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Article

Salinity and depth as structuring factors of cryptic divergence in *Moina brachiata* (Crustacea: Cladocera)

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With 4 figures, 4 tables and 3 supplementary tables

Abstract: Microcrustacean taxa in temporary waters are important contributors to aquatic biodiversity on the landscape scale even though much of the diversity at the molecular level is still undiscovered. Cladoceran species other than *Daphnia* are not frequently targeted in molecular investigations. We used nuclear allozyme polymorphisms as well as DNA sequence variation in mitochondrial 16 S and COI gene regions to reveal patterns of genetic differentiation among populations of a cladoceran species – *Moina brachiata* – that typically inhabits temporary aquatic habitats. Samples originated from 20 temporary to semi-permanent waterbodies in the Hungarian Great Plain of the Pannonian biogeographic region. We observed strong genetic differentiation in the phylogenetic analyses of the concatenated 16 S and COI genes, based on which *M. brachiata* was found to represent a complex of four cryptic lineages (A, B, C and D) with, however, one of these (lineage D) detected based on only one individual. Regarding the nuclear markers, diagnostic alleles of the PGM and MDH enzyme loci in complete linkage disequilibrium were observed separating the 'B' lineage from the rest. In addition, indirect evidence was provided by the AAT locus, where the AAT1 allele was found to be potentially diagnostic for lineage 'C'. The three phylogenetically defined lineages ('A', 'B', 'C') could be separated from each other along the first canonical axis of a multivariate analysis of occurrence, and this first axis was strongly correlated with depth and salinity of the ponds. There is a strong association between habitat depth and the occurrence of the 'B' lineage. Our results indicate that habitat depth and associated ecological characteristics driven by differences in hydroperiod likely are responsible for the present distribution of the lineages.

Key words: cryptic species, COI, 16 S, haplotypes, temporary waters, depth, salinity.

Introduction

Organisms of temporary aquatic habitats require special adaptation strategies to withstand recurrent dry periods. As a consequence, this type of ecosystem is characterised by a specialized and unique fauna and flora and thus is an object of intense research and conservation efforts (Ramsar 2002, Brendonck et al. 2010). Human made changes in hydrologic regimes, levee building for river flood prevention and agricultural expansion have had drastic effects on temporary waters during the last centuries worldwide. In

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addition, the effects of climate change threaten these aquatic habitats (Hulsmans et al. 2008). The severe decline of biodiversity in freshwater systems during the past decades (Dudgeon et al. 2006, Balian et al. 2008, Naiman 2008, Heino et al. 2009) underscores the importance of research and conservation efforts for these unique temporary habitats.

A number of studies have aimed to unravel biotic and abiotic conditions that locally determine species composition and community structure in temporary waters. Hydroperiod, i.e. the length of the inundated period, is known to structure invertebrate communities (Wellborn et al. 1996, Jenkins et al. 2003, Frisch et al. 2006, Medley & Havel 2007, Tavernini 2008). In a set of floodplain ponds, habitat depth was found to be a significant local variable influencing species richness and zooplankton community structure (Medley & Havel 2007) and the effect of water depth on species richness has also been shown for ostracods and cladocerans (Eitam et al. 2004). It has been shown that microcrustacean communities and species richness are influenced by salinity (Boronat et al. 2001, Frisch et al. 2006, Waterkeyn et al. 2008), and the indirect effect of salinity through biological interactions and interactions between certain physical and chemical factors has been emphasised (Williams et al. 1990). Furthermore salinity is known to affect the physiology of cladocerans, to the extent that different osmoregulatory abilities might develop among populations of the same species inhabiting waters of differing salinities (Aladin & Potts 1995). Salinity has also been shown to influence morphological traits in ostracods (Yin et al. 1999).

Beside local conditions, dispersal of adults or dormant propagules by flooding events, wind, waterfowl, human activity and ecto- and endozoochory (Allen 2007, Green et al. 2008, Waterkeyn et al. 2010, Havel & Shurin 2004) also affect community composition in temporary aquatic systems. Dispersal rates depend on the availability of source habitats, dispersal mode and the abundance of dispersal vectors.

Biodiversity estimates of the crustacean fauna in aquatic habitats are hindered by incomplete information on delineation of species (Adamowicz & Purvis 2005). With the recent advances of molecular tools and their broadening application, the number of discoveries of cryptic species in animal taxa increased exponentially in the last decades (Bickford et al. 2007, Pfenninger & Schwenk 2007), and cryptic lineages are frequently detected among crustaceans as well (Elías-Gutiérrez et al. 2008, Belyaeva & Taylor 2009, Petrusek et al. 2012). However, despite the recent technical developments, the estimated number of cladoceran species is still likely 2–4 times higher than the currently recognized 620 species (Forró et al. 2008). At the same time, possible forces driving speciation often remain unknown, while knowledge of ecological factors accounting for adaptive processes may provide a useful basis for planning and managing conservation projects.

Most species of the family Moinidae possess a high degree of physiological adaptation to temporary environments, and occur primarily or exclusively in temporary ponds and pools, including saline and alkaline waters (Goulden 1968). *Moina macrocopa* (Straus, 1820) is a frequently used model species in ecotoxicological research (Gama-Flores et al. 2007, Mangas-Ramírez et al. 2004) and microsatellite markers have been published recently for this species (Tatsuta et al. 2009). Cryptic species have been reported in *Moina micrura* Kurz, 1875 (Elías-Gutiérrez et al. 2008, Petrusek et al. 2004).

Research on *Moina brachiata* (Jurine, 1820) (Crustacea: Anomopoda), a widely distributed taxon throughout most of the Old World (Goulden 1968), is relatively scarce. Based on detailed investigation of life history parameters (development, reproduction and growth pattern) in *M. brachiata*, Maier (1992) concluded that the species has a relatively short egg development period and high egg production rates that are advantageous in unstable environments. The species has a closed brood chamber (Aladin & Potts 1995) making it capable of regulating the osmolarity of the embryonic environment.

