

Article

Three New Diatom Species from Spring Habitats in the Northern Apennines (Emilia-Romagna, Italy)

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Abstract: Using light (LM, including plastid characterization on fresh material) and scanning electron microscopy (SEM), as well as a thorough morphological, physical, chemical, and biological characterization of the habitats, the present study aims at describing three species new to science. They belong to the genera *Eunotia* Ehrenb., *Planothidium* Round and L. Bukht., and *Delicatophycus* M.J. Wynne, and were found in two contrasting spring types in the northern Apennines. The three new species described differ morphologically from the most similar species by: less dense striae and areolae, and the absence of a ridge at the valve face-mantle transition (SEM feature) [*Eunotia crassiminator* Lange-Bert. et Cantonati sp. nov.; closest established species: *Eunotia minor* (Kütz.) Grunow]; narrower and shorter cells [*Planothidium angustilanceolatum* Lange-Bert. et Cantonati sp. nov.; most similar species: *Planothidium lanceolatum* (Bréb. ex Kütz.) Lange-Bert.]; barely-dorsiventral symmetry, set off ends, and lower density of the central dorsal striae [*Delicatophycus crassiminutus* Lange-Bert. et Cantonati sp. nov.; most similar species: *Delicatophycus minutus* M.J. Wynne]. Two of the three species we described are separated from the closest species by dimensions. Their description improved knowledge on two taxa (*Eunotia minor* s.l. and *Planothidium lanceolatum* s.l.) likely to be only partially resolved species complexes. We could also refine knowledge on the ecological profiles of the three newly-described species. *Eunotia crassiminator* sp. nov., as compared to *Eunotia minor*, appears to occur in colder inland waters with a circumneutral pH and a strict oligotrophy as well with respect to nitrogen. The typical habitat of *Planothidium angustilanceolatum* sp. nov. appears to be oligotrophic mountain flowing springs with low conductivity. *Delicatophycus crassiminutus* sp. nov. was observed only in limestone-precipitating springs, and is therefore likely to be restricted to hard water springs and comparable habitats where CO₂ degassing leads to carbonate precipitation. Springs are a unique but severely threatened wetland type. Therefore, the in-depth knowledge of the taxonomy and ecology of characteristic diatom species is important, because diatoms are excellent indicators of the quality and integrity of these peculiar ecosystems in the face of direct and indirect human impacts.

Keywords: diatom; springs; size; *Eunotia*; *Planothidium*; *Delicatophycus*; taxonomy; ecology; plastids



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1. Introduction

Springs are characterized by rich species pools at the landscape level (γ diversity, [1]). They are unique habitats: multiple ecotones and extremely heterogeneous environments, offering to the sheltered organisms a wide range of environmental conditions [2]. They are also the systems where the utility of a deep integration of hydrogeological and ecological approaches becomes obvious (ecohydrogeology, [3]). However, these ecosystems are menaced by many threats, the main ones being the water-resource exploitation, and the reduction of precipitation and recharge due to climate change [4].

Springs have been classified in many ways, and a number of spring types have been recognized (e.g., [3]). Those relevant for the present paper are the two following, contrasting spring types: rheocrenic mountain springs with low conductivity, and limestone precipitating springs.

Rheocrenic mountain springs with low conductivity are typically high-ecological-integrity, oligotrophic systems with relevant discharge and current velocity. They provide a habitat to many threatened-Red-List and recently discovered species (e.g., [5]), but they are sensitive to disturbance from human activities and climate and environmental change (e.g., [6,7]).

Limestone precipitating springs (LPS) are a very peculiar kind of spring where hard water and CO₂ degassing lead to the precipitation of carbonates. They host relatively low-diversity assemblages that however include many highly-adapted and characteristic taxa. This is the only widespread spring type clearly recognized by nature-protection legislation in the EU, but these springs are nevertheless often affected by many impacts (e.g., [8]).

Diatoms are the most diverse groups of algae in springs, where they can be excellent indicators of environmental features and ecosystem integrity [2,9]. Many rare and Red List diatom species occur in springs. Many diatom species were described from springs, and it is easy to provide examples also with reference to one of the genera discussed in the present paper, namely *Eunotia*: *E. arcofallax* Lange-Bert., *E. braendlei* Lange-Bert. et Werum, *E. kruegeri* Lange-Bert., *E. palatina* Lange-Bert. et W. Krüger, and *E. pexii* Lange-Bert. [10], *E. glacialispinosa* Lange-Bert. et Cantonati [11], *E. cisalpina* Lange-Bert. and Cantonati, *E. fallacoides* Lange-Bert. and Cantonati, and *E. insubrica* Lange-Bert. and Cantonati [12].

The importance of size in diatom species delimitation has been stressed in classical diatom literature (e.g., [13]) and current articles combining molecular and morphological approaches (e.g., [14]). Krammer [13] proposes the ratio of maximal and minimal width as a reliable means to test the quality of taxa: if >1.5, this ratio would point to an unresolved species complex whilst it is <1.5 in well-defined species. In this context, Krammer [13] also recalls Geitler's [15] first rule on life-cycle form changes: over the population developmental cycle the apical axis is shortened not only absolutely but also relatively more strongly than the transapical axis, leading to smaller specimens that are comparatively wider than larger specimens. Many diatom species were separated from the most similar species mainly on the basis of dimensions, and it is easy to provide examples as well with reference to one of the genera discussed in the present paper, namely *Eunotia*: *E. nanopapilio* Lange-Bert., and *E. superpaludosa* Lange-Bert. [16].

The great biogeographic and conservation importance of the Apennines is confirmed by the uniqueness of subalpine and alpine belts forming these mountains, peculiar climatic characteristics, and complex paleogeographic and paleoclimatic history of the region, combined with high geodiversity [1]. The Northern Apennines differ from the central and southern ones historically, geographically, and morphologically. They have some scattered stands of alpine vegetation, dominated by the orophilous central-European, boreal and Eurasiatic species, as well as a few limited endemics. Owing to its floristic similarity to the Alps, the summit area of the Northern Apennines has been considered as the southernmost part of a larger phytogeographic unit that also includes the main central-European massifs [17]. The described factors formed the autonomy of the Apennine communities from the Alps and central European mountains. This situation might also have favoured the discovery of new diatom species in this geographic area [6,18]. The focus of this paper is on the uniqueness of some representatives from three genera found in the Northern Apennines: *Eunotia* Ehrenb., *Planothidium* Round et L. Bukht., and *Delicatophycus* M.J.Wynne.

Eunotia Ehrenb. [19] is one of the most diverse diatom genera, consisting of more than 800 species [20] and more than 150 taxa known for Europe [16]. The morphology of the *Eunotia* species is characterized by dorsiventral outline, and at least one rimoportula at one apex. The species in the genus have striae which are punctate externally and interrupted

near the ventral portion of the valve, and most representatives are characterized by a “rudimentary” raphe system [16,21]. Although some fossil *Eunotia* have been described from New Zealand marine sediments [20], the extant species of the genus are restricted to freshwater environments [16,22]. The genus includes many species from tropical and subtropical areas as well, inhabiting mainly oligotrophic waterbodies in the epiphyton and metaphyton [22–25]. The large majority of the *Eunotia* species have an ecological optimum in acidic, low conductivity, and oligo-dystrophic conditions [12,25–27]. New species of *Eunotia* are continuously described. Recent examples are Ruwer et al. [28]; *E. nupeliana* D.T.Ruwer, L.Rodrigues), and Luo et al. [21]; *E. mugecuo* F. Luo, Q.-M.You and Q.-X.Wang, *E. filiformis* F. Luo, Q.-M.You and G.-X.Wang), who worked on high elevation aquatic habitats.

The genus *Planothidium* F.E. Round and L. Bukhtiyarova [29] includes more than 110 names flagged as accepted taxonomically on the basis of the literature listed under the species name, according to Guiry and Guiry [30]. A search in DiatomBase [31] yielded 142 matching extant records, 67 of which have been verified by a taxonomic editor. The species of the genus have heterovalvar frustules that are usually solitary, with elliptic to lanceolate valves. *Planothidium* taxa are characterized by slightly concave raphe valves and have an asymmetrical central area and convex rapheless sternum valves with continuous (‘delicatulum’ type) or interrupted striae on one side showing a clear space in the central area [32,33]. Along with other morphological characteristics, the central area serves as a distinguishing feature for the taxa of the genus presenting a shallow depression (named sinus), a hood (also known as cavum), or the lack of both of these structures [34]. The genus is formed of species with a wide geographical distribution, from South and North America [33,35,36], Africa [37,38], Europe [39,40], Asia [34,41], to the Antarctic region (e.g., [42]). Most species are known from freshwater environments, although there are some representatives reported from brackish and marine environments, and also from aerial environments (e.g., [43,44]). The species belonging to this genus are predominantly epilithic, epipsammic, and epiphytic on aquatic plants and algae [33,34]. The species inhabit flowing and standing waters, with low to high conductivity, occur from circumneutral to alkaline waters, and seem to be tolerant up to mesotrophic conditions [33,34,45]. Examples of recently described *Planothidium* species are: *Planothidium hinzianum* C.E.Wetzel, Van de Vijver and L.Ector [34], *P. potapovae* C.E.Wetzel and L.Ector [34], *P. sheathii* Stancheva [33], *P. tujii* C.E.Wetzel and L.Ector [34], *P. californicum* Stancheva and N. Kristan [46], *P. nanum* Bak, Kryk et Halabowski [47,48], and *P. marganaiensis* Lai, L.Ector and C.E.Wetzel [40].

