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A new *Navicula* (Bacillariophyta) species from low–elevation carbonate springs affected by anthropogenic disturbance

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Abstract: Diatom assemblages in carbonate spring habitats at low elevations, with low discharge, and affected by direct human impacts were studied only recently and in some geographic areas. We describe and characterize the ecology of a new *Navicula* species from springs with these characteristics within the basin of the River Adige in the south–eastern Alps and Alpine foothills (Province of Verona, Italy). *Navicula veronensis* sp. nov. is in the group of species around *N. cincta* but has distinct outline, size, central area, and proximal raphe endings. The availability of data on the distribution of the new species in streams of the Island of Cyprus allowed to quantitatively compare the ecological preferences, niche position, and niche breadth of *Navicula veronensis* sp. nov. with those of allied species. The new species was found to be characteristic of medium–high conductivity freshwaters, rich in sulphates and chloride, often nitrate enriched and affected by marked discharge fluctuations and seasonal desiccation. Niche position however differed only slightly from those of morphologically–similar species, with the exception of *Navicula dealpina* that was found to be characteristic of oligotrophic, calcium–bicarbonate rich environments with medium(–low) conductivity. Canonical Correspondence Analysis clearly showed that the most influential environmental variables determining the diatom assemblages of low–elevation carbonate springs affected by anthropogenic disturbance were nitrate enrichment, spring morphology alteration (in particular for water abstraction), and low discharge.

Key words: diatoms, *Navicula veronensis* sp. nov., springs, nitrates, morphology

Introduction

The genus *Navicula* is often one of the most species–rich genera in spring habitats (Werum & Lange–Bertalot 2004; Żelazna–Wieczorek 2011; Cantonati et al. 2012a; Wojtal 2013). In springs high in nitrate and phosphorus it was even found to be the most species rich genus (Żelazna–Wieczorek 2011). The group of species around *Navicula cincta* (Ehrenberg) Ralfs is reported to be rare in springs (Werum & Lange–Bertalot 2004; Cantonati et al. 2012a; Wojtal 2013). However, as many as three new species belonging to this species group were described from springs in recent years (Żelazna–Wieczorek 2011; Reichardt 2012; Beauger et al. 2015).

Springs are heavily exploited to gain drinking water but they are also habitats with special features (Cantonati et al. 2012b). However, low–elevation carbonate springs in densely populated areas, directly affected by various types of impacts, have been less considered than near–natural mountain springs. Some information on this type of springs is nevertheless available from Poland (Wojtal 2013; Żelazna–Wieczorek 2011; Wojtal & Sobczyk 2012), Switzerland (Taxböck & Preisig 2007), France (Bertrand et al. 1999), and Spain (Aboal et al. 1998; Delgado et al. 2007, Maiorca). Small springs on carbonate substratum fed by local aquifers are often affected by marked discharge fluctuations and even are intermittent or seasonal. This discharge variability is likely to be enhanced by climate change in many geographic areas (Cantonati & Lange–Bertalot 2009).

The characteristics of spring habitats vary markedly according to spring types (e.g., Spitale et al. 2012). Among the most peculiar types of ambient springs are petrifying springs or Limestone Precipitating Springs (LPS) (Cantonati et al. 2016). In spite of being the sole widely–distributed spring type included in the European Union Habitat Directive (EU–HD 1992), LPS are affected by many impacts, the most important ones being water diversion, and inadequate management due to lack of appreciation. The charac-

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teristics of diatom assemblages of petrifying springs affected by nitrate enrichment were recently discussed by DENYS & OOSTERLYNCK (2015).

Building on the availability of a dataset on diatom assemblages in low–elevation carbonate springs affected by anthropogenic disturbance (ANGELI et al. 2010), the main objectives of this study were as follows: (1) to describe and characterize a new *Navicula* species; (2) to statistically assess the ecological preferences and niche of the new species; (3) to compare the optima of the new species for the main environmental factors with those of allied species; (4) to identify the most influential environmental variables determining the diatom assemblages of low–elevation carbonate springs affected by anthropogenic disturbance, providing iconography for the most frequent and abundant species.

METHODS

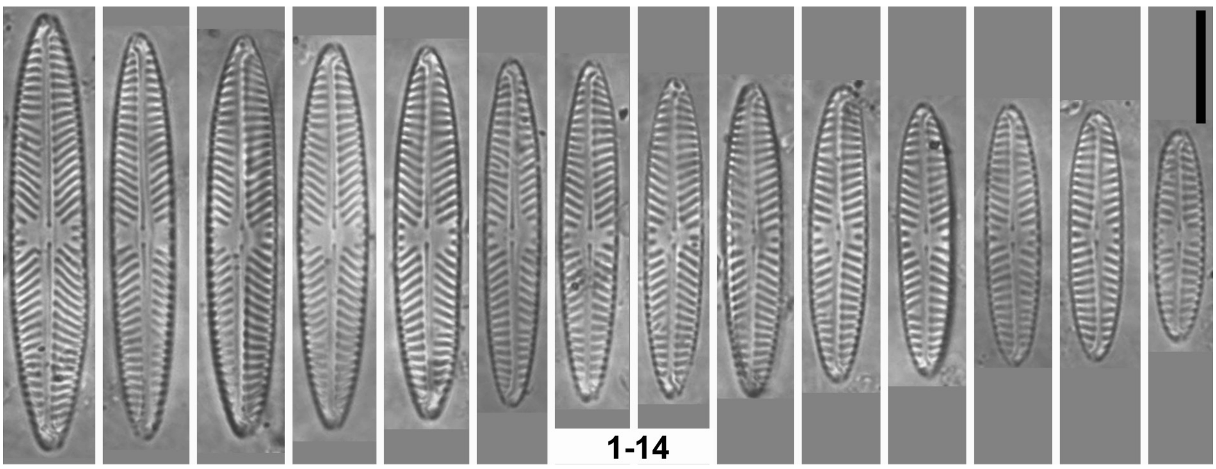
Dataset, study area, sampling. We studied 25 springs (named "CESSPA springs" in ANGELI et al. 2010) considering two main substrata: bryophytes and lithic materials. The springs were located within the basin of the River Adige in the Provinces of Verona, Trento, and Vicenza (south–eastern Alps and Alpine foothills in the Veneto Region + Autonomous Province of Trento, Italy), and distributed along an altitudinal gradient from 62 to 1266 m a.s.l. The Natural History Museum of Verona provided all samples. All details on the study area, sampling, field measurements (including the compound human–disturbances' index), and hydrochemical analyses are available in ANGELI et al. (2010).