In the present study, *Moina brachiata*, a common taxon from temporary waters of the Great Plain, was used to study the genetic structure of populations between different regions. We also aimed to investigate to what extent cryptic speciation may have occurred in this taxon. Finally, we also aimed at detecting the role of the abiotic environment in the occurrence of the genetically differentiated *M. brachiata* populations, since this information might be useful in the selection of habitats for conservation purposes and it might also indicate possible forces driving evolution.

Methods

Study area and habitats

The climate of the Hungarian Great Plain is continental, with a relatively long warm season, hot summers and cold winters. The amount of yearly precipitation is variable, but the larger part of it occurs during summer. Typically, the temporary pools in the area fill up twice a year, first after receiving snowmelt run-off in February to March, and later due to early summer

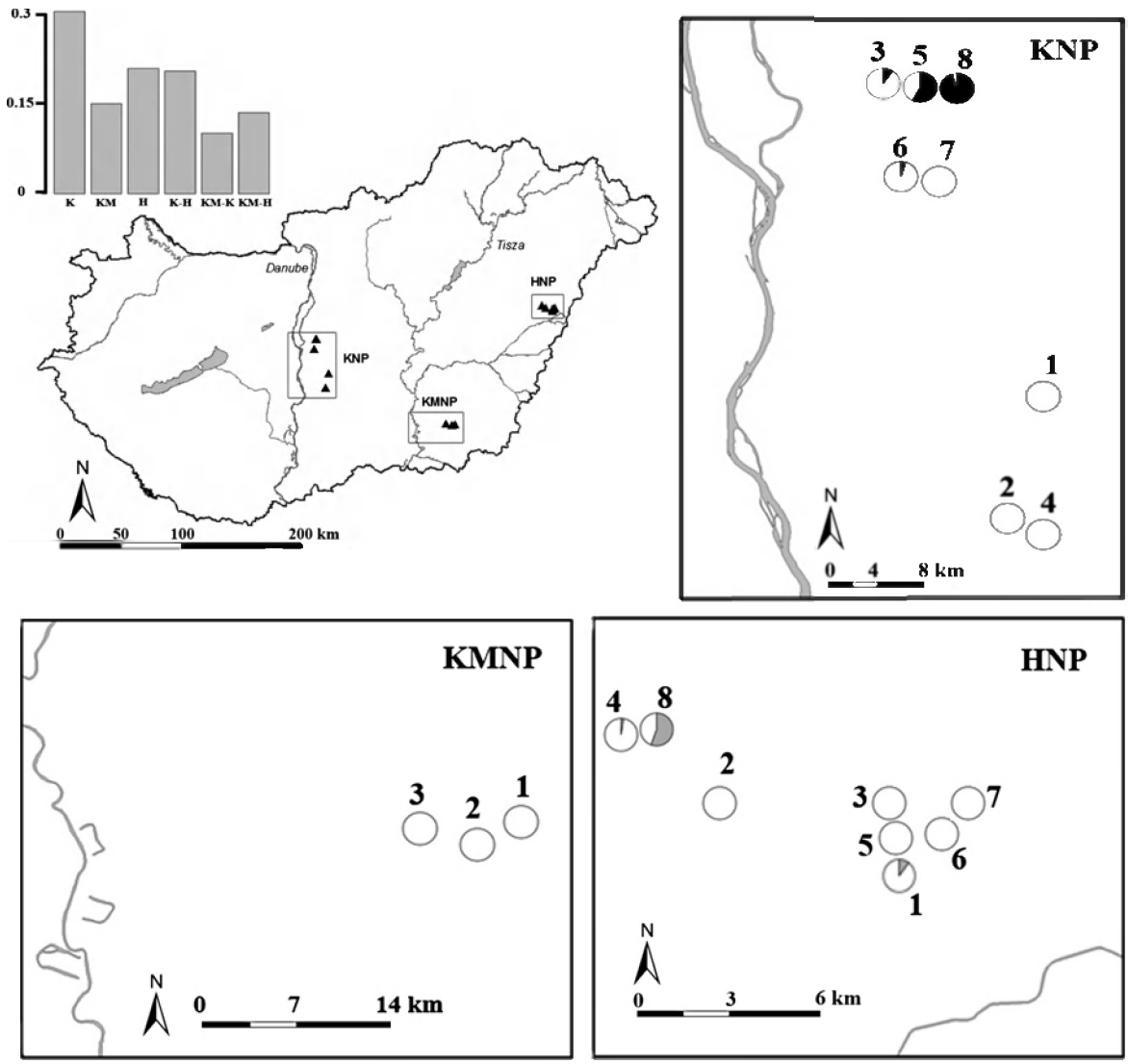

Fig. 1. Allozyme pattern associated with specific clades in the sampled populations on the map of Hungary. Top left barplot shows Fst values within lineage 'A' in the following order: K – within KNP, KM – within KMNP, H – within HNP, K-H – among KNP and HNP, KM-K – among KMNP and KNP, KM-H – among KMNP and HNP. Rectangle frame insets show the sampled regions, pie diagrams representing different ponds. Numbers refer to pond numbers (see Table 1). White color in the pie diagrams corresponds to lineage 'A'. Black color in the pie diagrams represents the frequency of the MDH1 allele (i.e. the 'B' lineage). Grey color in the pie diagrams corresponds to the frequency of the AAT1 allele (i.e. the putative 'C' lineage). KMNP- Körös- Maros National Park, KNP- Kiskunság National Park, HNP- Hortobágy National Park.

precipitation during June to July. By the end of the hot summer (August) most of the pools dry out and stay dried for the rest of the year. The hydrography of the Great Plain is mainly influenced by the Danube and Tisza rivers. Since the end of the 19th century, significant changes in the hydrological regime have taken place through levee building for flood prevention, building of irrigation channels, artificial river bed alterations and changes in land use, leading to a significant decrease in the number of temporary waterbodies, even though this type of habitat is still found relatively frequently.