Delicatophycus M.J.Wynne [49] is the correct name for the genus known as *Delicata* Krammer [50]. This name was invalid because it is a technical term and was amended by Wynne [49], who also noted that names ending in -phycus ($\phi\acute{\upsilon}\kappa\omicron\varsigma$, phykos), ought to be neutral, but were treated as masculine in accordance with tradition (International Code of Nomenclature for algae, fungi, and plants, Shenzhen Code) [51]. The current circumscription of the genus accounts for eight accepted species names [31], and 28 have been flagged as accepted taxonomically on the basis of the literature listed under the species name by Guiry and Guiry [30]. The morphology of the taxa belonging to *Delicatophycus* is characterized by dorsiventral valves with a lateral structure of the raphe, the presence of pseudosigmoids, the absence of apical pore fields and of stigmata, and foramina with a tendency to form undulated transapical structures externally. The species have strongly ventrally curved proximal raphe branches, distal raphe fissures deflected dorsally, with combination of the partly zig-zag shaped striae [50,52]. The representatives of the genus are found across a large climatic and geographical range: Europe [50], Asia [53,54], Africa [50], and South and North America [55,56]. The ecological preferences of the genus are still insufficiently known. However, common species of the genus are found in aerial habitats (dripping wet moss, wet rocks) and in the littoral of oligotrophic lakes [50]. Even within this relatively small genus new species are continuously described, a very recent example being *Delicatophycus liuweii* Y.-L. Li [57].

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 three new species from the genera *Eunotia*, *Planothidium* and *Delicatophycus* found in two contrasting spring types in the Northern Apennines.

2. Materials 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 [1]. Samples were collected by scraping 8–10 stones, and by collecting specimens of the dominant bryophyte species in three points of the spring area [9], and then digested using hydrogen peroxide (EN 13,946 2003 [58]). 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.

Materials (slides, prepared material, and aliquots of the original samples), including the holotype of the new species, are held at the Diatom Collection of the MUSE—Museo delle Scienze of Trento (TR) (Northern Italy). Isotype slides and aliquots of prepared material from the same locality and substratum were deposited at the Diatom Collection of the Botanical Garden and Botanical Museum of the Freie University of Berlin (B) (Germany) and at the Diatom Collection of the Academy of Natural Sciences of Drexel University (PH) (PA, USA).

If not otherwise stated, measurements on 30 different specimens representative of the size-diminution series were made to obtain ranges and averages of the morphological and ultrastructural features.

SEM observations were carried out at the University of Frankfurt using a Hitachi S-4500 (Hitachi Ltd., Tokyo, Japan) and at the MUSE—Museo delle Scienze (Trento) using a Zeiss-EVO40XVP, Carl Zeiss SMT Ltd., Cambridge, UK at high vacuum on gold-coated stubs.

Plastid characteristics and type were assessed using Cox [59]. Terminology to describe valve morphology is based on Round et al. [22].

As concerns the typification of the new species, we chose to use the entire slide as the holotype following article 8.2 of the International Code for Botanical Nomenclature [51]. The choice for the entire population on the slide is, in our opinion, more consistent with the fact that most diatom species show an extensive variability during their population cell cycle.

In an attempt to increase data on the distribution of the three new species, both diatom and environmental data of selected springs from a comprehensive dataset of the south-eastern Alps (CRENODAT Project, Biodiversity assessment and integrity evaluation of springs of Trentino—Italian Alps—and long-term ecological research, 2004–2008 [9]) were used. We looked for *Planothidium angustilanceolatum* sp. nov. in the epibryon samples from ten CRENODAT springs that were selected because counts included at least 100 valves of *P. lanceolatum* s.l. and because they had an ecomorphology/hydrochemistry consistent with the type locality of this species. We also looked for *Delicatophycus crassiminutus* sp. nov. in the epibryon slides from the five LPS included in the CRENODAT Project.

All the statistical analyses were performed within the R statistical environment [60]. To find out more about the ecological preferences of *Eunotia crassiminor* sp. nov. as compared to *Eunotia minor* (Kütz.) Grunow, we considered the 12 CRENODAT sites and the 4 EBERs sites in which both species occur. If necessary, by revisiting the slide, we carefully checked the relative abundances of the two species in each site. We then calculated weighted average, mode, percentiles, minimum, and maximum for each environmental parameter, tested differences between the two species for statistical significance using *t*-tests, and illustrated the preferences of the two species for each factor for which there was a significant difference with box plots (Figure 3a–l). The R packages used for these *Eunotia* analyses were *corrplot*, *weights*, and *ENmisc*. For the biometry part of the study, all relevant morphological

parameters listed in Table 2 were measured on 100 raphe-valves and 100 rapheless-valves of *Planothidium angustilanceolatum* sp. nov. and *P. lanceolatum* (Bréb. ex Kütz.) Lange-Bert., respectively. The R package *plotrix* was used for this *Planothidium* analysis.

3. Results

Eunotia crassiminor Lange-Bert. et Cantonati sp. nov. (Figures 1 and 2)