With regard to diatom sampling methods and the measurement of the main environmental parameters, CRENODAT methods were followed (CANTONATI et al. 2012a). Separate samples of all substrata considered for the analysis of diatom assemblages were taken. Epilithic diatoms were collected by brushing seven to ten cobbles or small boulders. For the epibryon, entire plantlets of the dominant bryophyte species (submerged or closest to the water) were collected, and identified to species level. The most common species was *Cratoneuron filicinum* (HEDW.) SPRUCE found in 9 springs (for a complete list see ANGELI et al. 2010).

Diatom preparation, identification, and counting. The diatom samples were treated with hydrogen peroxide and hydrochloric acid, and the valves thus freed from organic substance were mounted with Naphrax ® (Brunel Microscopes Ltd., Chippenham, Wiltshire, UK). For each sample three cover slips were prepared and mounted on a glass slide, and a total of 450–500 valve were counted to determine relative abundances. All slides were examined for a fixed time (3 hours) to try to find rare taxa with low relative abundance. The determination and nomenclature followed mainly KRAMMER & LANGE–BERTALOT (1986–1991), ROUND et al. (1990), LANGE–BERTALOT (2001), KRAMMER (1997a,b, 2002–2003), KRAMMER & LANGE–BERTALOT (2004), REICHARDT (1997), WERUM & LANGE–BERTALOT (2004), LEVKOV (2009), HOFMANN et al. (2011), LIU et al. (2012), LOWE et al. (2014), and JÜTTNER et al. (2015). Observations were performed with an optical microscope Zeiss Axioskop 2 equipped with phase contrast and a digital camera Axiocam (Carl Zeiss JSC, Milan, Italy). SEM observations on gold coated prepared material were made primarily at the University of Frankfurt using a Hitachi S–4500 (Hitachi Ltd., Tokyo, Japan). Further SEM observations were done at the Museo delle Scienze – MUSE in Trento using a LEO XVP (Carl Zeiss SMT Ltd., Cambridge, UK) at high vacuum. All of the observations and micrography referred to in the present note were done on epilithon samples.

All samples (original samples, suspensions of prepared material, and permanent mounts) have been catalogued and deposited in the collections of the Museo delle Scienze – MUSE (Trento) (access codes: cLIM007 DIAT 637–683) along with information about the abundance of the species found and the main environmental variables.

Data processing and statistical analysis. All the statistical analyses were performed within the R statistical environment (R CORE TEAM 2015). The optima and tolerance of species along the gradients were calculated assuming a Gaussian response curve with the package "analogue". Niche position and niche breadth were calculated using the OMI (Outlying Mean Index) analysis (DOLÉDEC et al. 2000) implemented in the "ade4" package. This method measures the marginality of species habitat distribution, i.e., the distance between the



Figs 1–14. LM micrographs showing a size diminution series for *Navicula veronensis* sp. nov. Scale bar 10 μm.

mean habitat conditions used by a species and the mean habitat conditions across the study area. Species that have high values of OMI have marginal niches (high niche position, since they occur in atypical habitats in a region), and those that have low values have non–marginal niches (low niche position, because they occur in typical habitats in a region). Tolerance index, or niche breadth, was defined as the variance term that measures the amplitude of the distribution of each species along the sampled environmental gradients. Species that have a wide niche breadth occur across a wide range of environmental conditions, and those that have low values occur only across a limited range of conditions.

The ecological preferences and niche of the new species were compared with those of morphologically similar species. To gain sufficient data on these species the following datasets had to be considered: CESSPA (Angeli et al. 2010), CRENODAT (Cantonati et al. 2012a), Cyprus_diatoms (Cantonati et al. 2015).

Canonical Correspondence Analysis (CCA) of the CESSPA springs diatom assemblages was calculated with the package "vegan" without prior transformation. CCA was selected over other ordination techniques, such as redundancy analysis (RDA), after evaluation of the length of the gradient. Model selection was performed with the "orddistep" function of "vegan", an automatic stepwise model building for constrained ordination method. Only the significant environmental variables were used for the final CCA model. Significance of the CCA model, terms, and constrained axes was tested with an ANOVA like permutation test (999 permutations). In the output biplots species were labelled using OMNIDIA (Lecointe et al. 1993) codes (new codes had to be generated for species not present in the OMNIDIA codes' list).

Results

***Navicula veronensis* Lange–Bertalot et Cantonati sp. nov. (Figs 1–14, 15–17)**

Description (compared to *Navicula cincta* (Ehrenberg) Ralfs; SEM outside view shown in Fig. 18 for comparison)

Light microscopy (Figs 1–14): Valves linear–lanceolate, becoming linear–elliptical–lanceolate to elliptical at the end of the size diminution cycle (smallest cell cycle stages). Ends becoming gradually wedge–shaped, finally obtusely rounded. Length 18.8–40 µm, width 4.2–6.6 µm. Length–to–width ratio = 4.2–6.0 (not 2.5–5.3). Raphe filiform, rather straight, with slightly expanded central pores that appear not noticeably deflected to the primary or secondary side of the valve; terminal fissures like a question mark. Axial area rather narrow, only very slightly expanded proximally. Central area broadly rectangular or transapically elliptical or bow–tie–shaped in large specimens, somewhat variable and less broad in smaller ones; 2–4 striae are distinctly shortened on either side (in comparison, the central area of *Navicula cincta* is distinctly smaller, the central striae are less and irregularly shortened). The other striae are rather strongly radiate in proximal parts, becoming abruptly convergent at the Voigt discordance; remarkable is the consistent number of 11–13 in 10 µm (not more variable, between 8–12 comparatively). Lineolae are not resolvable under the LM.