Temporary waters for our study were selected in three regions in the Great Plain: in the Kiskunság National Park (KNP), Körös-Maros National Park (KMNP) and Hortobágy National Park (HNP); see Fig. 1 for their relative position. Hydrological history of the regions is different. Selected sites in the KNP region lie directly on a former floodplain, therefore this region was drastically affected by river regulation. Sites in the HNP are located on an interfluvial plateau along the margin of the former floodplain while the KMNP sites lie on a loess plateau of the interfluve further away from the original floodplain area.

Sampled habitats were temporary or semi-permanent waterbodies (wheeltracks, puddles, pools, flooded depressions in the ground) surrounded by grassland, arable land or pastures. Interesting temporary habitat types in the Kiskunság National Park area are the bomb craters that were formed during the 1950's when the area was used as a military training ground.

Due to their depth, most of the bomb craters retain water for an extended period during summer and occasionally they may remain wet throughout the whole year.

Sample collection

Sampling site selection was accomplished based on prior knowledge of the occurrence of *M. brachiata*. Sampling was done between 5th April and 23rd June 2006. As 2006 was a very wet year, we aimed to select pools that were not connected to other waterbodies even during the highest water level. Sampling sites were arranged in three clusters corresponding to the Kiskunság (8 sites), Körös-Maros (4 sites) and Hortobágy (8 sites) National Parks (Fig. 1) referred to as 'KNP', 'KMNP' and 'HNP' regions, respectively.

Zooplankton samples were collected using a plankton net of 85 μm mesh size. Samples were placed into a white plastic tray and organisms were sorted in the field using magnifying glasses. Animals for cellulose acetate gel electrophoresis were placed into cryotubes (around 15 specimens per tube) and flash-frozen in liquid nitrogen for transport to the laboratory. We aimed to collect 60 individuals per population wherever this was possible. Additional individuals were preserved in 96 % ethanol and stored at 5 °C until use for DNA extraction. Egg-bearing parthenogenetic *M. brachiata* females were selected under the microscope in the laboratory directly before molecular analyses. Identification of the animals followed Goulden (1968). Four *M. macrocopa* individuals were also selected and sequenced to be used as the outgroup in the phylogenetic analyses.

Environmental variables were recorded for each pool (Table 1). We measured salinity and pH (WTW Multiline P3) and the geographical coordinates of the pools. Maximum water depth and the distance between the sampled pool and the nearest waterbody were measured with a measuring tape. Surface area at the time of the sampling was estimated based on direct measurements by tape measure for pools not exceeding 40 m in length, while surface area of pools over this size was estimated by eye.

mtDNA variation

DNA extraction procedures were conducted based on the H3 method (Schwenk et al. 1998). We used 100 μl H3 buffer and 20 μl proteinase K (Fermentas, 18 mg/ml) per individual in the reactions. In the case of parallel analyses of allozyme and mitochondrial DNA variation, we used 5 μl sterile ultrapure water to emacerate each individual and placed 1.5 μl of this homogenate into 20 μl H3 buffer with 6 μl proteinase K. The rest of the homogenate was diluted with 5 μl sterile ultrapure water to be used for cellulose acetate gel electrophoresis.

The universal primer LCO1490 (5'– GGTCAACAAATCATAAAGATA –3') (Folmer et al. 1994) and COI-H (5'– TCAGGGTGACCAAAAAATCA –3') (Machordom et al. 2003) were used to amplify an approximately 660 bp region of the mitochondrial cytochrome oxidase subunit I. For the 16 S region we used S1 (5'– CGG CCG CCT GTT TAT CAA AAA CAT –3') and S2 (5' – GGAGCT CCG GTT TGA ACT CAG ATC –3') (Schwenk et al. 1998).

PCR amplification of the 16 S region was conducted in 25 μl volumes containing 5 μl homogenate, 1 × reaction buffer+$(NH_4)_2SO_4$ (Fermentas), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of the primers and 0.5 U/μl Taq polymerase (Fermentas). Thermal cycle settings for the 16 S region were 94 °C for 5 min followed by 40 cycles of 93 °C for 45 s, 50 °C for 1 min, 72 °C for 2 min and an additional elongation step at 72 °C for 2 min.

Table 1. Summary of the environmental characteristics of the sampling sites and the number of individuals (N) per population used in the allozyme analyses. Pop: population identifier, EOVX and EOVY: geographical coordinates (Hungarian uniform national projection), Sal: salinity (g/l), depth (cm), near: distance to nearest pool (m), surface: surface area of the pond (m^2) at the time of sampling. Population KMNP4 could not be analysed for enzyme polymorphism.

Pop	EOVX	EOVY	N	Sal	pH	depth	near	surface
HNP1	220965	851346	29	0.5	8.4	10	2	120
HNP2	223394	845552	42	0	7.9	20	0.8	20
HNP3	222512	851228	39	0.3	8.7	10	16	4.5
HNP4	225404	842505	41	0.2	8.4	30	1	100
HNP5	221956	851306	23	1.1	8.6	30	26	2
HNP6	222557	852800	30	1.1	8.2	10	1	2.5
HNP7	223166	853424	37	3.2	9.0	10	1	0.45
HNP8	225455	842677	36	0	7.4	45	6	50
KNP1	169491	666722	55	0.1	7.8	30	7	150
KNP2	157646	664143	41	0	7.8	30	2	10.5
KNP3	197539	656022	31	2.5	8.8	50	4	300
KNP4	157432	664305	47	0.1	8.5	15	0.5	6
KNP5	197444	656530	33	2.6	9.6	120	10	113
KNP6	189725	654948	42	5.9	9.2	10	2	1
KNP7	189740	655000	32	1.6	8.6	10	2	0.8
KNP8	197380	656804	44	3.2	9.3	130	7	133
KMNP1	127248	770718	40	0.5	8.6	30	2	68000
KMNP2	126457	768297	16	0	8.8	15	1	18
KMNP3	127942	763462	40	0	8.6	25	25	300
KMNP4	126427	768660	–	0.4	8.3	30	25	40

PCR amplifications of the COI region were conducted in 25 µl volumes containing 10 µl homogenate, 1 × reaction buffer+$(NH_4)_2SO_4$ (Fermentas), 2 mM $MgCl_2$, 250 µM of each dNTP, 0.35 µM of the primers and 0.5 U/µl Taq polymerase (Fermentas). For the cytochrome oxidase I region we used 94 °C for 1 min followed by 40 cycles of 94 °C for 1 min, 40 °C for 1 min 30 s, 72 °C for 1 min 30 s and the final elongation at 72 °C for 6 min.