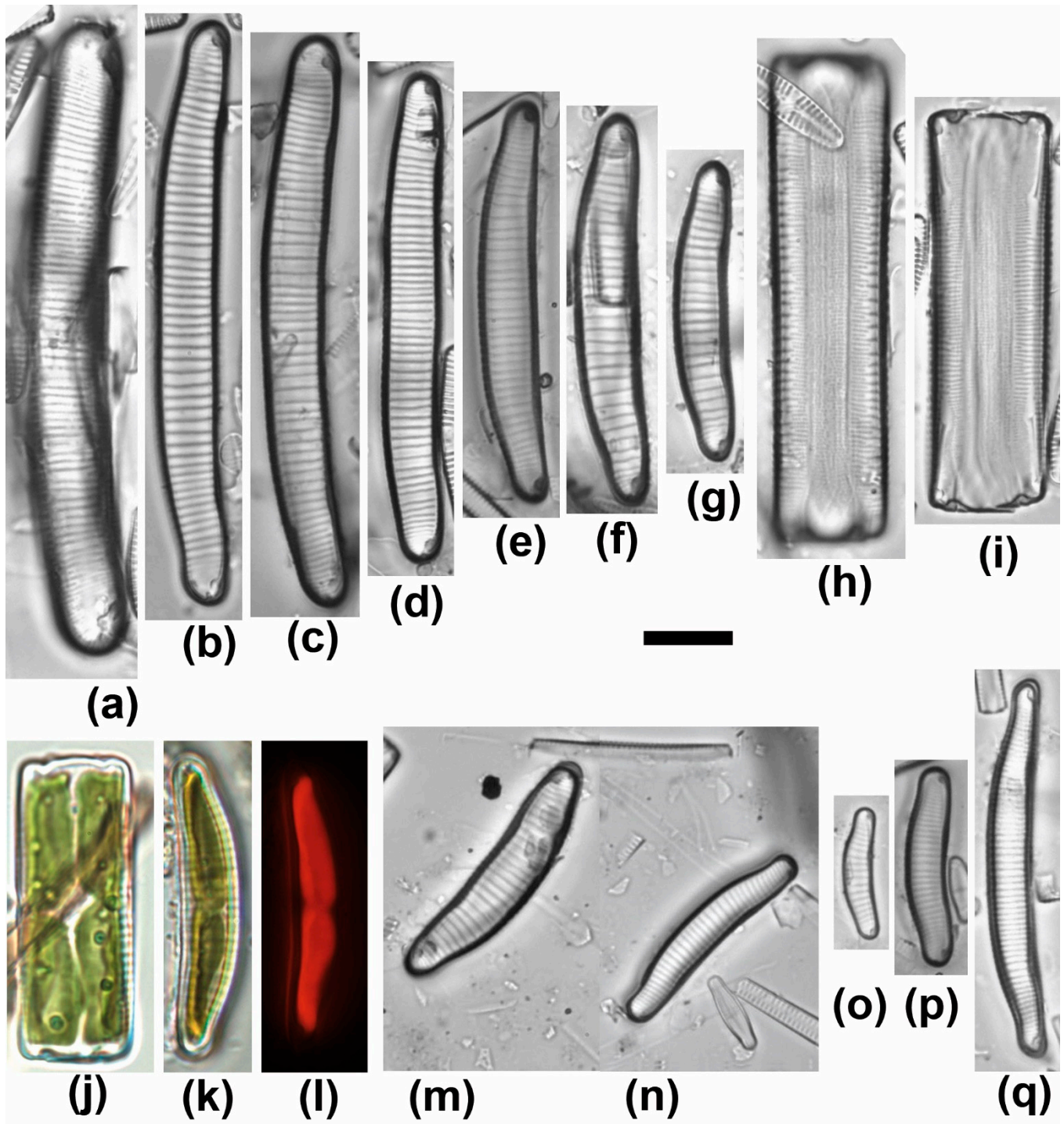


Figure 1. LM morphology of *Eunotia crassiminor* Lange-Bert. et Cantonati sp. nov. (a–g,k–q): valve views. (h–j): girdle views. (a): initial cell. (j,k): Chromoplast morphology. (i,m–q): *Eunotia minor* specimens shown for comparison. All micrographs bright field, with the exception of 12 which is based on chlorophyll autofluorescence. Scale bar 10 μ m.

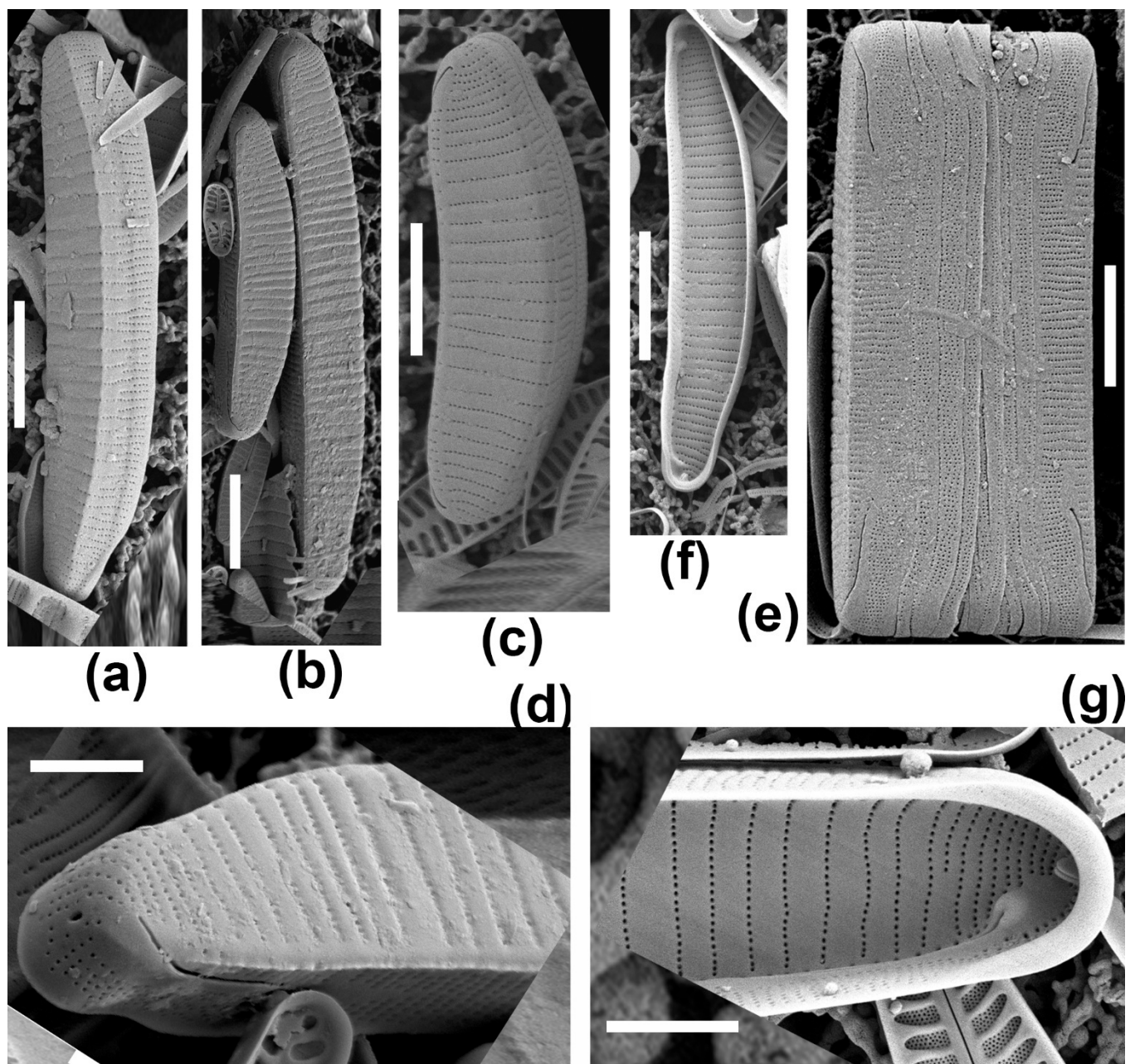


Figure 2. (a–g). SEM images of *Eunotia crassiminor* Lange-Bert. et Cantonati sp. nov. (a–e): External views. (f,g): internal views. Scale bars 10 μm (a–c,e,f), 4 μm (g), 3 μm (a–c,e,f).

Synonymy. *Eunotia minor* sensu Lange-Bert. et al. [16], Figure 159: 1–7.

To exclude from synonymy: *Eunotia minor* sensu Lange-Bert. et al. [16], Figure 159: 12–27.

Differential diagnosis versus *Eunotia minor* (Kütz.) Grunow [referred to *Himanthidium minus* Kütz., 1844, Material Kützing N. 30 from Jever, leg. Koch (=B.M. 17863), see [16], Figure 158: 14–17. Frustule morphology as in *E. minor* but specimens on average larger, appearing more strongly silicified. (Figure 1h,i provides a comparison for the girdle view, and Figure 1m,n for the valve view). Valve outline and shape variability during the cell cycle barely different. Length 28–63 (vs. 14–44) μm , breadth 6.0–8.0 (vs. 3.5–5.0) μm . Raphe course with terminal fissures not different. Transapical striae proximally 6–10 (vs. 10–17) in 10 μm , becoming rather abruptly much more densely spaced at the ends, 16–20 (vs.

becoming gradually denser up to 18–20) in 10 μm . Areolae precisely to count only with electron microscopical techniques.

As is typical for the genus, two elongate chromoplasts lying on the ventral side of the cell and extending onto the valve faces (Figure 1j,k).

SEM (Figure 2)

External view: Areolae 33–36 (vs. 39–41) in 10 μm . Sternum (=ventral area) broader in comparison. Most remarkable distinguishing character a delicate ridge on both valve margins at the junction between face and ventral/dorsal mantles (e.g., Figure 2a,b,d). Ridges lacking in *E. minor* (e.g., Figure 160: 1–2 in Lange-Bertalot et al. [16]) but present even in small cell-cycle stages of *E. crassiminor* (Figure 19).

Only one valve pole with a rimoportula in both taxa (Figure 2d,g). A faint pseudoseptum sometimes developed at the poles (Figures 23 and 24).

Other characters seen with light microscopy (e.g., striae becoming abruptly much denser towards the poles, e.g., Figure 2b) could be confirmed.

Type material. HOLOTYPE. Diatom collection of the MUSE—Museo delle Scienze, Trento, Italy, TR, slide cLIM007 DIAT 1971 (Mt. Penna spring, bryophytes). Collected by M. Cantonati on the 25th of July 2011. The holotype material is shown in Figure 1a–h,j–m and Figure 2a–g.

ISOTYPES. Diatom Collection of the Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA: ANSP GC14462 (slide), ANSP GCM15149 (cleaned material), ANSP GCM15150 (raw material); -Botanical Museum of the University of Berlin, Germany: B 40 0,041,535 (slide), B 40 0,041,536 (cleaned material), B 40 0,041,537 (raw material).

REGISTRATION.—<http://phycobank.org/102929>

Type locality. Monte Penna spring (EBERs Project code: MtPe_ShFS-Hi, [1]). Shaded (Sh) Flowing Spring (FS) with the crustose red alga *Hildenbrandia* (Hi). Coordinates: Longitude: 9°30'29.493" E, Latitude 44°29'6.029" N. 1324 m a.s.l. Lithology: ophiolite (basalts) hard rock aquifer.