Scanning electron microscopy (Figs 15–17: Figs 15–16 external views, Fig. 17 internal view): Areolae (lineolae) c. 50 (not 40 or less) in 10 µm.

Holotype (designated here): Torricelle spring epilithon (slide) deposited in the collections of the Museo delle Scienze – MUSE in Trento (Italy), accession code: cLIM007 DIAT 680. Since the amount of material in the epilithon sample was small, epibryon from the same spring is available as supplementary (paratype) material (suspension of prepared material + original fixed sample).

Isotypes: Diatom collection *Botanischer Garten und Botanisches Museum Berlin–Dahlem*, *Freie Universität Berlin* (Berlin, Germany), Codes: B 40 0041529 (slide), B 40 0041530 (cleaned material), B 40 0041531 (row material, bryophytes, paratype); and ANSP Diatom Herbarium (The Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA), Codes: ANSP GC 14460 (isotype slide), ANSP GCM 15145 (cleaned material), ANSP GCM 15146 (raw paratype material).

Iconotype: Fig. 11

Type locality: Torricelle (longitude: 11.004323, latitude: 45.469326, WGS84 system) is a partially–tapped spring within the urban area of Verona at an elevation of 220 m a.s.l. The spring discharges out of a tunnel in which boulders and cobbles are partly stabilized with concrete. The spring head receives light through heavy metal gratings. The species was found to occur both on lithic material and on bryophytes [*Eurhynchium pulchellum* var. *pulchellum* (Hedw.) Jenn.].

Etymology: The name refers to the geographic location of the type locality, a spring in the urban area of the town of Verona in northern Italy.

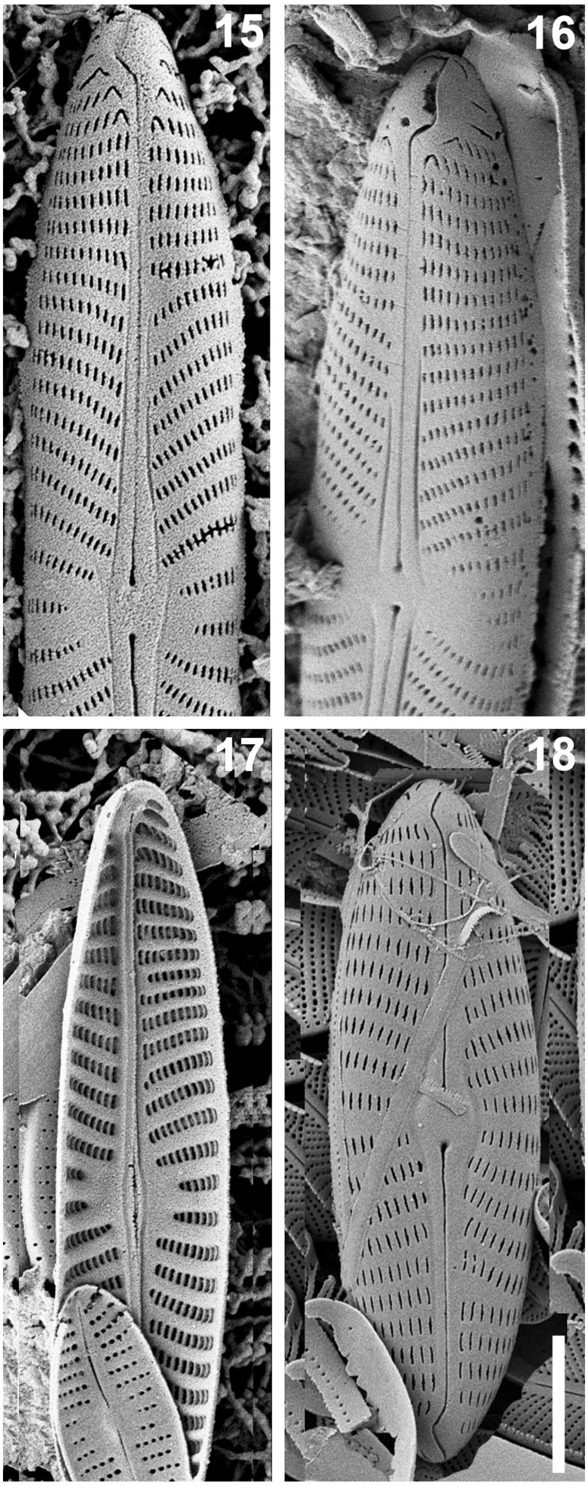
Taxonomic comments: Other more or less resembling European taxa (Table 1) are *Navicula cariocincta* Lange–Bertalot in Tsarenko et al. (2000) and *Navicula dealpina* Lange–Bertalot 1993, the latter one being significantly broader, 8–12 µm. Both are mainly distinguished by much coarser lineolae that are easy to resolve with the LM, c. 30 or c. 26 in 10 µm respectively. Also *Navicula cari* Ehrenberg possesses a similar complex of characters but differs by less densely spaced lineolae, 32–40 in 10 µm. *Navicula seibigiana* Lange–Bertalot has a different outline and lower stria and lineola density. *Navicula wiesneri* Lange–Bertalot has a different outline (in particular the obtusely–rounded ends), and a lower lineola density.

Ecological preferences and niche of *Navicula veronensis* sp. nov.

