Nupela troglophila sp. nov., an aerophilous diatom (Bacillariophyta) from the Bossea cave (NW Italy), with notes on its ecology

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Abstract: *Nupela troglophila* sp. nov. is described from epilithic and epiphytic samples collected around the artificial lighting system in the Bossea cave, SW Alps (NW Italy). The morphological features of the new species are described and documented through light microscopy and scanning electron microscopy analysis. A comparison with morphologically similar taxa is provided. *Nupela troglophila* belongs to the group of the small, elliptical–lanceolate species of the genus *Nupela*. The key feature that distinguishes it from all the other taxa belonging to this genus is the shape of the distal raphe fissures, always laying on the valvar surface, strongly deflected on the same side, but never reaching the valve mantle. The ecological preferences of the most abundant diatom species are explored through the ecological optimum calculation and the Outlying Mean Index (OMI) analysis. Within the Bossea cave, *N. troglophila* seems to prefer humid and warm sites. The highest abundance was reached on wet walls characterized by low light conditions. *Nupela troglophila* was generally associated with *Humidophila pyrenaica* that showed the same pattern of distribution in the cave and similar relative abundance. On the contrary, *N. troglophila* showed its lowest densities when *Humidophila contenta* was abundant. Until now, *N. troglophila* has been recorded only in the type locality.

Key words: artificial lighting system, autecology, biofilm, diatom flora, Naviculaceae, show cave

Introduction

The genus *Nupela* Vyverman et Compère is morphologically closely related to the families of the Brachysiraceae D.G. Mann and Diadesmidaceae D.G. Mann, sharing with them features related with i) the shape of the areolae (transapically elongated) and ii) their density (few areolae composing each stria), iii) a ridge or a hyaline area at the junction of valve face and mantle, and iv) simple or inconspicuous proximal raphe fissures (Vyverman & Compère 1991). Despite these common features, the genus *Nupela* presents unique characters. Cells of *Nupela* are generally solitary and small in size, mainly less than 20 µm. They can be iso– or heterovalvar with regards to raphe development (Spaulding & Edlund 2008) showing, in the first case, two fully developed raphe slits, while presenting reduced or absent raphe on the other valve in the second case. Valves of *Nupela* can be slightly asymmetric with respect to the longitudinal axis and consequently, they can show an asymmetric central area. In connective view, valve faces are generally flat, but sometimes frustules can appear slightly bent. The external opening of the areolae, often covered by hymenes that erode during the sample treatment (Potapova et al. 2003), are transapically elongated, while small and rounded or oval in the internal view. The areolae form striae of variable length and extend as a single row on the valve mantle (Siver et al. 2007). Raphe slit can be straight or undulated. In external view, the terminal fissures are clearly bent on the same side at both poles and they extend down onto the apical mantle. Proximal raphe ends are simple and sometimes slightly bent toward the secondary side of the valve. In internal view, terminal raphe fissures are straight and a helictoglossa is present. Proximal raphe ends can be simple, hooked or T–shaped. Sometimes, proximal raphe ends can be flanked by depressions and small areolae visible at light microscopy (LM) as refractive spots. Until now, a total of 60 species belonging to the genus *Nupela* have been described (see Wojtal 2009 and Table S1 of this paper for a complete checklist).