Etymology. Resembles *E. minor* but is larger and with a more robust structure.

Distribution. As yet critically observed with SEM and distinguished from *E. minor* in several springs with low conductivity in the south-eastern Alps and in the Northern Apennine but probably occurring elsewhere under appropriate conditions, waiting for critical differentiation from other morphodemes of *E. minor* sensu lato. At the type locality, the new species was more abundant in the epibryon than in the epilithon (relative abundance: 4.9 vs. 2.3%, respectively).

Ecology, co-occurring diatom species, and associated photoautotrophs. Environmental conditions at the type locality: Discharge (L s^{-1}): 3.5, Temperature ($^{\circ}\text{C}$): 5.3, conductivity ($\mu\text{S cm}^{-1}$): 62, alkalinity ($\mu\text{eq L}^{-1}$): 311, pH: 6.6, nitrate (mg L^{-1}): 1.2, TP ($\mu\text{g L}^{-1}$): 7 (see [1] for more details). As concerns photoautotrophs, in this very shaded source the competitive balance between large groups (algae, lichens, bryophytes, and vascular plants) is clearly favorable to the mosses, which cover almost all the lithic substrata [dominance of *Brachythecium rivulare* W.P. Schimper, both submerged and, in large portions, emerged, and a certain relevance of *Plagiomnium undulatum* (Hedw.) T.J. Kop. and *Rhizomnium punctatum* (Hook.) T.J. Kop.]. Vascular plants are not abundant (as cover), and *Adenostyles glabra* (Miller) DC. and *Saxifraga rotundifolia* L. can be mentioned among them. In terms of cover, bryophytes are followed by lichens. These include two species which are rarely reported in Italy: *Verrucaria madida* Orange, an amphibious species in frequently flooded sites on siliceous rocks, often in association with other aquatic lichens and bryophytes, and *Verrucaria aquatilis* Mudd., common both in springs and along streams, in conditions of perennial/frequent submersion. Benthic macroalgae are rare and mainly represented by the red freshwater alga *Hildenbrandia rivularis* (Liebmann) J. Agardh, which is characteristic of shaded springs with well-buffered waters and medium-high conductivity.

The main co-occurring diatom species at the type locality (at least 5% relative abundance in one of the slides): *Achnantheidium minutissimum* sp. gr., *Amphora inariensis* Krammer, *Amphora indistincta* Levkov, *Brachysira exilis* (Kütz.) Round and D.G.Mann *Cocconeis pseu-*

dolineata (Geitler) Lange-Bert., *Gomphonema elegantissimum* E.Reichardt and Lange-Bert., *Humidophila perpusilla* (Grunow) Lowe, Kocielek, J.R.Johansen, Van de Vijver, Lange-Bert. and Kopalová, *Planothidium angustilanceolatum* sp. nov., *P. frequentissimum* (Lange-Bert.) Lange-Bert., *P. lanceolatum*, *Psammothidium grischunum* Bukht. and Round.

Ecology (Table 1, Figure 3a–l). With reference to temperature, *E. crassiminator* has a lower optimum weighted average than *E. minor* (Table 1); consistently, *E. crassiminator* also seems to prefer sites which are more shaded (Figure 3b). As concerns pH (Figure 3e), interestingly, *E. crassiminator* appears to prefer circumneutral values whilst *E. minor* occurs at slightly acidic ones. *E. crassiminator* has a higher weighted average for sulphates whilst *E. minor* has a higher optimum for manganese (Table 1). In particular, with reference to nitrogen, *E. crassiminator* appears to be associated with more strict oligotrophy than *E. minor*.

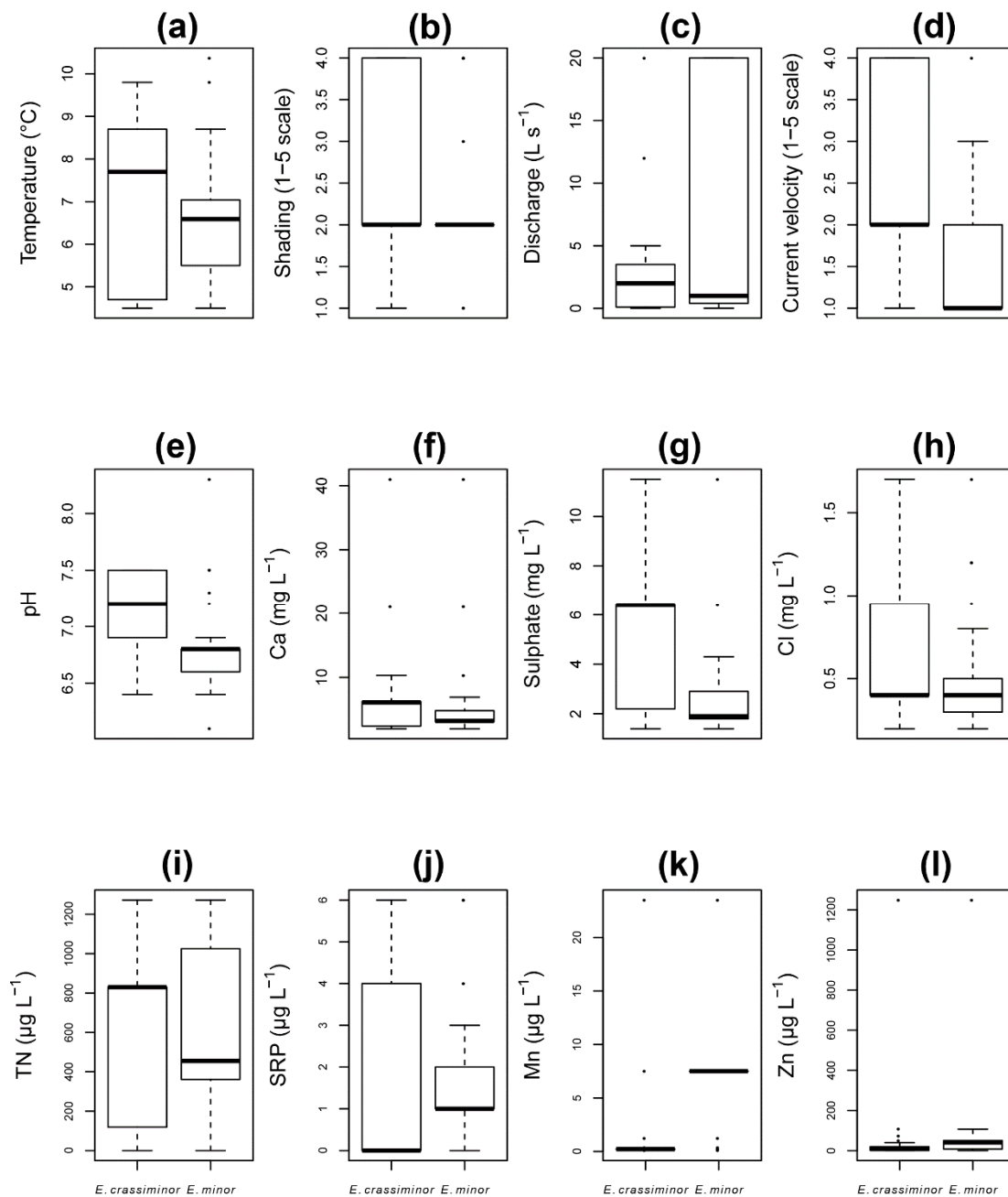


Figure 3. (a–l). Box and whisker plots showing the ecological preferences of *Eunotia crassiminator* as compared to *E. minor*. Only environmental factors/parameters for which statistically significant differences could be found are shown.

Table 1. Ecological preferences of *Eunotia crassiminator* as compared to *E. minor*. Only environmental factors/parameters for which statistically significant differences could be found are shown.