The type locality is a hard water (Cond. = 536 µS.cm^{-1}; pH = 8.0) ambient spring with low discharge (about 0.5

l.s^{-1}), high nitrate content (25 mg.l^{-1}), relatively high chlorides concentrations (11.7 mg.l^{-1}), and relatively–high temperature (12 °C). *N. veronensis* was observed also on stones at 10 stream monitoring stations on the Island of Cyprus (CANTONATI et al. 2015), always with very low abundances (usually < 0.2% and only once with 0.9%). These streams are both perennial (N = 6) and intermittent (N = 4), have low discharge (15–190 l.s^{-1}), alkaline pH (7.9–8.3, 8.9), high conductivity (465–683, 2850 µS.cm^{-1}), relatively–high temperature (8.4–18.3 °C), mostly high nitrate (1.5–20.4 mg L^{-1}) but mostly low total phosphorus (3–34 µg.l^{-1}), high sulphate (36–56, 118 mg.l^{-1}) and chloride (34–53 mg.l^{-1}). At the type locality the species was found more abundantly on lithic material (1.2% relative abundance) than on mosses (0.4%).

The ecology of *N. veronensis* was not clearly differentiated when compared to other morphologically similar species, *N. cincta*, *N. cariocincta*, *N. cari*, and *N. dealpina* (Table 2). All the species, with the exception of *N. dealpina*, occurred in quite similar habitats (Figs 19–24 boxplots). The analyses of niche parameters revealed that *N. veronensis* sp. nov. has a wider ecological tolerance compared to other similar species. The ecological niches of these species were largely overlapped with the exception of *N. dealpina*, which showed low optima for most of the environmental factors. *N. dealpina* was the sole species of the group with a quite distinct niche (Figs 25–30).



Figs 15–18. (15–17) SEM micrographs of *Navicula veronensis* sp. nov.; (18) *Navicula cincta* shown for comparison; (15–16, 18) outside view; (17) inside view. Scale bar 3 µm.

Diatom assemblage composition and main environmental determinants

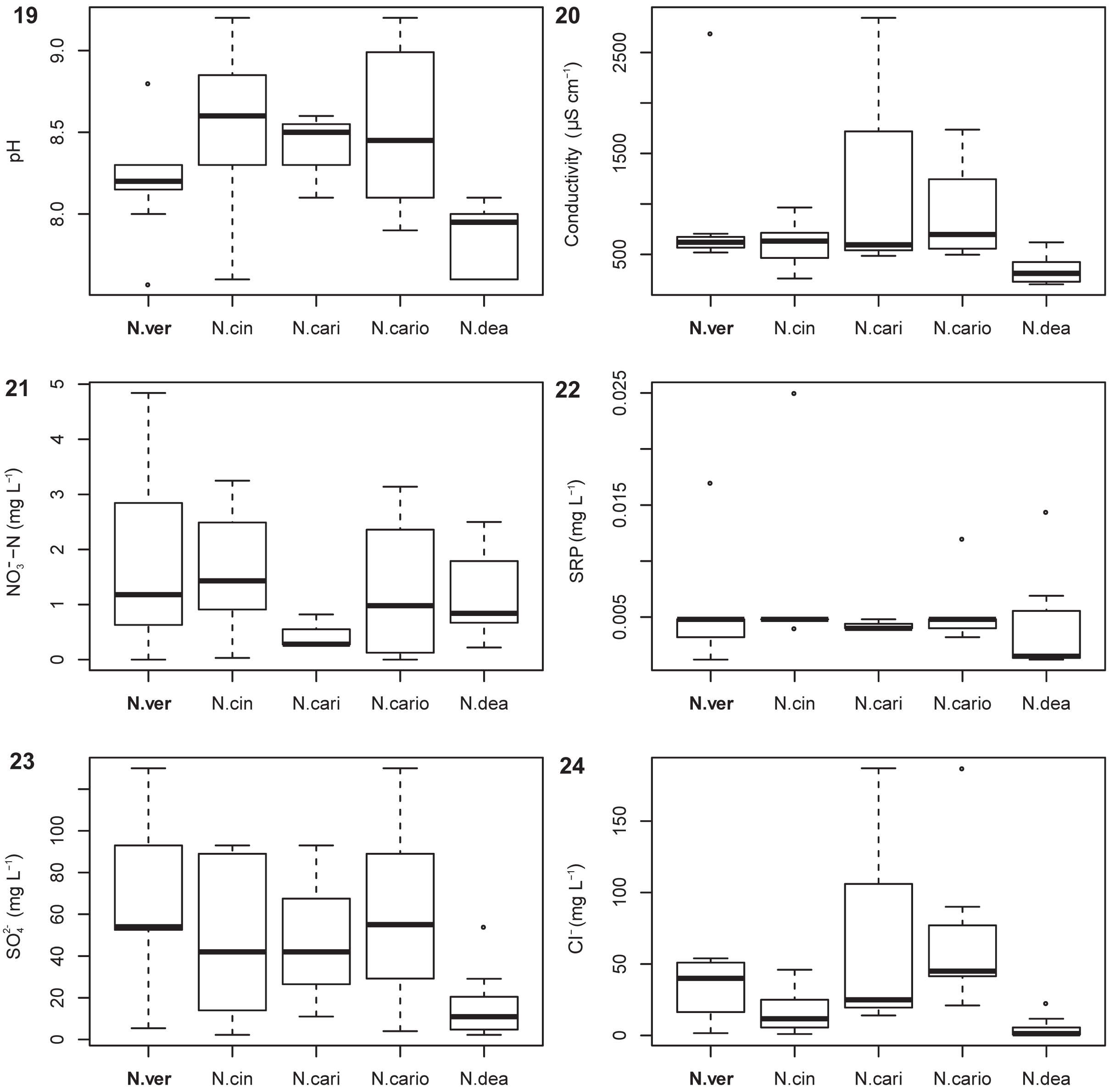
Overall, 24 samples of bryophytes, 21 of brushed lithic material, and 2 of macroalgae were examined. The analysis of diatoms (all substrata) of the springs studied allowed the identification of 139 taxa belonging to 40 genera (Supplementary Table S1). The 30 comparatively most abundant species are shown in Supplementary Fig. S1.

