Aerophytic diatoms from caves in central Moravia (Czech Republic)

Subaerické rozsivky jeskyní střední Moravy (Česká republika)

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This paper describes the first study of the diatom assemblages in caves in the Czech Republic. The study focused on subaeric habitats: rock faces within caves, walls at cave entrances and "lampflora" assemblages, in three cave systems, Mladeč, Javoříčko and Zbrašov, all in central Moravia. The morphological and cytological variability, ecology and life strategies of diatoms were studied in fresh samples, in Naphrax preparations, in cultures grown on agar plates and in monoclonal cultures. A total of 22 diatom species was identified, mostly aerophytic species and tolerant of low light intensities. *Luticola* and *Diadesmis* species complexes are discussed. Taxa *D. gallica* and *L. paramutica* var. *binodis* are new for the Czech Republic. Sexual reproduction was observed in *L. mutica* and *Orthoseira roeseana.*

Keywords: cave entrances, cytology, diatoms, ecology, lampflora, morphology, reproduction

Introduction

It has long been known that damp limestone surfaces often have visually conspicuous growths of cyanobacteria and algae (Golubić 1967). Aerophytic cyanobacteria and algae are particularly influenced by surface structure, temperature, light and moisture conditions (Pentecost 1992). Dry conditions permit growth of only the most desiccation-tolerant phototrophs, which include many cyanobacteria (Potts 1994). The representation of green algae and diatoms increases with increasing moisture. However, most caves, at least in Europe, are damp and the walls at the entrance covered with a green algal felt. At the entrance of limestone caves and on surfaces around electrical lights, cyanobacteria compete for light with other algae, bryophytes and ferns, but in the deepest recesses of caves they are usually the sole phototrophs (Round 1981). Remarkably little has been published on the environment of Czech caves (Dusan 1994, Kaštovský 1997, Balák et al. 1999) despite the popularity of caving as a sport. This contrasts with other European countries where the cave flora is better known (Maheu 1906, Palik 1964, 1966, Claus 1965, Mason-Williams & Benson-Evans 1967, Pentecost & Zhang 2004). Some caves have also been investigated by diatomists (VanLandingham 1965, 1966, 1967, Carter 1971, St. Clair & Rushforth 1976, Kashima et al. 1987).

This study reports the results of a study on the diatom flora of caves in central Moravia (Czech Republic), which focusses on the morphological and cytological variability of the diatoms and associated taxonomical problems.

Material and methods

Samples were collected from the walls and ceiling of caves at Mladeč, Javoříčko and Teplice nad Bečvou (Fig. 1, Table 1) from 2005 to 2006.

The underground system of the Javoříčko karst consists of a complex set of corridors, caves and abysses formed by the Špraněk stream inside an island of Devonian limestone. The Mladeč caves, situated close to the Javoříčko karst, represents a complex labyrinth of fissure corridors and caves inside the calcite hill of Třesín. The Zbrašov aragonite caves (Teplice), a part of the Hranice karst, are situated in the valley of the Bečva River, near the spa town of Teplice nad Bečvou. This is a unique cave system of European significance, formed simultaneously by atmospheric water and hot mineral water, moving upwards from great depths in the dolomite area during the Pleistocene. The bottom levels of the caves are permanently filled with carbon dioxide. The Zbrašov caves are the warmest underground spaces in the Czech Republic, with a constant temperature of 14 °C (Zimák & Štelcl 2003).

Algal crusts were scraped using a scalpel into labelled plastic bags and used directly for observation under a light microscope (LM) or as inocula for cultures on agar plates. Clonal cultures were obtained by isolation of colonies from agar plates, which were then transferred to liquid media in Petri dishes. Two different culture media were used for all samples, medium Z as described by Staub (1961) and diatom medium WC with silicate following Guillard & Lorenzen (1972). Cultures were maintained under two different conditions: (1) constant temperature of 17 °C, cool-white fluorescent lights providing 12 h light per day, 30 $\mu mol \cdot m^2 \cdot s^{-1}$; (2) night temperature 10 °C, day temperature 15 °C, with the same light and photoperiod. Environmental variables (temperature, light, humidity) were measured using digital thermohygrometer Testo 608 H2 and photoactinometer (Š. Kubín, AS CR Třeboň).

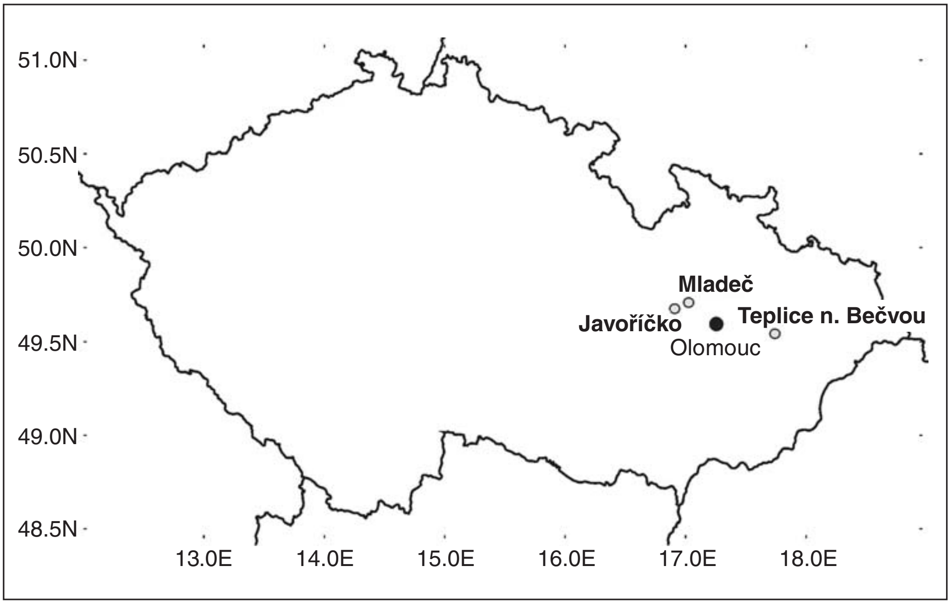

Fig. 1. – Map of investigated caves in central Moravia (Czech Republic).

Table 1. – List of sites sampled. * "natural cave" with an entrance, which is the only source of light, i.e. no electric light; distance from entrance given in meters; † in the area around electric lights.

