Botany Letters

ISSN: 2381-8107 (Print) 2381-8115 (Online) Journal homepage: www.tandfonline.com/journals/tabg21

Two new *Amphora* species from European rivers compared to morphologically similar species of the *Amphora copulata* complex (Catenulaceae, Bacillariophyceae)

David Heudre, Bart Van de Vijver, Marcus Werum, Horst Lange-Bertalot, Laura Moreau, Marco Cantonati, Nicola Angeli, Zlatko Levkov & Carlos E. Wetzel

To cite this article: David Heudre, Bart Van de Vijver, Marcus Werum, Horst Lange-Bertalot, Laura Moreau, Marco Cantonati, Nicola Angeli, Zlatko Levkov & Carlos E. Wetzel (15 Dec 2025): Two new *Amphora* species from European rivers compared to morphologically similar species of the *Amphora copulata* complex (Catenulaceae, Bacillariophyceae), Botany Letters, DOI: 10.1080/23818107.2025.2595624

To link to this article: https://doi.org/10.1080/23818107.2025.2595624

BOTANY LETTERS
https://doi.org/10.1080/23818107.2025.2595624

Taylor & Francis
Taylor & Francis Group

Two new *Amphora* species from European rivers compared to morphologically similar species of the *Amphora copulata* complex (Catenulaceae, Bacillariophyceae)

David Heudre[a], Bart Van de Vijver[b,c], Marcus Werum[d], Horst Lange-Bertalot[e], Laura Moreau[a], Marco Cantonati[f], Nicola Angeli[g], Zlatko Levkov[h] and Carlos E. Wetzel[i]

[a]Direction Régionale Grand Est, Office Français de la Biodiversité, Moulins-lès-Metz, France; [b]Research Department, Meise Botanic Garden, Meise, Belgium; [c]Department of Biology – ECOSPHERE, University of Antwerp, Wilrijk, Belgiumvvv; [d]Independant Researcher, Germany, Frankfurt am Main, Germany; [e]Institut fûr Ökologie, Evolution Und Diversität, Forschungsinstitut Senckenberg, Fb. Biowissenschaften der Goethe-Universität, Frankfurt am Main, Germany; [f]BIOME Lab, Department of Biological, Geological and Environmental Sciences–BiGea, Alma Mater studiorum–University of Bologna, Bologna, Italy; [g]Research & Collections (limnology & Phycology), Muse–Museo delle Scienze, Trento, Italy; [h]Institute of Biology, Faculty of Natural Sciences, Ss Cyril and Methodius University, Skopje, Republic of North Macedonia; [i]Luxembourg Institute of Science and Technology (LIST), Environmental Sensing and Modelling Unit, Biodiversity Monitoring and Assessment Group, Belvaux, Luxembourg

ABSTRACT

Amphora copulata is a widely reported diatom name in water quality surveys throughout Europe. Following a survey of 1 000 floristic lists from the French part of the Rhine-Meuse basin (2000–2021), seven samples with the highest abundance of this species were selected for detailed examination-based on light (LM) and scanning electron microscopy (SEM) observations. In addition, samples from the Netherlands and Germany with a high abundance of the *Amphora copulata* species complex were also studied. The specimens previously identified as *A. copulata* sensu lato could not be assigned to an existing species based on the currently available literature, and proved to belong to two taxa described here as new: *Amphora vanderwerffiana* sp. nov. and *Amphora lucectoreana* sp. nov. The two new species are illustrated using both light and electron microscopy and compared to morphologically similar species. As such, type materials of *A. eileencoxiae* and *A. neglectiformis*, and recent material from the type locality of *A. macedoniensis* are also illustrated and discussed.

ARTICLE HISTORY
Received 20 August 2025
Accepted 21 November 2025

KEYWORDS
Amphora; Europe; morphology; new species; type material analysis

Introduction

Amphora Ehrenberg ex Kützing (1844) is a widespread diatom genus in both marine and continental waters. It is primarily characterised by species possessing "cymbelloid" valves that are asymmetrical about the apical plane. The frustules have juxtaposed, mirror-image valves with a raphe system on the ventral side and well-developed connective bands on the dorsal side (Round et al. 1990). Currently, Algaebase lists more than 1000 taxon names described to date (Guiry and Guiry 2025), whereas other sources, such as Kociolek et al. (2025), include approximately 1700 names. Among large-celled European freshwater species of this genus, *A. libyca* Ehrenberg has been the most frequently cited in literature (Krammer and Lange-Bertalot 1986) along with the widely used name *A. ovalis* (Kützing) Kützing. Nagumo (2003) noted that *Amphora copulata* (Kützing) Schoeman & R.E.M.Archibald was often misidentified in Japanese freshwaters as *A. lybica*. Schoeman and Archibald (1986) and Lee and Round (1988) investigated the type material of *A. copulata* from the Kützing collection (sample BM 81,032), refining the morphology of the latter species. This and other studies on the freshwater representatives of the genus *Amphora* in the early 2000s culminated in a dedicated monograph in the *Diatoms of Europe* series (Levkov 2009), revealing a surprisingly high diversity of previously unrecognised species, particularly within taxa morphologically close to *A. copulata*. Despite the separation of several morphologically distinct taxa, *A. copulata* remains to date the most significant species of this group in the French freshwater biomonitoring survey, as it was registered and identified in more than 7000 French aquatic samples (https://naiades.eaufrance.fr/).

During a routine biomonitoring survey of rivers in France, Germany and the Netherlands, several populations of two unknown taxa belonging to the genus *Amphora*, initially identified as *A. copulata s.l.* were observed. They are morphologically similar to *A. copulata* and were thus obviously neglected and misidentified under that name in the past. Since one of the unidentified taxa resembled *Amphora eileencoxiae*

CONTACT David Heudre david.heudre@ofb.gouv.fr Direction Régionale Grand Est, Office Français de la Biodiversité, Chemin du Longeau, Moulins-lès-Metz 57160, France
Supplemental data for this article can be accessed online at https://doi.org/10.1080/23818107.2025.2595624

Published online 15 Dec 2025

Cantonati, Levkov & Lange-Bertalot, the type material of the latter was re-examined with scanning electron microscopy to illustrate ultrastructural features that were not clearly visible in Cantonati et al. (2019), such as details of the mantle areolae. Furthermore, original material of two other morphologically similar *Amphora* taxa is likewise investigated using light microscopy, comparing them with the unknown populations from France and the Netherlands: *Amphora neglectiformis* Levkov & Edlund (type material from Lake Prespa, Rep. North Macedonia) and *Amphora macedoniensis* Nagumo (recently collected material from the type locality of the species at Lake Dorjan, Rep. North Macedonia). The two unknown taxa were morphologically distinct from all previously known *Amphora* species and are therefore described here as new species: *Amphora vanderwerffiana* Van de Vijver, Werum, Lange-Bertalot & Heudre sp. nov. and *Amphora lucectoriana* Heudre, Moreau, Van de Vijver & C.E.Wetzel sp. nov. The morphology of both new species is described based on detailed light and scanning electron microscopy observations and compared with similar taxa. Notes on their ecology are added.

Material and methods

From approximately 4200 analyses conducted in the French part of the Rhine-Meuse basin (France) from 2000 to 2021, nearly 1000 floristic lists included at least one valve of *A. copulata s.l.* Seven samples containing the highest relative abundance of valves (i.e. between 1 and 12 %) identified as *A. copulata s.l.* were selected for the current analysis. One additional sample containing two unknown *Amphora* taxa, collected from the Netherlands was studied together with eight samples from Germany. All samples are listed in Table 1 together with some physical and chemical data (when available). Physical and chemical data for French locations were recovered from the Water Information System Rhine-Meuse (https://rhin-meuse.eaufrance.fr/?lang=en).

