

New *Amphora* and *Halamphora* (Bacillariophyta) species from springs in the northern Apennines (Emilia-Romagna, Italy)

Marco Cantonati^{1,*}, Nicola Angeli¹, Horst Lange-Bertalot² & Zlatko Levkov³

¹MUSE - Museo delle Scienze, Limnology & Phycology Section, Corso del Lavoro e della Scienza 3, I-38123 Trento, Italy

²Goethe Universität Frankfurt, Biologikum, Max-von-Laue Straße 13, 60438 Frankfurt, Germany

³Institute of Biology, Faculty of Natural Sciences, SS Cyril and Methodius University, Arhimedova 3, 1000 Skopje, North Macedonia

*Author for correspondence: marco.cantonati@muse.it

Background and aims – The detailed analysis of algae and cyanoprokaryotes in a heterogeneous group of spring habitats (including all the different typologies) of the northern Apennines (Emilia-Romagna Region, Italy) revealed a new *Amphora* species in a small mountain flowing spring with low conductivity, and a new *Halamphora* species in a large, inland-saline (Triassic gypsum), fast flowing spring (Poiano spring). The present study aims to describe in detail these two new species found in contrasting spring types.

Methods – This study is based on light microscopy (both fresh –for plastids – and prepared materials) and scanning electron microscopy observations, as well as a thorough morphological, physical, chemical, and biological characterization of the habitats.

Key results – *Amphora eileencoxiae* sp. nov. is most similar to *A. vetula* (and allied taxa), and is characterized by the outline with acutely rounded, moderately ventrally bent ends, by the dimensions, and by the well-defined, semi-elliptic dorsal area. *Halamphora poianensis* sp. nov. is most similar to *H. gasseae* but differs by the higher stria density, the clearly ventrally bent ends, and the strongly developed dorsal raphe ledge.

Conclusions – This is a contribution to the knowledge of the genera *Amphora* and *Halamphora* in mountain springs in understudied geographic areas and inland-saline springs, the species communities of which are likely insufficiently explored.

Key words – Diatoms, springs, inland saline habitats, new species, *Amphora eileencoxiae*, *Halamphora poianensis*, northern Apennines.

INTRODUCTION

Amphora Ehrenb. ex Kütz. s. lat. is a large, widely distributed, and diverse group of freshwater, brackish, and marine diatoms (e.g. Stepanek & Kociolek 2013). Recent important studies (e.g. Levkov 2009, Stepanek & Kociolek 2014, 2018, 2019) have advanced the understanding of this polyphyletic group, and of the evolution of amphoroid symmetry.

Many species in *Amphora* s. lat. are marine (e.g. Round et al. 1990, Stepanek & Kociolek 2013) but an increasing number of species are being described from inland habitats (e.g. Levkov 2009, Stepanek & Kociolek 2018). However, in

spite of recent revisions of *Amphora* s. lat. that included the elevation to the generic status of the subgenus *Halamphora* and the description of 63 new taxa from freshwater environments (Levkov 2009), the genera *Amphora* and *Halamphora* still appear to be insufficiently known in freshwater habitats (e.g. Stepanek & Kociolek 2013, 2015).

Freshwater ecosystems which are insufficiently studied are springs, important habitats (Cantonati et al. 2012a, 2015), multiple ecotones, biodiversity hotspots (Cantonati et al. 2016a, Taxböck et al. 2017), and ideal sites for long-term ecological research (Cantonati & Ortler 1998). Their diatom communities have only been studied in a more detail in the

last two decades (e.g. Cantonati et al. 2012b), and include species that occur preferentially in springheads and comparable habitats (e.g. Cantonati et al. 2010). The comprehensive study of their diatom communities is urgently needed, because they are often affected by human impact, in particular alteration of the morphology of the springhead and increased nitrate concentrations (e.g. Angeli et al. 2010).

There are a number of *Amphora* and *Halamphora* species described from springs. New *Amphora* species are mostly described from mountain and hill springs on both carbonate and siliceous substrata: *Amphora alpestris* Levkov from a spring on the Kozuf mountain (Macedonia; Levkov 2009); *Amphora polonica* Żelazna-Wieczorek & Lange-Bert. (Żelazna-Wieczorek 2011) from a spring in Central Poland; *Amphora sancti-naumii* Levkov & Metzeltin and several others from the St. Naum springs in Lake Ohrid (Macedonia) (Levkov et al. 2007). New *Halamphora* species have been described from mineral or thermo-mineral springs, such as *Halamphora gasseae* Levkov, from Sakallol hot spring (Kenya, Africa) (Levkov 2009), and *Halamphora thermalis* (Hust.) Levkov from mineral springs in Africa (Hustedt 1949).

The most common *Amphora* species in springs are *Amphora copulata* Schoeman & R.E.M. Archibald (Żelazna-Wieczorek 2011, Wojtal 2013), *Amphora inariensis* Krammer (Cantonati et al. 2012), *Amphora pediculus* (Kütz.) Grunow (likely including also *Amphora micra* Levkov; Werum & Lange-Bertalot 2004, Cantonati & Lange-Bertalot 2010, Cantonati et al. 2012, with significant preference for stones as substratum, Wojtal 2013), whilst the most common *Halamphora* species are *Halamphora normanii* (Rabenh.) Levkov and *Halamphora montana* (Krasske) Levkov (Werum & Lange-Bertalot 2004, Cantonati et al. 2016b).