Cave/coordinates	Sample no.	Sampling dates	Light/distance ($W \cdot m^{-2}$/m)	Temperature (° C)	Moisture (%)
Mladeč cave system					
49°42'43" N 17°00'45" E	M1 Podkova*	15.9.05	10.8/0	15.3	84.9
	M2 Podkova*	15.9.05	7.2/2	15.3	87
	M3 Podkova*	15.9.05	1.8/10	12.3	92.6
	M4 Podkova*	19.4.06	no data/2	10.5	85
	M5 Podkova*	27.6.06	11.7/0	19	79
	M6 Podkova*	27.6.06	7.3/3	14	89
Javoříčko cave system					
49°40'08" N 16°54'41" E	J1 Zátvořice*	12.8.05	2.8/2	14	70
	J2 Zátvořice*	12.8.05	2.4/2	13.8	70
	J3 Zátvořice*	12.8.05	2.8/4	14	70
	J4 Zátvořice*	12.8.05	2.1/10	12.4	87
	J5 Zátvořice*	27.6.06	2.9/4	15	78
	J6 Zátvořice*	27.6.06	5.9/2	15	75
	J7 Dóm gigantů	27.6.06	6.2/†	8	100
Zbrašov (Teplice) cave system					
49°31'45" N 17°44'27" E	T1 Netopýří*	14.7.05	7.3/2	23	59
	T2/A cave treshold	24.4.06	20.3/0	14	No data
	T2/B cave treshold	24.4.06	20.3/0	14	No data

Valves were cleaned in tubes by oxidation with 35% hydrogen peroxide and several crystals of potassium dichromate for 15 minutes, followed by several washings with distilled water. The washed valves were mounted on glass slides in Naphrax. Observations were carried out using an Olympus CH21 light microscope. Photomicrographs were taken using a Reichert Polyvar 2 photomicroscope fitted with a Polaroid DMC2 digital camera capable of 1600 × 1200 pixel resolution (images were captured using Optimas image analysis software, version 6.2; MediaCybernetics, Silver Spring, MD 20910, USA); bright field (BF) or differential interference contrast (DIC) optics were used at a maginification of ×100 (planapochromat lenses, nominal numerical aperture 1.32). In some cases, background noise and specks were removed digitally by image division (Bayer et al. 2001; http://rbg-web2.rbge.org.uk/algae/methods/removing_dust.htm).

For SEM observations a drop of cleaned sample was dried onto a cover-slip, which was then attached to an aluminium stub by a carbon tab. Stubs were coated with platinum for 2 min in an Emitech K575X sputter coater and examined using a LEO Supra 55VP Field Emission SEM operated at 5kV (6 mm working distance; aperture 20 µm). Images were captured as 3 MB TIFF files.

Results

A total of 22 diatom taxa were identified during this study. At well-illuminated sites outside caves and at their entrances, the algal flora was dominated by green algae (cca 27 species; *Apatococcus vulgaris* Meneghini, *Chlorella vulgaris* Beijerinck, *Klebsormidium flaccidum* A. Brown, *Muriella* sp.); deeper parts of caves were dominated by cyanobacteria (ca 22 species, *Gloeocapsa aeruginosa* Kützing, *Leptolyngbya* spp., *Nostoc commune* Vaucher ex Bornet et Flahault). Both dominants were accompanied by sparse populations of diatoms. The greatest algal species richness was observed in Mladeč and Zbrašov (Table 2). Comments on the most interesting taxa follow.

Table 2. – The list of taxa identified. * new species, it will be described elsewhere.

Taxon	Mladeč	Javoříčko	Zbrašov
Cyanobacteria	+	+	
Aphanocapsa parietina Nägeli			
Calothrix fusca Kützing ex Bornet et Flahault			+
Chroococcus minor (Kützing) Nägeli		+	+
Chroococcus varius A. Braun			+
Chroococcus spelaeus Ercegović	+	+	
Chroococcidiopsis cf. *kashayi* Friedmann	+		
Chroococcidiopsis sp.			+
Gloeocapsa aeruginosa Kützing	+	+	+
Gloeocapsa alpina (Nägeli) Brand		+	
Gloeocapsa punctata Nägeli	+		
Gloeocapsa sp. 1*	+		
Gloeocapsa sp. 2*			+
Gloeothece palea (Kützing) Rabenhorst	+		
Gloeothece sp.		+	
Leptolyngbya boryana Anagnostidis et Komárek			+
Leptolyngbya sp. div.	+	+	+
Nostoc commune Vaucher ex Bornet et Flahault	+	+	+
Nostoc sp.			+
Pseudocapsa dubia Ercegović	+		+
Schizothrix sp.		+	
Scytonema hofmanni Agardh ex Bornet et Flahault			+
Tolypothrix sp.	+		
Diatoms			
Achnanthes coarctata (Brébisson) Grunow	+	+	+
Achnanthidium minutissimum (Kützing) Czarnecki	+		
Anomoeoneis brachysira (Brébisson) Grunow			
Cyclotella meneghiniana Kützing			+
Cymbella silesiaca Bleisch	+		
Diadesmis aerophila (Krasske) D. G. Mann	+	+	
Diadesmis contenta (Grunow ex Van Heurck) D. G. Mann	+	+	+
Diadesmis gallica W. Smith		+	
Gomphonema angustatum (Kützing) Rabenhorst	+		
Gomphonema parvulum (Kützing) Kützing	+		
Hantzschia amphioxys (Ehreberg) Grunow	+		+
Luticola mutica (Kützing) D. G. Mann	+		+
Luticola nivalis (Ehrenberg) D. G. Mann	+		+
Luticola nivaloides (Bock) Denys et De Smet			+