The generitype species of *Nupela*, *N. giluwensis* Vyverman et Compère, was originally collected and described from shallow mountain lakes (tarns) on the Mount Giluwe (Papua New Guinea), characterized by peaty bottom, low conductivity and moderately low pH (Vyverman & Compère 1991). The genus *Nupela* is highly diversified in the Neotropics (Metzeltin & Lange–Bertalot 1998), where the genus is often present and abundant on periphytic communities in acid rivers of the Amazon basin (Wetzel et al. 2012), but several taxa have also been described from temperate zones (Potapova et al. 2003) or even from Arctic and Alpine regions (Potapova 2014). It has been detected in lentic (Siver et al. 2007; Bahls 2011; Mitrofanova & Genkal 2013) and in lotic freshwater ecosystems (Wojtal 2009), as well as in intermittent wet habitats as *Sphagnum* bogs (Kulikovskiy et al. 2009) and wet walls (Rushforth et al. 1984; Potapova 2011). The genus *Nupela* does not show substrate preferences and it has been found on rocks, wood and saturated soils (Potapova et al. 2003). In general, it is present in oligotrophic waters (Wojtal 2009; Mitrofanova & Genkal 2013), but exceptions occur. This is the case of *Nupela pardinhoensis* Bes, Torgan et Ector (in Bes et al. 2012), typical of moderate TP concentrations (ca. 0.1 mg.l^{-1}) (Bes et al. 2012), *N. carolina* Potapova et Clason in Potapova et al., abundant in rivers with 0.75 mg.l^{-1} P–TP (Potapova et al. 2003), and *N. wellneri* (Lange–Bertalot) Lange–Bertalot in Rumrich et al. and *N. lesothensis* (Schoeman) Lange–Bertalot in Moser et al. generally present in polluted rivers (Moser et al. 1998; Rumrich et al. 2000; Monnier et al. 2003). The genus *Nupela* does not show a pH preference, being present both in slightly acidic (Potapova 2013) and alkaline waters (Bahls 2011). Most species are found in low conductivity waters (Potapova et al. 2003; Mitrofanova & Genkal 2013; Potapova 2013; Sala et al. 2014), apart from *N. exotica* O. Monnier, Lange–Bertalot et J. Bertrand (conductivity = 1013 µS.cm^{-1}; Monnier et al. 2003) and *N. vitiosa* (Schimanski) Siver et P.B. Hamilton (conductivity = 92–206 µS.cm^{-1}; Siver & Hamilton 2005). In general, *Nupela* species show low relative abundances (ca. 1% for *N. potapovae* Bahls; ca. 4% for *N. decipiens* (Reimer) Potapova) (Bahls 2011; Potapova 2013), but also exceptions occur: for instance *Nupela carolina* reached 20% of relative abundance in Contentnea Creek (North Carolina) and *N. neglecta* Ponader, R.L. Lowe et Potapova in Potapova et al. represented 26.5% of the community in Coles Brook (New Jersey; Potapova et al. 2003). Recently, Wojtal (2009) suggested that light is not a limiting factor for *Nupela* populations growth. Despite this, from a literature review on diatom flora in caves (covering a temporal range from 1900 up to date), it is possible to conclude that till now only one species belonging to *Nupela* has been collected in subterranean environments, characterized by dim light (Falasco et al. 2014). Indeed, *N. thurstonensis* (Kaczmarska) Kulikovskiy, Lange–Bertalot et Witkowski was collected from wet walls in Thurston lava tube, Hawaii (Rushforth et al. 1984). The latter species was collected both on walls and bryophytes, both close to the entrance and the exit of the lava tube, but also near the artificial lighting system at the center of the tube.

In the framework of the CAVELAB project "From microclimate to climate change: caves as laboratories for the study of the effects of temperature on ecosystems and biodiversity", we performed the analysis of the diatom flora colonizing the wet and artificially illuminated walls of the Bossea cave, SW Alps (NW Italy). The Bossea cave has an important value both from the aesthetic and scientific point of view. It is a dynamic cave and the speleogenetic process is still in progress. Considering its high naturalistic value, the cave was equipped of two scientific stations for biological (opened in 1969) and physical (opened in 1980) studies (Peano & Fisanotti 1994). The Bossea cave was discovered in 1850 and, as first in Italy, opened to the public and equipped with artificial illumination in 1874. Even at a first sight, the walls in the cave are covered by green–brown patinas, especially in the areas surrounding artificial lights. The patinas are patchy and present a brighter color near the most illuminated part of the walls to become sparser and thinner moving towards the darkest zones. A very thick brown biofilm was observed at the "Monache" wall, a vast stone waterfall, artificially illuminated and never cleaned from the biofilm since the cave opened. In all the other parts of the cave, where the patinas are more evident, a cleaning procedure with bleach is carried out every year. The aim of this paper is to illustrate and formally describe a new *Nupela* species, highly frequent and abundant in the Bossea cave. Some first indications on its ecological requirements and distribution within the cave are provided.

Table 1. Environmental parameters: range of variation within the Bossea cave and ecological optima for *Diadesmis gallica*, *Humidophila contenta*, *H. pyrenaica* and *Nupela troglophila* in terms of light intensity (PAR), air humidity (Hmean), temperature (Tmean) and distance from the entrance (Distance.entrance).

	PAR (µmol.m^{-2}.s^{-1})	Hmean (%)	Tmean (°C)	Distance.entrance (m)
Cave range of variation	0.2–78.6	51.5–98.5	6.5–10.2	10–427
Optimum *Diadesmis gallica*	13.36	79.39	9.04	91
Optimum *Humidophila contenta*	19.13	66.71	8.91	256
Optimum *Humidophila pyrenaica*	4.15	75.69	9.03	205
Optimum *Nupela troglophila*	3.24	78.96	9.25	176

Table 2. Niche parameters of selected diatom species in Bossea cave [(Inertia) variance or weighted sum of squared distances to the origin of the environmental axes; (OMI%) percentage of variability of outlying mean index (marginality), or the deviation of a particular species' distribution from the overall mean habitat conditions (origin of outlying mean index axes), described by the environmental variables; (TOL%) percentage of variability of tolerance index, which is analogous to "niche breadth" or spatial variance of an organism's "niche" across the measured environmental variables – a function of all sampling sites with which the species is associated; (RTOL%) residual tolerance (%); p–value = frequency based on number of random permutations (out of 9,999) that yielded a higher value than the observed outlying mean index ($p < 0.05$ indicates a significant influence of the environmental variables for a species)].