The taxa found include also some rare taxa, e.g. *Geissleria gereckei* CANTONATI et LANGE–BERTALOT (Fig. S1, 24) that occurred with a considerable proportion (4.5%), for this rare and never abundant species, in the epilithon of the Veraghi spring. *Gomphosphenia holmquistii* (FOGED) LANGE–BERTALOT (Fig. S1–25) is a species rarely recorded in lakes, reservoirs, and their outlets, was found thriving on lithic material in Val di Canova spring, where the outflow (overflow) of the capturing concrete basin was sampled.

After the stepwise model building, the following factors were used into the CCA model: discharge (F = 3.62, P = 0.001), nitrate (F = 2.05, P = 0.004), and impacts (F = 2.12, P = 0.002) (Figs 31–32). The model was highly significant (F= 2.60, P = 0.001), and explained 17.4% of the total variance, whilst the first two axes explained a total of 76.7% (axis 1 = 46.8%, axis 2 = 29.7%). The CCA diagrams (Figs 31–32) show that high concentrations of nitrates were found in impacted springs, and that e.g., *Navicula veronensis* sp. nov., *Nitzschia frustulum* (KÜTZING) GRUNOW in CLEVE et GRUNOW preferred such springs. The third important gradient, orthogonal to the first two ones, was discharge. Springs with high discharge were colonized by species like *Navicula antonii* LANGE–BERTALOT, *Nitzschia fonticola* (GRUNOW) GRUNOW in VAN HEURCK and *N. lacuum* LANGE–BERTALOT.

Table 1. Comparison between *Navicula veronensis* sp. nov. and morphologically similar species.

Navicula	***veronensis* sp. nov.**	***cincta***	***cari***	***cariocincta***	***dealpina***	***seibigiana***	***wiesnerii***
Valve length (µm)	18.8–40	14–45	(13)20–40	30–50	25–86	25–35	13–38
Valve width (µm)	4.2–6.6	5.5–8	5.5–8	5.5–7	8.0–12.0	5.5–6.5	4.5–6
Valve outline	linear–lanceolate, becoming linear–elliptical–lanceolate to elliptical at the end of the cell cycle	elliptic to lanceolate to linear–elliptic–lanceolate	lanceolate to linear–lanceolate to linear	linear–elliptic–lanceolate	linear–lanceolate to almost elliptical	elliptic– to linear–lanceolate	elliptic– to elliptic–lanceolate
Valve apices	becoming gradually wedge–shaped, finally obtusely rounded	obtusely rounded, never protracted	more or less wedge–shaped, from obtuse to almost acutely–rounded	narrowed to a wedge, obtusely–rounded, never protracted	obtusely wedge–shaped	often slightly–protracted, very–obtusely to almost broadly–rounded	obtusely–rounded
Central area	broadly rectangular or transapically elliptical or bow–tie–shaped (large specimens), somewhat variable and less broad in smaller ones	small, irregular	always transversely rectangular	large, transversely rectangular to elliptical	almost symmetrical, transversely rectangular	irregular, almost rectangular	small, outline variable
Striae around central area	2–4 striae are distinctly shortened on either side	only–slightly and irregularly shortened	several (in extreme cases only 1–2) shortened on either side	irregularly shortened	distinctly but irregularly shortened	1–2 shortened striae on either side	irregularly shortened
Striae (in 10 µm)	11–13	8–12	9–12	10–12	8–10	9–11	11.5–14
Number of lineolae (in 10 µm)	c. 50 (SEM)	40 (SEM, LM difficult to resolve)	32–40 (SEM, LM indistinct even with oblique lighting)	c. 30 (LM, difficult to resolve)	c. 26 (LM)	c. 36 (LM, difficult to resolve)	37–40 (SEM)
Striation pattern	rather strongly radiate in proximal parts, becoming abruptly convergent at the Voigt fault	strongly radiate in the middle, abruptly changing to convergent at the Voigt fault	radiate and somewhat curved in the centre, parallel to convergent some distance from the poles	strongly radiate, the distal 7–8 parallel to convergent	strongly radiate in the middle and appearing slightly sigmoid in shape, converget at the poles	strongly radiate in the middle, then abruptly parallel and convergent	radiate, convergent at the ends
Habitat and ecology	eutrophic, electrolyte–rich (sulphates) waters; periodically wet microhabitats	mostly in eutrophic, electrolyte–rich waters; periodically wet habitats	eutrophic waters with average to higher electrolyte content	mostly in oligo–, mesotrophic waters with average electrolyte content	oligotrophic, calcium–bicarbonate–rich springs and lakes	eutrophic waters	eutrophic, electrolyte–rich waters