Luticola paramutica var. *binodis* (Bock) D. G. Mann			+
Melosira varians Agardh			+
Navicula tenelloides Hustedt			+
Nitzschia sp.			+
Pinnularia borealis Ehrenberg	+		
Planothidium lanceolatum (Brébisson) Lange-Bertalot	+		
Stauroneis undata Hustedt			+
Orthoseira roeseana (Rabenhorst) O'Mara	+	+	+
Chlorophyta			
Actinotaenium curtum (Brébisson) Teiling ex Růžička et Pouzar	+		
Apatococcus lobatus (Chodat) J. B. Petersen	+	+	+
Chlorella fusca Shihira et Krauss			+
Chlorella kessleri Fott et Nováková	+		
Chlorella vulgaris Beijerinck	+	+	+
Chlorella sp. div.	+	+	+
Chlorosarcinopsis minor (Gerneck) Herndon	+		
Coccomyxa confluens (Kützing) Fott	+	+	+
Coelastrella striolata Chodat			+
Desmococcus olivaceus (Persoon ex Archerson) Laudon			+
Klebsormidium crenulatum (Kützing) Ettl	+	+	+
Klebsormidium flaccidum (Kützing) Silva	+	+	+
Muriella decolor Vischer		+	+
Muriella terrestris J. B. Petersen	+	+	+
Muriella sp.			+
Myrmecia cf. *astigmatica* Vinatzer			+
Myrmecia sp.	+		
Oocystis sp.	+		
Scenedesmus sp.			+
Scotiellopsis terrestris (Reisigl) Punčochářová et Kalina	+		
Stichococcus bacillaris Nägeli	+	+	+
Stichococcus minutus Grintzesco et Péterfi	+	+	+
Tetracystis excentrica Brown et Bold		+	+
Trebouxia cf. *decolorans* Ahmadjian		+	
Trebouxia glomerata (Warén) Ahmadjian		+	
Trentepohlia aurea (Linné) Martius			+
Trentepohlia sp.	+		

Achnanthes coarctata (Brébisson) Grunow Fig. 2: 18, 19

This species occurred in all cave systems studied, and was most abundant in Zbrašov T2/A sample. When growing on agar plates it forms flat, geometric band-like colonies, formed by cells in girdle view. Isolated clones grew well in liquid WC medium.

Valves are elliptical or linear-elliptical sometimes with broadly subcapitate apices and more or less constricted at the centre of the valve. Raphe-bearing valves have central area expanded into a rectangular facia; rapheless valves have an off-center axial area. Valves were 24–38 µm long, 6–8 µm wide with 14–18 striae per 10 µm (Table 3). The morphological variability reported by Krammer & Lange-Bertalot (1991b, their Tafel 2: Figs 1–8), the varieties found in the Antarctic region (Ettl & Gärtner 1995) together with the cosmopolitan distribution of the species reported in the literature (Krammer & Lange-Bertalot 1991b) give rise to the supposition that *A. coarctata* is a species complex worthy of investigation.

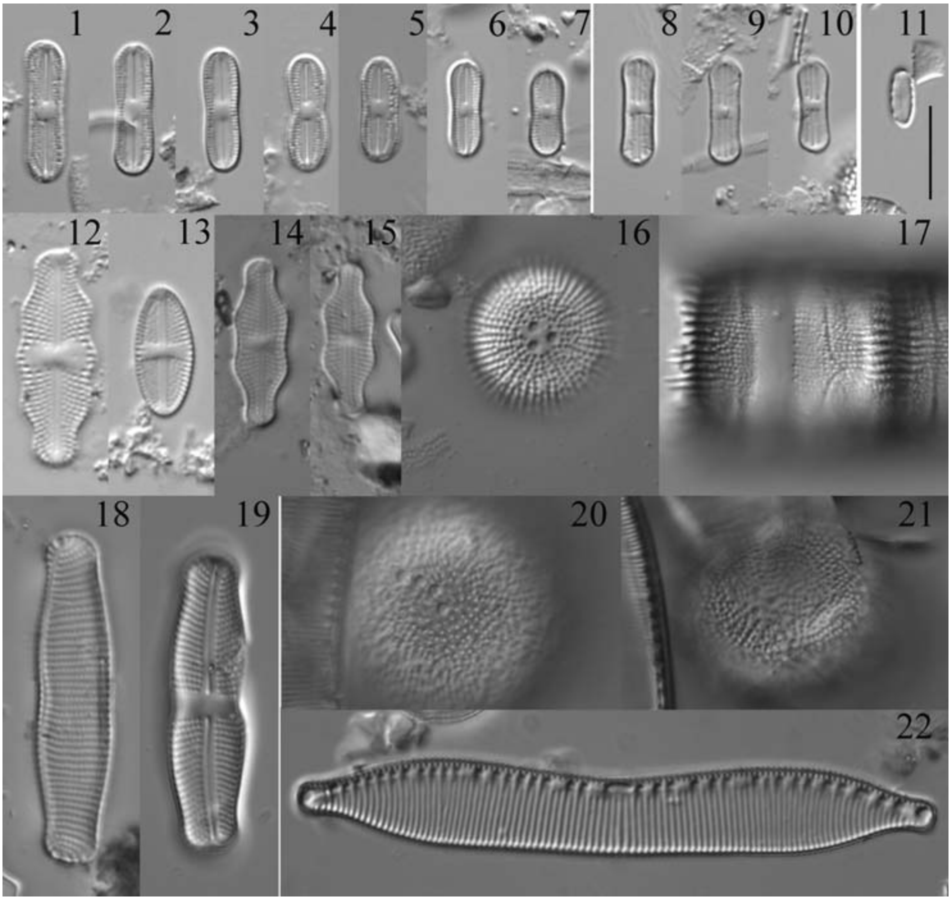

Fig. 2. – Aerophytic diatoms, light micrographs, DIC optics. Scale bar = 10 µm. 1–7 *Diadesmis aerophila*, 8–10 *D. contenta,* Mladeč cave system; 11 *D. gallica*, Javoříčko cave system; 12 *Luticola nivalis*; 13 *L. mutica*; 14–15 *L. paramutica* var. *binodis*, Zbrašov cave; 16, 17, 20, 21 *Orthoseira roeseana*, Javoříčko cave system, 16, 17 valve and girdle view; 20, 21 initial valves, Mladeč; 18, 19 *Achnanthes coarctata*, Mladeč; 18 rapheless valve, 19 raphe valve; 22 *Hantzschia amphioxys*, Mladeč.

Distribution in the Czech Republic: Recorded from nine localities in Bohemia and Moravia (Procházka 1924, Maloch 1937, Federle 1938, Poulíčková et al. 2004).

General distribution: Characteristic subaeric species (Krammer & Lange-Bertalot 1991b), found in mosses in the Alps, in Europe, USA (St. Clair & Rushforth 1976), on calcium rich soils in England (Ettl & Gärtner 1995), recorded even from extreme arid regions including the Namib desert, where it is accompanied by cyanobacteria (Dodd & Stoermer 1962) and in the Antarctic region (Broady 1979).

