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Halamphora ectorii sp. nov. – a new diatom species from saline spring Solenica in North Macedonia

Zlatko Levkov[1]*, Dušica Zaova[1]

[1] Institute of Biology, Faculty of Natural Sciences, Ss Cyril and Methodius University in Skopje, Arhimedova 3, 1000 Skopje, Republic of North Macedonia

* Corresponding author: zlevkov@pmf.ukim.mk

With 31 figures

Abstract: Saline springs are aquatic habitats inhabited by specific diatom flora. Recently several new species and genera have been described from such habitats. Spring Solenica is the only saline spring in Macedonia and it is not an exception. Observations of diatom assemblages in the spring reveal presence of several interesting species. One of them having unique morphological features is described here as *Halamphora ectorii* sp. nov. The new species has been observed by light and scanning electron microscope. It is characterized by semi-lanceolate valves with hyaline appearance, since the striae are densely spaced and composed of areolae with small, round external openings. Central area from both valve sides is broad and continues on valve mantle on dorsal side. *Halamphora ectorii* is compared with several similar species from saline inland and costal habitats and main differential features are provided. This study highlights the importance of rare habitats to the diversity of the broader region.

Keywords: diversity, halophile species, inland saline habitats, taxonomy

Introduction

The genus *Halamphora* (Cleve) Levkov was recently raised to genus level (Levkov 2009) comprising a part of the species included in the group *Halamphora* sensu Cleve (1895). This biraphid genus is characterized by strongly dorsiventral valves, a raphe lying on a raphe ledge near the ventral margin with dorsally curved terminal raphe fissures, internally proximal raphe ends fused into a central helictoglossa and numerous girdle bands with one to two rows of pores (Levkov 2009). Recent molecular analyses reveal that the genus *Halamphora* represents a monophyletic group (Wang et al. 2014, Stepanek & Kociolek 2019) with Surirellales as sister group (Ruck et al. 2016).

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Since its establishment, a large number of new species has been described in the genus *Halamphora* from various habitats (e.g. Stepanek & Kociolek 2018, Cantonati et al. 2019, Zhang et. al. 2019, Zidarova et al. 2022). Most of the species are inhabiting marine, brackish or freshwaters with elevated mineral content. Several species can also be found in subaerial habitats (Van de Vijver et al. 2014) and oligotrophic waters (You et al. 2015), while few species occur in hypersaline inland habitats (Levkov 2009, López-Fuerte et. al. 2020).

One of the habitats where *Halamphora* species are frequently observed, are saline springs (Beauger et al. 2023). Such habitats usually have a low species richness, but most of the observed species are very characteristic and have a very restricted distribution (Beauger et al. 2015, Beauger et al. 2022). These type of habitats are extremely rare in the RN Macedonia (Kotevski 1987) and their diatom composition has so far not been studied. Several new species have been described from saline habitats such as halomorphic soils (Levkov et al. 2017), showing a restricted distribution. Recently, a detailed survey on the diatom flora of RN Macedonia has been started, comprising observations of all types of water and wet habitats, including saline springs. In one of the springs, a new *Halamphora* species, observed as a dominant taxon in the diatom community, is described here as *H. ectorii* sp. nov.