Original (type) material from three previously described *Amphora* taxa was re-investigated to compare with the new species:

- *Amphora eileencoxiae* Cantonati, Levkov & Lange-Bertalot in Cantonati et al. (2019), Fontana del Vescovo (Municipality of Corniglio, Province of Parma, Italy, 44°22′44.767″N, 10°2′26.790″E, 1613 m a.s.l.), coll. date 2011, leg. M. Cantonati s.n., TR, slide cLIM007 DIAT1968 (type material)
- *Amphora neglectiformis* Levkov & Edlund in Levkov (2009), Bay of Konjsko, Lake Prespa, Rep. of North Macedonia, slide Acc. No. 479, leg. Z. Levkov (type material)
- *Amphora macedoniensis* Nagumo (2003), Lake Dojran, Rep. of North Macedonia, slide Acc. No. 9436, leg. Z. Levkov (recently collected material from the type locality)

Subsamples of the selected materials were cleaned by adding hot 37% hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl), and rinsed three times with distilled water. For light microscopy observations, the material was diluted with distilled water to avoid excessive concentrations of diatom valves. Cleaned diatom valves were mounted in Naphrax (refraction index 1.73) and analysed using an Olympus B×53 microscope at x1000 magnification (N.A. 1.30), equipped with Differential Interference Contrast (Nomarski) optics and the Olympus UC30 Imaging System, connected to the Cell Sense Standard program. For scanning electron microscopy (SEM), parts of the oxidised suspensions were filtered with additional deionised water through a 3 µm Isopore™ polycarbonate membrane filter (Merck Millipore), pieces of which were affixed on aluminium stubs after airdrying. Filters were coated with a 12 nm platinum layer using a Leica EM ACE600 sputter coater (Leica Microsystems GmbH, Germany). SEM analysis was performed with an ultra-high-resolution analytical field emission microscope (FE-SEM Hitachi SU-70, Hitachi High-Technologies Corporation, Tokyo, Japan) at 5 kV and a 10 mm working distance, using the lower secondary electron (SE-L) detector. The sample from the Netherlands and the type material of *A. eileencoxiae* were studied using a JEOL-JSM -7100F field emission scanning electron microscope at 1.5 kV and working distance of 4 mm.

Terminology used to describe valve morphology is based on Ross et al. (1979), Round et al. (1990), and Levkov (2009).

For the biometric data, the number of specimens (n) measured at random on the slides is indicated. Mean and standard deviation values of length and width are calculated and provided in square brackets. The stria density was measured on 10 µm with exclusion of the central area. Median values of striae are calculated and also provided in square brackets.

For typification of the species, we chose to use the entire slide as the type, following article 8.2 of the International Code of Nomenclature for algae, fungi, and plants (Turland et al. 2025). 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 best illustrate the taxon (see Figures). All novelties are registered proactively according to Art. 42.3 (Turland et al. 2025).

Table 1. Value of physicochemical parameters during the sampling period for the stations studied in this paper. Values for the stations from France correspond to the mean value during the sampling period (from June to August of the sampling year). For the other countries, when two values are provided, they correspond to the minimum and maximum values of the parameter during the growth period.

Sampling site ID	River	Municipality (Country)	Sampling date	Coordinates		pH	Conductivity ($\mu S.cm^{-1}$)	Total nitrogen ($mg.l^{-1}$)	NO_3^- ($mg.l^{-1}$)	Total phosphorus ($mg.l^{-1}$)	SO_4^{2-} ($mg.l^{-1}$)	Cl^- ($mg.l^{-1}$)
02001050	Rhine	Rhinau (F)	12/08/2014	7°42′48″ E	48°18′45″ N	8.2	296	1	4.4	0.018	23.7	9.7
02058000	Madon	Xeuilley (F)	16/07/2014	6°6′28″ E	48°34′32″ N	8.1	1268	2.5	7.4	0.14	429	68
02074000	Meurthe	Bouxieres (F)	25/07/2014	6°9′58″ E	48°44′46″ N	7.5	3031	2.9	7.4	0.22	75	1104
02082900	Seille	Metz (F)	23/07/2014	6°11′16″ E	49°6′1″ N	7.8	2675	1.1	5	0.15	430	536
02093100	Moselle	Manom (F)	04/08/2020	6°12′25″ E	49°22′18″ N	8	1800	–	1	0.15	130	410
02094900	Moselle	Sierck-les-Bains (F)	04/08/2020	6°20′48″ E	49°26′56″ N	8.6	1820	–	0.5	0.12	160	400
02099500	Sarre	Sarreinsming (F)	06/08/2009	7°6′28″ E	49°5′13″ N	7.9	807	2.3	5	0.24	141	63.1
2024EASFB00005	Hollands Diep	Bovensluis (Nl)	21/05/2024	4°28′42″ E	51°41′9″ N	7.6–8.5	–	1.1–4.1	1.1–3.5	0.02–1.1	29–70	–
2173	Wipper	Sachsenburg (Ger)	12/08/2020	11°9′41″ E	51°17′12″ N	8.1–8.4	4290–4730	3.4	2.2–3.2	0.14–0.25	512–641	1090–1200
4,100,025	Lippe	(L 20) oh Gersteinwerk, uh Herr. Bach (Ger)	16/08/2021	7°43′46″ E	51°40′19″ N	7.9–8.2	802–897	–	–	–	–	–
9,000,652	Lippe	(L 24) oh Seseke-Mdg. (Ger)	03/08/2021	7°31′55″ E	51°36′38″ N	8.1	895	6.1	4.7	0.05	75	95
9,000,653	Lippe	(L 25) uh Seseke-Mdg. (rechtes Ufer) (Ger)	03/08/2021	7°30′44″ E	51°37′0″ N	7.7–8.2	743–1050	–	2.7–5.5	0.10–0.15	59–93	53–130
9,000,885	Lippe	(L 25) uh Seseke-Mdg. (rechtes Ufer) (Ger)	28/07/2020	7°30′44″ E	51°37′0″ N	7.9–8.1	870–910	3.6	3.4	0.18	72	110
9,001,030	Südliche Umflut	Lippstadt (Ger)	31/08/2021	8°20′57″ E	51°40′8″ N	7.8	580–650	3.3–4.6	2.7–4.0	0.06–0.12	36–48	45–54
48,182,009	Allerkanal	Weyhausen (Ger)	06/08/2020	10°42′43″ E	52°27′9″ N	7.6	935	–	–	–	–	–
59,152,010	Elbe	Schnackenburg (Ger)	17/09/2020	11°34′10″ E	53°2′17″ N	8.2–9.1	648–1175	1.8–3.8	0.3–2.0	0.13–0.16	72–144	89–224
59,362,897	Rögnitz	Rosien (Ger)	01/10/2021	10°58′12″ E	53°18′4″ N	8.4	573	–	–	–	–	–
59,462,738	Neetze	Horburg (Ger)	01/10/2020	10°23′29″ E	53°21′46″ N	6.4	735	–	–	–	–	–

Results

Class Bacillariophyceae Haeckel
Subclass Bacillariophycidae D.G.Mann
Order Thalassiophysales D.G.Mann
Family Catenulaceae Mereschkowsky
Genus *Amphora* Ehrenberg ex Kützing

Amphora eileencoxiae Cantonati, Levkov & Lange-Bertalot in Cantonati et al. (2019) (Figure 1)

Light microscopy: Valves semi-elliptic, moderately dorsi-ventral with clearly convex dorsal and slightly concave ventral margin (Figure 1(a–h)). Valve dimensions ($n = 25$) length 24–59 µm [35 µm ±8], valve width 6–9 µm [7.1 µm ±0.9]. Valve apices broadly rounded, ventrally slightly bent. Axial area is narrow throughout on smaller valves but slightly expanded in larger specimens. Large semi-elliptic, closed dorsal area present, ventrally delimited by one row of 4 to 6 "ghost" areolae, extending to the margin on its dorsal side. Ventral fascia extending to the margin having