After cleaning of the PCR products (High Pure PCR Product Purification Kit, Roche Diagnostics) sequencing followed on an ABI 3130 Genetic Analyser according to the producers' instructions. The same primer pairs as for the PCR amplifications were used to sequence the regions from both directions. Multiple sequence alignments were carried out using BioEdit version 7.0.5.3 (Hall 1999) and checked by eye. Descriptive statistics for the obtained sequences were calculated with DnaSP v5 (Librado & Rozas 2009).

Every detected concatenated 16 S+COI *M. brachiata* haplotype and two *M. macrocopa* haplotypes (Mmac1 and Mmac2) were used for phylogenetic analysis with the outgroup *Polyphemus pediculus* (Linné, 1761) AY075048 for 16 S and AY075066 for COI (Cristescu & Hebert 2002). Model selection and phylogenetic analysis by maximum likelihood was done in TREEFINDER (Jobb 2008). Model selection by Akaike information criterion corrected for finite sample sizes (AICc) resulted in the transversion model (TVM) (Posada 2003) for the 16 S (475 bp) gene while the J2 model (Jobb 2008) was selected for the COI (605 bp) region. For both genes the discrete Gamma heterogeneity model with four rate categories was applied. Nonparametric bootstrap support to assess internal branch support was calculated by 1000 replicates, and the 50 % majority-rule consensus tree was built under the partitioned model. Branch support through posterior clade probability for the same partitioned dataset was assessed in MrBayes 3 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Since TVM and J2 are not implemented in this software, calculations were done under the GTR+G model with 4 rate categories for 16 S and the HKY+G model with 4 rate categories for COI. Two independent analyses were started from random starting trees and ran for 1001000 generations. The Markov chains were sampled each 100 generations, and final statistics were calculated after discarding the first 2500 trees as burn-in.

To illustrate the level of diversification within the *Moina* genus and to make a comparison to the well-known *Daphnia longispina* group, an additional phylogenetic reconstruction was carried out for the COI gene (604 bp) only. This second analysis contained one *M. brachiata* haplotype from each putative lineage detected in the prior analysis of the concatenated dataset. To compare divergence within *M. brachiata* to divergence within other *Moina* species we also included one *M. macrocopa* individual collected in Hungary and sequenced for the present study. The other *M. macrocopa* (GenBank accession number EU702249), three lineages of *M. micrura* from Mexico (GenBank accession numbers: EU702207, EU702239, EU702244) and one *Ceriodaphnia dubia* Richard, 1894 haplotype (GenBank accession number EU702080) used as outgroup were sequenced by Elías-Gutiérrez et al. (2008). *Daphnia longispina* (EF375862) and *Daphnia lacustris* (EF375863) were published by Petrusek et al. (2008). In addition, *Daphnia dentifera* (FJ427488) (Adamowicz et al. 2009), *Daphnia mendotae* (GQ475272) (Briski et al. 2011), *Daphnia galeata* (JF821192) and *Daphnia cucullata* (JF821190) (unpublished sequences downloaded from GenBank) were included in the analysis.

Model selection and phylogenetic reconstruction by maximum likelihood under the GTR+GI model with four rate categories was carried out in TREEFINDER (Jobb 2008). Nonparametric bootstrap analysis with 1000 replicates was done to assess internal branch support. Bayesian inference by two independent analyses from random starting trees was calculated in MrBayes (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) under the same model. Generation, sampling and burn-in settings for this analysis were set as for the concatenated dataset. Branch support was assessed through posterior clade probabilities.

Pairwise sequence divergence estimates of the COI gene under the K2 p model with uniform rates among sites were calculated in MEGA 5.01 (Tamura et al. 2011) for the same dataset.

A minimum spanning network at 99 % connection limit was generated with TCS 1.21 (Clement et al. 2000) based on the statistical parsimony cladogram estimation method (Templeton et al. 1992). The analysis was carried out for joined 16 S and COI regions. The network therefore includes only the animals (53) for which sequencing was successful for both genes.

Cellulose acetate gel electrophoresis

Cellulose acetate gel electrophoresis was used to reveal enzymatic variability within *M. brachiata* at five loci: supernatant aspartate amino transferase (sAAT; EC 2.6.1.1), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), supernatant malate dehydrogenase (sMDH; EC 1.1.1.37), mannose-6-phosphate isomerase (MPI; EC 5.3.1.8) and phosphoglucomutase (PGM; EC 5.4.4.2). Individuals were emacerated in 5 µl sterile ultrapure water before application to the gel (Helena Super Z-12 Applicator kit, Helena Laboratories, Beaumont, Texas). Gel electrophoresis was conducted at 270 V (ZipZone Chamber, Helena Laboratories). Individuals of a parthenogenetically reared *Daphnia pulex* Leydig, 1860 clone were used as markers in each run in the ninth position on the gel. The buffer system used for running PGM (15 min runtime), MPI (10 min) and PGI (15 min) was pH 8.7 tris-citrate (39 mM Tris, 0.001 M EDTA, 2.5 mM citric acid). To run MDH (15 min) pH 7.3 tris-citrate buffer (36.45 mM Tris, 14.3 mM citric acid) and to run AAT (25 min) LiOH-boric acid buffer (11.89 g boric acid and 1.175 g LiOH for 1000 ml buffer) was used. Staining procedures were carried out following the protocols described by Hebert & Beaton (1989). Migration lengths of the different alleles were measured from the application position.

Data analysis on population genetic structure and environmental factors

Standard population genetic characteristics (allele frequencies, heterozygosity) were quantified from the allozyme data using the software TFPGA (Miller 1997). In addition, we carried out hierarchical Wright's F statistics analyses on the dataset. Animals with missing alleles were excluded from the descriptive and hierarchical population genetic analyses. Wright's F statistics was calculated based on the method of Weir & Cockerham (1984).