	INERTIA	OMI%	TOL%	RTOL%	p–value
Diadesmis gallica	2.84	20.4	10.8	68.8	0.26
Humidophila contenta	6.57	14.8	30.0	55.2	0.01*
Humidophila pyrenaica	3.94	4.0	10.4	85.6	0.21
Nupela troglophila	3.27	11.7	22.6	73.1	0.19

Material and Methods

Study area. Bossea cave is part of the Site of Community Importance (SCI) IT1160026 (Faggete di Pamparato, Tana del Forno, Grotta delle Turbiglie e Grotte di Bossea) under the managing authority of the Marguareis Natural Park at Frabosa Soprana (836 m a.s.l.) (NW Italy). Bossea cave (44°14'31"N, 07°50'27"E, Corsaglia Valley near Frabosa Soprana, Piemonte region, Italy) is 2800 m long and is structured into two levels (for a total difference in height of 200 m). The lowest part goes from the entrance to the Ernestina Lake. It is characterized by a first corridor (ca. 100 m long), used as location for contemporary art expositions, followed by a succession of big rooms and walls with magnificent speleothems; a river is flowing at the bottom. This touristic part of the cave is artificially illuminated during the visits. The visitors' pathway ends with a waterfall flowing out from a small tunnel in the wall. The second part of the Bossea cave is not illuminated and scarcely frequented; it develops horizontally behind the waterfall and consists of a series of canyons carved by the river.

Sampling design. We randomly identified sampling sites distributed all along the touristic pathway, and characterized by the presence of incandescent light. The lighting system is manually regulated and switched on only during the visits. A preliminary mapping of the primary productivity was performed by means of the bbe BenthoTorch® fluorometer probe, developed by 'bbe Moldaenke GmbH' for lithic substrates (Schwentinental, Germany). This instrument quantifies and classifies algal chlorophyll–*a* through the analysis of red light fluorescence emitted from the excitation at different wavelengths. In this way, the bbe BenthoTorch provided chlorophyll–*a* concentrations of the three main photosynthetic groups colonizing the walls: diatoms, cyanobacteria and green algae. Three replicates per each site were performed. For diatom taxonomical analyses we selected only those sites in which the diatom chlorophyll–*a* concentration reached at least 0.5 µg.cm^{-2}. Following these criteria, 31 diatom samples were collected and treated following the standard procedure (UNI EN 13946:2005). Epilithic, and in some cases, epiphytic diatom samplings were performed by means of a toothbrush and preserved with ethanol (final concentration 50%). Parts of the samples were cleaned from ethanol with following centrifugations. Afterwards, the samples were digested by adding 37% H_2O_2 on a heater (ca. 80 °C for about two hours). The reaction was completed by addition of HCl (1N). Samples were cleaned again with centrifugations and every time rinsed with distilled water. Cleaned diatom material was mounted in Naphrax® for LM analyses.

For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered and rinsed with additional deionized water through a 3–µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were mounted on aluminium stubs and coated with platinum using a BAL–TEC MED 020 Modular High Vacuum Coating System for 30 s at 100 mA. An ultra–high–resolution analytical field emission (FE) scanning electron microscope Hitachi SU–70 (Hitachi High–Technologies Corporation, Tokyo, Japan) operated at 5 kV and 10 mm distance was used for the analysis. SEM images were taken using the lower (SE–L) detector signal.

In each sampling site we measured the following environmental parameters: distance from the entrance (m), distance from the light (m), photosynthetically active radiation (PAR; µmol.m^{-2}.s^{-1}) and luminous emittance (LUX; lux). PAR was detected by means of a DELTA OHM S.r.l. DO 9721 probe, LUX were detected by means of a photometric probe LP 471 Phot. Temperature (°C) and relative air humidity (%) were monitored by means of dataloggers (portable meters DO9847 Delta OHM S.r.l.), located near each wall point, at least for one week and set to record environmental variations every hour. Dataloggers in sites F17 and F24 were lost, for this reason they were excluded from the statistical analyses.

Autecology. Considering that sampling points are located inside a single cave, the ecological characterization of the new *Nupela* species was conducted at microhabitat level, with respect to the following ecological parameters: light (PAR), mean temperature (Tmean), mean humidity (Hmean) and distance from the entrance (Distance.entrance), which are expected to be the main drivers of diatom distributions in caves (Hoffmann 2002; Poulíčková & Hašler 2007; Roldán & Hernández–Mariné 2009; Czerwik–Marcinkowska & Mrozińska 2011).