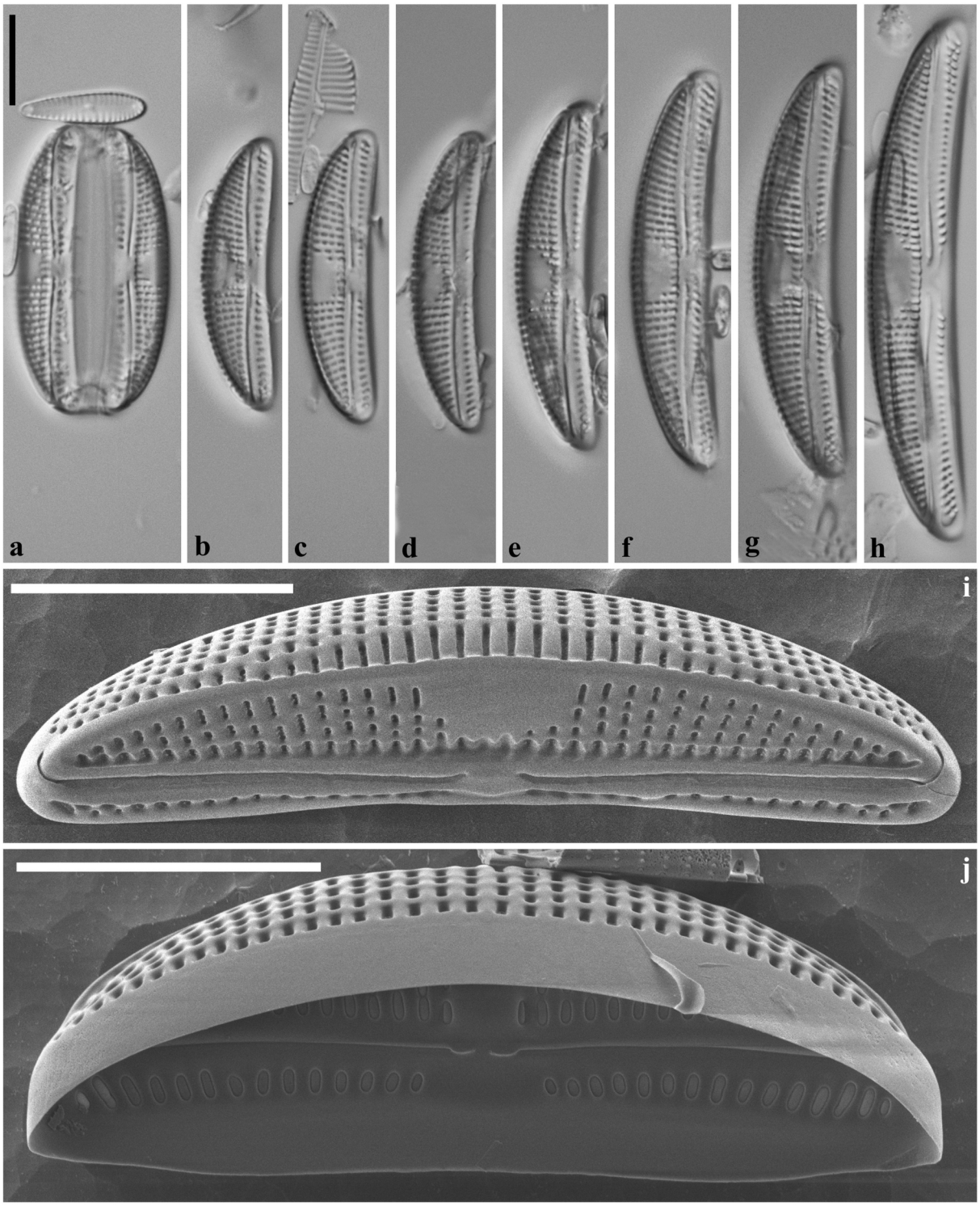

Figure 1. *Amphora eileencoxiae*, type material (raw material B 40 0043812). a. LM frustule view. b – h. LM valve views. i. Valve in external SEM view tilted toward the mantle. j. Valve in internal SEM view. Scale bars represent 10 µm.

more or less the same as the dorsal part of the dorsal fascia. Raphe branches are weakly arcuate to almost straight. The central raphe ends straight dorsally, slightly deflected. Dorsal striae slightly radiate, becoming more strongly radiate near apices, 12–13 in 10 µm [12 in 10 µm], usually composed of 4 distinct areolae (areola density ca. 16 in 10 µm). Ventral striae, slightly radiate near centre, becoming convergent near apices, 11–12 in 10 µm [12 in 10 µm], composed of one areola becoming two near apices on larger valves.

Scanning electron microscopy: Distinct marginal dorsal ridge is present running at valve face/mantle junction. Raphe ledge is continuous on the dorsal and ventral sides. Raphe branches are slightly arcuate. External central raphe endings are dorsally slightly deflected. Terminal raphe fissures elongated, dorsally deflected, continuing onto dorsal valve mantle. Dorsal and ventral striae distinctly areolate. The dorsal mantle striae composed of one transapically very elongated areola in the central part of the valve and up to five square to rectangular areolae (Figure 1(i)). Abvalvar is part of the mantle, forming a broad, hyaline band (Figure 1(j)). On the valve face, dorsal striae composed of up to 4 rectangular areolae with serrated areola foramina. Central area trapezium-shaped. Areolae dorsally bordering raphe ledge are located in a shallow depression. Ventral striae are composed of one transapically rectangular areola, two near the apices (Figure 1(i)). Internally, central raphe endings terminate onto elongated, raised helictoglossae. Areolae rectangularly rounded, with plain coverings (Figure 1(j)).

Amphora neglectiformis Levkov & Edlund in Levkov (2009) (Figure 2)

Light microscopy: Valves rather narrow, semi-elliptical to semi-lanceolate. Dorsal margin smoothly

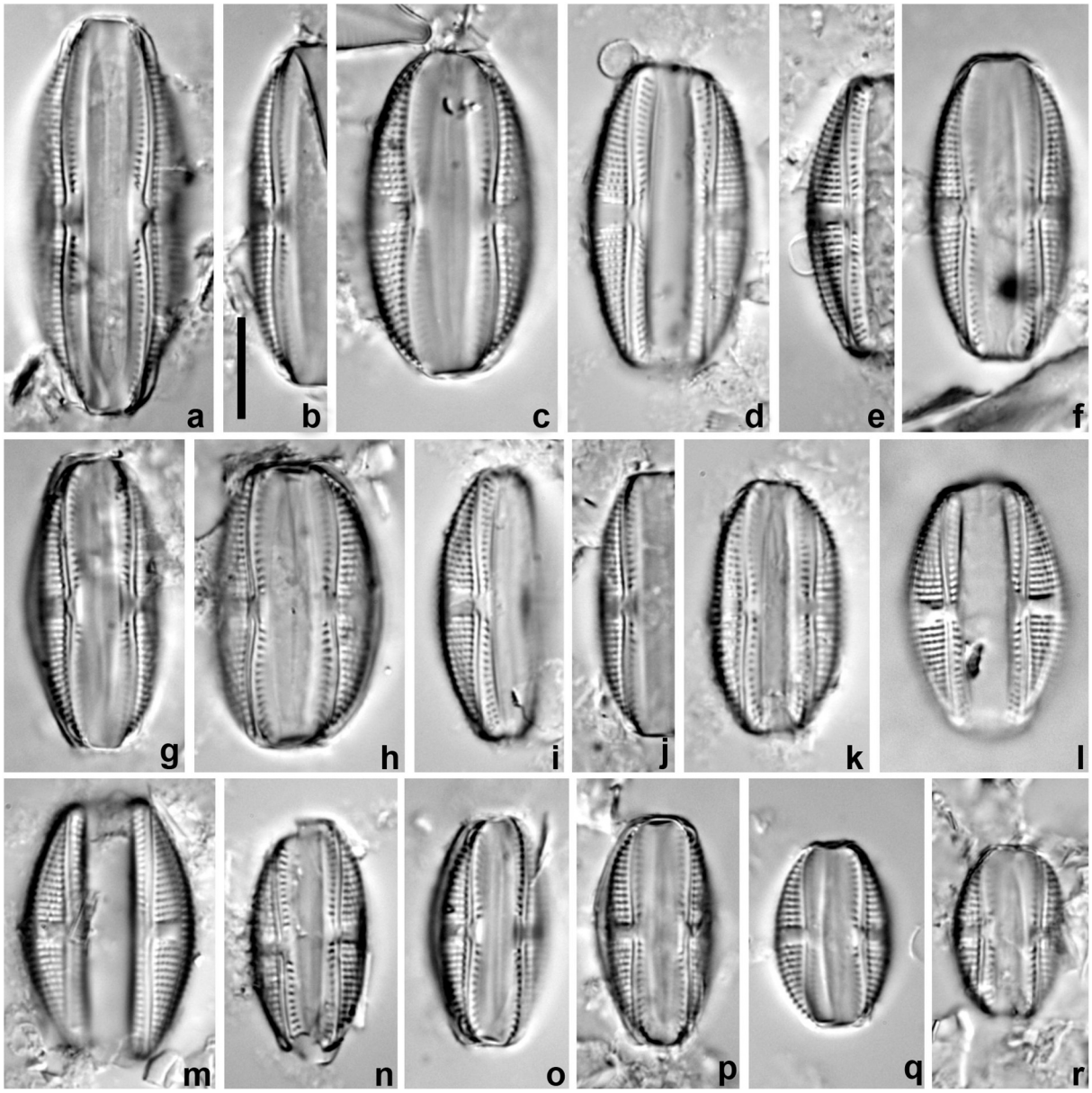

Figure 2. *Amphora neglectiformis*, type material (Bay of Konjsko, Lake Prespa, Rep. Of North Macedonia, slide Acc. No. 9436, leg. Z. Levkov). a – r. LM frustule and valve views. Scale bar represents 10 µm.