The salinity barrier dividing the freshwater from the marine realm was supposed to be virtually impassable but Alverson et al. (2007) showed that this did not apply to the thalassiosiroid diatoms. More recently, Ruck et al. (2016), working on the Surirellales and Rhopalodiales, proposed an interesting ‘stepping-stone’ hypothesis, based on comparative molecular-phylogeny evidence supporting the idea that the ancestrally-marine diatoms belonging to these groups would have used brackish waters as an intermediate habitat to which to adapt before invading freshwater environments. In the light of these papers, the most recent study by Stepanek & Kociolek (2019) suggests that inland saline habitats are of particular interest for such studies: for diatom groups, such as *Amphora* and *Halamphora*, that have representatives colonizing smaller inland waterbodies with elevated conductivity, these waterbodies might represent potential intermediate habitats from which the colonization of surrounding freshwaters could have taken place.

Using light microscopy (LM, both fresh and prepared materials) and scanning electron microscopy (SEM) observations, as well as a thorough morphological, physical, chemical, and biological characterization of the habitats, the present study aims to describe in detail two new species, one in the genus *Amphora* and one species of *Halamphora*. They were found in two contrasting spring types in the northern Apennines.

MATERIAL AND METHODS

The samples on which this study is based were collected during surveys for the EBERs (Exploring the Biodiversity of Emilia-Romagna springs, 2011–2013) project (Cantonati, unpubl. res.) Samples were collected by scraping 8–10 stones, and by collecting specimens of the dominant bryophyte species in three points of the spring area (Cantonati et al. 2012), and then digested using hydrogen peroxide (EN 13946 2003). The cleaned material was mounted in Naphrax (refractive index of 1.74). Relative abundances were determined by identifying and counting a total of at least 450 valves using a Zeiss Axioskop 2 (Zeiss, Jena, Germany) and x1000 magnification.

The following material of other species was compared with the new *Amphora* species: *Amphora lange-bertalotii* Levkov & Metzeltin (Acc. number MKNDC 011437), organic sediment, alkaline fen, Hanoj, below Mal Turchin, Shara Mountain; *Amphora lange-bertalotii* var. *tenuis* Levkov & Metzeltin (Acc. number MKNDC 003009), mosses, peat-bog, Ceripashina, Shara Mountain; *Amphora vetula* Levkov (Acc. number MKNDC 000694), mosses near the shore, St. Naum Bay, Lake Ohrid.

Material (slides, prepared material, and aliquots of the original samples), including the holotypes of the two new species, is held at the Diatom Collection of MUSE – Museo delle Scienze (TR) of Trento (Northern Italy). Isotype slides and aliquots of prepared material from the same locality and substratum were deposited at the Diatom Collection of the Natural History Museum, London (BM) (UK), and the Diatom Collection of the Botanical Garden and Botanical Museum of the Freie Universität Berlin (B) (Germany). If not otherwise stated, measurements on 15–20 different specimens representative of the size-diminution series were made to obtain ranges and averages of the morphological and ultrastructural features. Plastid characteristics and type were assessed using Cox (1996) and Levkov (2009).

SEM observations were made primarily at the University of Frankfurt using a Hitachi S-4500 (Hitachi Ltd., Tokyo, Japan [high vacuum, gold coated stubs]). Terminology to describe valve morphology is based on Round et al. (1990) and Levkov (2009).

RESULTS

Amphora eileencoxiae Cantonati, Levkov & Lange-Bert., **sp. nov.**

Figs 1–3

Type material – Italy, Northern Apennines, Province of Parma, Municipality of Corniglio, Rheocrenic mountain spring Fontana del Vescovo (“Bishop’s Fountain”) in the Upper Parma Valley (Alta Val Parma), Lithology: Siliciclastic sandstones, (44°22′44.767″N, 10°2′26.790″E, 1613 m a.s.l.), 31 Jul. 2011, *M. Cantonati* s.n. (holo-: TR, slide cLIM007 DIAT 1968, partly shown here in fig. 1A–O; iso-: B, slide B 40 0043810, cleaned material B 40 0043811, raw material B 40 0043812; BM, slide BM 101960).