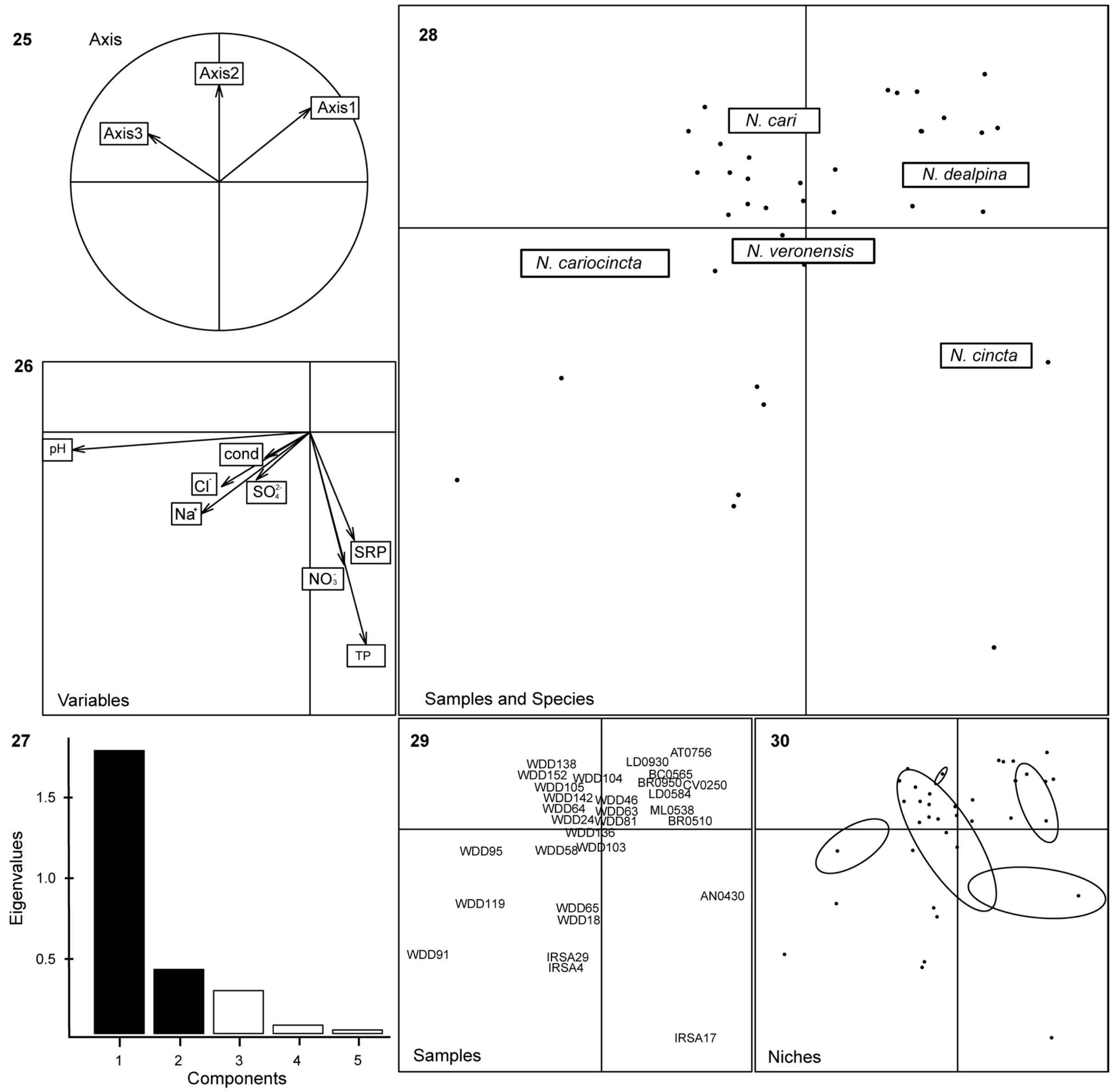


Figs 19–24. Box–and–whisker plots comparing the optima of *Navicula veronensis* sp. nov. and of allied taxa: (N.ver) *Navicula veronensis* sp. nov.; (N.cin) *Navicula cincta*; (N.cari) *Navicula cari*; (N.cario) *Navicula cariocincta*, (N.dea) *Navicula dealpina*.

DISCUSSION

Although signs of alteration due to human disturbance were evident, several rare taxa were observed, and even a diatom species new to science (*Navicula veronensis* sp. nov.) was described. The finding of new or rare species is most likely to be due to the high level of detail with which taxonomic studies were conducted (cf. WOJTAL 2013).

Three further species, that belong to the group of species around *Navicula cincta*, were described from central Europe, remarkably all of them from spring habitats. *Navicula domiciliensis* REICHARDT (2012) was described from a seepage spring in the Opalinuston spring horizon in Treuchtlingen–Bubenheim, Bavaria, Germany. It differs from *N. veronensis* by the smaller, clearly–unilaterally developed, central area, slightly lower stria density (10–11 instead of 11–13 in 10 µm), and less markedly radiate striae. *Navicula fontana* ŻELAZNA–WIECZOREK et LANGE–BERTALOT nom. prov. is still unpublished but was depicted by ŻELAZNA–WIECZOREK (2011). It was collected in a high–conductivity (400 µS cm^{-1}), alkaline spring in Central Poland, and differs from *N. veronensis* by the undifferentiated central area, the smaller width (3.8–4.8 instead of 4.2–6.6 µm), and proximal raphe endings shortly deflected to the same side of the valve. *Navicula sanctamargaritae* BEAUGER (BEAUGER et al. 2015), from an inland saline spring, differs by many features, the main ones being the small central area bordered by usually one (rarely 2–3) irregularly shortened striae, the much wider density range of the slightly–curved striae, and the proxi-