arched; ventral margin usually straight, occasionally slightly concave. Valve apices not protracted, acutely rounded. Valve dimensions ($n = 20$): length 16–53 µm [29 µm ±8], width 5–7 µm [5.5 µm ±0.5]. Axial area is narrow, slightly arched. Central area forming a wide, trapezoidal to rectangular fascia on the dorsal side; a broader fascia expanding towards the valve margin on the ventral side. Raphe branches clearly biarcuate with straight central endings and dorsally bent terminal raphe fissures (not clearly visible in LM). Striae on the dorsal side weakly radiate in the middle becoming more radiate towards the apices, coarsely punctate, composed of up to 4 areolae, 13–15 in 10 µm [15 in 10 µm]. Ventral striae radiate in the middle, more convergent towards the apices, 12–14 in 10 µm [13 in 10 µm], composed of one single areola near the central area, two areolae towards the apices (Figure 2(i)).

Amphora macedoniensis Nagumo (2003) (Figure 3)

Light microscopy:Valves semi-elliptical. Dorsal margin clearly convex, ventral margin usually concave, often slightly inflated at mid-valve. Valve apices ventrally bent, narrowly rounded, not protracted. Valve dimensions ($n = 20$): length 17–35 µm [22 µm ±4], width 3.5–7 µm [5 µm ±0.8]. Axial area narrow, dorsally slightly curved. Central area dorsally elliptical to almost rectangular, bordered by several elongated areolae. Ventrally central area forming a trapezium-shaped fascia, slightly broader than the dorsal area. Raphe branches biarcuate with straight to very weakly deflected central raphe endings. Striae dorsally radiate in the middle, becoming more strongly radiate towards the apices, 15–16 in 10 µm [15 in 10 µm], composed of 3–4 usually elongated areolae. Ventral striae slightly radiate in the middle, convergent towards the apices, 10–13 in 10 µm [13 in 10 µm], composed of one single areola.

Amphora vanderwerffiana Van de Vijver, Cantonati, Werum, Lange-Bertalot & Heudre sp. nov. (Figures 4 and 5, S1, S2)

Holotype

BR-4929 (Meise Botanic Garden, Belgium).

Isotype

Slide 474 (University of Antwerp, Belgium).

Type locality: Hollands Diep, Bovensluis (community of Moerdijk, prov. Noord-Brabant, The

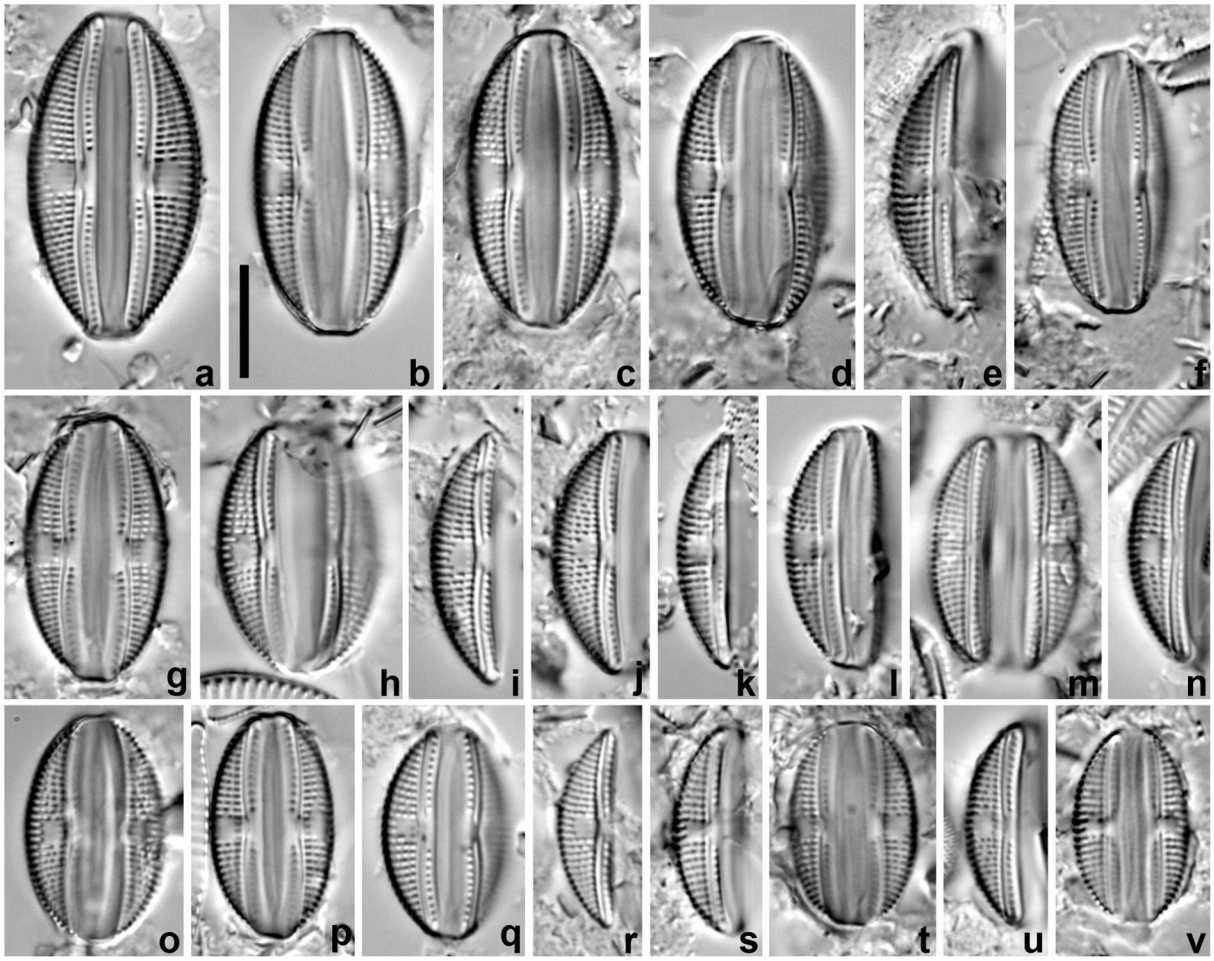

Figure 3. *Amphora macedoniensis*, recently collected material from the type locality (Lake Dorjan, Rep. of North Macedonia slide Acc. No. 479, leg. Z. Levkov). (a–r). lm frustule and valve views. Scale bar represents 10µm.