Table 3. – Cell dimensions and stria densities in natural populations of *Achnanthes, Diadesmis, Hantzschia* and *Luticola* species. Mean ± standard deviation, ranges in parentheses; n = number of measurements, for sample codes see Table 1; * spines; † measured in SEM, n = 1; ‡ central part measured.

Species/sample	n	Length (µm)	Breadth (µm)	Stria density (10 µm^{-1})	Fibulae/spines (10 µm^{-1})
A. coarctata	15	31.87±3.84	7.73±0.54	15.4±1.62	
Zbrašov /T2A		(24–38)	(6–8)	(14–18)	
D. aerophila	22	12.54±1.02	3.27±0.20	26.86±0.92	
Mladeč /M1		(10.4–14.2)	(2.8–3.8)	(25–29)	
D. contenta	13	10.81±1.68	3.35±0.57	40†	
Mladeč/M1		(7–13)	(2.5–4.0)		
D. contenta	16	10.09±1.81	2.78±0.39		
Zbrašov /T2A		(7–12)	(2.0–3.5)		
D. contenta	10	6.7±0.51	2.2±0.24		
Zbrašov /T2B		(6.0–7.5)	(2.0–2.5)		
D. gallica	12	8.88±1.06	3±0		12.83±1.91*
Javoříčko/J7		(7–10)	(3)		(10–16)
H. amphioxys	15	63.87±8.44	7.36±0.81	15.93±1.57	6.07±1.12
Mladeč/M1		(43–70)	(6–9)	(14–18)	(4–9)
L. mutica	10	15.06±2.22	6.67±0.36	19.2±1.33	
Zbrašov/T2A		(12.3–19.7)	(5.9–7.0)	(18–22)	
L. nivalis	30	22.07±1.41	8.5±0.48	16.73±1.59	
Zbrašov /T2A		(19–24)	(7.5–9.0)	(14–20)	
L. nivalis	30	16.35±2.52	7.63±0.72	17.33±1.74	
Zbrašov T2/B		(11–22)	(6–9)	(14–20)	
L. paramutica var. *binodis*	15	15.19±0.94	4.65± 0.19‡	19.62±0.92	
Zbrašov T2/A		(13.95–17.4)	(4.3–4.9)	(18–21)	

Diadesmis Kützing

Diadesmis is a small freshwater diatom, almost restricted to subaerial habitats like damp moss or rock. A genus easily separated from other naviculoid diatoms by the combination of stria and raphe structure, valve shape and often colonial habit (Round et al. 1990). Until recently, most *Diadesmis* species were thought to be extremely polymorphic (Granetti 1977, 1978, Werum & Lange-Bertalot 2004) and very widespread, being recorded as cosmopolitan. Although in extreme conditions (hot springs, rock faces, caves) there is "some kind of microalgal cosmopolitanism" within these particular habitat types (J. Komárek, personal communication), it is highly likely that the number of species in this genus is underestimated. Traditionally, diatom taxonomy depends on cell wall characteristics, but there is no consensus on where the species boundaries should be drawn. Two ways of investigating species-level variation are molecular methods and mating to test directly for potential gene flow, both depending on the availability of clonal isolates. So far, most studies indicate that diatom species often form heterogeneous complexes, containing several to many semicryptic or cryptic species (Mann et al. 2004).

Diadesmis gallica W. Smith Fig. 2: 11

Syn. *Navicula gallica* (W. Smith) Lagerstedt var. *gallica*

This is the only diatom species that occurs in the Javoříčko cave system in areas surrounding electric lamps.

Cells small, sometimes solitary but often forming band-like colonies in which cells are connected by interlocking spines on their valve margins. Individual cells lie in valve or girdle view with approximately equal frequency. Valves linear-elliptical with or without marginal spines, 7–10 μm long, 3 μm wide, spines 10–16 per 10 μm. Intraspecific phenotypic polymorphism in *D. gallica* was studied by Cox (2006). This species is unusual in producing two contrasting valves morphologies, one with a raphe system and non-spiny valves, the second without a raphe system but with peripheral linking spines, the latter being associated with colony formation Cox (2006).

Distribution in the Czech Republic: This species has not previously been recorded from the the Czech or Slovak Republics.

General distribution: Common in terrestrial biotopes including bryophytes, rock faces, soil (Krammer & Lange-Bertalot 1986), under low light conditions, such as in caves (Cox 1996); recorded from Austrian caves (Schagerl 1991).

Diadesmis contenta (Grunow ex Van Heurck) D. G. Mann Figs 2: 8–10; 3: 26

Syn.: *Navicula contenta* Grunow ex Van Heurck

This species was abundant in the Podkova natural cave in the Mladeč karst where it was accompanied by other *Diadesmis*, *Hantzschia, Orthoseira, Luticola, Achnanthidium* species; it was common also in the Zbrašov cave system. It grows in WC liquid medium.

Cells were solitary, small, bluntly linear, slightly swollen or constricted at the centre of the valve; cells from T2/B sample were shorter (Table 3). The species is similar in some respects to *D. paracontenta* Lange-Bertalot et Werum, recently described from freshwater in Siberia (Werum & Lange-Bertalot 2004), but both differ notably in stria density. *Diadesmis paracontenta* has typically 27–30 striae per 10 μm (easy visible in LM) and *D. contenta* about 40 striae (Van de Vijver et al. 2002). It is impossible to count striae at this density using LM (B. Van de Vijver, personal communication). The specimen on SEM image (Fig 3: 26) has 40 striae per 10 μm.

Distribution in the Czech Republic: Recorded from Bohemian soils (Rosa 1968, Lukešová & Komárek 1987, Lukešová 1993).

General distribution: Recorded from caves in Austria (Schagerl 1991), Scotland (Carter 1971), USA (St. Clair & Rushforth 1976, Dayner & Johansen 1991) and as a photobiont from lichens (Lakatos et al. 2004).

Diadesmis aerophila (Krasske) D. G. Mann Figs 2: 1–7, 3: 23–25

Syn. *Navicula aerophila* Krasske

Diadesmis from Mladeč are even more diverse, both in dimensions (Table 3) and shape (Figs 2: 1–7). Although some specimens are quite eroded (Fig. 3: 23) they are more likely to belong to *D. aerophila* (B. Van de Vijver, personal communication). Molecular methods could help to resolve the taxonomic problems in this genus.