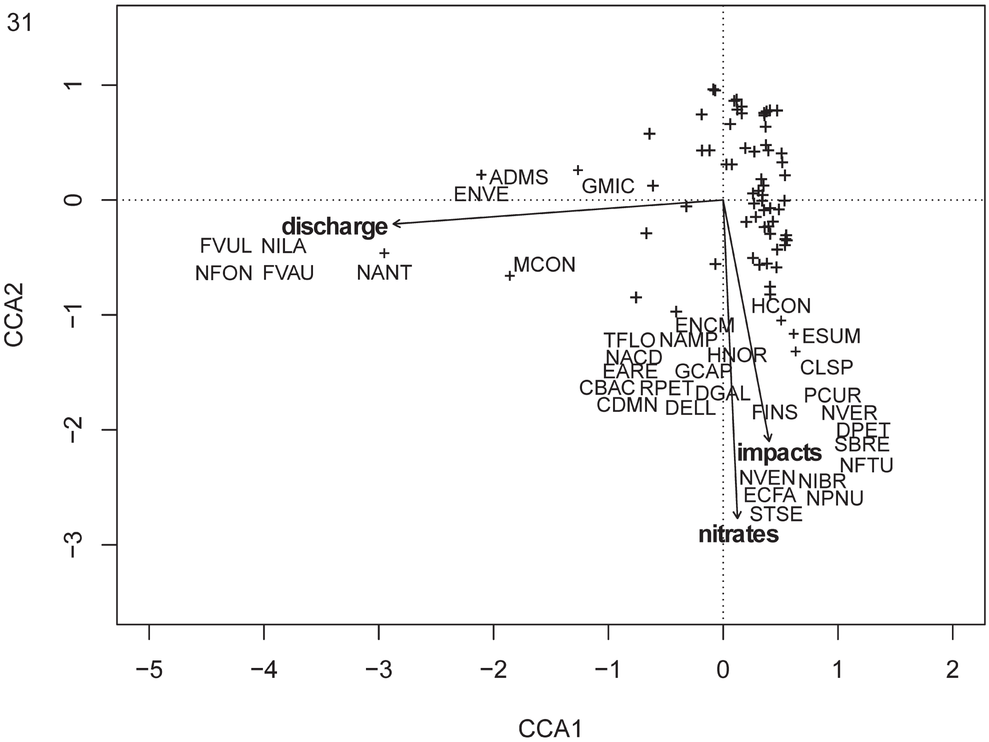
Figs 25–30. Niche parameters of *N. veronensis* sp. nov. and allied species: (25) projection of the first three PCA axes of the environmental variables on the first two axes of the OMI analysis; (26) canonical weights of environmental variables; (27) screen plot of the eigenvalues associated with each component; (28) distribution of the species on the first factorial plane of the OMI analysis. Crosses identify the position of species using the canonical weights of the environmental variables; (29) site labels for Fig. 28; 30. Centers of gravity of the species profiles (weighted average position of the species showed in Fig 28). In Fig. 29 „WDD“ and „IRSA“ samples (sites) are from the Cyprus_diatoms Project, other codes refer to the CRENODAT Project (more details in Cantonati et al. 2012a).

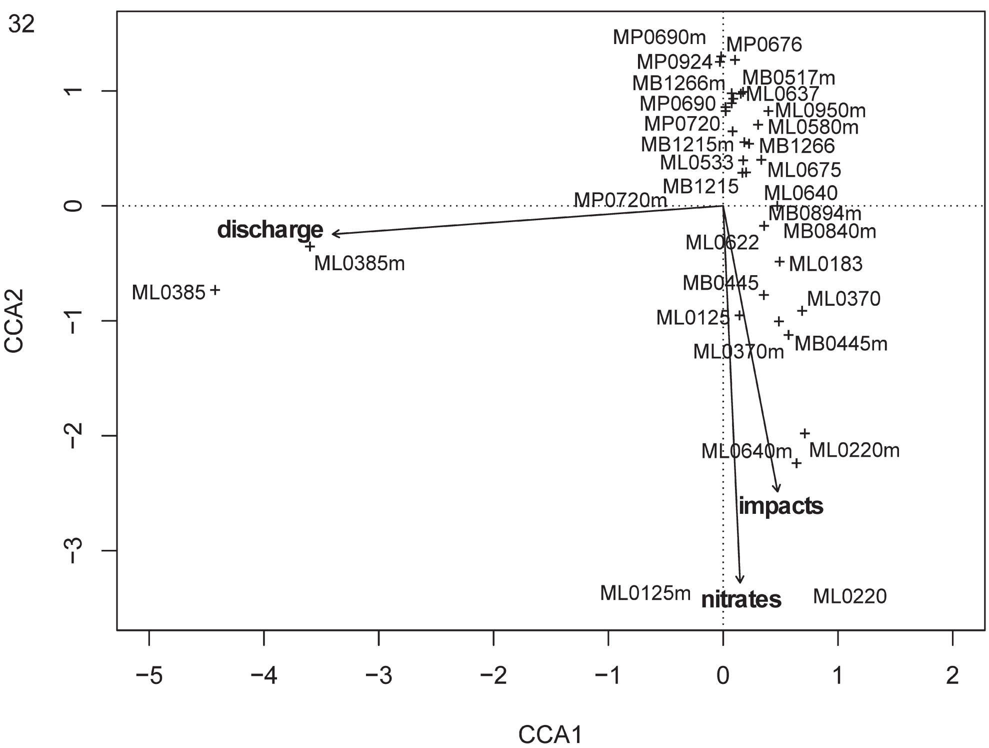
mal raphe endings clearly deflected towards the secondary side.

Navicula lauca Rumrich et Lange–Bertalot was described from a streamlet in the Chilean Andes (Rumrich et al. 2000), and is frequently reported from high–elevation freshwaters of Chile, Bolivia, and Argentina (Maidana et al. 2011). It differs from *Navicula veronensis* sp. nov. by the shape of the endings and of the central area.

N. veronensis sp. nov. was shown to be a species with clear preferences for alkaline waters, like the allied species considered in the present study. The restricted range of pH suitable for its colonization, indicated that it might be the most specialized species among those considered. In contrast, nitrate contents were somewhat variable, suggesting that the species can occur in a wide range of situations, though always in unpolluted waters. The niche breadth and niche position of the species revealed that the ecological preferences of *N. veronensis* sp. nov. don’t differ substantially from those of the allied species *N. cincta*, *N. cariocincta*, *N. cari*, and *N. dealpina.*

In general, the composition of diatom assemblages found in the springs studied shows analogies with recent studies on comparable environments (e.g., Żelazna–Wieczorek 2011; Wojtal & Sobczyk 2012;