Factor	<i>Eunotia crassiminator</i>			<i>Eunotia minor</i>			<i>t</i> -Tests	
	Weighted Average	Min	Max	Weighted Average	Min	Max	<i>t</i> -Value	<i>p</i> -Value
Temperature (°C)	5.6	4.5	9.8	7.8	4.5	8.7	7.58	5.99×10^{-11}
Shading (1–5 scale)	3	1	4	2	2	2	−9.08	1.20×10^{-14}
Discharge (L s ^{−1})	2.0	0.0	5.0	4.3	0.0	20.0	2.0	4.85×10^{-2}
Current velocity (1–5 scale)	3	1	4	2	1	3	−6.07	2.33×10^{-8}
pH	6.8	6.4	7.5	7.0	6.4	6.9	2.8	6.11×10^{-3}
Ca ²⁺ (mg L ^{−1})	4.9	2.0	10.3	8.5	2.0	6.9	2.20	3.11×10^{-2}
Sulphates (mg L ^{−1})	5.1	1.4	11.5	2.2	1.4	4.3	−7.39	1.45×10^{-11}
Cl ^{−1} (mg L ^{−1})	0.4	0.2	1.7	0.8	0.2	0.8	4.53	2.28×10^{-5}
TN (µg L ^{−1})	340	0	1272	649	0	1272	4.29	7.69×10^{-5}
SRP (µg L ^{−1})	2	0	6	2	0	3	−2.25	2.69×10^{-2}
Mn (µg L ^{−1})	0.6	0.2	0.2	9.8	7.5	7.5	4.95	2.59×10^{-5}
Zn (µg L ^{−1})	101	0	40	37	0	107	−2.12	3.56×10^{-2}

Taxonomic comments. The obvious heterogeneity of various morphodemes and problems of identity concerning type and typification of *Eunotia minor* (Kütz.) Grunow have been discussed at length by Lange-Bertalot et al. ([16], pp. 157–160, Figure captions of plates 158–164). Whilst the true identity of *Himantidium minus* Kütz. is not yet quite clear, *E. crassiminator* from the south-eastern Alps can be defined taxonomically and is well characterized from the morphological and ecological standpoints. It was possible to find associated in a single sample from a helocrenic spring in the Apennines (Elocrena Lago Scuro) *E. minor* and *E. crassiminator*, both never converging and easy to distinguish with the light microscope. *E. crassiminator* roughly resembles *E. pomeranica* Lange-Bert., Bağ et Witkowski [16] from peat bogs in north-western Poland. However, the latter differs by almost evenly spaced striae in the proximal and distal parts of the valve, 11–15 and 16–18 in 10 µm, respectively. The amount of areolae and striae in the new described species is less than in similarly compared species. Marginal ridges in SEM view are missing.

***Planothidium angustilanceolatum* Lange-Bert. et Cantonati sp. nov. (Figures 4 and 5)**

Differential diagnosis compared with an associated population of *Planothidium lanceolatum* (Bréb. ex Kütz.) Lange-Bert. Specimens with conspicuously narrower valves, concerning in particular medium-sized and smaller cell-cycle stages. Valves linear-elliptic with rounded ends (vs. broadly elliptic to elliptic-lanceolate with broadly rounded ends). Largest stages rhombic-lanceolate). Length 5–24 µm, breadth 2.5–4 µm (vs. 13–32 and 5–7.5 µm, respectively). Length-to-breadth ratio 2.8–5.8 (vs. 1.6–4.7). Areae, raphe, and striae are barely different in both taxa, stria density 12–16 in 10 µm, considerably variable in both taxa.

As is typical for the genus, one chromoplast, in girdle view lying against the more convexly curved valve but extending under the other one; only moderate indentation between the two shorter lobes (Figure 4m–o; *Planothidium lanceolatum* plastid shown for comparison in Figure 4a',b').

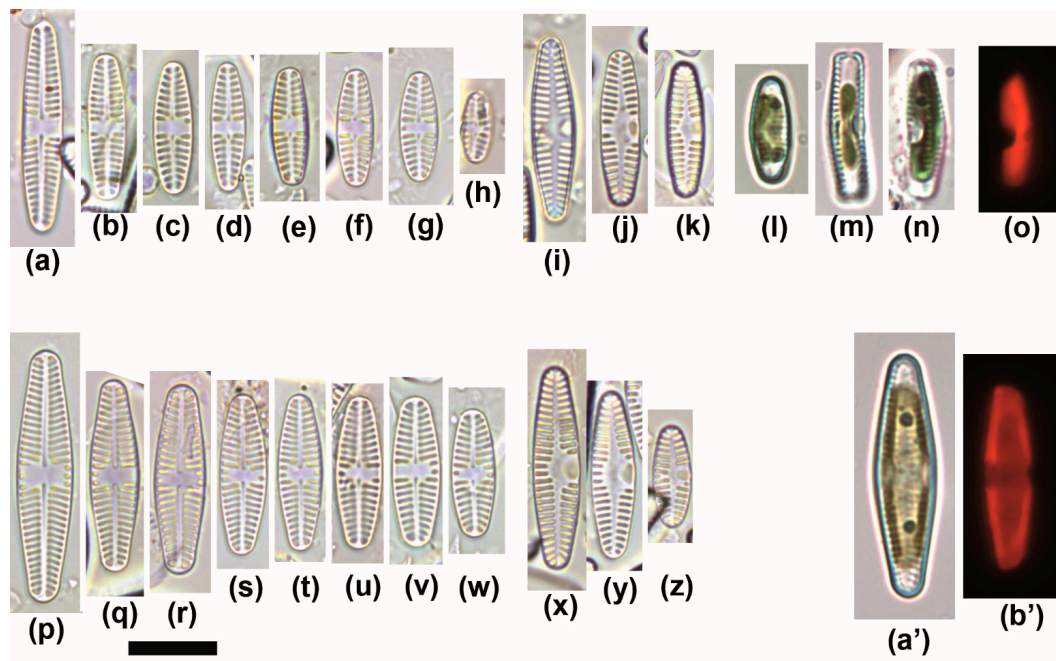


Figure 4. (a–h). LM morphology of *Planothidium angustilanceolatum* sp. nov. (a–o) as compared to a population of *Planothidium lanceolatum* co-occurring at the type locality (p–b’). All bright-field micrographs with the exception of (o,b’) which are based on chlorophyll autofluorescence. All valve views, with the exception of (m,n) that are girdle views. (a–h,p–w): Raphe valves. (i–k,x–z): Rapheless valves. (l–o,a’,b’): Chromoplast morphology. Scale bar 10 μ m.

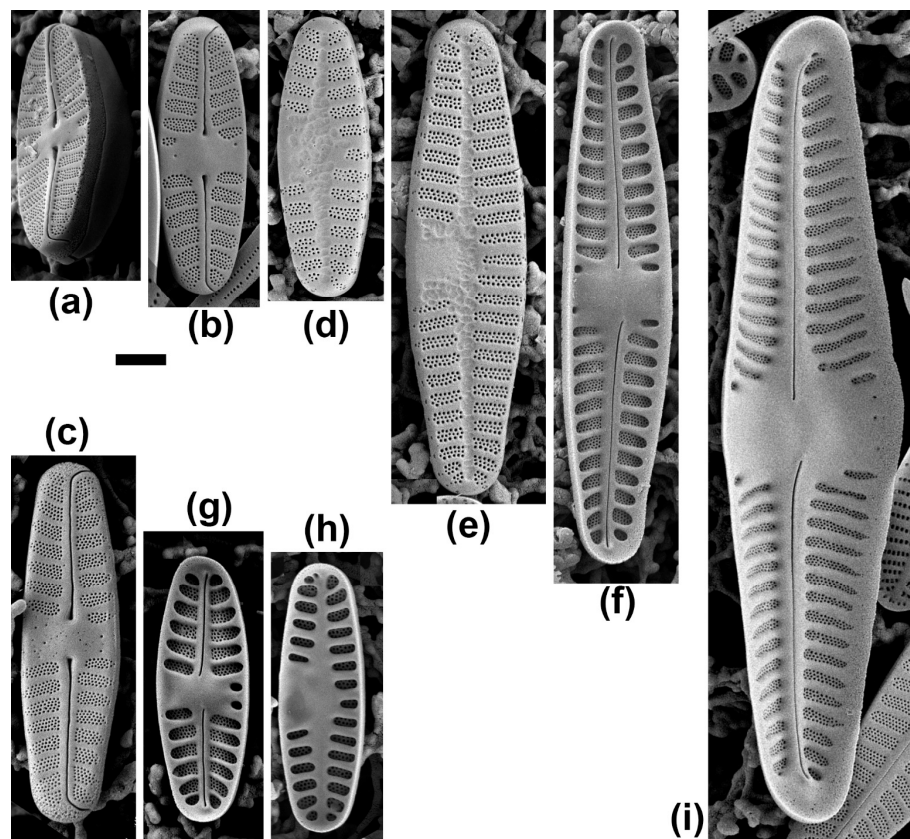


Figure 5. (a–i). SEM images of *Planothidium angustilanceolatum* Lange-Bert. et Cantonati sp. nov. (a–h), and of *P. lanceolatum* for comparison (i). (a–e): External views. (f–i): Internal views. (a–c,f,g,i): Raphe valves. (d,e,h): Rapheless valves. Scale bar 1 μ m.

SEM (Figure 5).