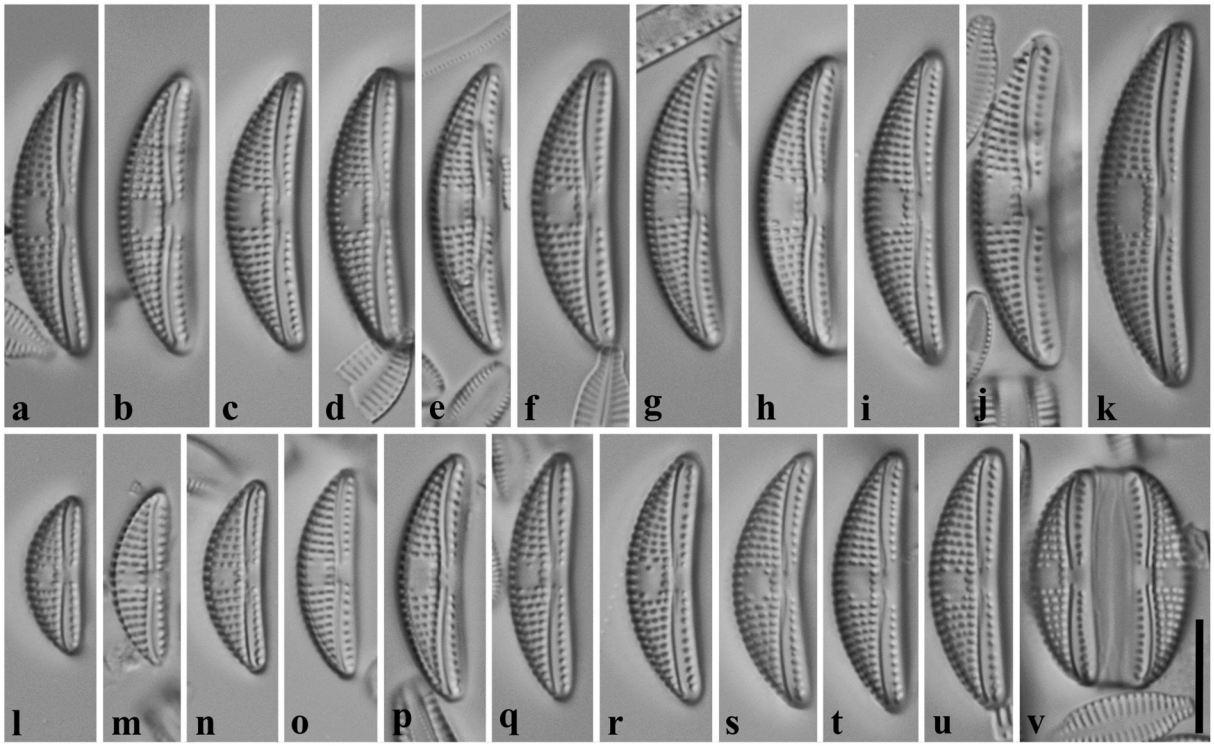

Figure 4. *Amphora vanderwerffiana*, type material (Material from Hollands Diep at Bovensluis (Nl), collected on 21 May 2023). a – u. LM valve views. v. LM frustule view. Scale bar represents 10µm.

Netherlands), coll. date 21 May 2023, leg. Arjen Ponger (Rijkswaterstaat).

Additional material: Sarre River, Sarreinsming, France, coll. date 6 August 2009 (Figure S1); Lippe, Seseke-Mdg. (right shore), Germany, coll. date 28 July 2020 (Figure S2).

Etymology

The species is named after Albert van der Werff (1903–1991), in honour of his impressive scientific contribution to the study of the morphology, ecology and biology of diatoms in the Netherlands.

Registration

http://phycobank.org/106379.

Description

Light microscopy: Valves semi-elliptic, moderately dorsi-ventral with slightly concave ventral margin (Figure 4(a–v)). Valve apices are acutely rounded, ventrally slightly bent. Valve dimensions ($n = 25$): length 14–32 µm [23 µm ±4.3], valve width 4.5–7.0 µm [6.0 µm ±0.7]. Raphe branches are weakly arcuate with straight to slightly dorsally deflected central endings. Axial area is narrow throughout, occasionally slightly expanded. Central area dorsally expanded, rectangular, separated from raphe ledge by a series of large, which may appear as ghost-like, areolae. Ventrally, the central area trapezium-shaped expanded up to the valve margin. Striae dorsally almost parallel to slightly radiate near the central area, becoming more strongly radiate near apices, 11–13 in 10 µm [13 in 10 µm], composed of up to 3 (rarely 4) distinct areolae. Areola density ca. 13 in 10 µm. Ventral striae, slightly radiate near centre becoming convergent near apices, 10–13 in 10 µm [11 in 10 µm], composed of one areola becoming transapically more elongated near apices.

Scanning electron microscopy: Thickened marginal dorsal ridge running along valve face/mantle junction. Dorsal mantle striae are composed of one or more elongated rectangular areola bordering the dorsal ridge along the length of the valve, and up to five large, square to rounded areolae (Figure 5(c)). Abvalvar is part of the mantle forming broad hyaline band (Figure 5(b, e)). Dorsal valve face striae composed of three, occasionally four rectangular areolae. Areolae are visible as slits in a rounded depression. Dorsal central area rectangular, almost not widening towards the dorsal margin, bordered by striae composed of four smaller areolae (Figure 5 (a, d)). Ghost-like areolae present at the raphe ledge. Ventral striae are located in long apically running groove. Ventral central area trapezium-shaped expanding. Raphe ledge continuous on both sides. Raphe branches slightly arcuate. External central raphe endings expanded, weakly dorsally deflected. Terminal raphe fissures are short, almost hooked towards dorsal margin, reaching the dorsal marginal ridge (Figure 5(a, c, d)). Internally, central raphe endings terminate onto fine, elongated helictoglossae (Figure 5(e, f)).

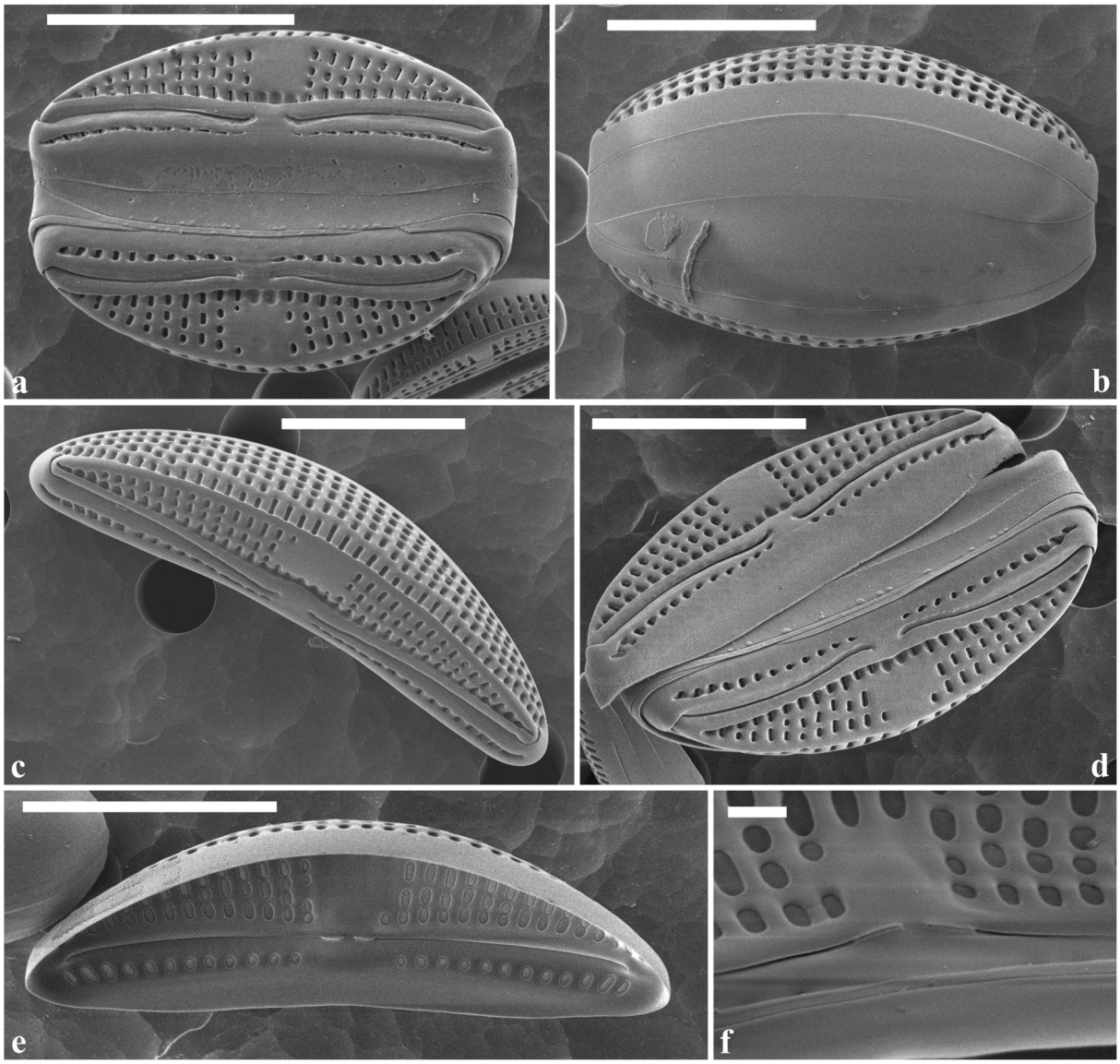

Figure 5. *Amphora vanderwerffiana*, type material (Material from Hollands Diep (Nl) at Bovensluis, collected on 21 May 2023). SEM views. a. Frustule in external view showing valves face. b. Frustule in external view showing girdle side. c. Valve in external view showing the mantle. d. Valves in external view. e. Valve in internal view. f. Close up of the raphe proximal endings in internal view. Scale bars represent 10 µm except for figure 5(f) where scale bar = 1 µm.

