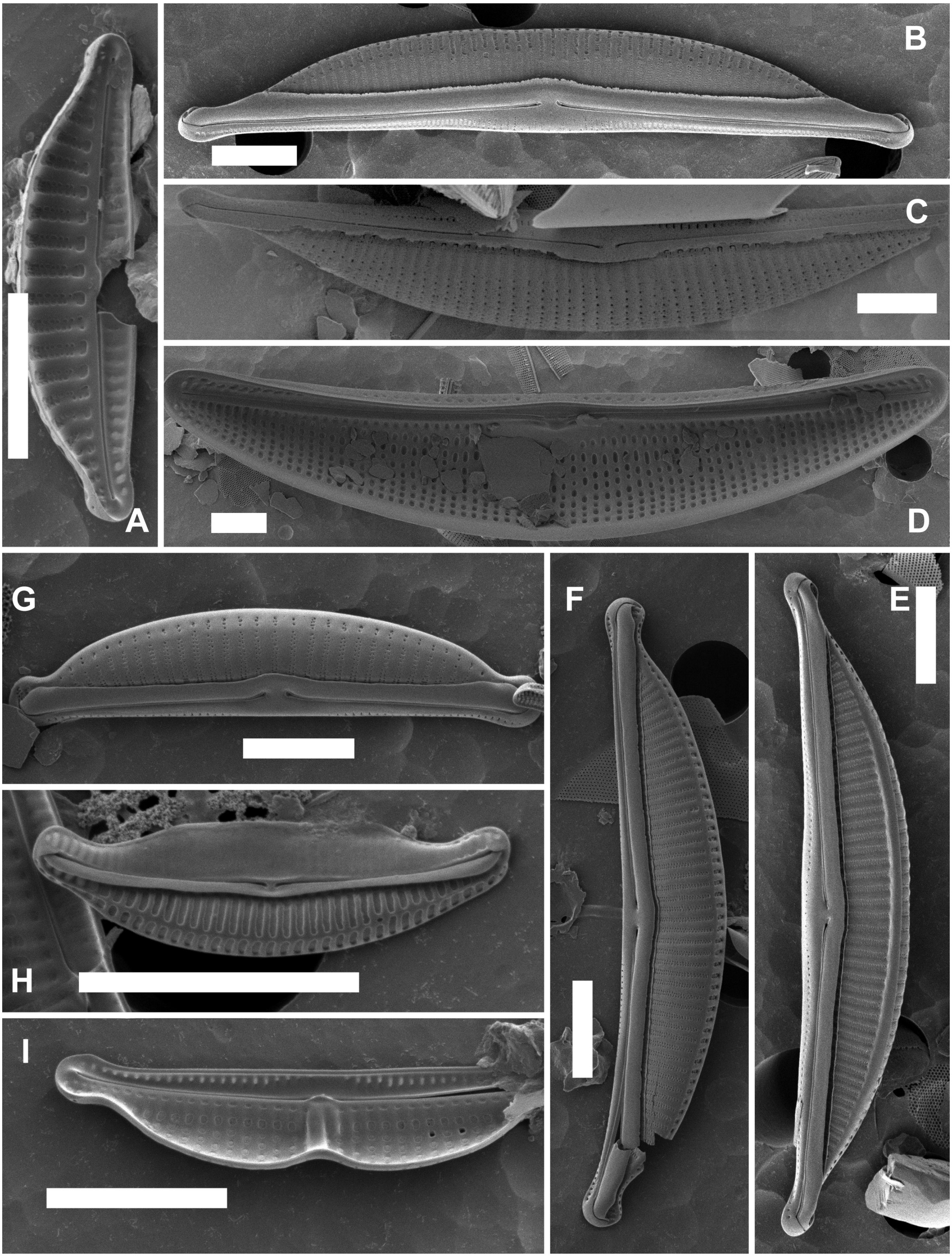

**FIGURE 8.** *Amphora* sensu lato taxa from Sozopol Bay under SEM. A. *Halamphora* sp. S10, internal view. B–C. *Halamphora* cf. *acutiuscula,* external view. D. *Amphora* cf. *proteus,* internal view. E–F. *Halamphora coffeaeformis,* external view. G. *Halamphora* sp. aff. *H. nagumoi,* external view. H. *Amphora (Halamphora)* sp. S21, external view. I. *Halamphora kolbei,* internal view. Scale bars = 5 µm.

**fig. 7B: *Amphora* aff. *pseudotenuissima*** Wachnicka & Gaiser

**Morphometric data:—**L: ca. 13.0 µm; W: n/a; DS: 32; VS: ca. 45.

**Notes:—**By dimensions, striation pattern, and the truncated raphe ledge near the apices, most similar species is *A. pseudotenuissima* (Wachnicka & Gaiser 2007). There is a possibility that this taxon represents the species depicted here in Figs 5I–L. Unfortunately, we observed only a single and oblique positioned valve under SEM, and this questions its identification.