Externally the areas of raphid valves are smooth (Figure 5b), whereas the araphid valves are covered by shallow irregular grooves on both the axial and the central area (Figure 5d,e), the latter more extended but not restricted unilaterally. The pluriseriate areolae (3–5 series) extend more or less clearly over the valve face margins onto the mantle. More in rapheless (Figure 5d) and less in raphid (Figure 5c) valves. The characteristic unilateral depression, “sinus”, of rapheless valves is very shallow comparatively (Figure 5d,e).

Type material. HOLOTYPE. Diatom collection of the MUSE—Museo delle Scienze, Trento, Italy, TR, slide cLIM007 DIAT 1971. The holotype material is shown in Figures 4a–o and 5a–h.

ISOTYPES. Diatom Collection of the Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA: ANSP GC14463 (slide), ANSP GCM15151 (cleaned material), ANSP GCM15152 (raw material); Botanical Museum of the University of Berlin, Germany: B 40 0,041,538 (slide), B 40 0,041,539 (cleaned material), B 40 0,041,540 (raw material).

REGISTRATION. <http://phycobank.org/102930>

Type locality. Monte Penna spring (EBERs Project code: MtPe_ShFS-Hi, [1]). Shaded (Sh) Flowing Spring (FS) with the crustose red alga *Hildenbrandia* (Hi) (see the description of *Eunotia crassiminor* for complete information).

Etymology. Resembles *P. lanceolatum* but is narrower.

Distribution. As yet critically observed exclusively at the type location in the Northern Apennines and in a spring with very similar hydrochemistry in the southeastern Alps (Belvedere spring epibryon: 3.7% *Planothidium angustilanceolatum* sp. nov., 15.6% *Planothidium lanceolatum*). At the type locality, the new species was clearly more abundant in the epibryon than in the epilithon (relative abundance: 13.6% vs. 5.4%, respectively).

Ecology, co-occurring diatom species, and associated photoautotrophs. See the description of *Eunotia crassiminor* sp. nov. for complete information.

The search for this new species in ten comparable CRENODAT springs allowed us to find it in the low-conductivity high-mountain (2056 m a.s.l.) spring Belvedere (CRENODAT Project code: OC2056). Environmental conditions at Belvedere spring: Discharge ($L s^{-1}$): 2, Temperature ($^{\circ}C$): 4.5, conductivity ($\mu S cm^{-1}$): 60, alkalinity ($\mu eq L^{-1}$): 360, pH: 6.9, nitrate ($mg L^{-1}$): 0.48, TP ($\mu g L^{-1}$): 5.

Taxonomic comments. Obviously *Planothidium lanceolatum* sensu stricto is the closest related taxon. A population, probably identical with the *P. lanceolatum* type, is associated in the samples from the type locality. Geitler [15] described the entire cell cycle of *Achnanthes lanceolata* (syn. *Planothidium lanceolatum*): length of the auxospores (apical axis) 32–36, rarely up to 40 μm ; copulating cells (gametes) 11–16, rarely up to 20 μm ; smallest cells length: 7 μm ; breadth, transapical axis, of post-initial cells 8–10 μm , of copulating cells 5–7 μm , of smallest specimens 4.5–5 μm , resulting in a length-to-breadth ratio of 4.1, 2.5, and 1.6, respectively. The cultured clones originate from the calcium-carbonate-rich, oligotrophic Lake Lunz in the Austrian northern Alps. The valve outlines documented by line drawings are broadly elliptical in smallest stages and elliptic-lanceolate to rhombic-lanceolate. All with broadly rounded ends. Valve outlines conforming to *P. angustilanceolatum* do not occur. Stria density 13–14 in 10 μm . On the other hand, various photographically documented specimens from all continents conform to Geitler’s description as far as *P. lanceolatum* sensu stricto is concerned, excluding many misidentified similar taxa. Examples are given by Rumrich et al. ([61], Figure 28: 11–16) from the Andes in Chile, 4000 m a.s.l., Sonneman et al. ([62], p. 15, Figure 10a–d) from Australia, Dorofeyuk and Kulikovskiy ([63], Figure 41: 1–8, 14–29, Figure 45: 1–4, 46: 1–6) from Mongolia, Metzelin et al. ([64], Figure 28: 1–8) from Mongolia, Blanco ([65], Figure 44: 1–22) from Spain, Wojtal ([66], Figure 138: 11–22) from Poland, Van de Vijner et al. ([67], Figure 23: 7–14, 24: 8–a) from the Ile Crozet Archipelago, Sub Antarctica.

Other, just roughly similar taxa with comparable size and outlines are: *P. frequentissimum* (Lange-Bert.) Lange-Bert. in Metzelin et al. ([64], Figure 28: 9–11), *P. aff. fragilarioides* sensu Lange-Bert. et Krammer ([68], Figure 88: 16–21), both distinguished mainly by the

presence of a horseshoe-shaped “cavum”, *P. aueri* (Krasske) Lange-Bert. distinguished by striae consisting of biseriatae areolae and a “cavum”.

Geitler ([15], p.41, Figure 64a–d) mentioned and displayed by line drawings smaller specimens that occurred together with *P. lanceolatum*, 8–10 µm long, 4 µm broad, resembling but not identical with *P. angustilanceolatum* sp. nov. Later on, [69] validly established *Achnanthes lanceolata* var. *minor* (Schulz) Lange-Bert. as an infraspecific taxon, with a type originating from the Botanical Garden of Vienna, Austria, distinguished by conspicuously lower dimensions. Length was 6.5–21 µm, breadth 3.2–6 µm; copulating cells (gametes) were 6.5–9.5 µm long, ca. 3.9 µm broad, primary (initial) cells 18.5–21 µm long and 5.5 µm broad. Very likely this taxon is not a synonym of *P. angustilanceolatum* sp. nov. The valves of primary cells possessed rhombic outlines, i.e., inflated in the central part, whereas 22 µm long stages of *P. angustilanceolatum* sp. nov. are distinguished by non-inflated linear to slightly linear-lanceolate outlines. Moreover, the distinctly contoured “horseshoe”-shaped depressions of the rapheless valves of *A. lanceolata* var. *minor* point to a covered “cavum” rather than to a shallow open depression (“sinus”). Photographically documented specimens identified by Reichard ([70], Figure 7: 13–15) as Geitler’s taxon display that character clearly. Thus, it appears to be more closely related to *P. frequentissimum* and *P. frequentissimum* var. *minus* (Schulz) Lange-Bert. rather than to *P. lanceolatum* sensu stricto.

Biometry of *Planothidium angustilanceolatum* sp. nov. at the type locality, compared with a co-occurring population of *P. lanceolatum* (Table 2, Figure 6a–e). By means of the biometry part of the study we could confirm that width (Table 2, Figure 6b) and length (Table 2, Figure 6a) differ in a statistically significant way between the *Planothidium angustilanceolatum* sp. nov. and *P. lanceolatum* population, being larger in the latter species. This is underlined also by the length—width relationships plotted in Figure 6e.

Delicatophycus crassiminutus Lange-Bert. et Cantonati sp. nov. (Figures 7 and 8)

Light microscopy. Valves rather weakly dorsiventral, lanceolate, ends distinctly protracted rostrate-subcapitate (Figure 7a–i). Length 18–27 µm, breadth 3.5–5 µm proximally, becoming ca. 2 µm below the expanded ends, maximum length-to-breadth ratio 6.2. Axial area narrow, curved, extended gradually to the valve centre, slightly displaced to the ventral side. No set off central area developed, since dorsal striae commonly not, occasionally very little shortened gradually, never abruptly and barely wider spaced than the subproximal striae (Figure 7d–g). Occasionally the area system even appears slightly constricted in the centre. Raphe strongly reverse-lateral towards proximal ends. Terminal fissures comma-shaped and dorsally bent (see SEM). Dorsal striae radiate throughout, 15–16 in 10 µm. Ventral striae are distinctly more narrowly spaced, 18–21 in 10 µm, the median ones at most very little radiate. Areolation of the striae is indistinctly resolvable in LM.

As is typical for the genus, one highly-lobed chromoplast lying with its centre against the ventral side of the girdle but extending under each valve with longitudinal indentations (H-shaped); pyrenoid on the dorsal side of the cell (Figure 7k,l).

SEM. External and internal views see Figure 8a,b. Basic pattern of the fine structures is principally the same as in other taxa of the genus, particularly *D. minutus* M.J.Wynne. However, the wavy appearance of fusing areolae externally is less expressed in our materials probably due to corrosion effects, see likewise Figure 8c for *D. minutus*. Areola density 35–44 in 10 µm. Proximal raphe ends deflected to the ventral side, distal ends strongly bent onto the dorsal mantle, sickle-shaped. Striae in the subcapitate ends become subparallel on the dorsal side, even convergent on the ventral side.

