

Epiphytic diatoms in lotic and lentic waters – diversity and representation of species complexes

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Abstract: Small streams and shallow ponds represent sensitive ecosystems and attached diatoms can serve as integrative indicator with fast response to environmental changes. Development of methods for ecological monitoring throughout Europe and their calibration for particular ecoregions are not finished yet and databases need to be filled by data from undersampled regions and overlooked substrates. The present study aims to explore the diversity of epiphytic diatoms in unexplored catchment areas with special attention to substrate specificity and distribution of unresolved diatom species complexes. Significant differences were found in diversity of both regions and water types (lotic/lentic). No significant differences were found in the case of substrates. CCA analysis showed significant influence of pH, water streaming (streaming/stagnant) and *Lemna* substrate to species composition. Surprisingly species complexes represent the majority of epiphytic assemblages with no significant differences between lotic and lentic waters or substrates except of *Lemna*. The high representation of complexes does not lead automatically to reduction of overall diversity of the sample.

Key words: diatoms, epiphyton, lotic and lentic waters, species complexes

INTRODUCTION

In Europe, most shallow lakes/ponds and rivers are strongly affected by human activities. The EU members in the frame of Water Framework Directive (WFD) developed standardized methods to assess the ecological status of surface waters using bioindicators. Diatoms are considered to be good indicator organisms in aquatic ecosystems (BLANCO et al. 2004, 2014). The cross–taxon congruence of six contrasting groups of organisms (vascular plants, bryophytes, fungi, diatoms, desmids and testate amoebae) in the same permanent plots were analysed in freshwater wetlands (HÁJEK et al. 2014). The main difference among different taxa corresponded clearly with body size and life span (micro versus macroorganisms), conforming the assumption of faster response of microorganisms to environmental changes. Generally, macroorganisms provide similar information, while diatoms behave most independently (HÁJEK et al. 2014). Diatoms occupy a variety of substrates in both lotic and lentic waters. Development of methods for ecological monitoring throughout Europe (KELLY et al. 2009) and their calibration for particular ecoregions are not finished yet and databases need to be filled by data from undersampled regions and less

sampled substrates. Moreover there are many problems with cryptic diversity and their ecological significance (POULÍČKOVÁ et al. 2008, 2014). Some diatom traditional morphospecies included in regional floras (*Sellaphora pupula*, *Achnantheidium minutissimum*, *Gomphonema parvulum* etc.) have long been considered cosmopolitan, ubiquitous, and morphologically highly variable taxa. However molecular methods revealed, that these diatoms are species complexes consisting of few or many species, whose identification in LM is difficult or impossible (POTAPOVA & HAMILTON 2007; MANN et al. 2008; POULÍČKOVÁ et al. 2010). The use of benthic diatoms in the context of ecological status assessment (KING et al. 2006; KELLY et al. 2007) seems to be broadly accepted, although more studies are dealing with running waters and epilithon (RIMET & BOUCHEZ 2012). Methodology for shallow lakes using epiphyton has been suggested quite recently (BLANCO et al. 2014). The present study aims to explore epiphytic diatoms of small ponds and streams covering main ecological gradients of Southeastern Moravia (Czech Republic). Special attention was paid to representation of diatom species complexes at different substrates and water types and its influence to epiphyton bioindication capacity.

MATERIAL AND METHODS

Samples were collected in summer 2013 and 2014 in 25 ponds and 13 streams of two sampling areas. Both regions (the Svitava region and the White Carpathian Mountains) belong to the Morava River Basin. The first one – the Svitava Highland is a part of the Svitava River basin (SB) and geologically belongs to the southeastern part of the Cretaceous Table. In the area prevail mesozoic (sandstone, marstone, marlstone, claystone) and quarternary (loam, loess, gravel, sand) sedimentary rocks. Annual mean temperature is around 6 °C and annual mean precipitation is around 600 mm (TOLASZ et al. 2007). Sites located in this area lay in elevation around 500 m a.s.l. (Fig. 1). The second area – the White Carpathian Mountains (WC) is situated on southeast of the Czech Republic (on the western margin of the Western Carpathians) along the border with Slovakia. Geological bedrock is formed by flysch belt, in which sandstone and claystone of variable calcium content alternate. Prevailing is marl, lime-rich claystone, limestone and calcareous sandstone (HÁJEK et al. 2002). Groundwaters are carbonatogenic and their dominant mineralization process is carbonate dissolution which leads to the calcium–(magnesium)–bicarbonate type of chemistry (RAPANT et al. 1996). This chemistry type supports cold water travertine (tufa) formation. Annual mean temperature is about 8°C and annual mean precipitation is about 700 mm (TOLASZ et al. 2007). Sites located in this area are situated in altitudes from 225 m up to 535 m above sea level. Basic characteristics of investigated localities are given in Table 1.

Epiphytic communities (in littoral part of ponds and/or streaming part of the rivers) growing on submerged macrophytes *Phragmites australis* (CAV.) STEUD.; *Poaceae* (incl. *Phalaris arundinacea* L., *Arrhenatherum elatius* (L.) J. PRESL et C. PRESL, *Poa* sp., *Dactylis* sp., *Glyceria* sp.); *Typha* sp.; *Lemna* sp.; *Salix* sp.; *Callitriche* sp. and *Sparganium* sp. were examined.

Diatom sampling methods followed those recommended by KELLY et al. (1998), diatom frustules were cleaned in hydrogen peroxide (TAYLOR et al. 2007) and moun-

ted in Naphrax. Four hundred individuals were identified in each sample to species level using literature (KRAMMER & LANGE–BERTALOT 1986; KRAMMER & LANGE–BERTALOT 1988; KRAMMER & LANGE–BERTALOT 1991; KRAMMER 2000; LANGE–BERTALOT 2001; KRAMMER 2002; KRAMMER 2003; KRAMMER & LANGE–BERTALOT 2004; LANGE–BERTALOT et al. 2011). Nomenclature has been unified following Algaebase (GUIRY & GUIRY 2015). Species complexes selection was based on actual list of species, recent molecular literature and own experience and are summarized in Table 2, although their list can expand in near future due to molecular studies boom. Few examples of species complexes representatives are documented in Fig. 2.