Links between genetic divergence of *M. brachiata* populations and abiotic characteristics were addressed by Constrained Analysis of Principal Coordinates (CAP) (Legendre & Ander-

son 1999). The distance matrix of the response variable for the analysis was generated with TFPGA (Miller 1997), calculating pairwise Nei's unbiased genetic distances (Nei 1978) between all population pairs that contained at least 14 specimens. In populations where multiple lineages co-occurred, we either removed individuals from lineages that occurred at low frequency (in practice AAT1 or PGM1 allele bearing individuals), or, if these lineages occurred in 14 or more individuals, we kept them separately and included them as separate populations in the analysis. Environmental data were $\log_{10}(x+1)$ transformed prior to the analysis to have approximate normally distributed random errors (Jongman et al. 1995). pH was omitted from further analysis since it displayed significant correlation with salinity (Spearman's $r_{pH\text{-}salinity} = 0.717$, $p < 0.01$). The environmental variables were first screened via forward selection procedure with Monte Carlo randomization tests (4999 runs, $\alpha = 0.1$) within CAP to determine a reduced set of significant variables for the final model. Furthermore AIC values were calculated for each step within the procedure to select the best fitting model to the observed genetic structure. We conducted variance partitioning procedure within CAP to determine the relative influences of selected environmental variables. Additional Monte Carlo permutation tests (4999 runs, $\alpha = 0.1$) were performed to determine the significance of individual environmental variables and canonical axes. To clarify the meaning of canonical axes we calculated Pearson correlations between the axes and significant environmental variables. Finally, we performed additional variance analyses (Kruskal-Wallis tests) to quantify the differences between the phylogenetically defined lineages ('A', 'B', 'C') along the canonical axes. Thus we used object scores projected on the canonical axes as explanatory variables and previously defined lineages ('A', 'B', 'C') as grouping variables. The statistical analyses were performed with software R ver. 2.14.0 (R Development Core team 2011) using the package 'vegan'.

Results

Deep lineage divergence and connection to environmental factors

Sequences of COI or 16 S or both were successfully acquired from 74 *M. brachiata* individuals altogether. A list of GenBank accession numbers obtained in this study can be found in Supplementary Table 1, along with allozyme data for individuals for which nuclear and mitochondrial markers were investigated from the same individual. For COI we obtained 627 bp fragments without alignment gaps or missing data for 57 *M. brachiata* specimens, representing 17 haplotypes. 109 variable sites were detected, 102 of them being parsimony informative. For the 16 S region 516 bp long fragments were acquired from 70 *M. brachiata* specimens, with one insertion-deletion site segregating for two indel haplotypes. Out of 40 variable sites 31 proved to be informative for parsimony.

Maximum likelihood (ML: -lnL = 3525.78) and Bayesian analysis (Bayesian best likelihood: -lnL = 3555.52) of the concatenated 16 S and COI genes resulted in the same topology (Figure 2), confirming the existence of four different clades ('A', 'B', 'C' and 'D') within the morphospecies *M. brachiata*.

Nuclear support for the deep lineage divergence comes from the allozyme electrophoresis. Detected allele frequencies and heterozygosities for each locus are listed in Supplementary Table 2 along with migration lengths of the different alleles observed under the applied electrophoretic conditions. Two alleles of different mobility (assigned as '1' and '2') were detectable at the MDH locus, while there were four alleles present at the PGM locus. The MDH1 and the PGM1 alleles occurred only in homozygous form and the multilocus genotypes of these two loci were in complete linkage disequilibrium (D = 1) for the entire dataset.

Allozyme and mtDNA investigations from the same individuals confirmed that the PGM1 and MDH1 alleles are diagnostic for the 'B' clade (Supplementary Table 1. individuals: 35, 36, 37, 38, 41, 43, 44).

The AAT1 allele was found in three populations (Supplementary Table 2 and Fig. 1), either as homozygotes or as heterozygotes combined with only one other allele (AAT2). AAT1 occurred at high frequency (0.556) only in the HNP8 population, where the mitochondrial 'C' lineage was also detected. Direct evidence (mtDNA investigation and allozyme analysis of the same individual) for an association between the AAT1 allele and the 'C' lineage was not provided, but putatively they are connected. Nuclear support for the separation of the 'D' clade was not found based on the investigated allozyme markers.

The model including the salinity and the depth of ponds could significantly (if $\alpha = 0.1$) explain the variability in the observed genetic structure and was best fitted to the genetic structure based on the forward selection procedure within CAP analysis (Table 2).

Table 2. Results of forward selection procedure with Monte Carlo randomization tests (4999 runs, $\alpha = 0.1$). Best fitting model to the observed genetic structure in bold. Abbrev.: sal: salinity (g/l), depth (cm), near: distance to nearest pool (m), surface: surface area of the pond (m^2).

	F-value	*p*-value	AIC
null model without env. variables			23.80
sal	5.253	0.018	20.68
sal+depth	2.957	0.081	19.47
sal+depth+near	0.615	0.499	20.72
sal+depth+surface	0.066	0.966	21.39
sal+depth+surface+near	2.172	0.099	22.66