Table 2. Morphological characteristics of *Planothidium angustilanceolatum* sp. nov. at the type locality, compared with a co-occurring population of *P. lanceolatum*.

	<i>P. angustilanceolatum</i>						<i>P. lanceolatum</i>						<i>t</i> -Tests					
	R-Valves			RL-Valves			R-Valves			RL-Valves			<i>t</i> -Value	R-Valves		RL-Valves		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max		<i>t</i> -Value	<i>df</i>	<i>p</i> -Value	<i>t</i> -Value	<i>df</i>
Length (µm)	14.9	7.9	24.5	14.0	7.9	24.1	18.8	12.8	29.1	18.9	11.7	27.4	5.12	99	1.51×10^{-6}	6.86	101	5.44×10^{-10}
Width (µm)	4.1	3.1	5.2	3.9	3.1	4.8	5.2	4.1	7.2	5.1	4.1	7.4	11.09	98	$<2.2 \times 10^{-16}$	13.41	100	$<2.2 \times 10^{-16}$
Length/Width	3.6	2.6	5.7	3.6	2.4	5.8	3.6	2.8	4.9	3.7	2.6	5.4	−0.09	75	9.28×10^{-1}	0.78	117	4.38×10^{-1}
Striae in 10 µm	13	12	14	13	12	14	13	12	16	13	12	14	1	88	1.84×10^{-1}	0.21	114	8.37×10^{-1}

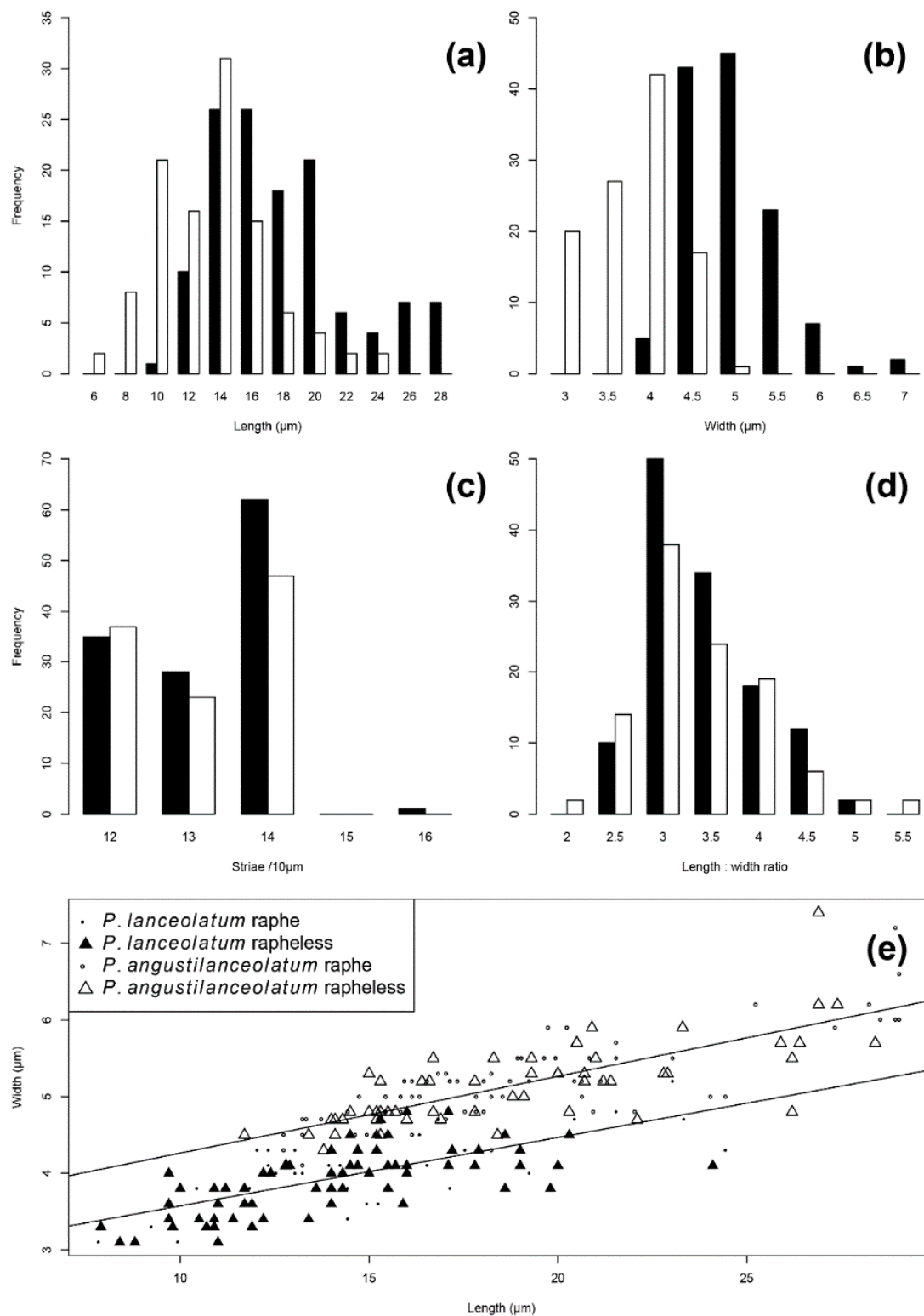


Figure 6. (a–e) Morphological characteristics of *Planothidium angustilanceolatum* sp. nov. at the type locality, compared with a co-occurring population of *P. lanceolatum*.

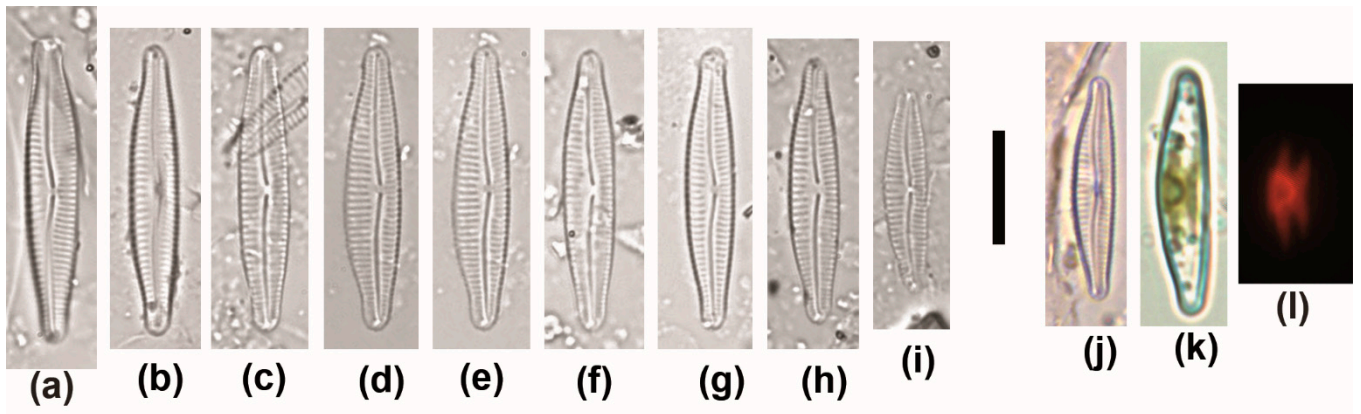


Figure 7. (a–l). LM morphology of *Delicatophycus crassiminutus* Lange-Bert. et Cantonati sp. nov. (a–i,k,l), and of *D. minutus* (j) for comparison. All bright-field micrographs with the exception of (l) which is based on chlorophyll autofluorescence. (k,l): Chromoplast morphology. Scale bar 10 μ m.