Registration – <http://phycobank.org/101111>

Description: LM – Frustule width (n = 5) is 13–22 μm (fig. 1P–S). Valves are semi-elliptic and moderately dorsiventral. The dorsal margin is smoothly arched. The ventral margin is slightly-concave to straight (fig. 1A–O). Valve length 21–62 μm , valve width 6.4–10.3 μm . The valve ends are broadly rounded and slightly bent ventrally. The raphe branches are weakly arcuate to straight with proximal raphe ends straight to slightly dorsally deflected. The axial area is narrow

throughout, slightly expanded in some larger specimens. A semi-elliptic closed dorsal area, delimited ventrally by one row of areolae, is present. The ventral fascia extends to the margin and has more or less the same width as the dorsal area. The dorsal striae, 11–14 in 10 μm , are distinctly areolate, slightly radiate to almost parallel near the centre and becoming more strongly radiate near the apices. Areola density is 12–19 in 10 μm . The ventral striae, 12–14 in 10 μm , are

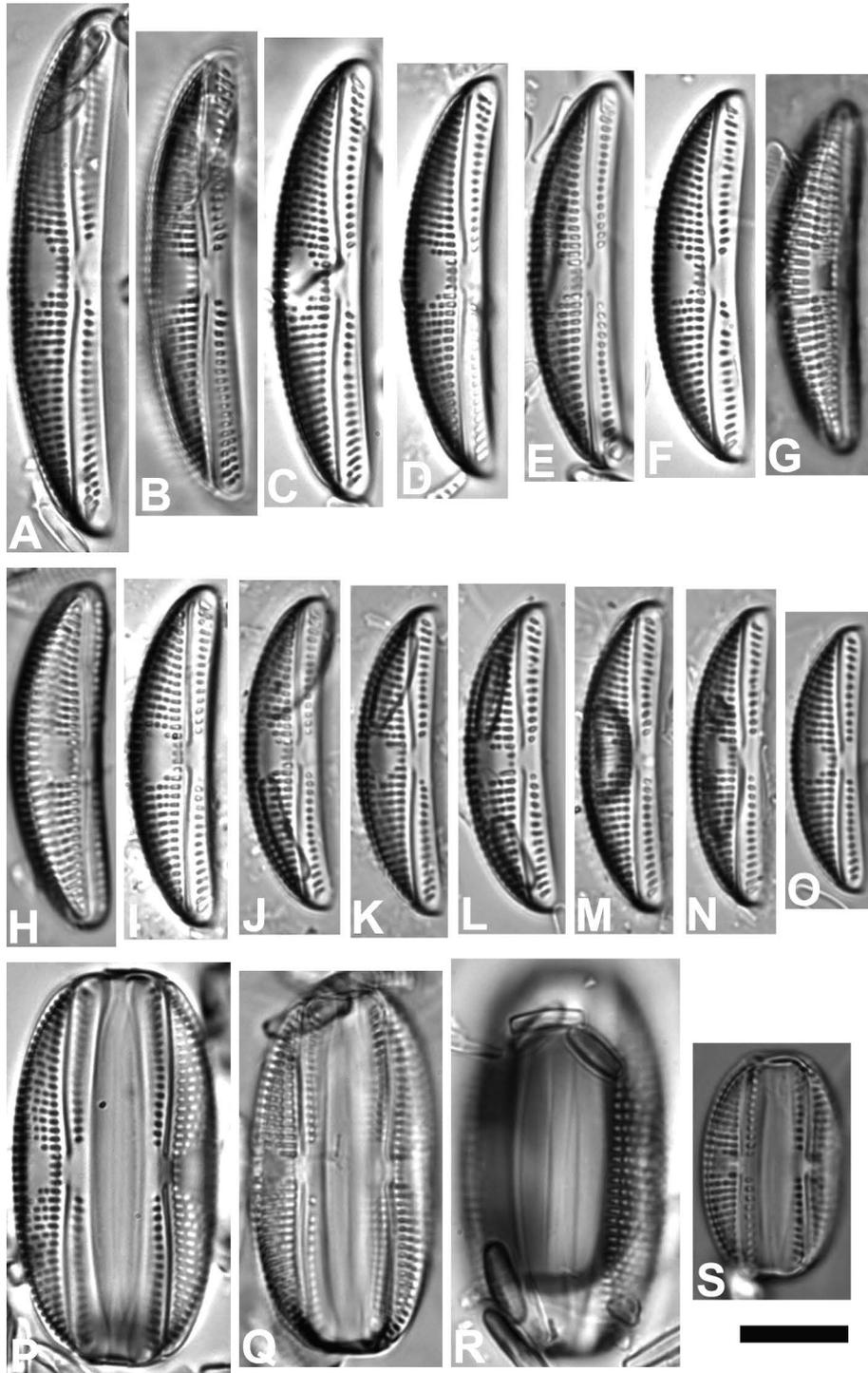


Figure 1 – *Amphora eileencoxiae*, LM: A–O, size diminution series of valve views (holotype: Slide cLIM007 DIAT 1968); P–Q & S, ventral views; R, dorsal view. Scale bar = 10 μm .