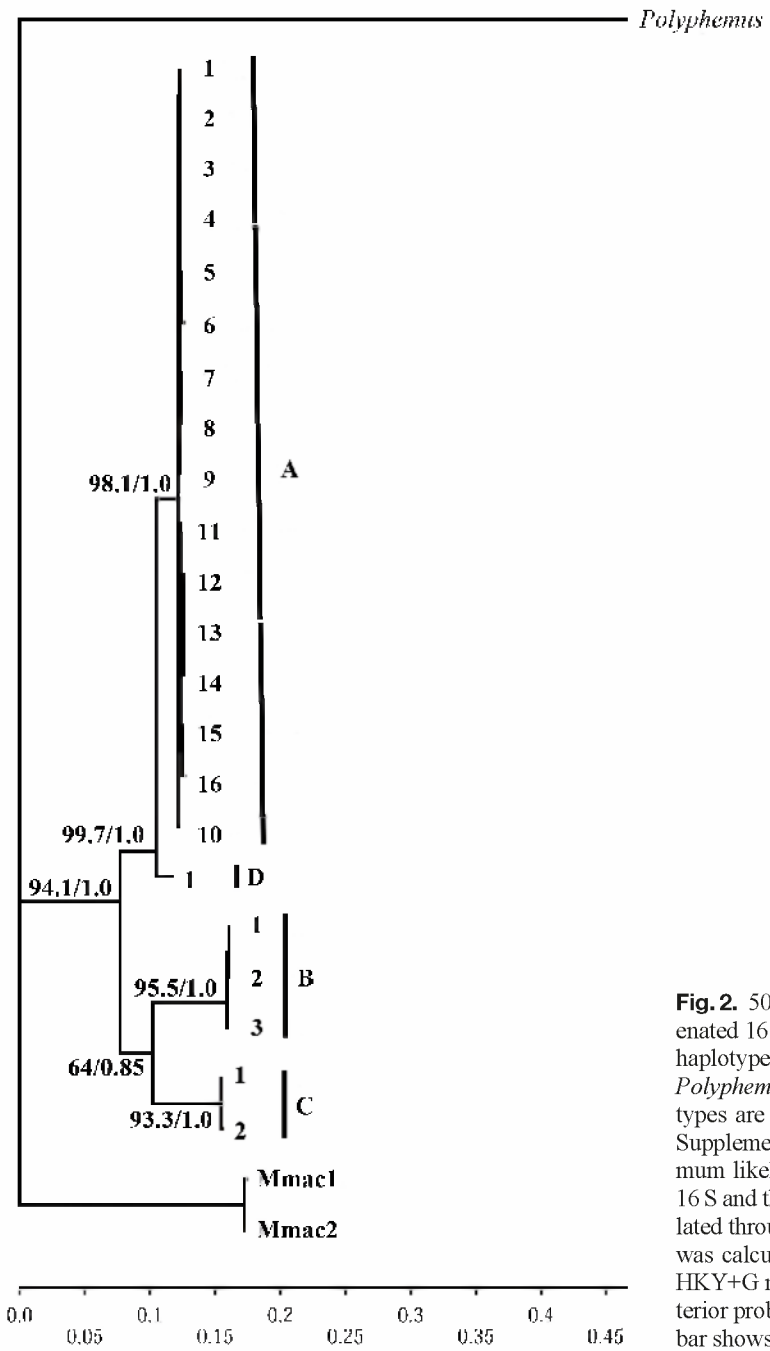

Fig. 2. 50 % majority rule consensus tree for the concatenated 16 S and COI regions (1080bp), for 22 *M. brachiata* haplotypes, two *M. macrocopa* (Mmac) haplotypes and *Polyphemus pediculus* as outgroup. *M. brachiata* haplotypes are encoded by the same numbers as on Fig. 4 and Supplementary Table 1. The tree has been inferred by maximum likelihood under the TVM+G substitution model for 16 S and the J2+G model for COI. Clade support was calculated through 1000 bootstrap replicates. Bayesian inference was calculated under the GTR+G model for 16 S and the HKY+G model for COI. Bootstrap support / Bayesian posterior probability is indicated over the main branches. Scale bar shows distances in substitution per site.

These two variables together explained 34.06 % of the total variance in genetic structure. The relative contribution to the total variability in genetic structure was higher for salinity (15.53 %) than for depth (11.47 %) based on variance partitioning procedure, and their effects were significant (if $\alpha = 0.1$) based on Monte Carlo permutation test (4999 runs) (Table 3). The first canonical axis (CAP1) explained 33.9 % of the total variability in the dataset, while this value was negligible in the case of the second canonical axis (CAP2) (Table 3). Monte Carlo permutation tests (4999 runs) indicated that the first and all canonical axes were significant (if $\alpha = 0.1$) with respect to the set of variables used (Table 3). The selected environmental variables positively and significantly correlated with CAP1 (salinity: $r = 0.51$, $p = 0.021$; depth: $r = 0.46$, $p = 0.039$), but did not with CAP2 (salinity: $r = -0.1$, $p = 0.683$; depth: $r = 0.11$, $p = 0.633$). Besides, the Kruskal-Wallis test on the objects score of CAP1 showed significant differences (df = 2, $\chi^2 = 7.33$, $p = 0.026$) among the

phylogenetically defined lineages ('A', 'B', 'C'), but this was not the case for the object scores on CAP2 (df = 2, χ^2 = 1.70, p = 0.43). Summarizing our findings, the three phylogenetically defined lineages ('A', 'B', 'C') were clearly separated from each other along the first canonical axes characterized by strong gradients in depth and salinity of the ponds.

M. brachiata divergence in comparison to divergence in the *Daphnia longispina* group

Genealogy of the COI gene (Fig. 3; ML: -lnL = 3886.29; Bayesian best likelihood: -lnL = 3888.68) illustrates the level of divergence within *M brachiata* compared to other taxa of the *Moina* genus, and to the well-known *Daphnia longispina* species group. The pairwise sequence divergence of COI (Supplementary Table 3) within *M. brachiata* ranged from 0.038 (between 'A' and 'D') to 0.132 (between 'A' and 'C') with an average of 0.105. Divergence detected between the Mexican and the Hungarian *Moina macrocopa* was 0.128. Divergence between different *Moina micrura* lineages from Mexico ranged from 0.115 to 0.124 (average 0.119). Mean sequence divergence between the *Moina brachiata* and the *Moina macrocopa* groups was 0.175 (minimum value: 0.163, maximum value: 0.195) while between *M. brachiata* and *M. micrura* it ranged from 0.154 to 0.198 with an average of 0.170. The lowest interspecific sequence divergence value

Table 3. The relative contributions of the canonical axes (CAP1, CAP2 and all axes) and the selected environmental variables to the observed variation in occurrence of cryptic taxa and the results of Monte Carlo permutation test (4999 runs, $\alpha = 0.1$) in the CAP analysis.