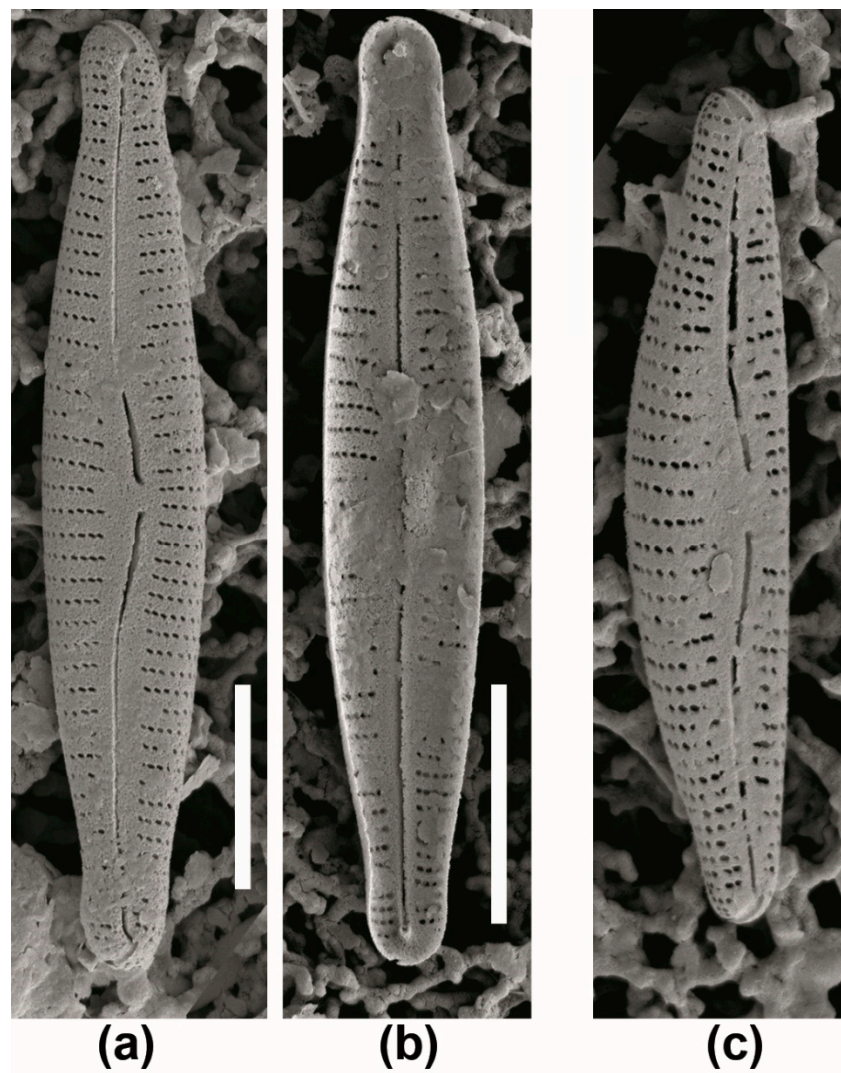


Figure 8. (a–c). SEM images of *Delicatophycus crassiminutus* Lange-Bert. & Cantonati sp. nov. (a,b), and of *D. minutus* for comparison (c). (a,c): External views. (b): Internal view. Scale bars 5 μ m.

Type material.

HOLOTYPE. Diatom collection of the MUSE—Museo delle Scienze, Trento, Italy, TR, slide cLIM007 DIAT 1962 (Carameto spring, epilithon). Collected by M. Cantonati on the 28th of July 2011. The holotype material is shown in Figure 7a–i,k,l and Figure 8a,b.

ISOTYPES. Diatom Collection of the Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA: ANSP GC14464 (slide), ANSP GCM15153 (cleaned material), ANSP GCM15154 (raw material); -Botanical Museum of the University of Berlin, Germany: B 40 0,041,541 (slide), B 40 0,041,542 (cleaned material), B 40 0,041,543 (raw material).

REGISTRATION.—<http://phycobank.org/102931>

Type locality. Carameto spring (EBERs Project code: Cara_LPS-sn, [1]). Small (s), near-natural (n) limestone-precipitating spring (LPS). Coordinates: Longitude: 9°45′35.165″ E, Latitude 44°39′50.840″ N. 758 m a.s.l. Lithology: limestones, flyschs; calcarenites.

Distribution. At the type locality, the new species was found only in the epilithon (relative abundance: 2.2%). Observations on CRENODAT materials confirmed that the species is restricted to LPS springs.

Ecology, co-occurring diatom species, and associated photoautotrophs. Environmental conditions at the type locality: Discharge (L s⁻¹): 0.07, Temperature (°C): 12.8, conductivity (µS cm⁻¹): 462, HCO₃⁻ (mg L⁻¹): 145, pH: 7.7, nitrate (mg L⁻¹): 0.12, TP (µg L⁻¹): 6 (see [1] for more details). The vegetation occurring in the Carameto spring belongs to the *Adiantum* alliance with dominance of the characteristic bryophyte species *Eucladium verticillatum* Bruch and W.P. Schimper, *Hymenostylium recurvirostrum* (Hedw.) Dixon, and *Pellia endiviifolia* (Dicks.) Dumort. Other taxa occurring at the type locality, but all with low cover, were the bryophytes *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer and Scherb. and *Palustriella commutata* Ochyra, and the vascular plants *Carex flacca* Schreb. and *Molinia caerulea* (L.) Moench. Species diversity was low (13 taxa), as expected in this kind of community [71].

Main co-occurring diatom species at the type locality (at least 5% relative abundance in one of the slides): *Achnantheidium minutissimum* sp. gr., *Achnantheidium trinode* Ralfs, *Delicatophycus minutus*, *Denticula tenuis* Kütz., *Encyonopsis lange-bertalotii* Krammer, *Gomphonema tenoccultum* E.Reichardt.

Taxonomic comments. Among the moderately few taxa of the genus, only *D. minutus* is actually similar (see [50], summarizing table of the taxa with illustrations (pp. 112–113). It differs mainly by more distinctly dorsiventral symmetry, missing set off ends, and presence of a central area dorsally. In SEM, external view ([50], Figure 137: 16), striae are distinctly radiate throughout; convergent striae on the dorsal side of the ends are lacking due to the simply rounded, not subcapitate ends. Moreover, *D. minutus* has a higher density of the central dorsal striae (16–21 vs. 15–16 in 10 µm).

4. Discussion

Springs are a unique but severely threatened wetland type. Therefore, the in-depth knowledge of the taxonomy and ecology of characteristic diatom species is important because diatoms are excellent indicators of the quality and integrity of these peculiar ecosystems in the face of direct and indirect human impacts.

The three new species described differ morphologically from the most similar existing species by: less dense striae and areolae, and absence of a ridge at the valve face-mantle transition (SEM feature) (*Eunotia crassiminor* Lange-Bert. et Cantonati sp. nov.; closest established species: *Eunotia minor*); narrower and shorter cells (*Planothidium angustilanceolatum* Lange-Bert. et Cantonati sp. nov.; closest established species: *Planothidium lanceolatum*); barely-dorsiventral symmetry, set off ends, and lower density of the central dorsal striae (*Delicatophycus crassiminutus* Lange-Bert. et Cantonati sp. nov.; closest established species: *Delicatophycus minutus*).

Two of the three species we described are separated from the most similar established species by dimensions. By applying Krammer's [13] ratio of maximal and minimal width as a reliable means to test the quality of taxa, we find that we have contributed to improve

knowledge on two taxa (*Eunotia minor* s.l. and *Planothidium lanceolatum* s.l.), which are likely to be only partially resolved complexes of species because they have a max-min width ratio of 2 and 2.2, respectively (data of minimum and maximum width taken from [72]).

We also could contribute to ameliorate knowledge on the ecological profiles of the three newly described species. In the case of *Eunotia crassiminor* sp. nov. data were sufficient to allow testing for statistical significance. *Eunotia crassiminor* sp. nov. as compared to *Eunotia minor*, appears to occur in colder inland waters with circumneutral pH, and strict oligotrophy also with respect to nitrogen. In the face of global warming, diffuse airborne nitrate pollution, and acidification risk, this realized ecological niche singles out *Eunotia crassiminor* sp. nov. as a species which is clearly more threatened than *E. minor*. This information is very useful as well for the generation and updating of diatom Red Lists that can provide excellent metrics for the conservation value of inland waters [5]. As far as the other two newly described species are concerned, data gained on the distribution were too few to allow for statistical treatment. However, they occur in spring types which are so peculiar that some generalization, though with caution, can be made. *Planothidium angustilanceolatum* sp. nov. was found only in two springs, one in the Northern Apennines and one in the south-eastern Alps, that however have almost identical morphological, physical, and chemical characteristics. It can thus be stated that the typical habitat of this species is oligotrophic mountain flowing springs with low conductivity (approximately $60 \mu\text{S cm}^{-1}$). *Delicatophycus crassiminutus* sp. nov. was observed only in LPS, and is therefore likely to be restricted to hard water springs and comparable habitats where CO_2 degassing leads to carbonate precipitation.

The correct knowledge of the taxonomy and ecology of the species occurring in mountain aquatic habitats is of great importance to use diatoms as reliable indicators of environmental and climate change. Mountain ecosystems are sensitive and reliable indicators of climate change (e.g., [73]). There are many good reasons for protecting these freshwater habitats [74]: they are relatively scarce, provide clean water for many uses, harbour a large number of Red List taxa [compare [7,74], and they are sensitive to disturbance from human activities [7,12].

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Conflicts of Interest: The authors declare no conflict of interest.

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