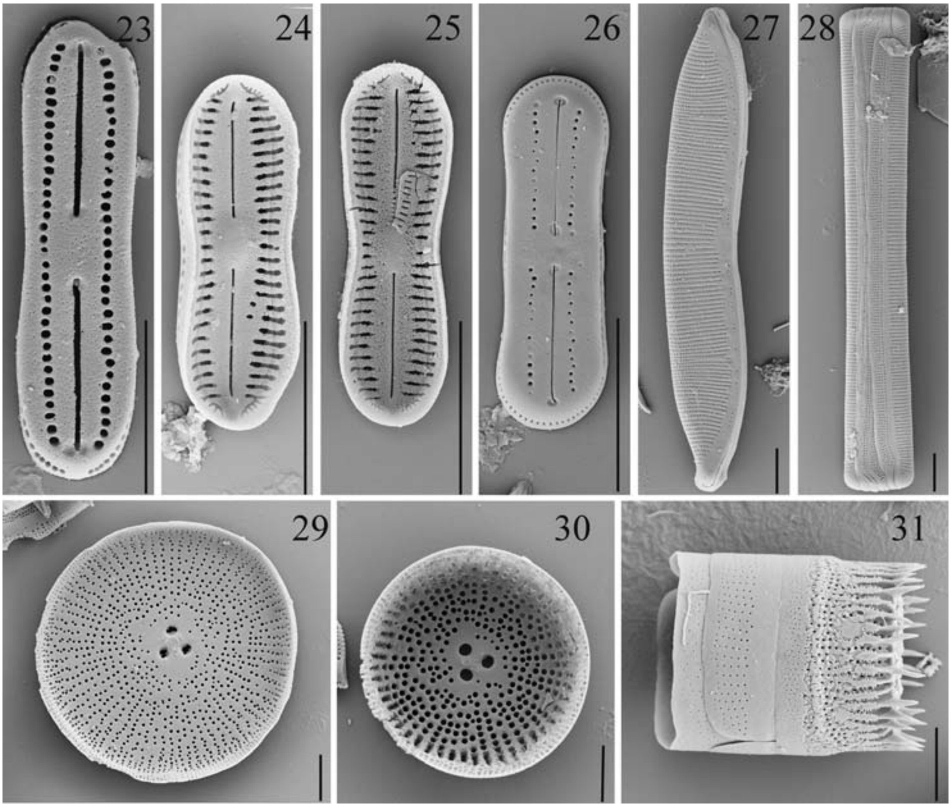

Fig. 3. – Aerophytic diatoms from Mladeč cave system, SEM micrographs. Scale bars = 5 µm. 23–25 *Diadesmis aerophila*; 23 outer view, 24–25 inner view; 26 *D. contenta* outer view; 27–28 *Hantzschia amphioxys* valve and girdle view; 29–31 *Orthoseira roeseana* valve outer view, valve inner view, girdle view.

Distribution in the Czech Republic: The only recorded locality for *D. aerophila* from the Czech Republic is a puddle in Brno (Bílý 1932)

General distribution: Cosmopolitan, with ecological optimum at sites at the water/air boundary, very often accompanied by *D. gallica* (Krammer & Lange-Bertalot 1986); recorded from Austrian caves (Schagerl 1991).

Luticola D. G. Mann

A fresh or slightly brackish water genus, commonest in soils or subaerial habitats and in estuaries (Round et al. 1990). The combination of raphe and pore structure, and the unique type of stigma, serves to separate *Luticola* from *Diadesmis.*

Luticola is an interesting and difficult species complex. Morphological variation within this complex is similar in many respects to that in *Sellaphora pupula* sp. comp. The vast majority of the species have similar ecological requirements and are often found in the same habitats. Three of these species occurred in the caves studied.

Although many species, varieties, and forms have been described (Van de Vijver et al. 2006) in the *Luticola* complex, there is no agreement as to what constitutes genetically based differences in form and what proportion of morphological variability is due to environmentally induced plasticity (Johansen 1999).

Luticola mutica (Kützing) D. G. Mann Figs 2: 13

Syn.: *Navicula mutica* Kützing

Species rarely found in the Podkova natural cave in the Mladeč system and in the Netopýří natural cave in the Zbrašov system and in Zbrašov T2 sample, usually accompanied by the more abundant species *L. nivalis,* observed also in cultures on agar plates. Single chloroplast, lobed from valve view, looks H-shaped from girdle view. In cultures, three of eight clones exhibited homothallic reproduction.

Cells are solitary, naviculoid, usually lying in valve view. Valves are elliptical, 13–19 µm long, 6–7 µm wide (Zbrašov T2 natural populations). Striae uniseriate, 18–22 per 10 µm. Our material was compared with Kützing's material on slide BM 78010 and Van Heurck's material slide BM 26425 deposited in The Natural History Museum, London. Kützing described this species in 1844 from a puddle mixed with seawater in Wargerooge (Germany) (Kützing 1844). Material from the type locality does not exist in NHM, London, and the only Kützing's material, BM 78010 from Alencon (France), more closely resembles var. *goeppertiana* M. Bleisch 1861, separated later as species *Navicula goeppertiana* (Bleisch) H. L. Smith, and more recently as *Luticola goeppertiana* (Bleisch in Rabenhorst) D. G. Mann. On the other hand specimens collected by Van Heurck (BM 26425) correspond well with *Luticola mutica* sensu Mann 1990, *Navicula mutica* sensu Krammer & Lange-Bertalot (1986) and our own material (no images from NHM London available).

Distribution in the Czech Republic: Common in soil samples (Rosa 1968, Lukešová & Komárek 1987, Lukešová 1993), mineral springs with high conductivity near Františkovy Lázně and Chomutov (Sprenger 1931, Brabez 1941), rivers (Poulíčková et. al. 2004).

General distribution: Very common in brackish conditions and freshwater, which is prone to dry out, e.g. sublittoral sites and damp surfaces; less tolerant of pollution than *L. goeppertiana* (Cox 1996), common in soils (Ettl & Gärtner 1995) and caves (Carter 1971, St. Clair & Rushforth 1976).