Statistical analysis. Our hypothesis assumes that diatom distribution among sampling sites is influenced by measured environmental variables. Prior to main statistical analyses we disproved correlation between geographical position and environmental variables (Spearman's correlation coefficient, pH: $r_s = 0.587$, conductivity: $r_s = 0.033$). Multivariate analysis using Canoco 4.5 (TER BRAAK & ŠMILAUER 2002) was carried out to test relationships between identified diatoms and environmental variables (pH, conductivity, host plant, streaming/stagnant water). First, *length* of the gradient was computed using Detrended correspondence analysis (DCA, detrending by segments, without transformation, length of the gradient on the first axis = 5.258, species data explain 11.8% on the first axis). Protocol of the Canonical correspondence analysis (CCA) was carried out as follows: imported data included diatom occurrence (%) and environmental variables (pH, conductivity, flowing/stagnant water, host plant), then biplot scaling focused on inter-species distances, Log transformation ($Y' = \log(A*Y+B)$, $A=1$, $B=1$) with downweighting of rare species, Monte-Carlo permutation test was used (reduced model, 499 permutations), forward selection of environmental variables (both automatic and manual selection) were performed. Analysed environmental variables did not show any collinearity. Their VIF ranged from 1.82 to 5.92. Results of CCA were visualized using Canoco Draw

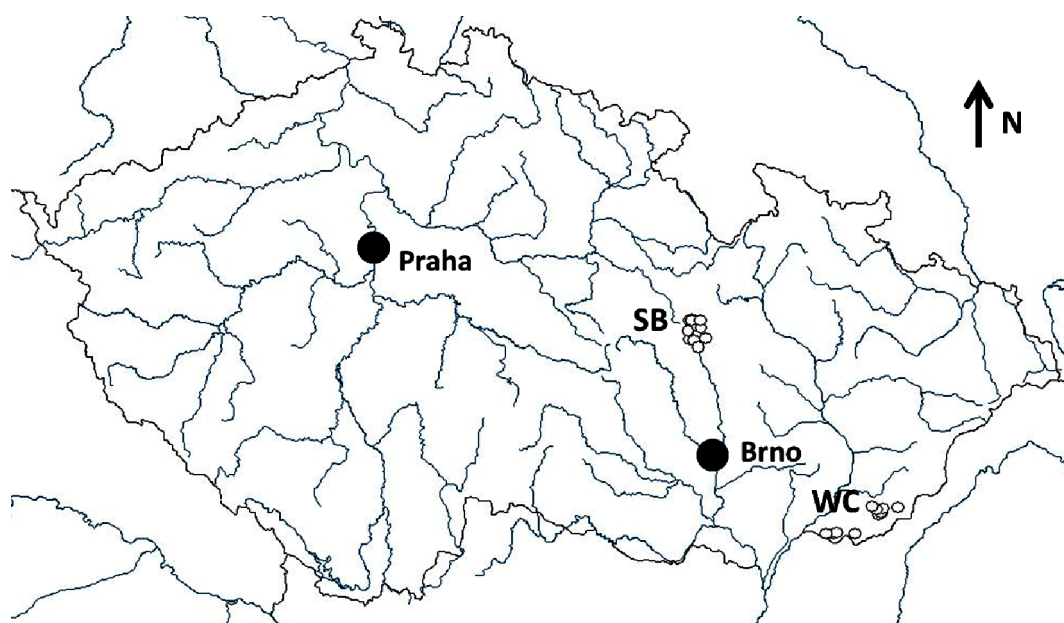


Fig. 1. Sampling sites in White Carpathians (WC) and the Svitava river basin (SB).

Table. 1. Basic characteristics of investigated localities, (Cond) conductivity ($\mu\text{mS}\cdot\text{cm}^{-1}$).

Area	Sample	Locality (Cadaster)	GPS coordinates	pH	Cond	Pond/ stream	Macrophyte
WC	LOK1	Lučina (Tvarožná Lhota)	48°51'46.14"N 17°23'41.04"E	8.19	505	P	<i>Poaceae</i>
WC	LOK2	Kejda (Kněždub)	48°52'3.84"N 17°24'37.26"E	8.13	470	P	<i>Poaceae</i>
WC	LOK3	Radějovka (Radějov)	48°51'38.16"N 17°20'32.70"E	8.32	546	S	<i>Poaceae</i>
WC	LOK4	Hrubý potok (Javorník)	48°51'49.56"N 17°31'54.24"E	8.36	414	S	<i>Poaceae</i>
WC	LOK5	Rasová (Komňa)	48°58'32.40"N 17°48'43.86"E	8.24	317	P	<i>Typha</i>
WC	LOK6	Lubná (Suchá Loz)	48°56'47.88"N 17°40'54.60"E	7.93	451	P	<i>Sparganium</i>
WC	LOK7	Lubná (Suchá Loz)	48°56'32.16"N 17°40'55.80"E	8.09	523	S	<i>Poaceae</i>
WC	LOK8	Basin on the Hradec- ký járek (Suchá Loz)	48°57'1.68"N 17°42'14.58"E	7.79	368	P	<i>Typha</i>
WC	LOK9	Hradecký járek (Suchá Loz)	48°57'8.52"N 17°42'5.88"E	7.92	408	S	<i>Salix</i>
WC	LOK10	Nivnička (Suchá Loz)	48°58'13.20"N 17°42'36.30"E	8.06	539	S	<i>Poaceae</i>
WC	LOK11	Basin near Čupák (Suchá Loz)	48°57'53.52"N 17°40'23.34"E	8.13	430	P	<i>Typha</i>
WC	LOK12	Nivnička (Nivnice)	48°58'48.00"N 17°38'30.84"E	8.45	547	S	<i>Poaceae</i>
SB	Ra1	Radiměřský potok (Radiměř)	49°41'31.163"N 16°27'26.189"E	6.2	175	S	<i>Poaceae</i>
SB	HnS1	Dolní hradecký ryb- níček (Hradec nad Svita- vou)	49°41'7.646"N 16°28'57.071"E	7.85	743	P	<i>Phragmites</i>
SB	HnS2	Horní hradecký ryb- níček (Hradec nad Sitavou)	49°41'8.094"N 16°28'55.294"E	4.80	534	P	<i>Lemna</i>
SB	HnS4	Řeka Svitava (Hradec nad Svita- vou)	49°41'6.744"N 16°28'51.934"E	7.00	540	S	<i>Poaceae</i>
SB	Sy1	Lánský rybník (Svitavy – Lány)	49°44'35.760"N 16°28'8.220"E	6.3	385	P	<i>Phragmites</i>
SB	Sy2	Svitavský rybník (Svitavy – Lačnov)	49°45'58.719"N 16°27'37.682"E	6.60	401	P	<i>Phragmites</i>
SB	Sy3	Rosnička (Svitavy – Předměstí)	49°46'15.313"N 16°27'5.582"E	7.98	506	P	<i>Phragmites</i>
SB	Sy6	Svitavy (Svitavy – Lány)	49°44'36.775"N 16°28'41.705"E	6.90	528	S	<i>Phragmites</i>
SB	Sy8	Lačnovský západní rybník (Svitavy – Lačnov)	49°46'25.950"N 16°28'7.276"E	5.75	293	P	<i>Typha</i>
SB	Sy12	Outlet at Lačnovský západní rybník (Svitavy – Lačnov)	49°46'24.584"N 16°28'10.762"E	6.00	251	P	<i>Typha</i>

Table 1 Cont.