The autecology of the new *Nupela* species was analyzed by the mean of two different approaches: the ecological optimum and the Outlying Mean Index analysis

(OMI). The former method measures the optimal conditions for each environmental parameter, while the second method defines the ecological niche occupied by the species.
The ecological optimum was calculated for each selected environmental variable in accordance with the weighted average method proposed by Birks et al. (1990):

$$VOp = \frac{\sum Vp_i * A_i}{\sum A_i}$$

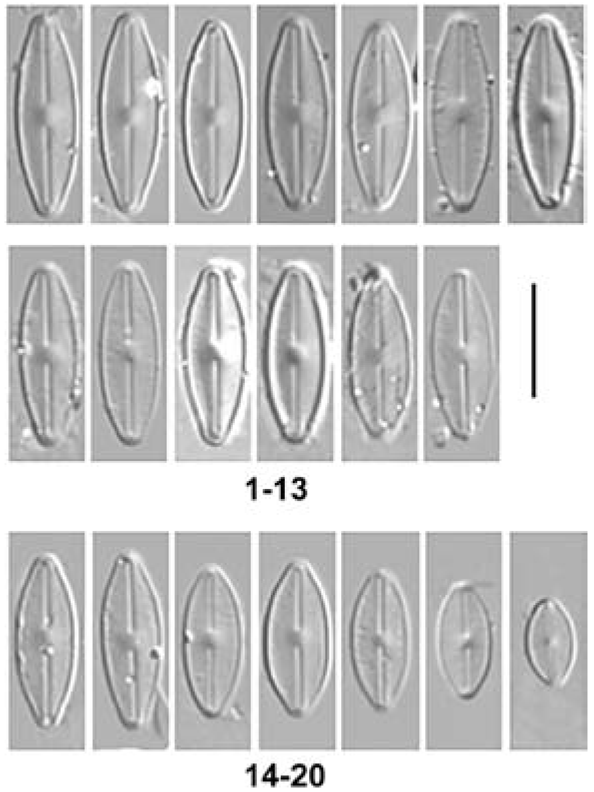
where VOp is the optimum value for the parameter p, Vpi is the value of the parameter p at point i and Ai is the relative abundance of the species at point i.
The ecological optimum was calculated not only for the new *Nupela* species, but also for other three frequent diatom species.

The Outlying Mean Index analysis (OMI) (Doledec et al. 2000) was used in order to explore the amplitude of the ecological niche of the new *Nupela* species in relation to the other dominant species of the cave. The OMI analysis is a two–table ordination technique which places the sampling units in a multidimensional space as a function of the environmental parameters. The distribution of species in this hyperspace represents their realized niches and both marginality and tolerance can be evaluated. The marginality corresponds to the distance between the mean habitat conditions used by a species and the mean habitat conditions across the study area, while the tolerance represents the niche breadth, which means the amplitude in the distribution of each species along the sampled environmental gradients.

For each species, the statistical significance of the marginality was tested by a Monte Carlo test with 9999 random permutations. The frequency of random permutation with values greater than the observed marginality was used as an estimated probability of rejecting the null hypothesis that the environmental gradient does not constrain species distribution. The OMI analysis was performed via the function "niche" in the package ade4 (Dray & Dufour 2007) for the R software (R Core Team 2013), considering the same environmental parameters and species as for the ecological optima.

Table 3. Environmental variables normed scores on the two axes of OMI analysis [(CS1) first axis; (CS2) second axis)].