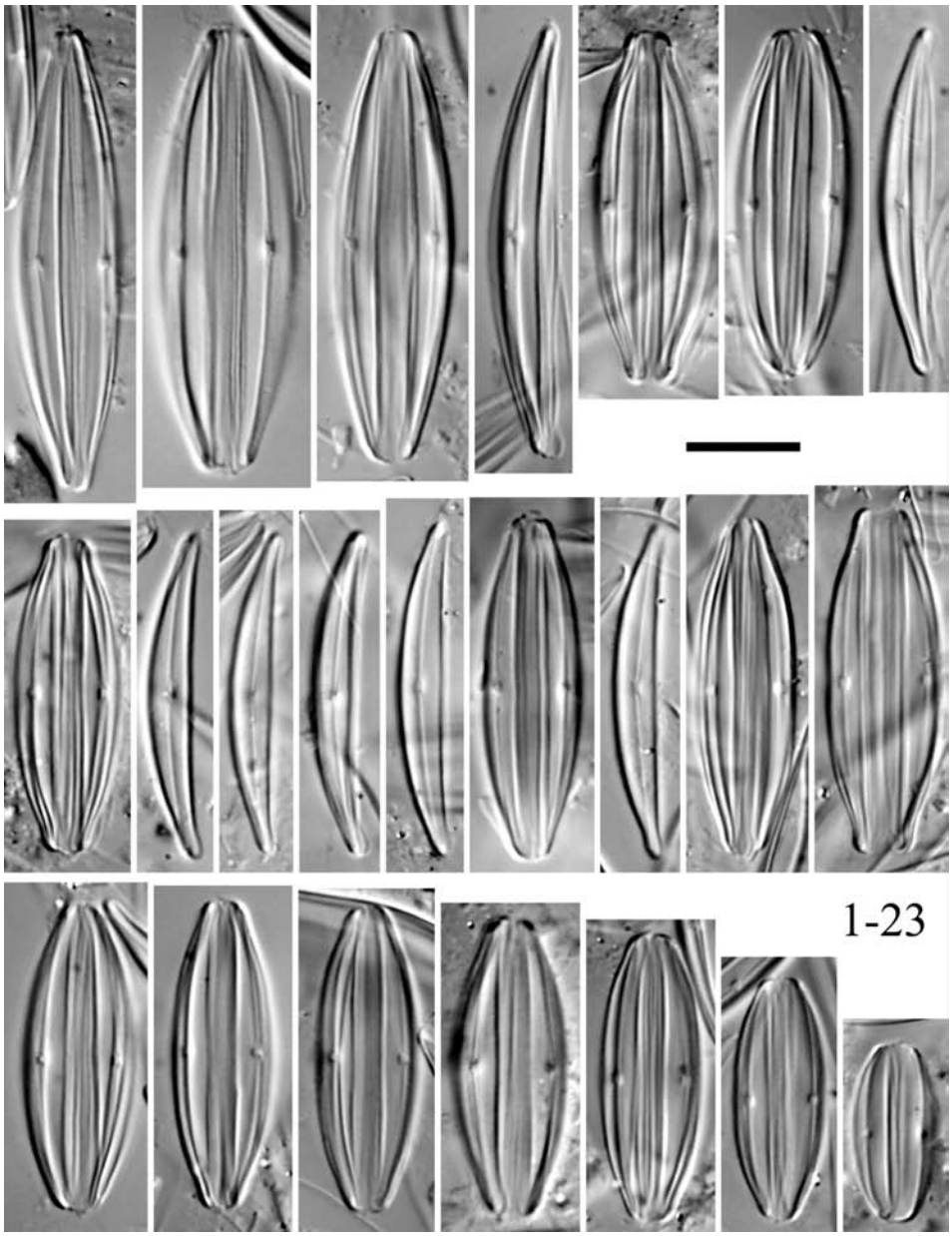
Material and Methods

Spring Solenica is located near the village of Slatinsko, Makedonski Brod, on right side of river Slatinska Reka (Fig. 1). The spring is captured with concrete building in a form of semi-closed reservoir with small pipe outlet. The water is characterized by high concentrations of salts (NaCl) 5.9 g dm^{-3}, a slightly alkaline pH (= 8) and a more or less constant temperature throughout the year (12 °C) (Kotevski 1987). The water discharge is constant throughout the year and very low (0.03 dm^3/s). Water from the spring is discharged through a pipe and before entering the river Slatinska, a small wetland is formed. Diatom samples from the reservoir, pipe and wetland were collected in July 2020. Samples are treated with $KMnO_4$ and HCl and heated at 80 °C for 30 min, and then rinsed with distilled water five times and subsequently centrifuged during 10 minutes at least five times. Permanent slides were prepared using Naphrax and observed under oil immersion at 1500× magnification with a Nikon Eclipse 80i microscope. Photomicroscopy was done using a Nikon Coolpix P6000 camera. The slides were deposited in the Macedonian National Diatom Collection (MKNDC) at the Institute of Biology, Faculty of Natural Sciences, Skopje. Terminology used for description of the morphological features and species is based on Levkov (2009).