SB	V1	U Rybníčku (Vendolí)	49°43'33.575"N 16°26'39.028"E	6.30	148	P	<i>Lemna</i>
SB	Po2	Fishpond (Pohledy)	49°41'46.008"N 16°33'39.107"E	6.30	252	P	<i>Phragmites</i>
SB	KH1	Fishpond (Kamenná horka)	49°44'18.342"N 16°31'43.116"E	5.80	459	P	<i>Typha</i>
SB	K1	Pool (Koclířov)	49°46'20.363"N 16°31'21.760"E	5.88	332	P	<i>Phragmites</i>
SB	BnS1	Svitava (Březová nad Svita- vou)	49°39'25.761"N 16°30'27.857"E	6.10	552	S	<i>Poaceae</i>
SB	Br1	Svitava (Brněnec)	49°37'23.628"N 16°31'26.769"E	7.65	518	S	<i>Phragmites</i>

Table 2. Species complexes occurring in the White Carpathians and the Svitava Basin, their trophic preferences, (nd) no data available.

Species complex	References	Trophic state (VAN DAMM et al. 1994)
<i>Achnanthydium minutissimum</i>	POTAPOVA & HAMILTON 2007	euryvalent
<i>Planothidium lanceolatum</i>	VAN DE VIJVER et al. 2013	eutrophic
<i>Amphora pediculus</i>	BRUDER 2006, WANG 2014	eutrophic
<i>Cocconeis pediculus</i>	JAHN et al. 2007	eutrophic
<i>Cocconeis placentula</i>	JAHN et al. 2009	eutrophic
<i>Encyonema minutum</i>		nd
<i>Eunotia bilunaris</i>	VANORMELINGER et al. 2013	euryvalent
<i>Ulnaria ulna</i>	WILLIAMS 2011	euryvalent
<i>Fragilaria capucina</i>	KAHLERT et al. 2009	euryvalent
<i>Staurosirella pinnata</i>	MORALES et al. 2013	euryvalent
<i>Gomphonema parvulum</i>	ABARCA et al. 2014	eutrophic
<i>Navicula cryptocephala</i>	POULÍČKOVÁ et al. 2010	euryvalent
<i>Nitzschia palea</i>	KAHLERT et al. 2009; TROBAJO et al. 2009	hypertrophic
<i>Nitzschia paleacea</i>		eutrophic
<i>Sellaphora pupula</i>	MANN et al. 2004, 2008; VANORMELINGEN et al. 2013	mesotrophic

4.0. Shannon diversity indexes of diatoms were computed (then sorted according to: host plants, sampling site, streaming/stagnant water, geographical location of sampling sites). Variation of Shannon diversity indexes among sampling sites and streaming/stagnant water was analysed with One-Way ANOVA. Because of unequal number of host plants sampled, variation of Shannon diversity index was analysed with non-parametric Kruskal-Wallis multiple comparison test (NCSS statistical package, HINTZE 2007). With respect to different plant habitus and physiology, difference in Shannon's diversity was analysed for *Lemna minor* versus group of other submerged vascular plants. Response of diatoms to the best fitting environmental variables was analysed using T-value statistics (CanocoDraw 4.0).

RESULTS

A total of 131 diatom species was found during the study. Species richness ranged from 1 to 34 species per sample. Species richness was higher in the West Carpathians (19–34) than in the Svitava river basin (1–15 per sample). The highest number of diatom species was found on *Poaceae* and *Typha*. The dominant diatom species was *Achnanthydium minutissimum* agg. creating up to 88% of the community. The most frequently occurring species were *Gomphonema parvulum* agg. with representation 1–48% and *Cocconeis placentula* with representation 1–100%. Frequent species for both regions were also *Nitzschia palea* and *Ulnaria ulna*. Planktonic diatoms (*Cyclotella*, *Aulacoseira* and *Asterionella*) frequently occurred in ponds.

Surprisingly species complexes (Table 2) represent the majority of epiphytic assemblages (lotic/lentic: 67,1% and 66,5% respectively; substrates: *Poaceae* 63%, *Typha* 60%, *Sparganium* 79,2%, *Salix* 74,9%, *Phragmites* 75%, *Callitriche* 89,8%, *Lemna* 25%). However, differences in percentage of species complexes among sampled host plants were not significant ($F = 1.91$, $P = 0.1341$). There was no close correlation between Shannon's diversity and percentage of species complexes among sampled host plants as well (Pearson correlation coefficient: $r = 0.4113$) by other words: the high representation of complexes does not automatically lead to reduction of overall diversity of the sample.

Canonical correspondence analysis spread sampling points through the ordination space with respect to their geographical position (West Carpathians and Svitava river basin) and ecological nature (stream and pond). Sampling sites in the Western Carpathians, both ponds and streams, form more coherent clusters than in the Svitava river basin (Fig 3). Significant differences in Shannon's diversity index were found between sampling sites in the Svitava river basin and Western Carpathians (Fig. 6, $F = 5.88$, $p = 0.0204$). Sampling sites located in the Western Carpathians (Fig. 3, squares) possess statistically higher Shannon diversity (1.95 ± 0.55) than those in the Svitava river basin (1.43 ± 0.68). Similarly, statistically significant differences were found between sampling sites from streaming (2.08 ± 0.47) and stagnant water bodies (1.33 ± 0.63 ; Fig. 5, $F = 13.94$, $p = 0.0006$).