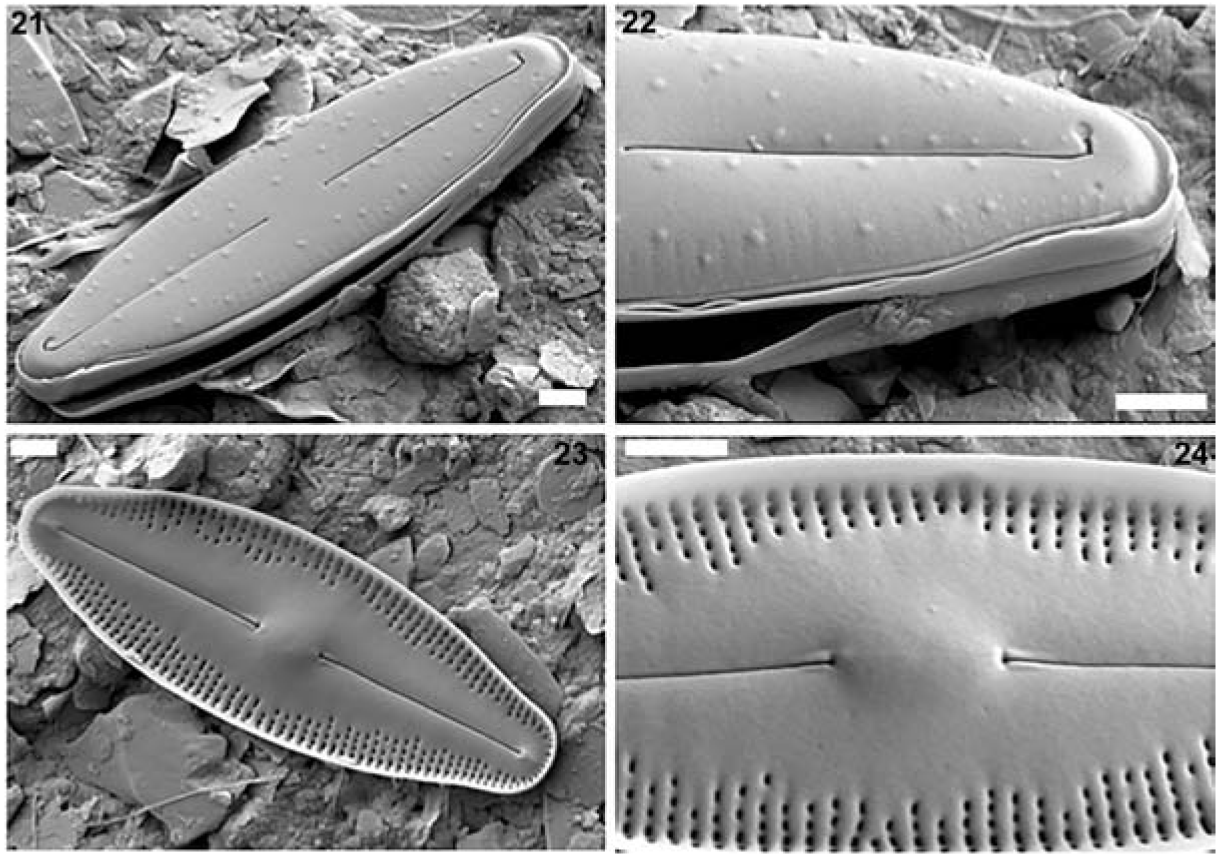
	CS1	CS2
PAR	0.6570644	0.61645780
Hmean	–0.5939306	0.21250915
Tmean	–0.2437798	–0.07832491
Distance.entrance	0.3950750	–0.75411196



Figs 1–20. Light microscope images of *Nupela troglophila* from sample 11Y (Holotype BR–4391) (1–13) and 4C (14–20). Scale bar 10 µm.

Results and Discussion

***Nupela troglophila* Falasco, C.E. Wetzel et Ector sp. nov. (Figs 1–30)**
Description
LM observations (Figs 1–20): Frustules isovalvar. Valve lanceolate to elliptical–lanceolate, presenting an oval outline in the smallest individuals. Length 5.0–13.1 µm, width 3.1–4.6 µm. Poles rounded or showing slightly protracted apices. Raphe slits straight, distance between the proximal ends slightly variable within the same population. At LM, fine structure of the valvar surface and ornamentations are not detectable.
SEM observations (Figs 21–30): Externally, outer openings of the areolae generally covered by hymenes, that, in some cases, can be partially eroded as a consequence of the treatment process. Proximal raphe fissures are straight and simple, sometimes slightly expanded. Distal raphe fissures are strongly deflected on the same side and terminate on the valve surface, never reaching the mantle. Internally, striae are generally short and composed of 3–4 small areolae, usually rounded or oval. In general, areolae occupy ⅓ of each hemivalve, leaving a wide longitudinal area. Central striae are slightly radiate, becoming parallel towards the apices, 42–50 in 10 µm. The pattern of striation is not constant, and sometimes striae appear wavy and disorganized in the middle portion of the valve. Proximal raphe ends are straight, generally simple, sometimes "T–shaped". Distance between the central fissures is generally quite constant within a population, ranging from 0.75 to 1.69 µm. Distal raphe



Figs 21–24. Scanning electron microscope images (21–22 external view; 23–24 internal view) of *Nupela troglophila* from sample 11Y (Holotype BR–4391). Detail of the valve apices in the external view (22) with distal raphe fissures strongly deflected on the same side and never reaching the mantle. Detail of the proximal raphe fissures in internal view, straight and simple (24). Scale bars 1 µm.

fissures are straight, terminating in a small and linear helictoglossa.