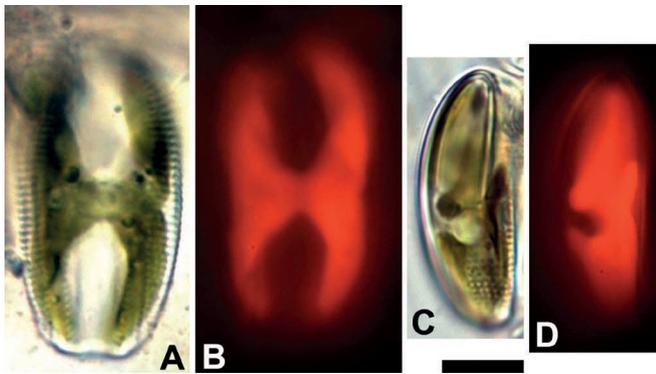


Figure 2 – *Amphora eileencoxiae*, LM: live specimens illustrating the position and shape of the plastid: A–B, dorsal view; C–D, lateral view; A & C, bright field; B & D, chlorophyll autofluorescence. Scale bar = 10 μ m.

slightly radiate near the center and composed of one (rarely two) areolae, becoming convergent near the apices. Single, H-shaped, ventrally-appressed chromoplast with central bridge (fig. 2).

Description: SEM – The raphe branches appear slightly arcuate, with external proximal ends slightly dorsally deflected, as are the external distal ends. The raphe ledge is continuous on the dorsal (fig. 3A) and ventral (fig. 3B) side. The dorsal

and ventral striae are distinctly areolate (cross section visible in fig. 3E). Intercostal ribs form round to ovoid areolae openings internally, whilst the areolae have transapically-oriented slit-shaped openings externally (fig. 3A). The dorsal area is well visible externally and internally (fig. 3A–C & E).

Etymology – The new species is named after our colleague Dr Eileen J. Cox in recognition of her outstanding contributions to diatom research.

Similar taxa – We think that *Amphora eileencoxiae* is conspecific with *Amphora* aff. *vetula* (in Levkov 2009: figs 54: 8–16). Our plate of the latter taxon (fig. 4A–D) includes initial and post-initial cells, and this might suggest that this is a larger species but all other characters are the same and suggest conspecificity. The most similar taxa are thus *Amphora vetula* (fig. 4E–H) which has more broadly rounded and less ventrally bent ends, *Amphora lange-bertalotii* var. *tenuis* (fig. 5A–D) which has more acute and sometimes protracted ends, and *Amphora lange-bertalotii* (fig. 5E–H) which is obviously larger.

Distribution – The distribution is currently unknown because the species has previously not been distinguished from other *Amphora* taxa. So far known only from the type locality and from Lake Stechlin (north-eastern Germany; reported as *Amphora* aff. *vetula* in Levkov 2009: figs 54: 8–16). At the type locality, the new species was found both in the epilithon and in the epibryon, but was more abundant in the former.