	% var. example	Monte Carlo permut. test	
		F-value	*p*-value
Canonical axes			
CAP1	33.86	8.729	0.006
CAP2	0.20	0.052	0.984
all axes	34.06	4.391	0.015
Selected variables			
salinity	15.53	4.004	0.042
depth	11.47	2.957	0.081
salinity+depth (shared effect)	7.06		

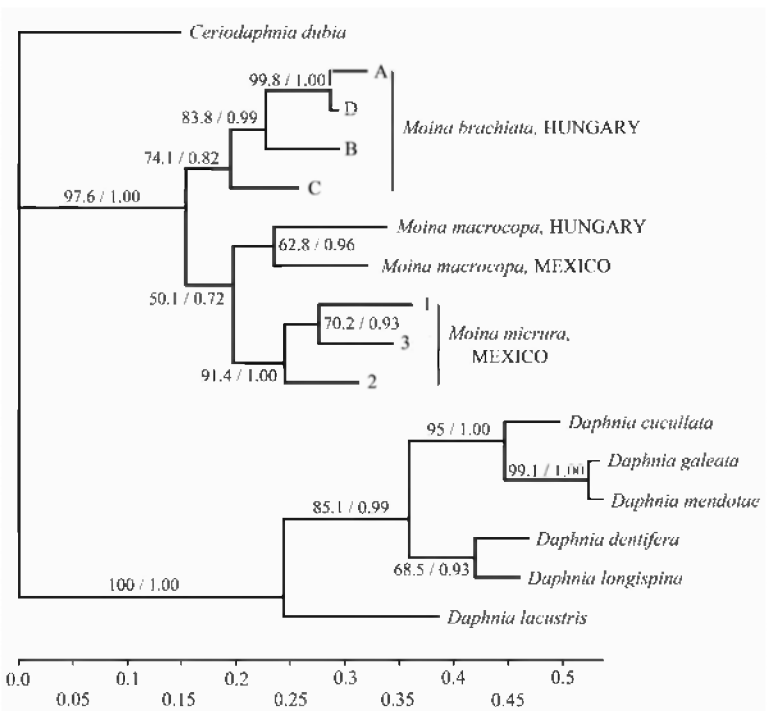

Fig. 3. 50 % majority rule consensus tree of COI (604 bp) for certain *Moina* and *Daphnia* haplotypes with *Ceriodaphnia dubia* as outgroup. The tree was inferred by maximum likelihood under the GTR+GI substitution model: branch support was assessed by 1000 bootstrap replicates. Bootstrap support / Bayesian posterior probability is indicated on the branches. Scale bar shows distances in substitution per site.

(0.142) within the *Moina* genus was detected between *M. macrocopa* and *M. micrura* while the maximum value between these species was 0.166 and the mean 0.161.

Within the *Daphnia cucullata – galeata – mendotae* complex of the *longispina*-group sequence divergences ranged from 0.022 (between *Daphnia galeata* and *Daphnia mendotae*) to 0.116 (between *D. cucullata* and *D. galeata*) with an average value of 0.084. Sequence divergence between *Daphnia dentifera* and *Daphnia longispina* was 0.088. Sequence divergence estimates between *the D. cucullata – galeata – mendotae* complex and the *D. dentifera – longispina* group were 0.144, 0.184, 0.165 (minimum, maximum, mean).

Genetic structure in space and genetic variation within the most common lineage

Haplotype network analysis of the pooled COI +16 S regions for 53 animals revealed 22 different haplo-