Results

Halamphora ectorii sp. nov. (Figs 1–31)

Light microscopy (Figs 1–23): Frustules elliptical with rounded to truncate apices. Valves narrow semi-lanceolate with dorsally strongly arched, convex margin and concave ventral margin and slightly tumid in mid-valve. Valve ends narrowly rounded, weakly to moder-



Figs 1–23. LM photographs of *Halamphora ectorii* sp. nov., type population (Acc. No. MKNDC 13028) showing the size range of the species. Scale bar 10 µm.

ately protracted and ventrally deflected. Valve length 16.0–41.0 µm, valve breadth 3.0–3.5 µm (n= 40). Axial area very narrow expanding into small round central area, more expressed on ventral side. Raphe positioned near median line margin with proximal raphe ends slightly dorsally deflected. Both ventral and dorsal striae not visible in LM.

Scanning electron microscopy (Figs 24–31): Marginal ridge absent and transition valve face/mantle gradual (Figs 24, 25). Ventral valve side very broad, occupying almost half of valve width (Figs 24–26). Raphe ledge distinct on dorsal valve side, moderately wide, continuous along whole valve length, slightly dorsally expanded and constricted in middle. In some specimens raphe ledge expanded near apices (Fig. 27). Axial area from ventral side moderately wide, expanded in broad irregularly-shaped central area (Figs 26, 30). Raphe branches almost straight with expanded and dorsally deflected central raphe ends (Fig. 30). Terminal raphe fissures strongly dorsally deflected. Striae entirely uniseriate composed of small, round areolae. Dorsal striae 30–35 in 10 µm, ventral striae 40–50 in 10 µm. Areolae near raphe ledge located in small depressions. Ventral striae comprised by irregularly arranged round areolae (Figs 24, 25). Internally, raphe located along a median line (Figs 28, 29, 31). Proximal raphe ends terminating onto a small fused central helictoglossa (Fig. 31). Distal raphe ends slightly deflected ventrally, terminating onto poorly developed helictoglossae (Figs 28, 29). Both, dorsal and ventral striae uniseriate, separated by thin virgae. Central dorsal virgae slightly wider than the others (Fig. 31). Areolae occluded by hymenes (Fig. 31).

Holotype: MKNDC 13028 (Macedonian National Diatom Collection, Skopje, R. North Macedonia).