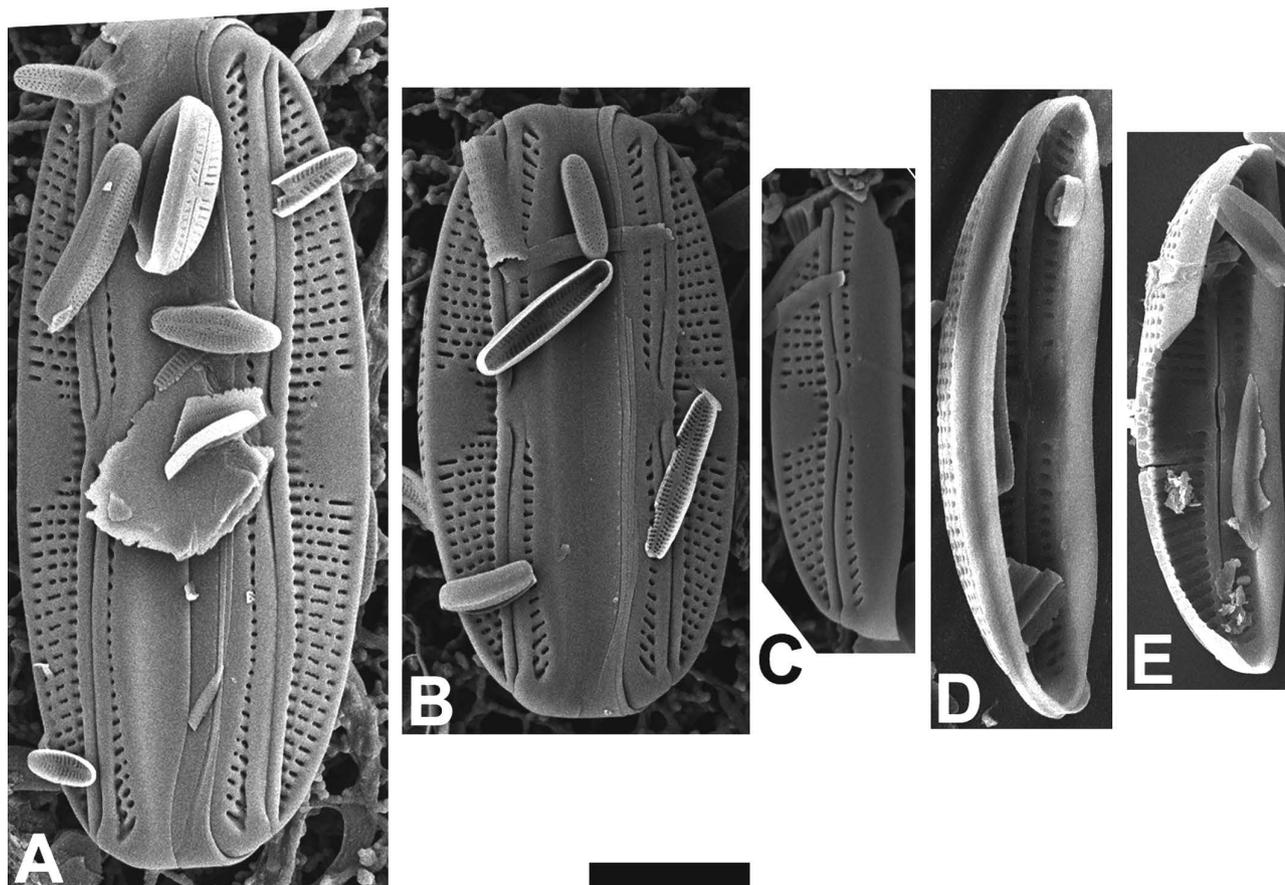


Figure 3 – *Amphora eileencoxiae*, SEM: A–C, external views; D–E, internal views. Scale bar = 10 μ m.

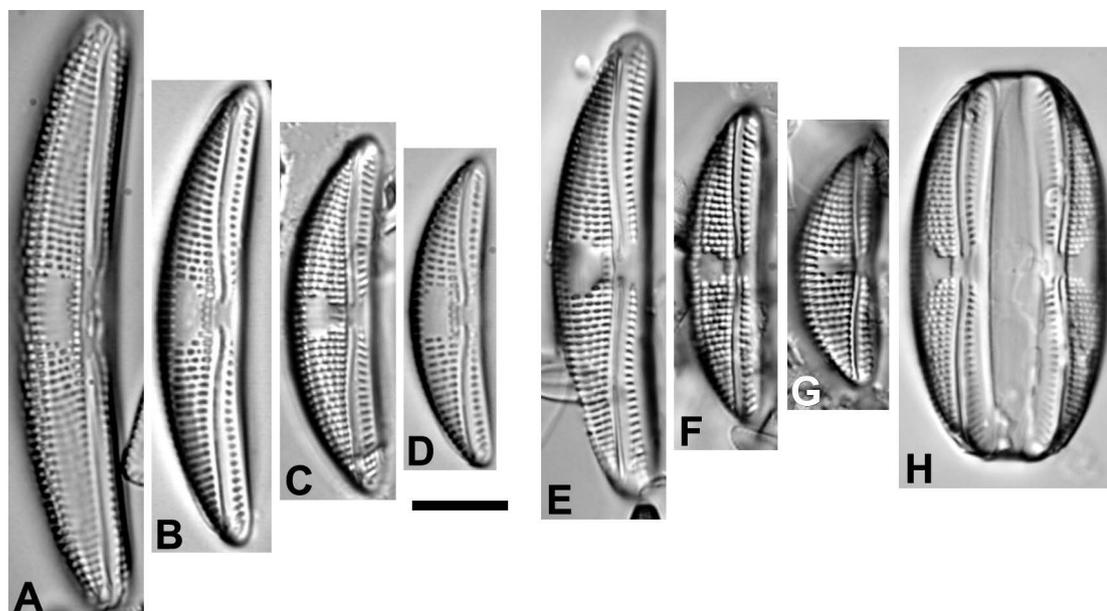


Figure 4 – LM: A–D, *Amphora* aff. *vetula*; E–H, *Amphora vetula*. Scale bar = 10 μm .

Ecology and co-occurring diatom species – The habitat is an almost unshaded (only shaded by tall grasses), rheocrenic spring with moderately flowing water, characterized by low electric conductivity and circumneutral pH. Environmental conditions at the type locality: discharge: 1 L s⁻¹, temperature: 4.7°C, conductivity: 57 $\mu\text{S cm}^{-1}$, pH: 7.3, nitrate: 1.1 mg L⁻¹, TP: 14 $\mu\text{g L}^{-1}$, TP-filtered: 4 $\mu\text{g L}^{-1}$, SRP: 3 $\mu\text{g L}^{-1}$, sulphate: 1.8 mg L⁻¹. With regards to trace elements and metals, arsenic, silver, antimony and uranium had above average values (Cantonati, unpubl. res.). Macroscopic structures and colourings were formed mainly by aquatic lichens, and by the following cyanobacteria: *Chamaesiphon polonicus* (Rostaf.) Hansg., *Phormidium favosum* Gomont, *Tapinothrix janthina* (Bornet & Flahault) Bohunická & J.R.Johans. The most common and abundant bryophyte species at the type locality were the mosses *Palustriella falcata* (Brid.) Hedenäs and *Brachythecium rivulare* Schimp. The vascular plants with the highest cover were *Caltha palustris* L. and *Saxifraga rotundifolia* L. (Cantonati, unpubl. res.).