## Discussion

The genus *Amphora* (sensu lato) is known with the notorious difficulties in taxa identification under LM (Stepanek & Kociolek 2018). Although SEM analyses were not possible for all the taxa in the study, the taxa we observed, even those with the smallest valve dimensions, are sufficiently morphologically different one from another to be separated even during LM observations, at least at local (i.e. Sozopol Bay) level. Taking into account the available literature data for benthic diatoms from the Black Sea, we were surprised to find such a high diversity of *Amphora* sensu lato species at a single site, on a single type of (artificial) substratum, and in a single season. For comparison, Temniskova-Topalova *et al.* (1994) recorded only 26 *Amphora* sensu lato taxa in more than 40 samples from the entire Bulgarian coast. On the northern coasts of the Black Sea, the reported number of *Amphora* sensu lato species greatly varies between the studies—from only a few taxa observed on artificial substrata (e.g. Milchakova *et al.* 2012, Lokhova 2012, Ryabushko *et al.* 2017), to more than 30–40 species in other studies (e.g. Guslyakov *et al.* 1992, Petrov & Nevrova 2013, Nevrova 2014b, 2015a). Once again, these latter studies, reporting high diversity, included a much higher number of samples and/or much larger areas under study: Petrov & Nevrova (2013) found 42 *Amphora* sensu lato taxa in 93 samples; Nevrova (2014b) observed 31 taxa from 16 sites in the entire Balaklava Bay; Nevrova (2015a) summarized the information from the existing literature and her own data for the shores of Karadag, where 34 taxa were recorded, whereas Guslyakov *et al.* (1992) listed more than 40 taxa in their atlas, based on observations of numerous samples from the Black Sea coast between Danube River and Crimea, including the data provided earlier by Proshkina-Lavrenko (1963). Finally, Nevrova (2015b) estimated that among 1094 diatom taxa present on the northern coasts of the Black Sea, 85 taxa belong to *Amphora* (excluding *Halamphora*). It would be unrealistic to assume that between a third and almost a half of this diversity could be found at a single site of our coast. In addition, the number of *Amphora* sensu lato taxa in our samples from Sozopol Bay accounts for 1/5 to 1/6 of the entire diatom diversity of the site (Zidarova *et al.,* unpubl. res.). Diversity in *Amphora* sensu lato in the Black Sea coastal waters seems to be greatly underestimated, which is most likely a result of the repeated incorrect identifications of the taxa with complicated taxonomic histories and the lack of new research and relevant new data from the region. Although we tried to identify the taxa in our samples based on the currently available literature, most of the species do not fit or match known species, neither we could find most of the taxa in the existing literature for the Black Sea, with few exceptions (e.g., *Amphora crassa, A.* cf. *helensis*, *Halamphora kolbei*, *Seminavis* cf. *robusta*). Many of the taxa have small valve dimensions, and the equipment insufficiency might have prevented their recognition by earlier explorers. Or, as a result of the historical confusion in the identities of many *Amphora* (sensu lato) species, these taxa might have been included within the range of other, similar species under LM. Many species, described in the early literature, were accompanied with sparse descriptions, whereas the illustrative material did not provide sufficient details for species separation, resulting in their different interpretations through the years (e.g. Danielidis & Mann 2002, Levkov 2009, Stepanek & Kociolek 2018). The earlier broad species concept used to summarize the high diversity in *Amphora* sensu lato (Levkov 2009) added to the repeated misidentifications, even for taxa with larger dimensions. Examples exist from the Black Sea as well. Both *Amphora proteus* and *Halamphora coffeaeformis* are reported among the most abundant diatoms in the Black Sea benthic habitats (Petrov & Nevrova 2007). *Halamphora coffeaeformis* has been repeatedly recorded on the northern coasts of the sea in the past 60 years (Proshkina-Lavrenko 1963, Guslyakov *et al.* 1992, Ryabushko *et al*. 2005, 2017, Nevrova 2013a, b, 2014a, b, 2015b, Petrov & Nevrova 2013, Nevrova & Petrov 2019a, Ryabushko 2020, either as *Amphora coffeiformis, Halamphora coffeiformis, H. coffeaeformis* or *Amphora coffeaeformis*), including from the Bulgarian coast (Temniskova-Topalova *et al*. 1994). Many taxa share similar valve outline (and biseriate striae, Stepanek & Kociolek 2018), and *H. coffeaeformis* has often been misindentified (Archibald & Schoeman 1984, Levkov 2009). It is possible that in earlier works some of the small-celled species we observed (*Halamphora* sp. aff. *H. nagumoi* on figs 4E–G, *H.* sp. S4 on figs 4I–M, *H.* sp. aff. *H. borealis* on figs 5A–E*, H.* sp. S14 on figs 5F–H) have been included within the range of *H. coffeaeformis*, especially if no SEM has been applied. Similarly, numerous records of *Amphora proteus* exist from the Black Sea (e.g. Proshkina-Lavrenko 1963, Guslyakov *et al.* 1992, Ryabushko *et al.* 2005, 2017, Nevrova 2013a, 2014a, b, 2015a, b, Petrov & Nevrova 2013, Balycheva & Ryabushko 2017, Nevrova & Petrov 2019a, etc.). The identity of the latter species has been confused for almost 130 years. Schoeman & Archibald (1986) noted that Gregory (1857) mistakenly included within the range of his taxon valves that belong to a smaller taxon with a finer striation, *A. marina* W.Smith. Proshkina-Lavrenko (1963) also found that the valves of *A. proteus* from the Black Sea samples are morphologically variable. Further, twenty records of infraspecific taxa, related to *A. proteus* exist in DiatomBase (Kociolek *et al*. 2021). Several of them were described from the Black Sea (e.g. *A. proteus* f. *ambigua* Proshkina-Lavrenko in Proshikina-Lavrenko 1963 and *A. proteus* var. *oculata* f. *nana* Bodeanu in Bodeanu 1976). Since no studies were done on the old materials from the Black Sea, it is unclear whether these taxa indeed form part of *A. proteus*. The real identity of *Amphora angusta,* another species reported from the Black Sea (e.g. Proshkina-Lavrenko 1963, Guslyakov *et al*. 1992, as *Cymbella angusta*), has also been unknown for almost 140 years, although the species has been reported numerous times by different researchers (Danielidis & Mann 2002). Our findings suggest that the species observed in earlier works from the Black Sea is actually a species of *Seminavis,* and not *A. angusta*. Finally, *Amphora exigua* (*Halamphora exigua*) is another taxon with a still unknown identity (Levkov 2009) and all its records from the Black Sea should be considered doubtful (e.g. in Guslyakov *et al.* 1992, Ryabushko *et al*. 2005, Nevrova 2014a, Petrov & Nevrova 2013, Ryabushko 2020). Unfortunately, in the recent literature for benthic diatoms from the Black Sea often only lists of taxa are given and these are rarely accompanied by microphotographs of (at least some of) the recorded species (as discussed by Nevrova & Petrov 2019b), with a few exceptions (e.g. Nevrova 2013a, b, 2014b, 2016, Nevrova & Petrov 2019a). Especially taxa with small valve dimensions, which are difficult to document under LM, are rarely shown. This makes difficult to trace the historical species identifications in the studies and no good comparisons between the different reports could be made.