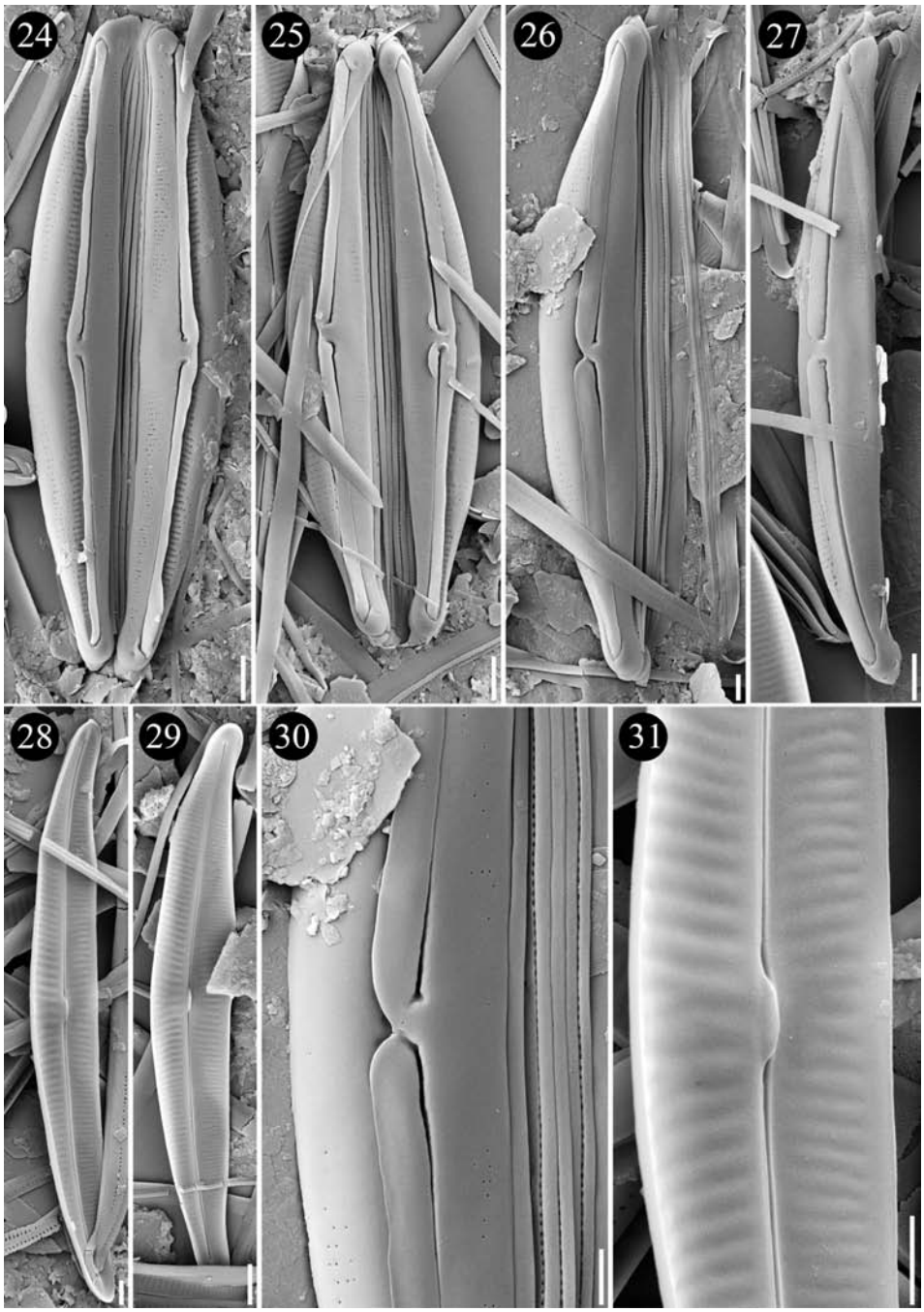
Isotype: BM 88038

Type locality: Spring Solenica near village Slatinsko, Makedonski Brod, sample Acc. No. MKNDC 13028 (41.5795100 N, 21.1835256 E, collection date: 12.07.2020, leg. Z. Levkov).

Etymology: The species is named in honor of our late friend and one of the most prominent diatomists Luc Ector.

Ecology and associated flora: Halophile species, in the type material is a dominant species with abundance of almost 99%. The spring Solenica is a hypersaline habitat with a high content of NaCl (5.9 g dm^{-3}) and a slightly alkaline pH (= 8) (Kotevski 1987). Other species in the sample include *Achnanthes adnata* Bory, *Navicula digitoconvergens* Lange-Bertalot and *Halamphora* sp. So far, *Halamphora ectorii* was observed only from the type locality.

Figs 24–31. SEM photographs of *Halamphora ectorii* sp. nov, type population. (Acc. No. MKNDC 13028). Figs 24, 25. External view of ventral side of whole frustule. Figs 26, 27. External view of whole valves. Figs 28, 29. Internal view of whole valve. Fig. 30. External detailed view of mid-valve showing the raphe ledge and proximal raphe ends. Fig. 31. Internal detailed view of mid-valve showing fused central helictoglossa and areolae occluded by hymenes. Scale bars = 2 µm (24, 25, 27, 29), 1 µm (26, 28, 30, 31).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Discussion