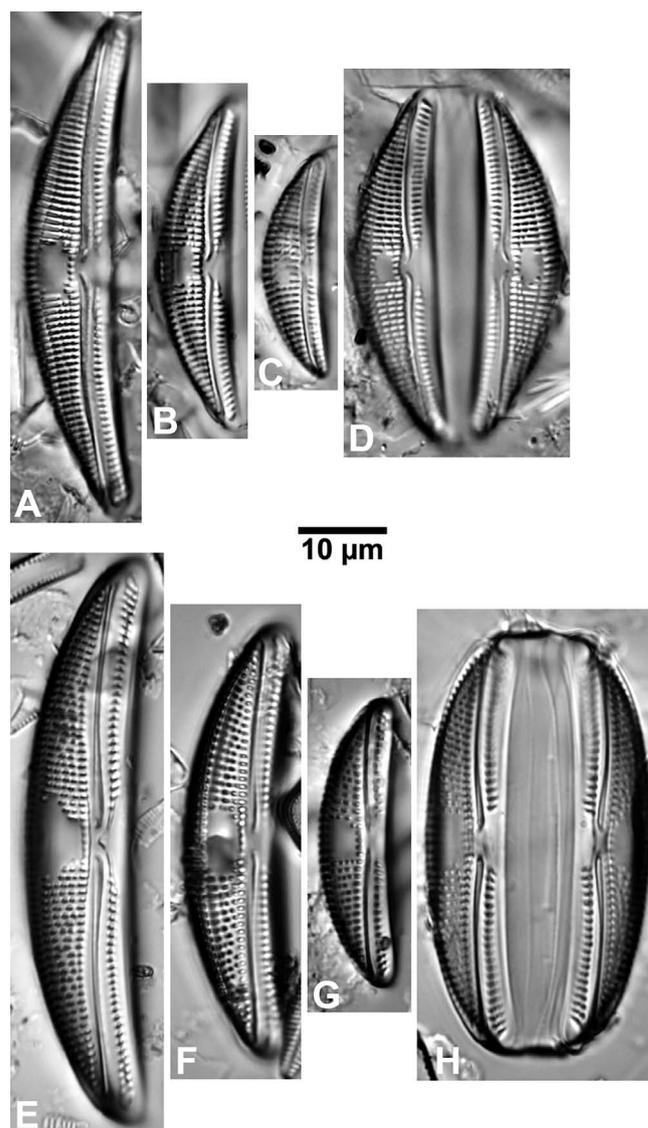
Associated diatom species were *Achnanthis minutissimum* (Kütz.) Czarn., *A. lineare* W.Smith, *Planothidium lanceolatum* (Bréb. ex Kütz.) Lange-Bert., *Amphora indistincta* Levkov, *A. inariensis* Krammer, *Navicula exilis* Kütz., and *Odontidium mesodon* (Kütz.) Kütz.

***Halamphora poianensis* Cantonati, Levkov & Lange-Bert., sp. nov.**

Figs 6–7

Type material – Italy, Northern Apennines, Province of Reggio Emilia, Municipality of Villa Minozzo, Rheocrenic inland-saline spring Poiano – PoianoS, EBERs Project, epibryon, Lithology: Triassic gypsum, (44°23'20.168"N, 10°26'21.274"E, 1613 m a.s.l.), 28 Jul. 2011, M. Cantonati s.n. (holo-: TR, slide cLIM007 DIAT 1978, partly shown

► **Figure 5** – LM: A–D, *Amphora lange-bertalotii* var. *tenuis*; E–H, *Amphora lange-bertalotii*. Scale bar = 10 μm .



here in fig. 6A–K; iso-: B, slide B 40 0043813; BM, slide BM 101961).

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Description: LM – Valves are semi-lanceolate with a smoothly arched dorsal margin and a straight to very slightly convex ventral margin. Valve length 18–25 μm , valve width 3.2–4.1 μm . The valve ends are protracted, rostrate to subcapitate, and bent ventrally. The raphe is smoothly arched with nearly straight raphe branches, with proximal raphe ends that terminate close to each other and are slightly bent dorsally. The distal raphe ends are difficult to see in the LM. The axial area is very narrow, a semi-elliptic ventral area in the center (difficult to observe with LM). The dorsal striae are very fine, 24–32 in 10 μm , very finely areolate, and slightly

radiate throughout. Areola density ($n = 3$) is 66–86 in 10 μm . A broad raphe ledge, seen as ‘longitudinal line’ or ‘shadow’ across the dorsal striae, can be visible in some specimens in LM. The ventral striae ($n = 6$) are very dense, 32–40 in 10 μm . Single, broadly H-shaped, ventrally-appressed, chromoplast with central bridge (fig. 6S–V), flanked by two conspicuous droplets, one towards each cell apex (fig. 6S).