Luticola nivalis (Ehrenberg) D. G. Mann Figs 2: 12

Syn.: *Navicula nivalis* Ehrenberg

Species occurrs in the Podkova natural cave at Mladeč and Netopýří natural cave at Zbrašov and in Zbrašov T2/A and Zbrašov T2/B samples accompanied by *Diadesmis, Hantzschia, Achnanthes* and *Orthoseira*, grows well on agar plates and in both Z and WC liquid media. Flat, geometric colonies on agar plates are formed by cells in girdle view. Although cells are very small no sexual reproduction was observed either within monoclonal cultures or in crossing experiments between eight isolated clones.

Cells solitary, valves linear with three undulations, 11–24 µm long, 6–9 µm wide with 14–20 striae per 10 µm (Table 3).

Distribution in the Czech Republic: *Luticola nivalis* is recorded from only four localities in Bohemia (Sprenger 1926, 1930, Bílý 1964, Lukešová & Komárek

1987); our finding represents the first recent record from Moravia, all previous records being older than 70 years (Fischer 1920, Bílý 1925, 1929).

General distribution: *Luticola nivalis* is a common subaerial species recorded from caves in Hungary (Claus 1955, 1964, Nagy 1965) and Kentucky (Barr 1968).

Luticola paramutica var. *binodis* (Bock) D. G. Mann Fig. 2: 14, 15

Syn. *Navicula paramutica* Bock

This species occurred in material from Zbrašov T2. Valves are linear-elliptical with twice undulated margins and capitate ends. Cell dimensions are given in Table 3. Axial area was narrow, linear. Central area is rectangular with stigma.

Distribution in the Czech Republic: *Luticola paramutica* var. *binodis* is a new species for the Czech Republic, there are no records from the Slovak Republic.

General distribution: *Luticola paramutica* var. *binodis* is a rare subaerial species (Krammer & Lange-Bertalot 1986). It is recorded from a small cave in the Subantarctic region (Van de Vijver et al. 2002). The accompanying flora point to a more brackish environment.

Orthoseira roeseana (Rabenhorst) O'Mara Figs 2: 16, 17, 20, 21; 3: 29–31

Syn.: *Melosira roeseana* Rabenhorst

Species abundant in the Podkova natural cave at Mladeč, accompanied by *Hantzschia, Diadesmis, Achnanthes* and *Luticola*, and in the Zátvořice natural cave in the Javoříčko karst, accompanied by *Diadesmis* and *Achnanthes*; it is rare in the Netopýří natural cave at Zbrašov. This species grows well on agar plates forming long string-like chains, both in WC and Z media, but isolated cells in liquid media grow very slowly. Living cells contain numerous small, discoid plastids. A live auxospore was observed in a natural population in the Zátvořice cave. Rounded auxospore was 31.7 µm in diameter, parental valves 12.2 µm wide. Average valve diameter decreased in the population from Javoříčko during our investigation (Table 4). Initial valves were found on slide from Mladeč.

Cells cylindrical in girdle view and linked by spines into short to long chains. Although valves that are wider (diameter) than they are long (valve high) are characteristic for the species (8–70 µm in diameter, 6–13 µm high; Cox 1996), our specimens from samples and cultures had a larger width/length ratio (Table 4). This ratio is influenced by the stage within the life cycle (Roemer & Rosowski 1980). Post-auxospore valves vary 19.5–39.5 µm in width and 8.0–18.5 µm in length, whereas pre-auxospore valves vary from 5–12 µm in length, but only 5–13 µm in width. Hence the decrease in cell size in *C. roeseana* (recently *O. roeseana*) is due to a much more rapid reduction in width than in length (Roemer & Rosowski 1980). In contrast to their measurements obtained from a clonal culture, our data were obtained mostly from natural samples, which included a mixture of cells at different stages of the life cycle, as revealed by the wide variation in valve diameter (Table 4). Chains in natural material were short, probably broken by scraping, but in cultures 20-celled chains were common. Valve faces were flat, with radial, unequally long rows of pori (called striae in Table 4).On the valve face, there are 1–4 (mostly 3) coarse pori (carinoportulae). Valves of initial cells were hemispherical (Fig. 2: 20, 21). The centre of an initial valve resembles that of a normal vegetative valve. Size and surface pattern accord with previous records (Roemer & Rosowski 1980). Although the genus *Orthoseira* Thwaites sensu Round et al. 1990 has been studied intensively (Spaulding & Kociolek 1998) its taxonomic status is still unclear and needs revision (Houk 2003).

Distribution in Czech Republic: Frequent in freshwater (Poulíčková et al. 2004) and wet rock faces (Dvořák & Nováček 1926, 1933), present in the J. Bílý slide collection (Skácelová & Konečná 2006). *Orthoseira* is included on the red list of endangered plants of the Slovak Republic as vulnerable (Baláž et al. 2001).

General distribution: Common aerophytic diatom of rock faces, bryophytes and trees all over the world (Krammer & Lange-Bertalot 1991), especially in alkaline areas (Wehr & Sheath 2003). Species recorded from Hungarian and American caves (Kol 1964, St. Clair & Rushforth 1976) and as a photobiont from lichens (Lakatos et al. 2004).

Table 4. – Cell dimensions, number of carinoportulae and stria densities in natural populations and cultures of *Orthoseira roeseana*. Mean ± standard deviation, ranges in parentheses; n – number of measurements; * measured from girdle view – cells mostly in chains; ‡ not visible on initial valves; † both valves together. For sample codes see Table 1.