Etymology: The epithet of the new species refers to the troglodyte habitat in which it was collected; in ancient Greek: τρώγλη, troglê = cavern.
Type locality: NW Italy, SW Alps, Piemonte Region, Cuneo Province, Corsaglia Valley, Frabosa Soprana, Grotta di Bossea – Bossea cave. 44°14'31"N, 07°50'27"E. Epilithic samples collected on 26th November 2012, on the "Monache wall" by Dr. Elisa Falasco.
Holotype (designated here): BR–4391 (Botanic Garden Meise, Belgium).
Isotypes (designated here): BM–101 774 (the Natural History Museum, London), BRM–ZU9/85 (Hustedt Collection, Bremerhaven, Germany).

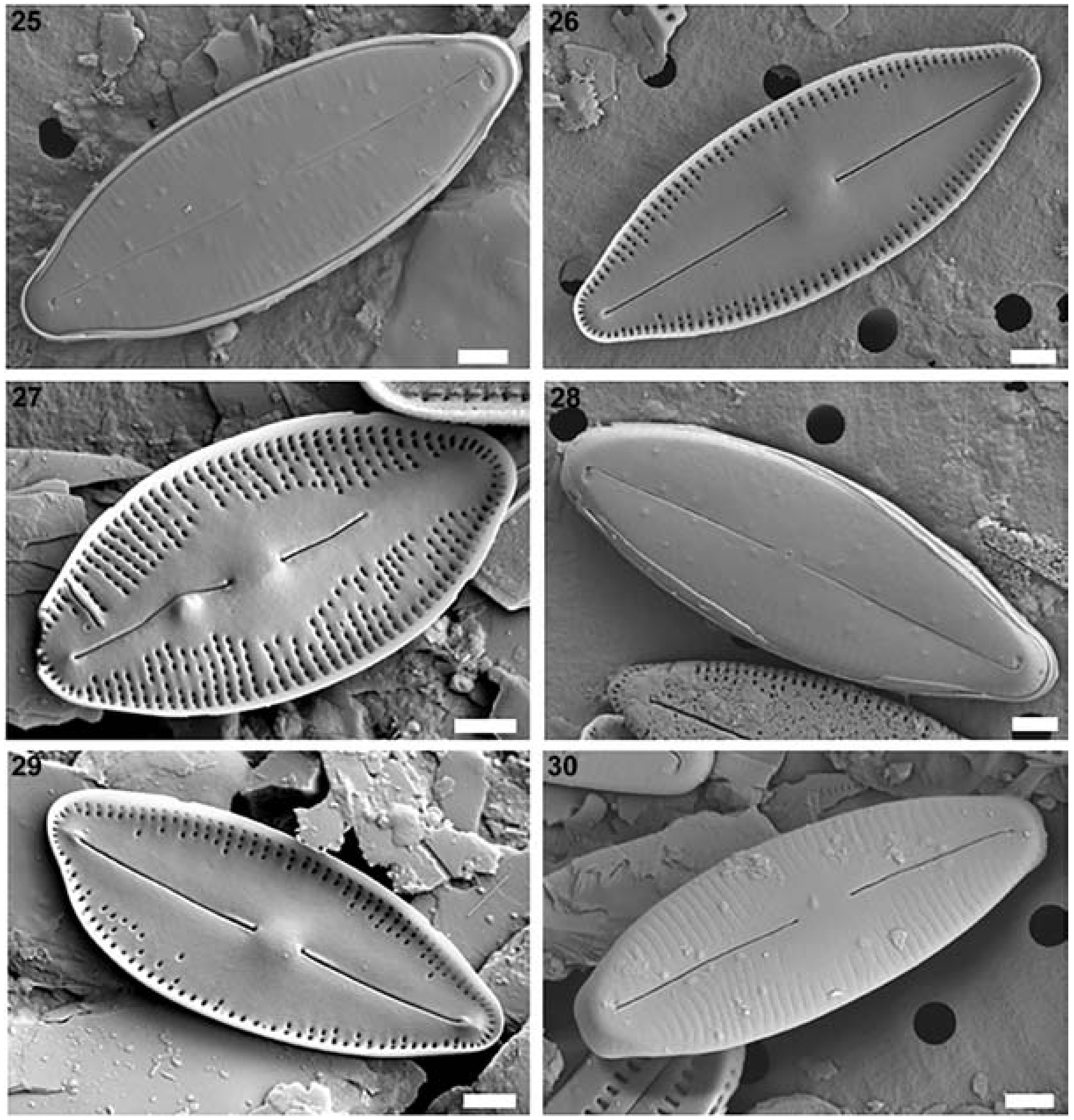
Ecology and associated taxa: *Nupela troglophila* was collected in different sections of the Bossea cave, both close to the main entrance (corridor) and in the deepest sections, on artificially illuminated walls. Environmental parameters and corresponding relative abundance of *N. troglophila* in the whole diatom communities are shown in Table S2.