Description: SEM – The raphe is slightly bi-arcuate to almost straight, with external proximal ends deflected dorsally, and the external distal ends deflected dorsally past the raphe ledge. The dorsal raphe ledge is very broad (fig. 7A). The dorsal striae are crossed by many, irregularly-spaced vimines, forming round to ovoid areolae. The detailed internal view (fig. 7C) reveals that the rows of areolae adjacent

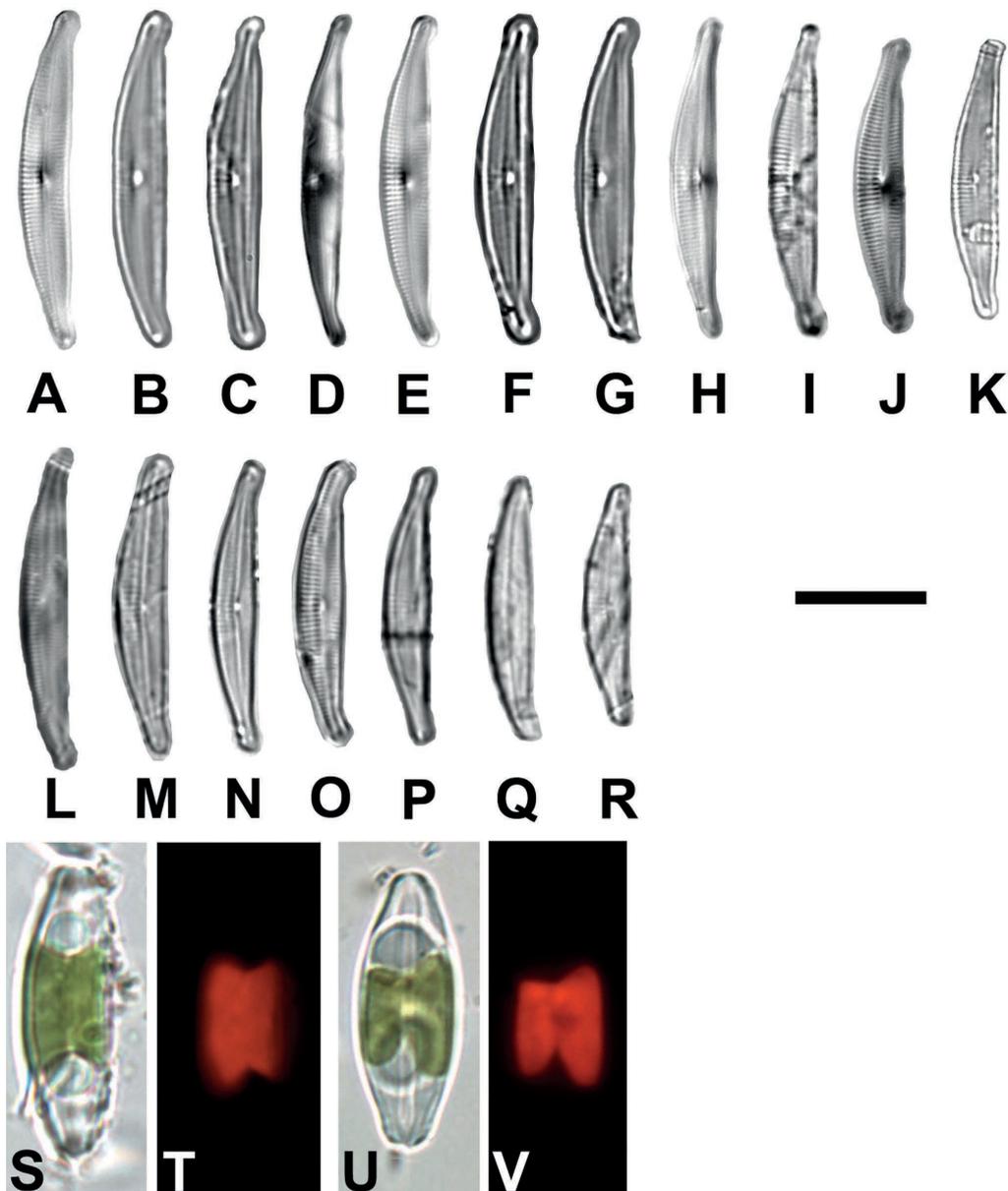


Figure 6 – *Halamphora poianensis*, LM: A–R, valve views; A–K, epibryon (holotype: slide cLIM007 DIAT 1978); L–R, epilithon; S–V, live specimens (girdle views) illustrating the position and shape of the plastid; S & U, bright field; T & V, chlorophyll autofluorescence. Scale bar = 10 μm .

to the axial area on the dorsal side are biseriate and slightly elongate in contrast to the single areolate structure of the rest of the striae. These biseriate rows of areolae are covered externally by the dorsal raphe ledge, and are thus recognizable only internally. The internal proximal raphe ends are turned ventrally.

Etymology – The new species is named after the type locality, the renowned inland-saline springs of Poiano.

Similar taxa – *Halamphora gasseae* has differently-shaped outline and ends (typically straight ventral margin and ends that are not or only slightly bent ventrally), and a lower dorsal stria density. *Halamphora abuensis* (Foged) Levkov, *Halamphora aponina* (Kütz.) Levkov, and *Halamphora borealis* (Kütz.) Levkov have different valve shapes, lower dorsal stria density, and differently shaped endings (longer in the latter two, that are brackish water species). *H. abuensis* is also broader than *Halamphora poianensis* sp. nov.

Distribution – Not yet known because the species has previously not been distinguished from other *Halamphora* taxa. So far known only from the type locality. At the type locality, the new species was more abundant in the epibryon (2.1%) than in the epilithon (1.2%).