Terminal raphe endings terminating onto very small helictoglossae (Figure 5(e)). Girdle composed of three open, broad, plain copulae (Figure 5(b)).

Amphora lucectoriana Heudre, Moreau, Van de Vijver & C.E.Wetzel sp. nov. (Figures 6 and 7, S3)

Holotype

BR-4930 (Meise Botanic Garden, Belgium).

Isotype

Slide 475 (University of Antwerp, Belgium).

Type locality: Sarre River, Sarreinsming, France, coll. date 6 August 2009.

Additional material: Hollands Diep (community of Moerdijk, prov. Noord-Brabant, The Netherlands), coll. date 21 May 2023, leg. Arjen Ponger (Rijkswaterstaat) (Figure S3).

Etymology

The species is named after our colleague and friend Luc Ector (1962–2022) who passionately and enthusiastically devoted his life to teaching and studying the morphology, ecology and biology of diatoms.

Registration

http://phycobank.org/106380.

Description

Light microscopy: Valves semi-elliptic, moderately dorsi-ventral with convex dorsal and slightly concave ventral margin (Figure 6(a-c)). Valve apices acutely rounded, ventrally slightly bent. Valve dimensions ($n = 30$): length 11–23 µm [17.0 µm ±3.1], valve width 4–5 µm [4.5 µm ±0.3]. Axial area narrow throughout. Rectangular to occasionally slightly elliptical, closed dorsal central area present, ventrally

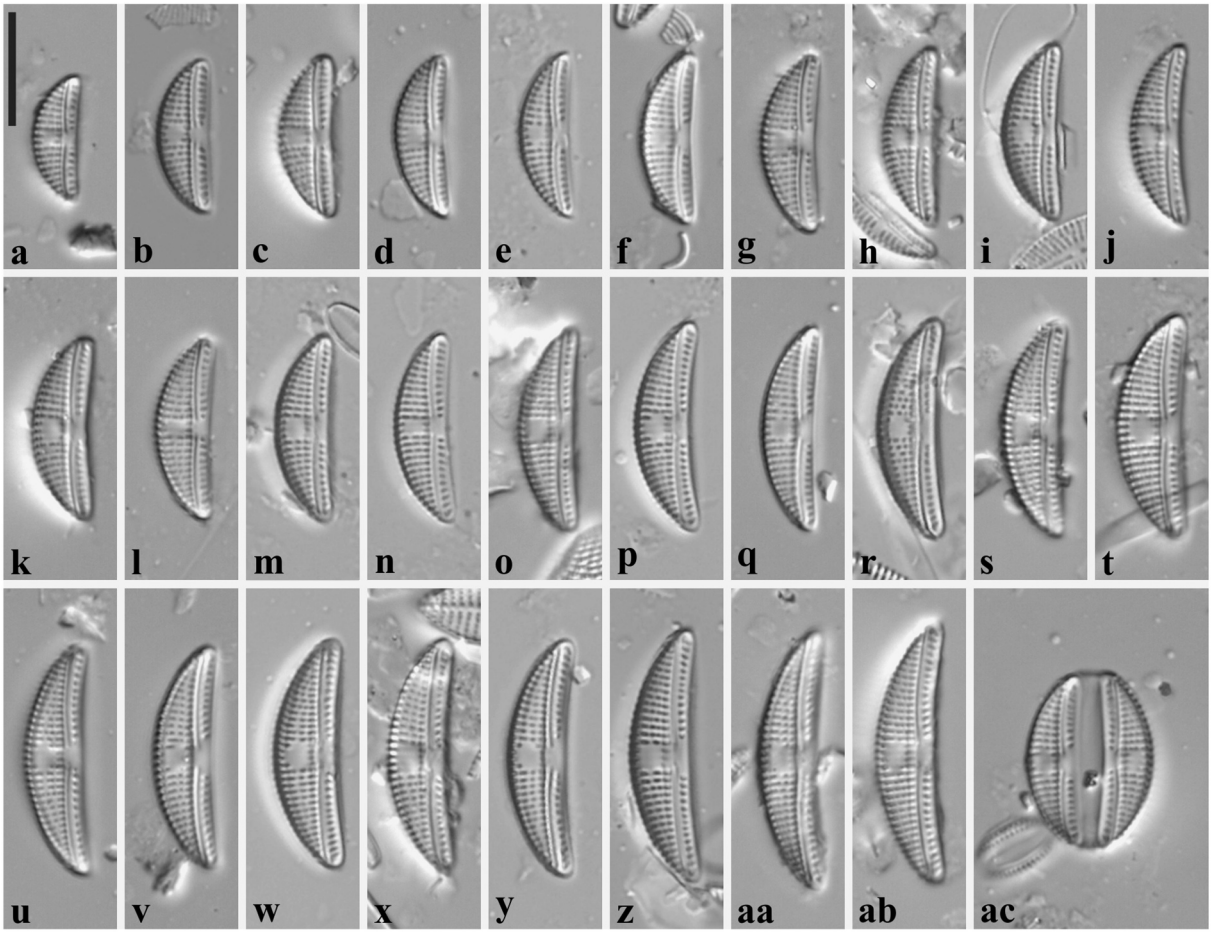

Figure 6. *Amphora lucectoreana*, type material (Sarre River at Sarreinsming, France, Collected on 06 August 2009). a – ab. LM valve views. ac. lm frustule view. Scale bar represents 10µm.

delimited by one row of two to four ghost-like areolae. Ventral fascia extending to margin, more or less as broad as dorsal central area. Raphe branches are weakly arcuate. Central raphe endings straight, rarely ventrally slightly deflected. Dorsal striae almost parallel in mid-valve, becoming slightly radiate near apices, 16–21 in 10 µm [18 in 10 µm], composed of 3–4 distinct areolae. Areola density ca. 15 in 10 µm. Ventral striae slightly radiate near centre becoming convergent near apices, 15–20 in 10 µm [16 in 10 µm], composed of one areola.

Scanning electron microscopy: Dorsal marginal ridge distinct, running along dorsal valve face/mantle junction (Figure 7(b)). Areolae dumbbell-shaped throughout entire valve (Figure 7(b-d)). Dorsal mantle striae composed of one more elongated areola close to dorsal ridge, and several (up to 4) shorter, weakly elongated areolae (Figure 7(b)). Abvalvar edge of dorsal mantle broad, plain (Figure 7(c)). Dorsal central area typically square-shaped. Dorsal striae on valve face composed of usually three, rarely four areolae. Series of areolae bordering raphe ledge partly covered by this ledge. Ventrally, striae composed of only one areola, located in shallow, slit-like groove, covering most of areolae. Raphe ledge continuous on dorsal and ventral side. Raphe branches slightly arcuate. External central raphe endings simple, weakly dorsally deflected. Terminal raphe fissures variable in length, short to weakly elongated, dorsally deflected (Figure 7 (b, d)) with raphe ledge continuing to mantle margin (Figure 7(c)). Internally, central raphe endings terminating onto clearly elongated helictoglossae (Figure 7(a)). Terminal raphe endings short, helictoglossae almost absent.

Ecology of the new species and associated diatom flora: These two new taxa were often observed in the same samples, collected from waterbodies presenting a wide range of environmental conditions, varying in both mineralisation and trophic levels (Table 1). However, the seven French stations seem to have some characteristics in common: carbonated, meso-eutrophic and rather warm waters, and the presence of notable clogging (siltation) of the sampled supports. Dominating taxa of the diatom assemblages in selected samples are *Amphora pediculus* (Kützing) Grunow, *Navicula cryptotenella* Lange-Bertalot, *Nitzschia inconspicua* Grunow and *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot. The sample from the Netherlands was collected from the Hollands Diep, a large river that is part of the Meuse-Rhine estuary. The sample was characterised by high numbers of *Amphora*

