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 (Siever 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 Woetal 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
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); determined TOL% percentage of variability of tolerance index, which is analogous to “niche breadth” or spatial variation of an organism’s “niche” across the mean environmental conditions; (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.]

<table>
<thead>
<tr>
<th>Species</th>
<th>INERTIA</th>
<th>OMI%</th>
<th>TOL%</th>
<th>RTOL%</th>
<th>p–value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diadesmis gallica</td>
<td>2.84</td>
<td>20.4</td>
<td>10.8</td>
<td>68.8</td>
<td>0.26</td>
</tr>
<tr>
<td>Humidophila contenta</td>
<td>6.57</td>
<td>14.8</td>
<td>30.0</td>
<td>55.2</td>
<td>0.01*</td>
</tr>
<tr>
<td>Humidophila pyrenaica</td>
<td>3.94</td>
<td>4.0</td>
<td>10.4</td>
<td>85.6</td>
<td>0.21</td>
</tr>
<tr>
<td>Nupela tropoglopha</td>
<td>3.27</td>
<td>11.7</td>
<td>22.6</td>
<td>75.1</td>
<td>0.19</td>
</tr>
</tbody>
</table>

first indications on its ecological requirements and distribution within the cave are provided.

**Material and Methods**

**Study area.** Bossea cave is part of the Site of Community Importance (SCI) IT11600026 (Faggette di Pamparato, Tana del Forto, Giotta delle Turbiglie and Grotte di Bossea) under the managing authority of the Maresurie Natural Park at Frasosa Suprap (836 m a.s.l.) (NW Italy). Bossea cave (44° 35' 12.7'' N, 9° 25' 42.3'' E), Corallina Valley near Frasosa Suprap, Piemonte region, is 2800 m long and is structured into two levels (for a total difference in height of 200 m). It consists of a vast stone waterfall, artificially illuminated and distributed all along the touristic pathway, and characterized by the presence of incandescent light. The lighting system is performed by means of the bbe BenthoTorch® fluorometer (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 20 m. 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 nm 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 determined by means of a DELTALOH S.DI 09721 probe, LUX were determined 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 variables every hour. Dataloggers DO9847 & MED 020 Modular High Vacuum Coating System were leased for the experiment, 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 (°C), mean emittance (LUX) and distance from the entrance (Distance,entrance). These parameters were expected to be the main drivers of diatom distributions in caves (HUSSMAN 2002; PROTHERoe & ROUSSET; ROUSSET & BRUNEAU–MARIN 2009; CEBROWSKA–MARCINIAWSKA & MOŻDZIŃSKI 2011).

The ecology of the new Nupela species was analyzed by the mean of two different approaches: the ecological optimum and the Outlying Mean Index analysis.
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) (Dolédec 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 optimum.

**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. Polys 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 valve 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 ½ 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 striaation 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 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: τρωγλη, troglê = cavern.

**Type locality:** NW Italy, SW Alps, Piemonte Region, Cuneo Province, Corsaglia Valley, Frabosa Soprania, 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. The range of variation of environmental parameters within the Bossea cave overlaps with the *N. 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.

**Staining and observation:** Whole mount of the *N. 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.

**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 Biou et al. (1998):

\[
V_{p} = \frac{\sum p_{i} \cdot A_{i}}{\sum A_{i}}
\]

where \(V_{p}\) is the optimum value for the parameter \(p\), \(A_{i}\) is the value of the parameter \(p\) at point \(i\) and \(A_{i}\) is the relative abundance of the species at point \(i\).
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 Weum) 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* (Grnov ex Van Helckx) 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 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. Smitt 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* Kulikovsk & Lange–Bertalot et W. Smith 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 *N. exotica* and *N. carolina* and *N. troglophila*) and shape of the longitudinal area (narrow and straight in *N. 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 striate 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 (Rushfort 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, Rushfort et al. (1984) noticed abnormalities in the horse-shoe area (or “sinus”) in *Planothidium lanceolatum* (Brewson ex Keyzing 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* (Grnov) Round et Bukhtiyarova, *Encyonema minutum* (Huse in Ramsdorff) D.G. Mann, *Eunotia praerupta* Ehmcke & E. tenella (Grnov) Hustedt, *Adlafia hyspyala* (J.B. Püttem) Gerz Moser, *Lange–Bertalot et Metzeltin* and *Pinnularia leptosoma* (Grnov) Cleve. Deformed valves and irregularities in the distribution of protoperithecia were already observed in *Diadesmis gallica* and related to unstable environmental conditions, such as wide temperature

![Fig. 31. Projection of environmental variables on the axis of OMI analysis and representation of ecological niches of the four analyzed species.](image-url)
Acknowledgements

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