Nupela troglophila shows a preference for wet walls, where a thin biofilm of water moistens the surface, even if it was also detected on dry sites. It is mainly epilithic, but it was also recorded on mosses and ferns in the corridor. The environmental optima for *N. troglophila* are shown in Table 1.

The range of variation of environmental parameters within the Bossea cave overlaps with the *Nupela troglophila* ones. The new species is not limited by light intensity; indeed its optimum shows low values of PAR and LUX. It does not seem to be limited by temperature, with an optimum of 9.25 °C and it does not require high air humidity.

Compared to other studies (Falasco et al. 2014) the flora of Bossea is not very rich, but the species collected from its walls are very peculiar and interesting. In total, 23 taxa were identified, so as the corresponding teratological forms, that were observed and recorded (see Table S3).

Most of the recorded species are considered typical of subaerial environments and do not present a limitation in terms of light. The most abundant and widely distributed species belong to the genus *Humidophila* R.L. Lowe et al. (Lowe et al. 2014). Even from a first analysis at LM, it appeared clear that co–



Figs 25–30. Scanning electron microscope images of *Nupela troglophila* from sample 32X (25 external view; 26 internal view) and example of teratological forms, respectively from samples 32B, 32X, 4C and 28Y. Scale bars 1 µm.

occurrence of different taxa existed. In particular, from the analysis of the relative abundances, it was possible to observe a similar trend for *N. troglophila* and *Humidophila pyrenaica* (Lange–Bertalot et Werum) R.L. Lowe et al. in terms of distribution in the cave and relative abundance within the communities. On the opposite, the relative abundance of *N. troglophila* showed maximum values in those samples in which the percentage of *Humidophila contenta* (Grunow ex Van Heurck) R.L. Lowe et al. was minimal, and *vice versa*. These observations were statistically confirmed by the niche analysis, performed on these three taxa.

The OMI analysis results are reported in Table 2. The first two axes of the OMI analysis were selected. They accounted for 96.4% (73.7% for the first and 22.7% for the second axis) of the total explained variability. The first axis is negatively correlated with mean humidity and mean temperature, while it is positively correlated with light and distance from the entrance. The second axis is negatively correlated with the distance from the entrance and positively correlated with light, while its relationship with mean temperature and mean humidity is very weak (Table 3 and Fig. 31). The OMI analysis revealed different levels of niche overlap/differentiation among the four species. Figure 31 shows that the niche of *N. troglophila* overlaps with the niche of *H. pyrenaica* as both seem to prefer humid and warm sites with low light intensities. *Humidophila*

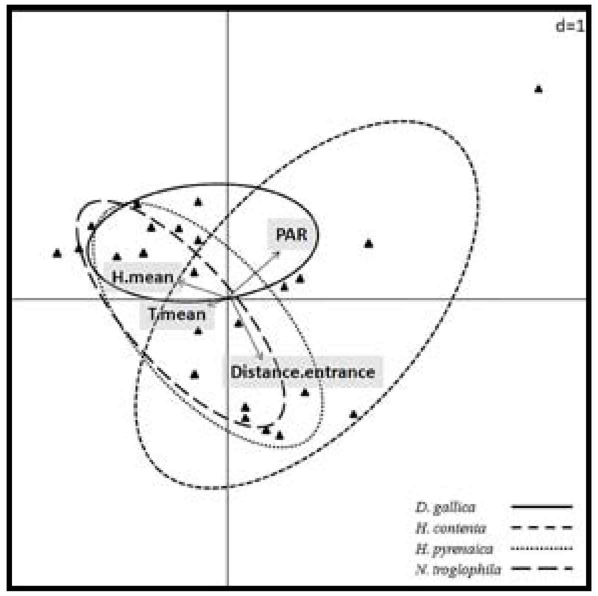


Fig. 31. Projection of environmental variables on the axis of OMI analysis and representation of ecological niches of the four analyzed species .

contenta shows opposite ecological preferences in relation to mean humidity and mean temperature, thus being found in colder and drier sites, but it seems not to have particular preferences for distance from the entrance and light intensity. As shown from the graph in Fig. 31 and in Table 2, *H. contenta* is the most tolerant species and it is the only species whose mean habitat requirements significantly differ from the mean habitat values of the Bossea cave (Monte Carlo permutation test: p = 0.03). *Diadesmis gallica* W. Smith is mainly found in sites with high light intensities and far from the cave entrance, while it shows no particular preference for temperature and humidity.

The total percentage of teratological forms is quite high, reaching a peak of 9.9 at the site close to the river (see sample 2X in Table S2). With the exception of sample F4, the highest percentages (> 2% of teratological forms) were found in the inner part of the cave.

Distribution: Until now only found in the type locality (Bossea cave) in Italy.