As for the small-celled *Amphora* taxa from the Black Sea, Guslyakov (1987) and Guslyakov in Guslaykov *et al.* (1992) recognized and described a number of small-celled species from the region. We were not able to match our small-celled species with the species they described. Based on our literature search so far, these taxa seem not to be reported by other researchers after their discovery; with certainty, these taxa are not recorded in the recent floristic studies (e.g. Ryabushko *et al*. 2005, Nevrova 2013a, b, 2014a, b, 2015a, Petrov & Nevrova 2013, Nevrova & Petrov 2019a, Ryabishko 2020, etc.). No precise LM observations and descriptions of these taxa are available with their original descriptions, and for some of them only a few SEM pictures were provided with the descriptions (e.g. *A. genkallii* and *A. lydiae* in Guslyakov 1987*; A. chadjibeiensis* and *A. pontica* Guslyakov non Mereschkowsky in Guslyakov *et al.* 1992). The poor quality of the older reprints, together with the lack of re-examination of the materials with the currently available equipment and in the current taxonomy of the genus (e.g. Levkov 2009, Stepanek & Kociolek 2018) all make the recognition of these taxa impossible at present, whereas their identities remain unclear. The observed high number of small-celled taxa that do not match any known taxa suggests that undescribed species are still present. Several new taxa were recognized and described recently in other genera, such as *Lyrella* (Nevrova *et al*. 2013) and *Navicula* (Witkowski *et al*. 2010), showing the potential for new discoveries. Unfortunately, with the current war in Ukraine it is unknown whether the old materials of Guslyakov, known to be preserved in the Collection of the Odessa State University (E. Nevrova, pers. comm.), will remain available for re-examination and comparisons.

## Conclusion

The diversity of *Amphora* sensu lato taxa in the Black Sea benthos is underestimated, and still a lot of confusion exists for the identities of the species from the Black Sea, while some of the past records are doubtful (e.g., as in the case of *Amphora angusta, A. arcus* and *A. exigua*). A lot more efforts, with re-examination of the original materials of the taxa, described from the Black Sea and a revision of the existing data, combined with new research is needed, in order to better understand the species identities and real diversity.

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