Species data explain 11.8% of variability on the first and 19.8% on the second ordination axis ($p = 0.002$, $F = 3.792$). Diatom ordination is significantly influenced by pH ($F = 3.84$, $p = 0.002$), water hydrodynamism (streaming/stagnant, $F = 2.59$, $p = 0.002$) and *Lemna minor* as a host plant ($F = 1.97$, $p = 0.018$, for details see Table 3, Fig. 4). Species such as *Amphora pediculus*, *Cocconeis pediculus*, *Cymbella excisiformis*, *Encyonopsis cesatii*, *Encyonopsis microcephala*, *Eunotia arcus*, *Gomphonema pumilum*, *Nitzschia palaeformis* or *Nitzschia sinuata* prefer significantly higher pH than *Mayamaea atomus*, *Planothidium ellipticum*, *Planothidium lanceolatum* or *Nitzschia palea*, which prefer lower pH value within investigated scale (4.80–8.45). Species preferring stagnant water bodies include *Fragilaria brevistriata*, *Encyonopsis microcephala*, *Eunotia arcus* and *Denticula tenuis*. On the other hand, streaming water prefer *Cocconeis pediculus*, *Gomphonema angustatum* and *Navicula tripunctata*. Diatom assemblages among sampled host plants possess almost the same diversity. *Lemna minor* showed the lowest variability of Shannon diversity (1.37 ± 0.17) in contrast to other host plants (*Phrag* = 1.56 ± 0.72 , *Poac* = 1.82 ± 0.53 , *Salix* = 1.54 ± 1.38 , *Typha* = 1.50 ± 0.59). However, Shannon's diversity did not show any statistically significant differences among host plants (Table 4). Similarly, difference in Shannon's diversity

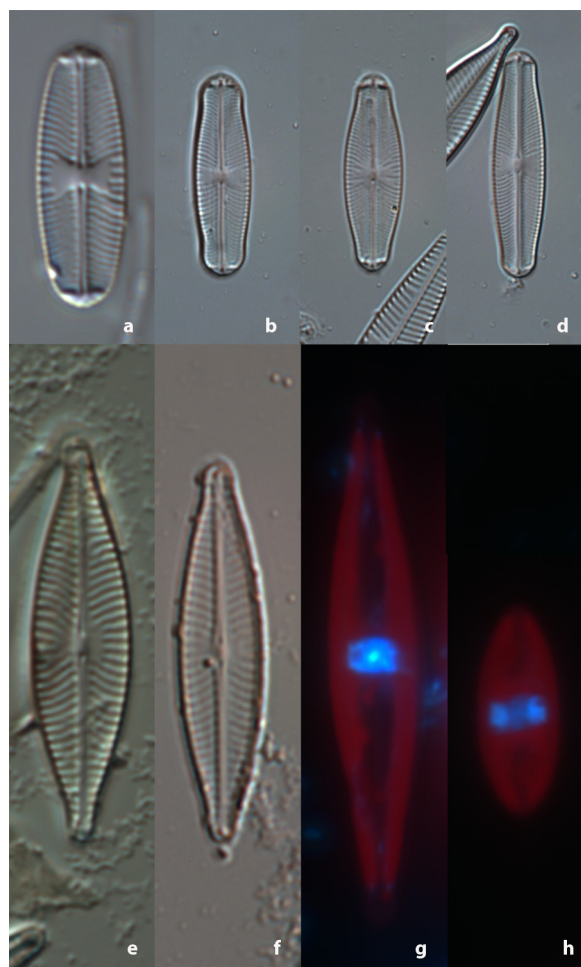


Fig. 2. Examples of species complexes in the Czech Republic: (a–d) *Sellaphora pupula* sensu lato differ in frustule morphology, (e–h) *Navicula cryptocephala* sensu lato differ in interphase nuclei structure.

between *Lemna minor* and other vascular submerged plants was not significant as well ($z = 0.874$, $\alpha = 0.05$). Diatoms showed low specificity to host plants except of *Lemna minor* ($F = 1.97$, $p = 0.018$). Species such as *Fragilaria brevistriata*, *Staurosirella pinnata* or *Nitzschia palaeformis* avoid *Lemna minor* as a host plant. Surprisingly, *Lemnicola hungarica* as a diatom typical for *Lemna minor*, inhabited also *Phragmites australis* in the Svitava river basin.

DISCUSSION

Small streams and shallow ponds represent ecosystems sensitive to environmental changes. It can be demonstrated by much higher nutrient variation in shallow than deep waters (JEPPSEN et al. 2000). In comparison with physicochemical variables, attached diatoms seem to be more integrative indicators with fast response to environmental changes (BLANCO et al. 2004; HÁJEK et al. 2014). Diatoms are able to inhabit all available substrates, which are mostly represented by