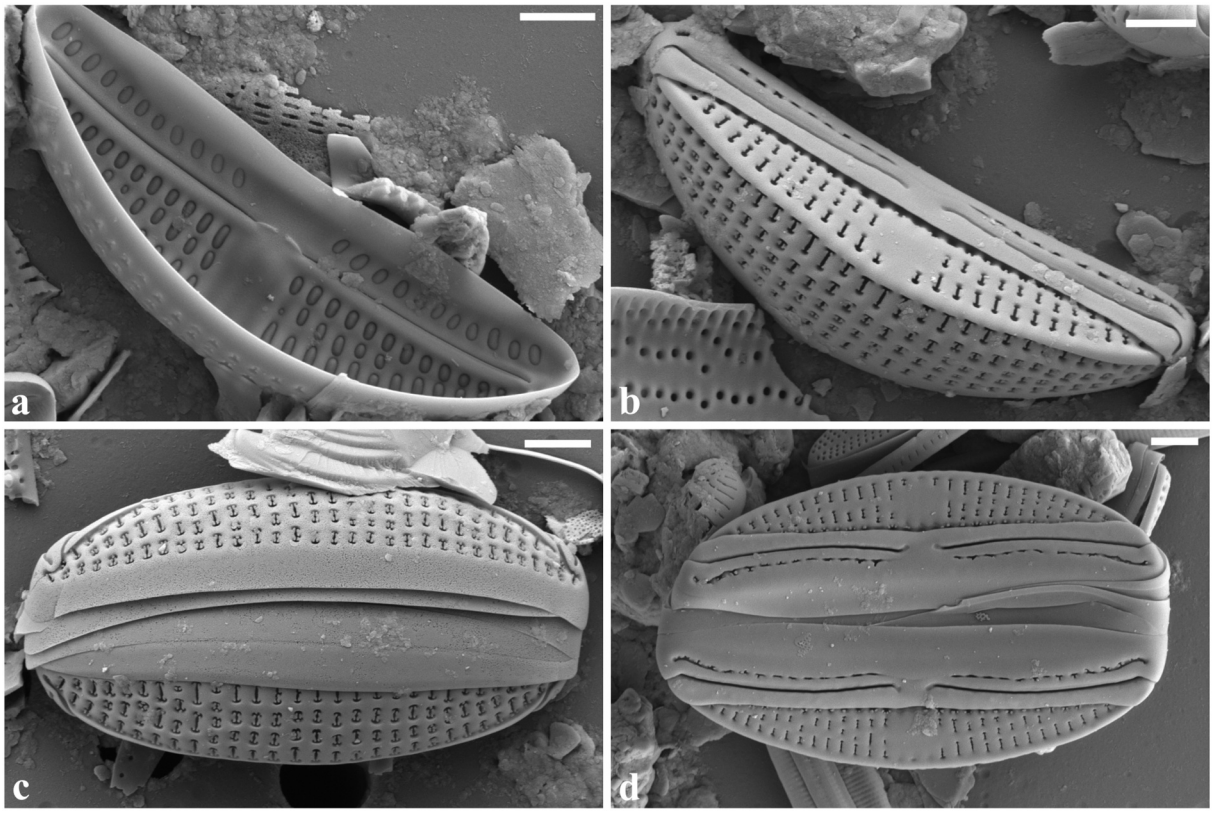

Figure 7. *Amphora lucectoreana*, type material (Sarre River at Sarreinsming, France). SEM views. a. Valve in internal view. b. Valve in external view showing the mantle. c. Frustule in external view showing girdle side. d. Frustule in external view showing valves face. Scale bars represent 2µm.

pediculus, *Rhoicosphenia abbreviata*, *Karayevia ploenensis* (Hustedt) Bukhtiyarova, and *Nitzschia amphibia* Grunow.

The diatom compositions of the investigated samples all point to alkaline, very electrolyte-enriched freshwater habitats with higher nutrient contents and a saprobity level up to α-mesosaprobic (Lange-Bertalot et al. 2017). This is consistent with the measured physical and chemical data showing high pH values (7.5–8.5), higher total-N, total-P and nitrate concentrations and higher conductivities (Table 1).

Discussion

Both new *Amphora* species had been "force-fitted" in the past during routine biomonitoring surveys in

Table 2. Morphology and morphometric details of species showing some similarity with *A. vanderwerffiana* and *A. lucectoreana*.

	Amphora baotuensis	*Amphora copulata*	*Amphora eileencoxiae*	*Amphora lange-bertalotii*	***Amphora lucectoreana* sp. nov.**	*Amphora macedoniensis*	*Amphora neglectiformis*	***Amphora vanderwerffiana* sp. nov.**	*Amphora vetula*
Valve length (µm)	14.5–38	19–42	24–59 [35 ± 8.0]	38–85	11–23 [17 ± 3.1]	13–30	18–53	14–32 [23 ± 4.3]	30–62
Valve width (µm)	4–6	5–7.5	6–9 [7.1 ± 0.9]	9.5–16	4–5 [4.5 ± 0.3]	3–6	5–7	4.5–7 [6 ± 0.7]	7.5–11.5
Number of dorsal striae in 10 µm	13–16	14–16	12–13 [12]	11–13	16–21 [18]	16–18	13–15	11–13 [13]	12–13
Number of ventral striae in 10 µm	13–16	12–14	11–12 [12]	10–12	15–20 [16]	10–13	12–14	10–13 [11]	11–12
Areolae number in dorsal striae	3 to 5	3 or 4	Mostly 4	Mostly 5	3 or 4	3 or 4	3 or 4	3 (rarely 4)	Mostly 4
Areolae number in ventral striae	1	1	1, 2 near apices	1, 2 near apices	1	1	1, 2 near apices	1	1, 2 near apices
Areolae (external view)	Elliptical	Elongated loculate	Rounded square to slit-shaped	–	Transapically-oriented dumbbell-shaped	Oblong	Round to oblong	Rounded square to slit-shaped	Round to oblong
Width of dorsal area (number of areolae)	3–5	3–4	4–6	4–7	2–4	3–4	2–4	3–6	4–6
Reference	Li et al. (2022)	Levkov (2009)	This study	Levkov (2009)	This study	Nagumo (2003)	Levkov (2009)	This study	Levkov (2009)

known species such as *Amphora copulata*, *A. eileencoxiae*, *A. vetula* Levkov, *A. lange-bertalotii* Lange-Bertalot & Metzeltin, or *A. neglectiformis*. A detailed comparison with the morphology of the latter five species shows that a separation of both taxa as new species is justified. Table 2 shows the morphological comparison of all the discussed species.

Although often found in the same samples, the two new species are easily separated even under LM, by their width (4.5–7 µm for *A. vanderwerffiana* vs 4–5 µm for *A. lucectoreana*), their stria density (always under 13 in 10 µm for *A. vanderwerffiana* and above 15 in 10 µm for *A. lucectoreana*) and more arcuate raphe branches and wider central area in *A. vanderwerffiana*. In SEM, the typical dumbbell-shaped areolae of *A. lucectoreana* make it easy to identify this species.

Amphora vanderwerffiana has been regularly identified as *A. eileencoxiae*. The morphological re-analysis of the type material of the latter showed that both taxa can be separated, not only based on morphological criteria, but also on habitat and ecological preferences. *Amphora eileencoxiae* can be distinguished (in SEM) by the typically strongly elongated first row of areolae bordering the valve face/mantle junction, a feature that was less distinct in *A. vanderwerffiana*. Both taxa can also be separated by the shape and size of the central area, especially on the dorsal side. In *A. eileencoxiae*, this central area is typically trapezium-shaped, clearly expanding towards the dorsal margin. In *A. vanderwerffiana*, on the contrary, the dorsal central area is rectangular to elliptical, lacking the trapezium-shaped widening, a feature clearly visible in LM. Additional differences include the number of areolae per stria (usually 5 in *A. eileencoxiae* versus 3, rarely 4 in *A. vanderwerffiana*), the ventral striae that are regularly composed of 2 areolae near the apices in *A. eileencoxiae* whereas in *A. vanderwerffiana*, only one areola is visible, smaller valve dimensions in *A. vanderwerffiana* (valve length 14–32 µm vs 24–59 µm, valve width 4.5–7.0 µm vs 6–9 µm), and the more acutely rounded ends and also more arcuate raphe branches in *A. vanderwerffiana*. The larger-celled *A. lange-bertalotii* can be separated not only by its much larger valve dimensions (length 38–85 µm, width 9.5–16 µm) giving the valves a more robust outlook, but also by the much larger dorsal trapezium-shaped central area, the higher number of areolae per stria (up to 7 dorsally) and more broadly rounded apices (Levkov 2009, 72, plate 52). *Amphora vetula* is different by its larger valve dimensions, its less concave ventral margin, the trapezium-shaped dorsal central area, and the higher number of areolae per stria (up to 6) (Levkov 2009, 144, plate 49). The ecology of *A. vanderwerffiana* (large rivers) also seems to differ from *A. eileencoxiae* (springs) and *A. vetula* (lakes) (Levkov 2009; Cantonati et al. 2019). *A. vanderwerffiana* also resembles *A. copulata*, but can be differentiated by its dorsal stria density (11–13 vs 14–16 in *A. copulata*), and by the external areola ultrastructure being rounded square to slit-like instead of elongated loculate as seen in Lee and Round (1988, figs 1–6). *A. vanderwerffiana* is very similar in light microscopy to *A. baotuensis* Yuhang Li, Nagumo & Kuidong Xu as it has the same valve size and overlapping striae density, but can be differentiated by its rectangular instead of fan-shaped dorsal area, its different external areola ultrastructure (elliptical in *A. baotuensis* vs rectangular in *A. vanderwerffiana*) and in SEM by the absence of a large coastated dorsal marginal ridge on the valvar face (Figure 5(a, c, d)) characteristic of *A. baotuensis* (Li et al. 2022, figs 10–15). Also, *A. baotuensis* seems to have a different ecology as it was described from a sample collected in a karstic source in Asia.