Ecology and co-occurring diatom species – The habitat is a shaded, rheocrenic fast-flowing, mineral (chloride, sulphate, sodium, calcium) spring, characterized by very high electric conductivity and slightly alkaline pH. Environmental condi-

tions at the type locality: discharge: 50 L s⁻¹, temperature: 9.6°C, conductivity: 11560 µS cm⁻¹, pH: 7.4, nitrate: 0.56 mg L⁻¹, TP: 33 µg L⁻¹, TP-filtered: 12 µg L⁻¹, SRP: 7 µg L⁻¹, sulphate: 2090 mg L⁻¹. With regards to trace elements and metals, lithium, copper, molybdenum, silver, cadmium, caesium, antimony, bismuth, and uranium had above average values (Cantonati, unpubl. res.). The main cyanobacteria and algae except diatoms forming macroscopic structures and colourings were:

- the cyanoprokaryotes: *Leptolyngbya* cf. *komarovii*, *Rivularia* sp. aff. *bullata*;
- the green algae: *Cladophora glomerata* (L.) Kütz., *Mougeotia* spp.;
- the red alga: *Chrootheca richteriana* Hansg.

The most common and abundant bryophyte species was the moss *Hymenostylium recurvirostre* (Hedw.) Dixon (Cantonati, unpubl. res.). Associated diatom species were *Achnanthisidium minutissimum* and another, still undescribed *Achnanthisidium* species, *Crenotia rumrichorum* Wojtal, *Fragilaria famelica* (Kütz.) Lange-Bert., and *Nitzschia frustulum* (Kütz.) Grunow in Cleve & Grunow.

DISCUSSION

The recent investigation by Stepanek & Kociolek (2019) offers further support to the separation of *Halamphora* as an independent genus as proposed by Levkov in 2009. *Halamphora* is recognized as a distinct, monophyletic lineage, an amphoroid taxon with a single, ventrally-appressed, broadly H-shaped chromoplast (Stepanek & Kociolek 2019). It is thus very important to consider also plastid structure and arrangement, in diatom taxonomy in general (Cox 1996), and in particular when dealing with amphoroid taxa. The *Halamphora* species studied has a Type 2 chromoplast morphology according to Mereschkowsky (1903) in Levkov (2009) flanked by two conspicuous droplets, one towards each cell apex (Cox 1996), whilst the *Amphora* species has a Type 1 chromoplast morphology according to Mereschkowsky (1903) in Levkov (2009). The *Amphora* and *Halamphora* species investigated can be differentiated from other similar species by characters or character combinations that can be seen in LM.

Stepanek & Kociolek (2019) used comparative molecular phylogenies to show that the ancestral inferred habitat type for *Halamphora* is coastal marine, and that this genus, unlike *Amphora* that exhibits a pattern more closely aligned with a salinity barrier model (Mann 1999), appears to have carried out several independent incursions into inland and freshwater systems. Inland-saline spring habitats and other high conductivity inland waters appear to be of special interest for studying the biodiversity and geographical distribution of *Halamphora* taxa in particular, and for the understanding of diatom lineage movement between freshwater and marine systems in general.

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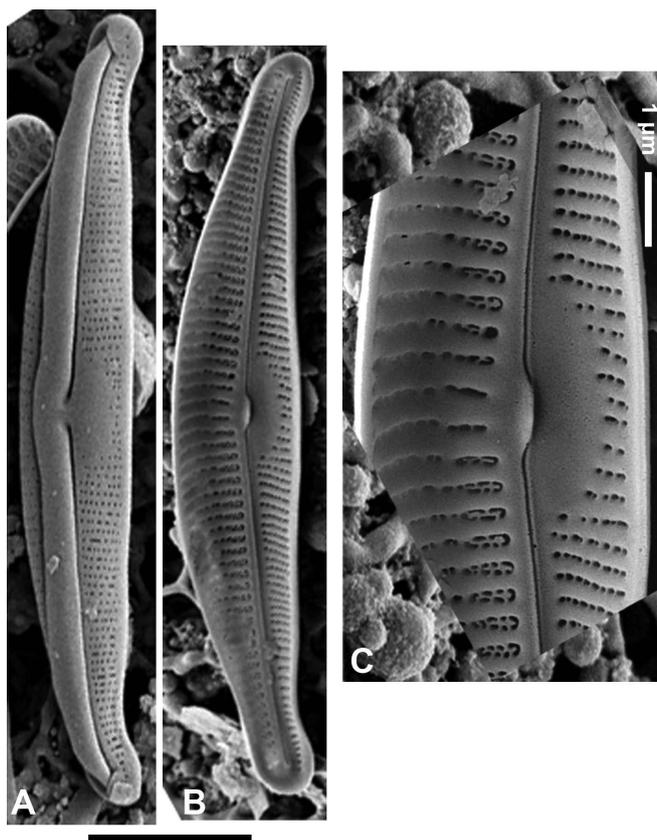


Figure 7 – *Halamphora poianensis*, SEM: A, external view; B & C, internal views. Scale bars: A & B = 5 µm; C = 1 µm.

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