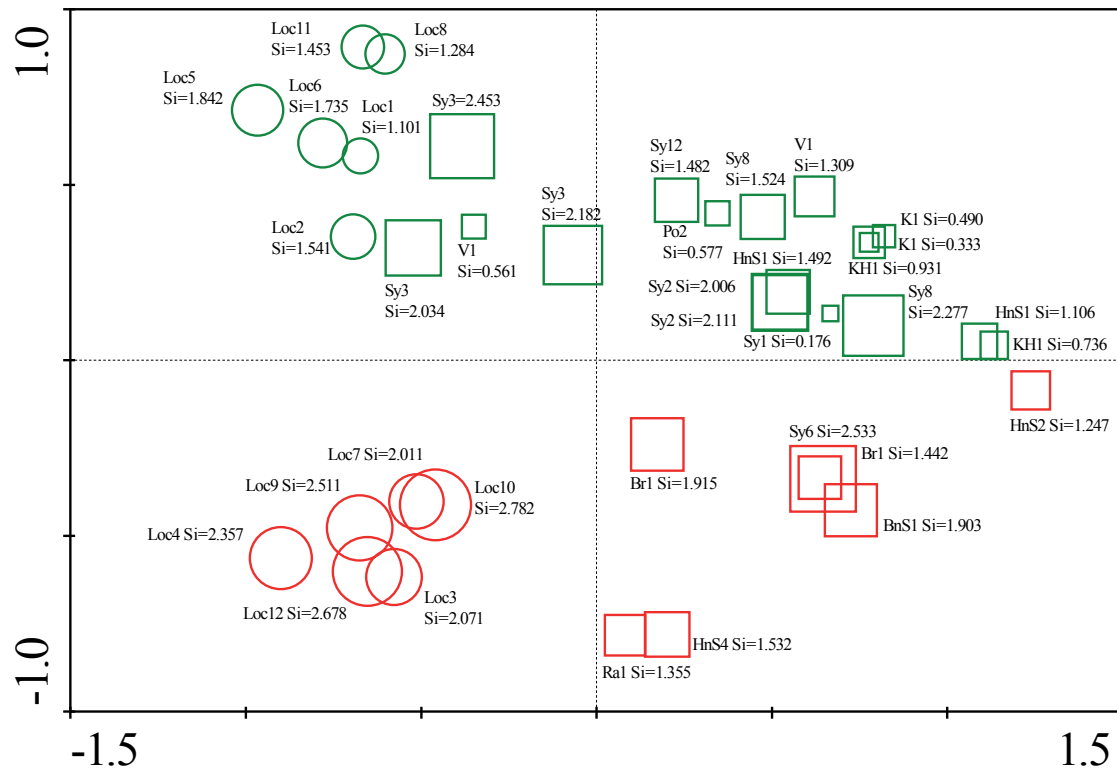
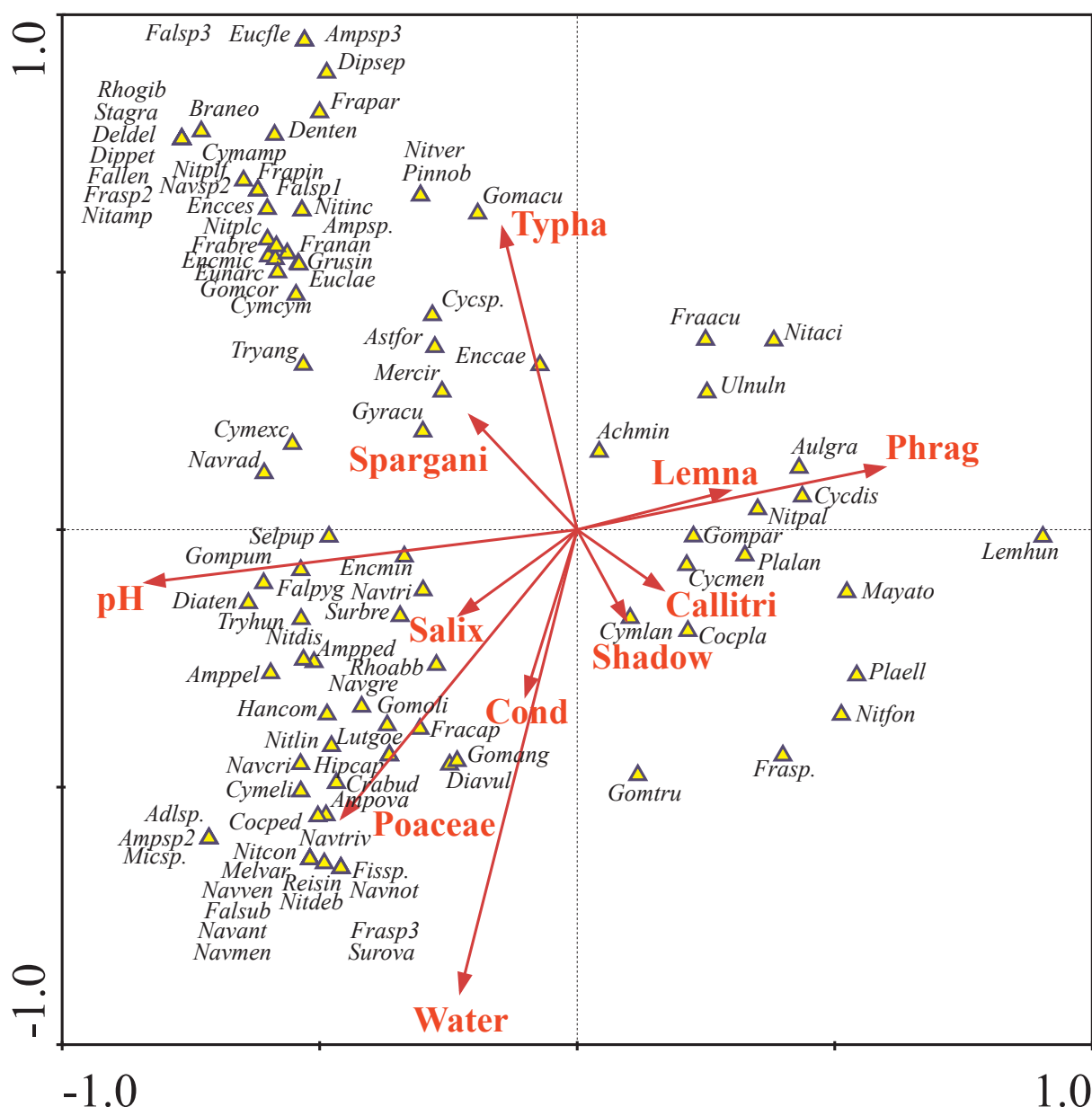


Fig. 3 CCA ordination diagram: investigated sites, (circles) Western Carpathians, (squares) Svitava river basin, (green) ponds, (red) streams; symbol size corresponds to value of Shannon's diversity index (Si).