The second new species, *Amphora lucectoreana*, can also be differentiated from *A. copulata* by its smaller valve dimensions (valve length 11–23 µm vs 19–42 µm, valve width 4–5 µm vs 5–7.5 µm), its higher stria density (dorsally 16–21 vs 14–16 striae in 10 µm, ventrally 15–20 vs 12–14 in 10 µm), less arcuate raphe branches and by the external areola structure in SEM showing the typical dumbbell shape. These dumbbell-shaped areolae (i.e. pinched in the middle) are unique in European *Amphora* species. Analysis of the published images of all *Amphora* species in Levkov (2009) revealed that the only species with similar areola morphology is *Amphora paracopulata* Levkov & Edlund in Levkov et al. (2009, pl. 183, figs 1–5) from Lake Hovsgol, Mongolia. *Amphora cruciferoides* Stoermer & Yang from Lake Michigan, South Haven, USA' as illustrated in Levkov et al. (2009, pl. 51, figs 15–19 and pl. 189, figs 1–4) also show similar patterns on the dorsal areolae (while eroded). Illustrations from *Amphora cruciferoides* by Stoermer & Yang (1971, figs 3a,b) only show LM images, so the areolae pattern in the type material remains unknown. Similar dumbbell areolae shape can also be found in *Amphora arenicola* Grunow ex Cleve illustrated by Nagumo (2003, pl. 16, figs 1–3) and *Amphora liriope* Nagumo (2003, pl. 64, fig. 2). *Amphora lucectoreana* was often identified as *A. neglectiformis* but can be separated (apart by its dumbbell-shaped areolae) by its smaller valve dimensions (length 11–23 µm vs 18–53 µm, width 4–5 µm vs 5–7 µm), and stria density (dorsal 16–21 vs 13–15 striae in 10 µm, ventral 15–20 vs 12–14 striae in 10 µm). Finally, *A. lucectoreana* can be separated from *A. macedoniensis* by a higher stria density (dorsal 16–21 vs 16–18 striae in 10 µm, ventral 15–20 vs 10–13 striae in 10 µm), its dumbbell-shaped

areolae and ventral margin shape of large specimens (slightly concave to straight in *A. lucectoreana* vs concave with the central part convex in *A. macedoniensis*).

Given the observed differences in morphology of several populations for both new taxa, with all currently known species and based on the observed ecological differences of the new species (being more typical for meso-eutrophic, electrolyte-enriched large rivers), the description of both taxa as new species can be justified. As the two species were mostly misidentified as *A. copulata sensu lato*, further investigations will be necessary to refine our knowledge of their ecology and distribution.

Acknowledgment

Parts of the results of this paper have been presented during the 40ème Colloque de l'Association des Diatomistes de Langue Française (ADLaF), 13–15 September 2022, in Clermont-Ferrand, France, and during the 43ème Colloque de l'Association des Diatomistes de Langue Française (ADLaF), 09–12 September 2025, in Rouen, France. Dr René van Wezel (Rijkswaterstaat) is thanked for the permission to use the sample from Bovensluis and for providing us with the corresponding physico-chemical data. Mr. Arjen Ponger (Rijkswaterstaat) has collected the sample. We also thank the two anonymous referees for their time and constructive comments.

Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ORCID

David Heudre http://orcid.org/0009-0003-5134-5332
Bart Van de Vijver http://orcid.org/0000-0002-6244-1886
Marcus Werum http://orcid.org/0009-0000-4938-2896
Horst Lange-Bertalot http://orcid.org/0000-0002-9513-3310
Laura Moreau http://orcid.org/0009-0007-1020-6378
Marco Cantonati http://orcid.org/0000-0003-0179-3842
Nicola Angeli http://orcid.org/0000-0003-0927-2830
Zlatko Levkov http://orcid.org/0000-0002-1184-2356
Carlos E. Wetzel http://orcid.org/0000-0001-5330-0494

Author contributions

David Heudre: samples collection, generation and analyses of LM material, plates editing, physicochemical data treatment, discussion of results, development, writing, revision and editing of the manuscript.

Bart Van de Vijver: generation and analyses of LM and SEM material, plates editing, discussion of results, development, revision and editing of the manuscript.

Marcus Werum: discussion of results, development, revision and editing of the manuscript.

Horst Lange-Bertalot: discussion of results, development, revision and editing of the manuscript.

Laura Moreau: samples collection, discussion of results, development, revision and editing of the manuscript.

Marco Cantonati: discussion of results, development, revision and editing of the manuscript.

Nicola Angeli: discussion of results, development, revision and editing of the manuscript.

Zlatko Levkov: analysis of the Macedonian populations, plates editing, discussion of results, development, revision and editing of the manuscript.

Carlos E. Wetzel: generation and analyses of SEM material, discussion of results, development, revision and editing of the manuscript.

References

Cantonati M, Angeli N, Lange-Bertalot H, Levkov Z. 2019. New *Amphora* and *Halamphora* (Bacillariophyta) species from springs in the northern Apennines (Emilia-Romagna, Italy). Plant Ecol Evol. 152 (2):285–292. https://doi.org/10.5091/plecevo.2019.1605

Guiry MD, Guiry GM. 2025. AlgaeBase. University of Galway. https://www.algaebase.org

Kociolek JP et al. 2025. DiatomBase. https://doi.org/10.14284/504

Krammer K, Lange-Bertalot H. 1986. Bacillariophyceae 1. teil: naviculaceae. In Ettl H, Gerloff J, Heynig H, Mollenhauer D, editors. Süßwasserflora von Mitteleuropa. Vol 2/1. Gustav Fischer Verlag; p 1–876.

Kützing FT. 1844. *Die kieselschaligen Bacillarien oder Diatomeen*. Förstemann.

Lange-Bertalot H, Hofmann G, Werum M, Cantonati M. 2017. Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessment. English edition with updated taxonomy and added species. Koeltz Botanical Books.

Lee K, Round FE. 1988. Studies on freshwater *Amphora* species. II. *Amphora copulata* (Kütz.) Schoeman & Archibald. Diatom Res. 3(2):217–225. https://doi.org/10.1080/0269249X.1988.9705034

Levkov Z. 2009. *Amphora* sensu lato. Diatoms of Europe 5. Koeltz Botanical Books.

Li Y, Nagumo T, Xu K. 2022. Morphology and molecular phylogeny of *Amphora baotuensis* sp. nov. a new freshwater benthic diatom from a karst spring in China. Diatom Res. 37(2):145–153. https://doi.org/10.1080/0269249X.2022.2063390

Nagumo T. 2003. Taxonomic studies of the subgenus *Amphora* Cleve of the genus *Amphora* (Bacillariophyceae) in Japan. Bibl Diatom. 49:1–265.

Ross R et al. 1979. An amended terminology for the siliceous components of the diatom cell. Nova Hedw Beih. 64:513–533.

Round FE, Crawford RM, Mann DG. 1990. The diatoms: biology & morphology of the genera. Cambridge University Press.

Schoeman FR, Archibald REM. 1986. Observations on *Amphora* species (Bacillariophyceae) in the British Museum (Natural History). V. Some species from the subgenus *Amphora*. South Afr J Bot. 52(5):425–437. https://doi.org/10.1016/S0254-6299(16)31507-1

Turland NJ et al. 2025. *International code of nomenclature for algae, fungi, and plants (Madrid Code). Regnum Vegetabile*. Vol 162. The University of Chicago Press.