Locality/ sample	n	Valve diameter (μm)	Cell height (μm)	Stria density (10 μm^{-1})	Number of carinoportulae
Mladeč/M1	33	14.86±4.50 (9–28)	22.94±3.58 (15–30)	9.9±1.51 (6.4–13.4)	2.9±0.54 (1–4)
Mladeč/M1 initial valves	14	28.4±3.3 (23.4–34.2)	‡	‡	‡
Mladeč/M4 culture	62	14.62±3.91 (8.5–18.5)	26.77±4.71 (18–38)	no data*	no data*
Javoříčko/J1 2005	10	17.1±3.76 (12.5–24.0)	26.35±3.69 (22–34)	9.43±0.75 (8.3–10.9)	3±0.45 (2–4)
Javoříčko/J6 2006	30	15.94±3.24 (10.9–21.9)	25.01±3.02 (19.5–31.2)	no data*	no data*
Zbrašov/T1	12	19.04±3.19 (13.5–24.0)	25.08±4.55 (18–33)	no data*	no data*

Discussion

The epilithic algae of bare rock surfaces have received very little attention apart from the classic studies of Jaag (1945) of rocks in the Swiss Alps and of Golubić (1967) of calcareous rocks in Yugoslavia, yet all rock surfaces in moist climates probably have some algal colonists and even in the tropics the crevices in the surface of rocks support unicellular algae. In this habitat fungi also occur, and it is here that primitive lichen-like associations can be found (Lakatos et al. 2004). Algae occur not only in habitats subject to extremes of wetness and desiccation, but also in habitats where the temperatures are several degrees higher than ambient air temperature (Jaag 1945). Petersen (1915, 1935) described 196 diatom taxa from subaerial habitats. Schorler (1914) recognized five subassociations of diatoms in European habitats: *Fragilarietum virescentis, Pinnularietum borealis, Pinnula-*

rietum appendiculatae, Frustulietum saxonicae and Melosiretum roeseanae. However, the classification of algal assemblages is not as common as that of vascular plants. In Finland, Cedercreutz (1941) found 37 species in rock face habitats. Where the rock face is enriched by water seeping from above, a number of diatoms (*Melosira, Diatoma, Achnanthes coarctata, Navicula mutica, Pinnularia globiceps* and *Nitzschia palea*) occur (Round 1981). Very similar assemblages are recorded growing on rocks and stones the world over (Round 1981). An interesting aspect of the algae living on calcareous rock faces is their ability to deposit crystals of calcium carbonate in their sheaths. Several kinds of algae, particularly some species of cyanobacteria and diatoms, are closely asssociated with travertine crusts and are considered to play an important role in the formation of some travertines (Pentecost 1998).

Solution of rock by algal growth is probably rare or unknown on acidic rocks. More frequently algae penetrate the fine cracks in the surface of the rock and grow endolithically. Some algae occur more frequently on acid rocks surfaces, e.g. desmid species, *Eunotia* spp., *Pinnularia borealis*, while on alkaline surfaces *Melosira roeseana* is common (Jaag 1945).

Rock surfaces within caves where there is sufficient light are also colonized by algae, and again *Cyanophyta* tend to be dominant (Golubić 1967). Diatoms are not recorded from dry stone surfaces (Schlichting 1975).

Cave diatom species richness, diatom life stages and reproduction

About 340 diatom taxa are reported from soils, 400 from rock substrates and 130 from mosses (Johansen 1999). The systematics of aerial diatoms present problems. Many are small and difficult to study using LM. Diatom species richness recorded from caves is low (Table 5), except in a few cases (Carter 1971, St. Clair & Rushforth 1976, Bahls 1981). But these studies (particularly Carter 1971) include the vast majority of non-aerophiles, common benthic or planktonic species and no description of sites or sampling methods is given in these papers. Small pools occur in many caves, including those studied in Montana, Utah, Oregon and Kentucky (St. Clair & Rushforth 1976, Bahls 1981). All cave diatom records are strongly dependent on the sampling method and subsequent laboratory treatment. Some authors sample only dark sites and some only sites surrounding electric lights ("lampflora"). Sampling of rock faces at cave entrances and outside the caves is uncommon. Using all three sampling methods, we identified 22 diatom species in relatively small cave systems, which is comparable to numbers of other cyanobacteria and algae (Table 5). In contrast, most authors identify diatoms from cultures only, whereas we studied original samples and Naphrax preparations using LM. Furthermore previous studies have used culture media specifically designed for green algae, whereas we used a range of culture conditions, including a special diatom culture medium. On the basis of our experience, we assume, the biodiversity of cave floras is still underestimated.

Although some resting stages are recorded in freshwater diatoms (McQuoid & Hobson 1996), they can hardly be distinguished from vegetative cells in dry samples from rock faces. Consequently we cannot be sure whether diatom specimens present in samples were vegetative cells or resting stages. However, there is no doubt about their vitality, because they grew rapidly on agar plates. Three clones of *Luticola* isolated from Mladeč exhibited homothallic reproduction within monoclonal culture and an auxospore and the initial cells were found in natural material of *Orthoseira*. Although the first references to sexual repro-

Table 5. Number of taxa identified from caves in this study (*) and comparison to data published previously.

Source	*Cyanophyta*	*Chlorophyta*	*Bacillariophyceae*
Bahls 1981	–	–	43
Barr 1968	11	11	5
Carter 1971	–	–	94
Claus 1964	17	9	3
Claus 1965	20	7	2
Dayner & Johansen 1991	4	5	14
Hajdu 1966	7	0	2
Jones 1965	11	12	0
Kol 1964	9	11	1
Mason & Williams 1967	13	19	5
Nagy 1965	2	2	1
Palik 1966	4	12	4
Pentacost & Zhang 2001	3	1	0
Rushforth et al. 1984	–	–	49
Schagerl 1991	8	7	7
St. Clair & Rushforth 1976	–	–	26
Mladeč cave system *	12	16	14
Javoříčko cave system*	9	14	5
Zbrašov cave system*	17	18	13