Fig. 4. CCA ordination biplot diagram: species vs. environmental variables: (Achmin) *Achnanthes minutissimum* (KÜTZING) CZARNECKI, (Adlsp) *Adlafia* sp., (Ampel) *Amphipleura pellucida* (KÜTZING) KÜTZING, (Ampova) *Amphora ovalis* (KÜTZING) KÜTZING, (Amppe) *Amphora pediculus* (KÜTZING) GRUNOW ex A. SCHMIDT, (Ampsp) *Amphora* sp., (Ampsp2) *Amphora* sp. 2, (Ampsp3) *Amphora* sp. 3, (Astfor) *Asterionella formosa* HASSALL, (Aulgra) *Aulacoseira granulata* (EHRENBERG) SIMONSEN, (Braneo) *Brachysira neoexilis* LANGE-BERTALOT, (Cocped) *Cocconeis pediculus* EHRENBERG, (Cocpla) *Cocconeis placentula* EHRENBERG, (Cabud) *Craticula buderi* (HUSTEDT) LANGE-BERTALOT, (Cyccom) *Handmannia comta* (EHRENBERG) KOCIOLEK et KHURSEVICH, (Cedis) *Cyclotella distinguenda* HUSTEDT, (Cycmen) *Cyclotella meneghiniana* KÜTZING, (Cycsp) *Cyclotella* sp., (Cymeli) *Cymatopleura elliptica* (Brébisson) W. SMITH, (Cymcym) *Cymbella cymbiformis* C. AGARDH, (Cymex) *Cymbella excisiformis* KRAMMER, (Cymlan) *Cymbella lanceolata* (C. AGARDH) KIRCHNER, (Cymamp) *Cymbella amphicephala* (NÄGELI) KRAMMER, (Deldel) *Delicata delicatula* (KÜTZING) KRAMMER, (Denten) *Denticula tenuis* KÜTZING, (Diaten) *Diatoma tenuis* agg., (Diavul) *Diatoma vulgare* BORY DE SAINT-VINCENT, (Dippet) *Diploneis petersenii* HUSTEDT, (Dipsep) *Diploneis separanda* LANGE-BERTALOT, (Enccae) *Encyonema caespitosum* KÜTZING, (Encmin) *Encyonema minutum* agg., (Encces) *Encyonopsis cesatii* (RABENHORST) KRAMMER, (Encmic) *Encyonopsis microcephala* agg., (Eucfle) *Eucocconeis flexella* (KÜTZING) MEISTER, (Euclae) *Eucocconeis laevis* (ØSTRUP) LANGE-BERTALOT, (Eunarc) *Eunotia arcus* EHRENBERG, (Fallen) *Fallacia lenzii* (HUSTEDT) LANGE-BERTALOT, (Falpyg) *Fallacia pygmaea* (KÜTZING) A.J. STICKLE et D.G. MANN, (Falsp1) *Fallacia* sp., (Falsp3) *Fallacia* sp. 3, (Falsub) *Fallacia subhamulata* (GRUNOW) D.G. MANN, (Fissp) *Fistulifera* sp., (Fraacu) *Fragilaria acus* (KÜTZING) LANGE-BERTALOT, (Frabe) *Fragilaria brevistriata* GRUNOW, (Fracap) *Fragilaria capucina* DESMAZIÈRES, (Ffran) *Fragilaria nanana* LANGE-BERTALOT, (Frapar) *Fragilaria parasitica* (W. SMITH) GRUNOW var. *parasitica*, (Frasp) *Fragilaria* sp., (Frapin) *Staurisirella pinnata* (EHRENBERG) D. M. WILLIAMS et ROUND, (Frsp2) *Fragilaria* sp. 2, (Frsp3) *Fragilaria* sp., (Gomacu) *Gomphonema acuminatum* EHRENBERG, (Gomang) *Gomphonema angustatum* (KÜTZING) RABENHORST, (Gomcor) *Gomphonema coronatum* (EHRENBERG), (Gomoli) *Gomphonema olivaceum* (HORNEMANN) BRÉBISSE, (Gompar) *Gomphonema parvulum* (KÜTZING) KÜTZING, (Gompum) *Gomphonema pumilum* (GRUNOW) E. REICHARDT et LANGE-BERTALOT, (Gomtru) *Gomphonema truncatum* EHRENBERG, (Gyracu) *Gyrosigma acuminatum* (KÜTZING) RABENHORST, (Hipcap) *Hippodonta capitata* (EHRENBERG) LANGE-BERTALOT, METZELTIN et WITKOWSKI, (Lemhun) *Lemnicola hungarica* (GRUNOW) F.E. ROUND et P.W. BASSON, (Lutgoe) *Luticola goeppertiana* (BLEISCH) D.G. MANN, (Mayato) *Mayamaea atomus* (KÜTZING) LANGE-BERTALOT, (Melvar) *Melosira varians* C. AGARDH, (Mercir) *Meridion circulare* (GREVILLE) C. AGARDH, (Micsp) *Microcystus* sp., (Navant) *Navicula antonii* LANGE-BERTALOT, (Navci) *Navicula cryptotenelloides* LANGE-BERTALOT, (Navgre) *Navicula gregaria* DONKIN, (Navmen) *Navicula meniscus* SCHUMANN, (Navnot) *Navicula notha* WALLACE, (Navrad) *Navicula radiosa* KÜTZING, (Navsp2) *Navicula* sp. 2, (Navtri) *Navicula tripunctata* (O.F. MÜLLER) BORY DE SAINT-VINCENT, (Navtriv) *Navicula trivialis* LANGE-BERTALOT, (Navven) *Navicula veneta* KÜTZING, (Nitaci) *Nitzschia acicularis* (KÜTZING) W. SMITH, (Nitamp) *Nitzschia amphibia* GRUNOW, (Nitang) *Tryblionella angustata* W. SMITH, (Nitcon) *Nitzschia constricta* (KÜTZING) RALFS, (Nitdis) *Nitzschia dissipata* (KÜTZING) RABENHORST, (Nitfon) *Nitzschia fonticola* (GRUNOW) GRUNOW, (Nithun) *Tryblionella hungarica* (GRUNOW) FRENGUELLI, (Nitfru) *Nitzschia inconspicua* GRUNOW, (Nitlin) *Nitzschia linearis* W. SMITH, (Nitplf) *Nitzschia palaeiformis* HUSTEDT, (Nitdeb) *Nitzschia palea* var. *debilis* (KÜTZING) GRUNOW, (Nitpal) *Nitzschia palea* (KÜTZING) W. SMITH, (Nitplc) *Nitzschia paleacea* GRUNOW, (Nitsin) *Grunowia sinuata* (THWAITES) RABENHORST, (Nitver) *Nitzschia vermicularis* (KÜTZING) HANTZSCH, (Pinnob) *Pinnularia nobilis* (EHRENBERG) EHRENBERG, (Plaell) *Planolithidium ellipticum* (CLEVE) ROUND et BUKHTIYAROVA, (Plalan) *Planolithidium lanceolatum* (BRÉBISSEON ex KÜTZING) BUKHTIYAROVA, (Reisin) *Reimeria sinuata* (GREGORY) KOCIOLEK et STOERMER, (Rhoabb) *Rhoicosphenia abbreviata* (C. AGARDH) LANGE-BERTALOT, (Rhogib) *Rhopalodia gibba* (EHRENBERG) OTTO MÜLLER, (Selpup) *Sellaphora pupula* (KÜTZING) MERESCHKOWSKY, (Stagra) *Stauroneis gracilis* EHRENBERG, (Surbre) *Surirella brebissonii* var. *kuetzingii* KRAMMER et LANGE-BERTALOT, (Surova) *Surirella ovalis* BRÉBISSEON, (Ulnuln) *Ulnaria ulna* (NITZSCH) P. COMPÈRE. Variables: (Water) water streaming 0/1, (Cond) conductivity, pH, (Shadow) percentage of shade 0%/50%/100%; substrates: *Phragmites*, *Poaceae*, *Typha*, *Lemna*, *Salix*, *Callitriche*, *Sparganium*.