Remarks – Several *Nupela* species present morphological affinities with *N. troglophila.* A detailed morphometric and morphological comparison with the most similar taxa is shown in Table S4.

Despite the morphological and morphometric affinities, *N. neglecta* is distinguishable from *N. troglophila* even at LM, since strongly heterovalvar; indeed raphe slits on the convex valve of *N. neglecta* are distinctly shorter than on the concave valve. On the contrary, *N. troglophila* is always isovalvar. *Nupela matrioschka* Kulikovskiy, Lange–Bertalot et Witkowski differs from *N. troglophila* by the valvar shape (oval and never showing protracted apices) and by the larger width of the valves (4.3–6.3 vs 3.1–4.6 µm). *Nupela exotica* and *N. carolina* resemble *N. troglophila* both for shape and morphometric characters. The features distinguishing these taxa are clearly detectable only under SEM: shape of the distal raphe fissures (with typical *Nupela* arrangements in *Nupela exotica* and *N. carolina*) and shape of the longitudinal area (narrow and straight in *Nupela exotica* and *N. carolina*, while large in *N. troglophila*). *Nupela thurstonensis* is very similar to *N. troglophila* both for ecology and morphology. Morphologically, both of them are isovalvar, but *N. thurstonensis* shows differences with *N. troglophila* in terms of striae density [(30)35–45 vs 42–50 in 10 µm] and in the shape of the longitudinal area, narrow and linear in *N. thurstonensis.*

Conclusions

Nupela troglophila belongs to the group of the small, elliptical and elliptical–lanceolate species of the genus *Nupela*. The main characteristic feature of *N. troglophila* is the shape of the distal raphe fissures, always laying on the valvar surface, strongly deflected on the same side, but never reaching the valve mantle. This morphological character distinguishes *N. troglophila* from all the other species belonging to the genus *Nupela*.

Nupela troglophila could be confused with *N. thurstonensis* both for ecology and morphology. Indeed, *N. thurstonensis* was found and described in the Thurston lava tube, Hawaii (Rushforth et al. 1984), highlighting its affinities for the aerial habitats and the ability to grow also under natural and artificial light system.

In this study, we found a rather high percentage of teratological forms of *N. troglophila*. Indeed, diatom teratological forms in unstable microhabitats are quite common (Falasco et al. 2009). For instance, Rushforth et al. (1984) noticed abnormalities in the horse–shoe area (or "sinus") in *Planothidium lanceolatum* (Brébisson ex Kützing) Lange–Bertalot collected from walls in lava tubes. The same authors found some atypical morphological characters in several populations collected in this same site, if compared to the classical species descriptions. This is the case of *Rossithidium pusillum* (Grunow) Round et Bukhtiyarova, *Encyonema minutum* (Hilse in Rabenhorst) D.G. Mann, *Eunotia praerupta* Ehrenberg, *E. tenella* (Grunow) Hustedt, *Adlafia bryophila* (J.B. Petersen) Gerd Moser, Lange–Bertalot et Metzeltin, and *Pinnularia leptosoma* (Grunow) Cleve. Deformed valves and irregularities in the distribution of pores and spines were already observed in *Diadesmis gallica* and related to unstable environmental conditions, such as wide temperature oscillations, moisture and light conditions (Lund 1945, 1946; Granetti 1978).

The analyses of the samples collected from walls around artificial lamps in the Bossea cave revealed a very peculiar flora, with diatom species dominating the community mostly unknown. Potential new taxa belonging to the genera *Achnanthidium* Kützing and *Sellaphora* Mereschkowsky were observed during the sample analysis and they will be described in further papers. A deeper knowledge of the flora inhabiting this cave and its colonization pattern will play a key role for the elaboration of specific management guidelines and devices.

Acknowledgements

This contribution is part of the CAVELAB project "From microclimate to climate change: caves as laboratories for the study of the effects of temperature on ecosystems and biodiversity", funded by Compagnia di San Paolo and University of Turin.

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Supplementary material

the following supplementary material is available for this article:

Table S1. Ecology and distribution of 14 new *Nupela* species additional to the list of Wojtal (2009).

Table S2. Environmental parameters and corresponding relative abundance of *Nupela troglophila* (normal and teratological cells) in the analyzed communities.

Table S3. Checklist of the diatom species and corresponding distribution on cave walls in the Bossea cave.

Table S4. Comparison of the small–celled *Nupela* species. Data on *Nupela troglophila* are from the present study. Data on *Nupela astartiella*, *N. carolina*, *N. exotica*, *N. frezelii*, *N. matrioschka*, *N. neglecta*, *N. pardinhoensis*, *N. thurstonensis* and *N. vyvermanii* are taken from the original description.

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

Received September 17, 2014
Accepted November 4, 2014