Figs 31–32. CCA ordination diagram with environmental variables represented by arrows. Only species with score > |1| on the CCA axes were showed to avoid labels overlapping in the origin of the biplot. Few site labels were removed when completely overlapped. Species abbreviations (OMNIDIA codes): (ADMS) *Adlafia minuscula*; (CBAC) *Caloneis bacillum*; (CDMN) *Cymbella diminuta*; (CLSP) *Caloneis* sp.; (DELL) *Diploneis elliptica*; (DGAL) *Diadesmis gallica*; (DPET) *Diploneis peterseni*; (EARE) *Ellerbeckia arenaria*; (ECFA) *Encyonopsis falaisensis*; (ENCM) *Encyonopsis microcephala*; (ENVE) *Encyonema ventricosum*; (ESUM) *Encyonopsis subminuta*; (FINS) *Fallacia insociabilis*; (FVAU) *Fragilaria vaucheriae*; (FVUL) *Frustulia vulgaris*; (GCAP) *Gomphonema capitatum*; (GMIC) *Gomphonema micropus;* (HCON) *Humidophila contenta*; (HNOR) *Halamphora normannii*; (MCON) *Meridion constrictum*; (NACD) *Nitzschia acidoclinata*; (NAMP) *Nitzschia amphibia*; (NANT) *Navicula antonii*; (NFON) *Nitzschia fonticola*; (NFTU) *Nitzschia frustulum*; (NIBR) *Nitzschia bryophila*; (NILA) *Nitzschia lacuum*; (NPNU) *Nitzschia perminuta*; (NVEN) *Navicula veneta*; (NEVER) *Navicula veronensis* sp. nov.; (PCUR) *Psammothidium curtissimum*; (RPET) *Rossithidium petersenii*; (SBRE) *Surirella brebissonii*; (STSE) *Stauroneis separanda*; (TFLO) *Tabellaria flocculosa*. In the site (sample) codes the number is referred to the elevation of the spring, and the final "m" denotes a bryophyte ("moss") sample (more details in Table 1 in ANGELI et al. 2010).

Table 2. Results of the niche analysis of *Navicula veronesis* sp. nov. and allied species [(OMI) Outlying Mean Index, high values means high niche position (species occur in particular habitats); (Tol) tolerance, or niche breadth. Obs and Std.Obs are the observed and the standard deviation of the OMI index under a permuted distribution computed to test the statistical significance of the species marginality (marginality is defined as the ecological distance between the species optimum and the mean habitat within the background data)].

	OMI	**Tol**	**Obs**	**Std.Obs**	***P***
N. veronensis	0.497	0.877	0.497	–0.630	0.851
N. cincta	4.164	1.280	4.164	–0.042	0.207
N. cari	2.055	0.162	2.055	–0.507	0.774
N. cariocincta	4.506	0.667	4.506	–0.157	0.267
N. dealpina	2.824	0.065	2.824	3.213	**0.014**

Delgado et al. 2013; Wojtal 2013). The assemblages of the springs studied also show analogies with those of petrifying springs affected by nitrate enrichment (Denys & Oosterlynck 2015), which is consistent with the fact that the springs studied included limestone precipitating springs. The most characteristic algae (species involved in biocalcification processes) of LPS appear to be less sensitive to nitrate than to phosphate enrichment (Cantonati et al. 2016).

These springs, although impacted, still include in their diatom assemblages widely–distributed crenophilous species [e.g., *Caloneis fontinalis* (Grunow) A.Cleve, *Cymbella diminuta* (Grunow) Reichardt, *Odontidium mesodon* (Ehrenberg) Kützing, *Diploneis fontanella* Lange–Bertalot, *D. krammeri* Lange–Bertalot et Reichardt, *Eunotia arcubus* Nörpell–Schempp et Lange–Bertalot, *Fragilaria amphicephala* (Kützing) Lange–Bertalot, *Gomphonema elegantissimum* Reichardt et Lange–Bertalot, *Meridion circulare* (Greville) C.Agardh, *Rossithidium petersenii* (Hustedt) Round et Bukhtiyarova], mixed with species that are well–known indicators of nutrient enrichment [e.g., *Sellaphora nigri* (De Notaris) C.E.Wetzel et L.Ector, *Navicula veneta* Kützing, *Nitzschia frustulum*, *N. inconspicua* Grunow)] (cf. Cantonati 1998; Cantonati & Spitale 2009).

Besides other anthropogenic pressures (nitrates, artificial morphology of the site of emergence), an important driver is the low discharge, that is likely to cause extensive parts of the substrata to dry out during low flow. Accordingly, applying the moisture index of van Dam et al. (1994), 24.6% of the taxa were found to belong to categories 4+5, i.e. those including species occurring in temporarily dry places or outside water bodies. The large majority of the taxa (50.8%) were in category 3, i.e. taxa occurring in water bodies but regularly found also found in damp places (Angeli et al. 2010). Examples of well–known pseudaerial / euaerial species found in the springs studied are: *Adlafia minuscula* var. *muralis* (Grunow) Lange–Bertalot, *Diadesmis gallica* W.Smith, *Eunotia arcubus*, *Humidophila aerophila* (Krasske) Lowe et al., *H. contenta* (Grunow) Lowe et al., *H. paracontenta* (Lange–Bertalot & Werum) Lowe et al., *H. perpusilla* (Grunow) Lowe et al., *Luticola nivalis* (Ehrenberg) D.G. Mann. To this regard, also the finding of *Geissleria gereckei*, a species described from carbonate springs with extremely low discharge, is significant, in particular in the light of a recent work (Vidaković et al. 2016) that showed that this species can colonize also environments affected by nutrient enrichment, and that the most determinant driver of its distribution is strongly–fluctuating discharge causing the desiccation of large parts of the substrata within the wetted perimeter of springs and streams.

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Supplementary material

the following supplementary material is available for this article:

Fig. S1. LM micrographs of the 30 most abundant species.

Table 1**.** Diatom taxa list (all substrata) with frequency and maximum relative abundance.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

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