stones and sediments in streams and by macrophytes and sediments in shallow lakes and ponds. Homogeneity of periphytic communities and their composition are more related to chemical characteristics of the surrounding environment than to the substrate type, particularly in eutrophic systems (EMINSON & MOSS 1980; CATTANEO et al. 1998; KITNER & POULÍČKOVÁ 2003; POULÍČKOVÁ et al. 2004; CEJUDO-FIGUEIRAS et al. 2010). However, substrate specificity has been described in some oligotrophic waters (EMINSON & MOSS 1980; BLINDOW 1987; BUCZKÓ 2006; CANTONATI 1998; POULÍČKOVÁ et al. 2004). Our results did not confirm substrate specificity, except of specific assemblage growing on *Lemna* sp., similar to more complex study already published (BUCZKÓ 2007). In contrast to other periphytic assemblages, epiphytic assemblages include lower number of planktonic diatom taxa and

suspended particulates (KELLY et al. 1998, POULÍČKOVÁ et al. 2004, 2008, 2014). Planktonic diatoms were not frequent and were represented by *Aulacoseira granulata* (SB), *Asterionella formosa* and *Cyclotella* sp. (WC). In general, the relationship between epiphyton and water chemistry has been demonstrated many times (ÁCS et al. 1991, 1994; KITNER & POULÍČKOVÁ 2003; BLANCO et al. 2004; POULÍČKOVÁ et al. 2004; HÁJKOVÁ et al. 2011) and submerged macrophytes have been recommended for routine monitoring (KELLY et al. 1998; BLANCO et al. 2004). We found significant relationship to selected environmental variables (water streaming, pH) in congruence with other studies (POTAPOVA & CHARLES 2003; KITNER & POULÍČKOVÁ 2003; KOVÁCS et al. 2006; FRÁNKOVÁ et al. 2009; YANG & FLOWER 2012). However, the results of this method of water quality status assessment are strongly influenced by following

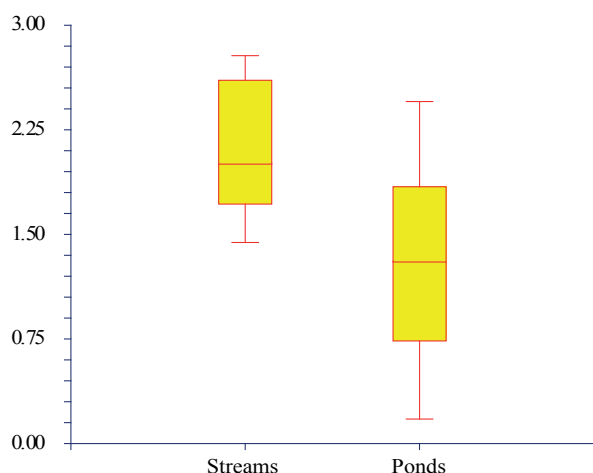


Fig. 5. Box plot of diatom Shannon diversity: comparison of habitat (streams, ponds; $F=13.94$, $p=0.0006$).

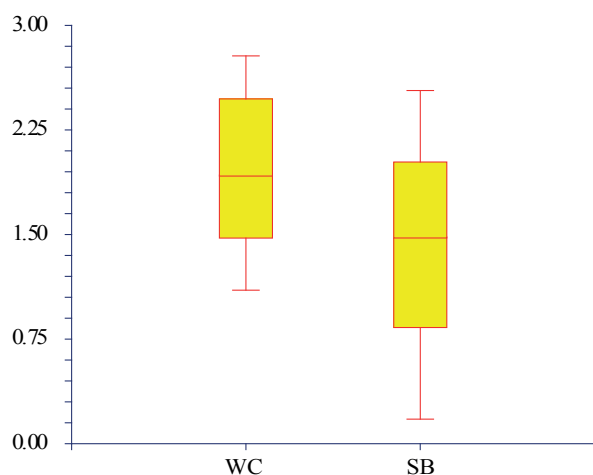


Fig. 6. Box plot of diatom Shannon diversity: comparison of sampling sites (SB) Svitava river basin, (WC) Western Carpathians ($F=5.88$, $p=0.0204$).

two factors: 1) trophic indices are working in ecoregions where they were intercalibrated (PRYGIEL et al. 2002; POULÍČKOVÁ et al. 2004; KOVÁČ et al. 2006) and 2) some traditional „euryvalent and cosmopolitan species“ represent species complexes consisting of few or many ecologically differentiated biological species (so called cryptic species), whose distinguishing in the LM is difficult or even impossible (MANN et al. 2008; KAHLERT et al. 2009; POULÍČKOVÁ et al. 2010).

Although many studies noticed that attached diatoms in wide spectrum of ecological conditions are dominated particularly by *Achnanthes minutissimum* (Kützinger) Czarnecki 1994 (BLANCO et al. 2004, CEJUDO-FIGUEIRAS et al. 2010), the assessment what is the proportion of species complexes within attached diatom assemblages has not been specified yet. SIGEE (2005) summarized dominant diatom species along a river course with increasing nutrient pollution. First zone (clean water) is characterized by small-celled

species directly attached to stone surface (*Eunotia exigua*, *Achnanthes microcephala*). Zones 2 and 3 are dominated by *Fragilaria capucina*, *Achnanthes minutissimum*, *Encyonema minutum* and *Cocconeis placentula*. Eutrophic zone 4 is characterized by *Gomphonema parvulum* and highly eutrophic zone 5 by *Nitzschia palea* (SIGEE 2005). In fact the majority of these „indicator dominants“ represent species complexes (Table 2; KWANDRANS et al. 1998; KAHLERT et al. 2009). If species complexes as a whole are not ecologically differentiated and create the majority of assemblage composition, it means that in this field is a great potential for trophic indices improvement. The ongoing progress with identification of cryptic diversity is in motion with implementation of molecular methods. Following six examples demonstrates the importance of species complexes investigation.

Sellaphora pupula agg.

An extreme example of species complexes seems to be *Sellaphora pupula* agg. (Fig. 2; MANN et al. 2008) with almost 50 morphospecies (demes), some of them already confirmed using molecular methods (EVANS et al. 2007, 2009; WETZEL et al. 2015). This diatom is typical for epipellic rather than epiphytic assemblages, with high representation in British lakes. It creates up to 40% of epipellic assemblages in lakes/ponds of Great Britain, while its representation in Czech and Hungarian ponds does not exceed 3% (POULÍČKOVÁ et al. 2008, ŠPAČKOVÁ et al. 2009). Although their identification is difficult particularly in the LM, some of them seem to be ecologically differentiated (POULÍČKOVÁ et al. 2008). Many lakes contain several different morphospecies, the greatest numbers of coexisting demes occurred in eutrophic Blackford Pond, Great Britain (POULÍČKOVÁ et al. 2008). Five morphospecies inhabiting Czech pond Bezedník (temperate zone) showed seasonal dynamics with significant correlation of their occurrence with temperature (ŠPAČKOVÁ et al. 2009).