duction in diatoms were published 150 years ago (Thwaites 1847, Griffith 1855, Smith 1856), followed by further observations by Geitler (1973), our knowledge of this aspect of their biology is still poor. As well as resulting in genetic recombination, sexual reproduction restores original cell size (asexual division is accompanied by a gradual reduction in size). Although nothing is known about cave diatom reproduction, one might imagine that a sexual phase might be absent within aerophytic communities, because of the sensitivity of sexual stages to environmental conditions (Poulíčková & Mann 2006). In spite of this, the presence of sexual reproduction was expected from the broad variation in size in *Orthoseira* observed in 2005. Oogamy in centric diatoms always involves fertilization of a large, nonmotile egg by a small, anteriorly uniflagellate sperm. However, auxospores can also be formed through automixis or asexually (Chepurnov et al. 2004). Auxosporulation within monoclonal cultures was observed by Roemer & Rosowski (1980) in *Melosira roeseana,* but they give no information on nuclei behaviour necessary for determining the type of reproduction (paedogamy, autogamy, apomixis). Thus the observation of auxospores in samples is not evidence of sexual reproduction in the speleoenvironment. Although, sexual reproduction of *Luticola* was observed in cultures, we cannot be sure, whether they can reproduce in the same way in natural conditions. Ecology of diatom life history strategies is reviewed by Edlund & Stoermer (1997). Most reports of sexuality in nature are for taxa that show synchronous sexuality when growth conditions are favourable. This is common in epiphytic and epilithic diatoms in springs and in planktonic species. The cue for sexual induction is probably tied to seasonal signals (light and/or temperature). When the development of an actively growing population is suddenly arrested some diatoms will undergo sexuality in response to environmental stress (nitrogen limitation), and show synchronous sexuality under poor growth conditions, such as *Ditylum brightwellii* (West) Grunow (Edlund & Stoermer 1997). Low numbers of cells participating in sex over long period during periods of normal growth characterizes a third sexual strategy (asynchronous sexuality under favourable growth conditions). This strategy may be more common in slow-growing taxa. It minimizes risk by allowing only a small proportion to become sexual at any given time. The fourth sexual strategy (asynchronous sexuality under poor conditions) is characterized by a small number of cells, a situation that makes this strategy the most poorly understood type. Waite & Harrison (1992) suggest that this strategy may be advantageous in an unpredictable and constantly fluctuating environment as reproductive costs are minimized. Speleoenvironments offer extreme but relatively stable conditions to diatoms, and are a good model system for future studies on their reproductive strategies.

Cave diatoms and their requirements

The speleoenvironment is harsh and limiting in a number of ways. This makes it difficult to determine which environmental factors are most significant in determining species distribution. Moisture availability has frequently been thought to be the most important limiting factor (Camburn 1982), although exposure to long periods of desiccation can be more crucial (Johansen 1999). Limestone caves and temperate sandstone seeps have similarly moist regimes, temperature extremes and low nutrient availability, but markedly different flora due to differences in pH; *Diadesmis gallica* and *D. laevissima* are especially confined to neutral to alkaline caves (pH 6.5–8.0), while *D. contenta* and *Eunotia exigua* (Brébisson) Rabenhorst occur primarily on acid seeps (pH 3.7–6.0; Johansen 1999). On the other hand *Hantzschia amphioxys* is less sensitive to pH, with a published pH range of occurrence between 5.6 and 8.5. Substrate is also a critical limiting factor. The euterrestrial species *H. amphioxys, Luticola mutica* and *Pinnularia borealis* are rare on lithic substrates, but more common on mosses (Reichardt 1985). Most aerial diatom species are indicative of low nutrient availability (Van Dam et al. 1994). Elevated conductivity is a feature of many aerial habitats, where evaporation is high, and halophilous species (*Luticola mutica, L. nivalis*) are commonly collected from these habitats (Johansen 1999). Our samples were collected from ± dry surfaces of limestone (alkaline) caves. Environmental variables measured are summarized in Table 1.

It is uncertain whether heterotrophy or mixotrophy occur in diatoms, although living cells were found in complete darkness (Poulíčková 1987). Furthermore, diatoms tolerate low light conditions (Poulíčková et al. 2005). Most the diatoms collected, moreover, were found on rock faces near cave entrances (Table 1), or in areas surrounding electric lights, where conditions provide photoautotrophic organisms with a sufficient source of energy.

The origin of cavernicole diatoms

Aerophytic cyanobacteria and algae in Mladeč and Javoříčko caves were studied previously by Kaštovský (1997). Diatoms were represented by 12 freshwater species, e.g. euplanktonic *Asterionella formosa* Hassall, and their origin was interpreted as allochthonous. He did not find typical aerophytic or “cavernicole” species. The occurrence of euplanktonic forms in caves was previously discussed by Jones (1965). Although 55 euplanktonic algae are recorded from six caves all over the world, none of the previous authors attributed any significance to these algae and did not account for their presence. Tourism is one of the most likely sources of "contamination" in the caves we studied, because of the absence of surface ponds and streams over the cave system. Tourists frequently visit Czech caves, particularly in Javoříčko and Teplice. Claus (1964) proposed that the algal flora of caves originated during the time of their formation and in many instances have a pre-glacial or glacial relict character. Further support for this "relic" theory is supplied by Suba (1957) who found several thermophilic species in the cave at Pálvolgy, which was formed by thermal springs excavating the dolomite layer. One argument against this theory is the extremely wide ecological range occupied by most of these algae. In two recent papers (Finlay 2002, Finlay et al. 2002) it is proposed that all microbes, including eukaryotes such as microscopic algae and diatoms, are ubiquitous. The scientific basis for this rests principally on the analysis of the distribution of morphologically defined species (morphospecies). However, *Navicula cryptocephala* Kützing, classified by Finlay et al. (2002) as one of the cosmopolitan diatoms, seems to be either extremely polymorphic with respect to some reproductive and cytological features, or is in fact a complex of many separate species, which have been combined under the same name, on the basis of their similarity in overall cell morphology and the stria pattern on the valve (Poulíčková & Mann 2006). The many new species described from caves (VanLandigham 1965, 1966, 1967) might be endemic forms since a cave environment is very unique. One may speculate on the species forming effect of such an environment. Since the surface algal flora of the Earth is poorly known, one may suppose that the "endemic forms" described from caves also occur in other terrestrial habitats but have as yet not been found.

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Souhrn

Rozsivková flóra jeskyní v České republice nebyla dosud systematicky studována. Zaměřili jsme se na subaerická stanoviště, viditelné nárosty na vlhkých stěnách a stropech přírodních jeskyní i okolí umělého osvětlení v jeskynních systémech Mladeč, Javoříčko a Zbrašov. Pro studium jsme využili všech dostupných metodik, přímé prohlížení odebraných vzorků v nativním i trvalém preparátu, izolaci a kultivaci rozsivek na tuhých i tekutých médiích v laboratorních podmínkách. Zajímalo nás nejen druhové složení, ale i morfologická variabilita, cytologie, autekologie a životní strategie aerofytických rozsivek. Práce shrnuje veškerou dostupnou literaturu k problematice, diskutuje existenci druhových komplexů v rodech *Luticola* a *Diadesmis* a uvádí nové taxony pro Českou republiku (*Diadesmis gallica*, *Luticola paramutica* var. *binodis*). U dvou druhů (*Luticola* sp. a *Orthoseira roeseana*) bylo pozorováno pohlavní rozmnožování.

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