In most *Halamphora* species, the raphe is located near the ventral margin and the raphe branches are usually straight or weakly curved. To date, only a few species have raphe branches located in the median part of the valve or closer to the dorsal margin, thus having a wide ventral valve side (e.g. Wachnicka & Gaiser 2007, Levkov 2009, Stepanek & Kociolek 2018). Valves of *H. ectorii* are comparable in outline and size as *H. mosensis* J.G.Stepanek & Kociolek (2018, Figs 61: 9–12; 64: 1–3), with a raphe also located near the median valve line. Differences between these two species can be noticed in the stria morphology (striae clearly visible with LM in *H. mosensis*), stria density (26–28 in 10 µm in *H. mosensis*) and structure (partly biseriate near the raphe ledge in *H. mosensis*). Additional differences might be noticed in the shape and size of the central area that is absent on the dorsal side and narrowly elliptical at the ventral valve side. Central dorsal virgae are thickened in *H. mosensis*, whereas in *H. ectorii* this feature is absent. *Halamphora halophila* J.G.Stepanek & Kociolek (2018, Figs 50: 1–4; 51: Figs 3–6) is characterized by semi-elliptical valves with broad ventral valve side, length 29.0–33.0 µm) width 4.5–5.0 µm and finely areolate dorsal striae 21–22 in 10 µm (Stepanek & Kociolek (2018, p. 48). Although the size and shape of *H. halophila* is comparable with *H. ectorii*, several differences can be noticed between these two species such as: stria density (21–22 in 10 µm vs. 30–35 in 10 µm), shape of the central area (absent from dorsal side, weakly expressed on ventral side in *H. halophila* vs. large central area present on both valve sides in *H. ectorii*), partly biseriate striae in *H. halophila* opposite to entirely uniseriate striae in *H. ectorii*. In *H. caribaea* (Wachnicka & E.E.Gaiser) Rimet & R.Jahn (= *Amphora caribaea* Wachnicka & E.E.Gaiser 2007, Figs 35–37), raphe is more dorsally displaced, but it can be easily differentiated from *H. ectorii* by the stria density (11–17 in 10 µm in *H. caribaea*) and valve breadth (6–8 µm in in *H. caribaea*). *Halamphora ectorii* has a comparable valve size (length 29–45 µm) and ultrastructure as *H. atacamana* (R.M.Patrick) Levkov (entirely uniseriate striae composed of small, round areolae), but it can be separated from the latter, by the size of the raphe ledge (very narrow in *H. atacamana*), the central area (semi-elliptical present on dorsal valve side) and stria density (25–28 in 10 µm). *Halamphora poianensis* Cantonati, Levkov & Lange-Bertalot (in Cantonati et al. 2019, Figs 6, 7) has a comparable valve size (length 18–25 µm, width 3.2–4.1 µm), shape (semi-lanceolate with a smoothly arched dorsal margin) and wide ventral valve size and comparable ecology (observed in rheocrenic spring, with very high electric conductivity and slightly alkaline pH). Differences between *H. poianensis* and *H. ectorii* can be noticed in stria morphology (areolate and clearly seen in LM in *H. poianensis*), stria density (24–32 in 10 µm in *H. poianensis*) and stria structure (partly biseriate near raphe ledge in *H. poianensis*).

Most of the species morphologically similar to *H. ectorii* are characteristic for saline habitats. According to Stepanek & Kociolek (2019), the ancestral habitat for *Halamphora* is coastal marine, and there are several independent incursions into inland and freshwater systems. Inland saline habitats can be found on each continent (Williams 2005) and are relatively frequent in Europe, represented by smaller or larger lakes or ponds (Stenger-Kovács & Lengyel 2015) fed by saline water from deep-layer aquifers. Water in the So-

lenica spring has the same origin (Kotevski 1987), but due to a quite low water flow, only a very small wetland is formed (few square meters). But even in such a small habitat still specific diatoms might occur. Recently interest in saline springs has increased over Europe that resulted in description of several new species (e.g. Żelazna-Wieczorek 2020, Beauger et al. 2017) and new genera (Wojtal 2013, Beauger et al. 2023). Some of these species might have broader distribution in Europe [e.g. *Navicula fontina* Żelazna-Wieczorek, *H. poianensis*], but several have been observed only from type locality or from very few localities (e.g. *Chamaepinnularia salina* Beauger, C.E.Wetzel, Allain & Ector in Beauger et al. 2022). The three possible explanations: 1) in the past newly described species were misidentified and confused with similar species; 2) saline springs have received only poor scientific attention, especially from a taxonomical point of view, compared with other inland habitats such as rivers, lakes, mountain springs, etc and 3) they might be really restricted to particular habitat due to the specific environmental conditions in saline springs. *Halamphora ectorii* has so far only been observed at the type locality (Solenica spring), despite detailed observations of several saline habitats in Macedonia have been performed (pers. observ.).

This study is part of a larger survey on the diatom flora and highlights the importance of rare habitats to the diversity of the broader region of Macedonia. The Solenica spring is considered a rare and specific habitat and is classified as threatened due to climate changes and perceived precipitation decline.

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