Achnanthes minutissimum agg.

Although molecular methods have not been used in this case yet, the opinion that previously described varieties within *A. minutissimum* can represent ecologically differentiated species seems to be evident (POTAPOVA & HAMILTON 2007). Moreover this species complex has been recorded as the most frequent dominant of epilithic and epiphytic assemblages in both lotic and lentic freshwaters (PONADER & POTAPOVA 2007; POTAPOVA & HAMILTON 2007). Morphometric study (POTAPOVA & HAMILTON 2007) revealed 6 morphological groups, however authors were not able to draw clear boundaries between them. These morphospecies differed significantly in their ecology and could serve as indicators of water quality (POTAPOVA & HAMILTON 2007). However, an analysis of the results of 25 diatomists participating in intercalibration exercise showed, that even experienced diatomists have problem to recog-

Table 3. CCA forward selection of environmental variables: influence of environmental variables on species distribution in the Svitava river basin and the White Carpathian Mts [(Water) streaming/stagnant water, (Cond) conductivity, (Lemna) *Lemna minor*, (Spargani) *Sparganium* sp., (Shadow) shadow/half-shadow/light, (Typha) *Typha* spp., (Phrag) *Phragmites australis*, (Callitri) *Callitriche* sp.].

Conditional Effects					Marginal Effects		
Variable	Var.N	LambdaA	p	F	Lambda1	p	F
pH	1	0.32	0.002	3.84**	0.32	0.002	3.84**
Water	4	0.21	0.002	2.59**	0.22	0.004	2.50**
Cond	2	0.16	0.010	2.10**	0.13	0.090	1.41
Lemna	8	0.15	0.018	1.97**	0.17	0.024	1.88**
Spargani	11	0.10	0.254	1.27	0.11	0.358	1.21
Shadow	3	0.08	0.278	1.14	0.09	0.406	1.1
Typha	7	0.08	0.314	1.7	0.13	0.078	1.51
Poaceae	6	0.08	0.434	1.1	0.18	0.004	2.3**
Salix	9	0.08	0.452	1.00	0.09	0.464	0.99
Phrag	5	0.03	0.920	0.41	0.20	0.002	2.33**
Callitri	10				0.05	0.796	0.57

** statistically significant

Table 4. Kruskal–Wallis Multiple–Comparison Z–Value Test (Dunn’s Test), differences of Shannon’s diversity index among host plants [(Lemna) *Lemna minor*, (Poac) *Poaceae*, (Phrag) *Phragmites australis*, (Salix) *Salix* spp., (Typha) *Typha* spp.].

	Lemna	Poac	Phrag	Salix	Typha
Lemna	0.0000	1.1867	0.8036	0.5970	0.4306
Poac	1.1867	0.0000	0.6897	0.4315	1.1457
Phrag	0.8036	0.6897	0.0000	0.0269	0.5636
Salix	0.5970	0.4315	0.0269	0.0000	0.3141
Typha	0.4306	1.1457	0.5636	0.3141	0.0000

Regular Test: Medians significantly different if z–value > 1.9600

Bonferroni Test: Medians significantly different if z–value > 2.8070

nize varieties/morphospecies of *A. minutissimum* in the LM (KAHLERT et al. 2009), due to small size close to the LM resolution limits (length 5 – 25 µm, width 2.5 – 4 µm and dense striation 26–30/10µm; HOFMANN et al. 2013). Improvement in bioassessments in this case strongly depends on application of molecular methods.

Gomphonema parvulum agg.

The name *G. parvulum* represents a diatom species which is relatively small in size (length 10–36µm, width 5–8µm) and has cosmopolitan distribution (HOFMANN et al. 2013). In fact it has been used as a collective name for a species complex for two centuries. Morphologically highly variable diatom occurs in wide range of water qualities (PATRICK & REIMER 1975; HUDSTEDT 1985; KRAMMER & LANGE–BERTALOT 1997). Molecular as well as morphological data obtained during the recent studies (KERMAREC et al. 2013; ABARCA et al. 2014) resulted in separation of at least four taxa based on their biogeography.

Eunotia bilunaris agg.

E. bilunaris sensu lato is a good candidate for studies on semicryptic species diversity in diatoms. It is a cosmopolitan and common epiphytic diatom in oligotrophic, mainly acidic freshwater bodies (KRAMMER & LANGE–BERTALOT 1991; VANORMELINGEN et al. 2008). Based on its phenotypic plasticity, a number of species have been described (LANGE–BERTALOT et al. 2011). Moreover morphological, molecular and reproductive data suggest the existence of several reproductively isolated species (VARNORMELINGEN et al. 2008).

Navicula cryptocephala agg.

N. cryptocephala is a common benthic diatom of moderate size (20–40 µm long, 5–7 µm wide; LANGE–BERTALOT 2001).

In contrast to species complexes with broad morphological variation, *N. cryptocephala* represents a complex with almost identical valve morphology. However, it has been found to be polymorphic with respect to interphase nuclear structure (Fig. 2; GEITLER 1951, 1952a,b, 1958; POULÍČKOVÁ et al. 2010). Phylo-

genetic analyses of 52 strains confirmed the existence of two genetically distinct lineages within *N. cryptocephala* that coexist sympatrically and are widely distributed, occurring in European and Australian ponds (POULÍČKOVÁ et al. 2010).

Nitzschia palea agg.

N. palea is believed to be a widely distributed diatom in lotic and lentic freshwater habitats (FINLAY et al. 2002; POTAPOVA & CHARLES 2007). In general, the genus *Nitzschia* is difficult for identification and discrimination between members, particularly in the section *Lanceolatae* Grunow (HUSTEDT 1930). Moreover identification is complicated by morphological variability during the life cycle and phenotypic plasticity due to environmental conditions. On the base of their results (morphological, genetic and mating diversity) TROBAJO et al. 2009 concluded that *N. palea* is not a simple, homogeneous taxon and that this complex will probably have to be split into three or more species. At least two of them appear to be geographically widespread. Ecological preferences and potential indicator value need to be further investigated (TROBAJO et al. 2009).

In conclusion, species complexes are important, because of their common occurrence, frequent dominance and difficulties with their distinguishing.

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