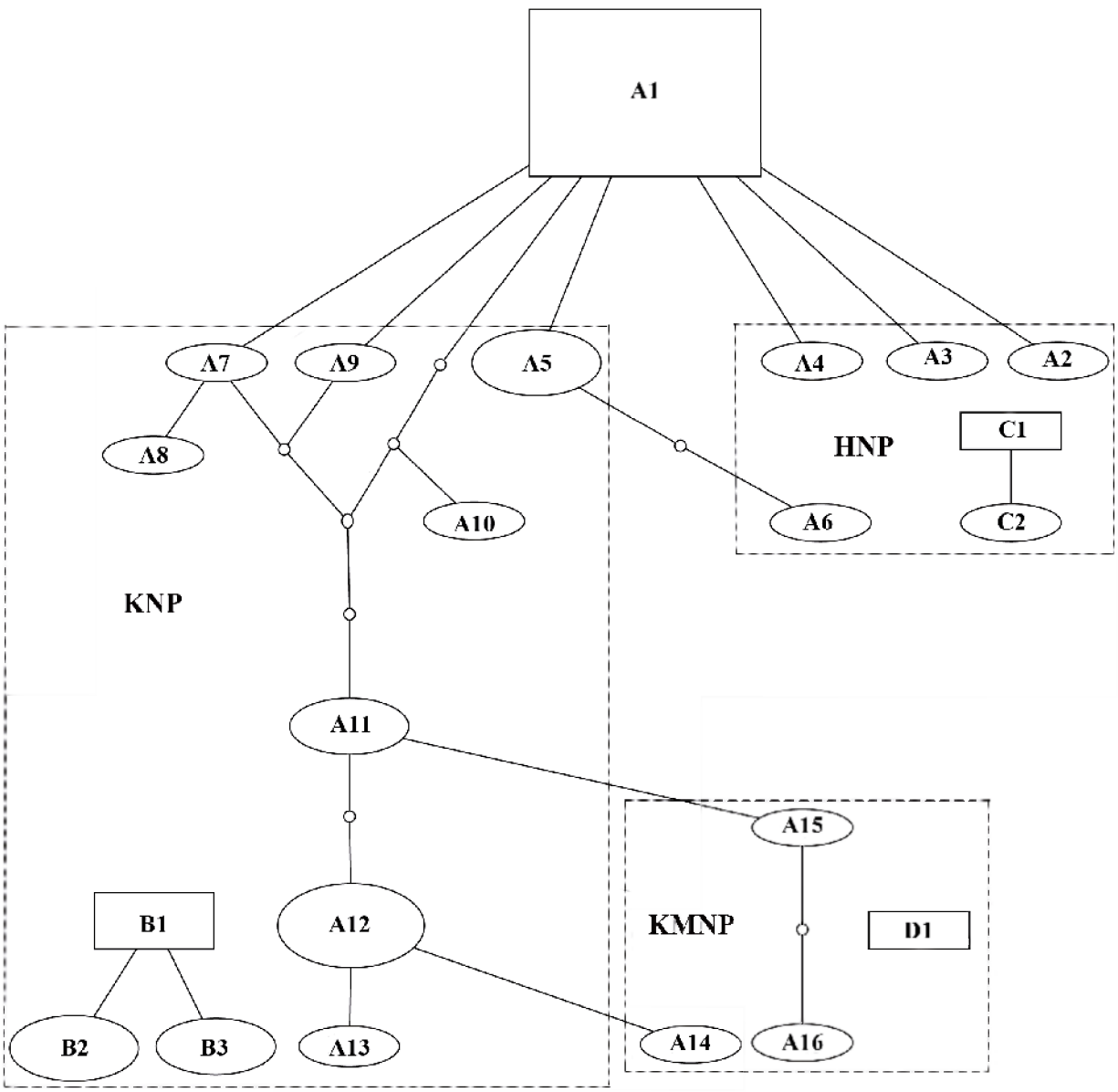

Fig. 4. Minimum spanning network of the joined COI and 16 S regions for 53 *M. brachiata* specimens. Size of the geometric figures in the network is proportional to the number of times the haplotype was observed. Haplotype A1 occurred in every region; haplotypes in hatched line rectangle frames occurred only in the marked region (KNP, KMNP or HNP). Letters (A, B, C and D) in the haplotype identifiers correspond to the different lineages while numbers identify the individual haplotypes. Haplotype identifiers are the same as in Supplementary Table 1 and on Fig. 2.

types, which clustered into four separated networks ('A', 'B', 'C' and 'D', Fig. 4). The clades revealed by the phylogenetic analyses based on the pooled 16 S and COI correspond to the four different network clusters in the haplotype network analysis (Figs 2 and 4). The most widespread and common haplotype 'A1' showed the highest number of connections of the whole dataset. Furthermore this is the only haplotype that occurred in all of the three investigated regions. Other haplotypes in the haplotype network cluster 'A' were found only in one of the regions (Fig. 4). Haplotype network cluster 'B' includes three haplotypes ('B1', 'B2' and 'B3') that were found only in the KNP region. Haplotype network cluster 'C' consists of two haplotypes ('C1' and 'C2') occurring only in the HNP region. Haplotype network 'D' was represented by one individual found in the KMNP.

Figure 1 illustrates the distribution of the MDH1 and AAT1 alleles in the three national parks. Individuals with the MDH1 allele were observed only in the KNP, while the occurrence of the AAT1 allele was restricted to HNP. The allele frequency of the MDH1 allele was high in two bomb crater habitats (KNP5 and KNP8), while it also occurred in a pool on a pasture (KNP3) and in a wheeltrack (KNP6) at low frequency (Supplementary Table 2 and Fig. 1). The AAT1 allele occurred at high frequency in the HNP8 habitat while in the HNP1 and HNP4 populations its frequency was low (0.103 and 0.024, respectively).

In the enzyme polymorphism analysis of individuals of the 'A' clade only (and with exclusion of populations KNP8 and HNP8 because of the small number of individuals), the MDH and AAT loci are monomorphic and the resulting dataset contains data for three loci (PGI, MPI and PGM) on 588 individuals from 17 temporary waterbodies. Wright's F-statistics calculated for this dataset are listed in Table 4. Genetic differentiation was high (overall Fst 0.25) between populations but negligible (overall Frt 0.08) between the three regions (KNP, HNP and KMNP). Average Fst values within the three regions and among pairwise region comparisons (KNP – HNP, KNP – KMNP, KMNP – HNP) are plotted in Fig. 1.

Table 4. Wright's F-statistics for the studied populations of lineage 'A'. Fit: overall inbreeding coefficient of an individual relative to the total set of individuals studied, Fst: coancestry coefficient for individual populations relative to the total set of individuals studied, Frt: coancestry coefficient for regions (KNP, KMNP, HNP) relative to the total set of individuals studied, Fis: inbreeding coefficient for individuals within populations. S.D.: standard deviation, C.I.: upper and lower bounds of 95 % confidence intervals after bootstrapping (5000 replicates) over loci.

Locus	Fit	Fst	Frt	Fis
PGI	0.17	0.23	0.14	–0.09
MPI	0.34	0.31	0.01	0.04
PGM	0.23	0.22	0.07	0.01
Overall	0.24	0.25	0.08	–0.02
average	0.24	0.25	0.08	–0.02
S.D.	0.05	0.03	0.04	0.04
C.I.	0.34; 0.17	0.31; 0.22	0.14; 0.01	0.04; –0.09

Discussion

Cryptic taxa

Based on the results presented in this study, we can conclude that *M. brachiata* is a complex of at least four cryptic lineages (clades 'A', 'B', 'C' and 'D'). Whether these lineages represent different biological species is not unambiguous for each lineage, but a number of arguments support the separation at the species level for most of the lineages. It is true that the sequence divergence under the K2 p model among the detected cryptic lineages of *M brachiata* do not reach the molecular threshold of species delimitation in crustaceans (Lefébure et al. 2006) but sequence divergence between *Daphnia cucullata* and *Daphnia galeata* in our investigations equaled that between *Moina brachiata* 'C' and 'B', while divergence between the *M. brachiata* 'A' and 'B', 'A' and 'C', 'C' and 'D' taxon-pairs was even higher than this value. The species status of lineage 'B' is strongly supported by the separation in complete linkage disequilibrium for two nuclear loci (MDH and PGM), proving the existence of reproductive isolation between lineages 'A' and 'B' that were found to coexist in some habitats. The association of the nuclear and mitochondrial markers was clear, as we used the same individuals for assessing enzyme polymorphisms and sequencing. Elías-Gutiérrez et al. (2008) considered the three *Moina micrura* lineages detected by them as different species and the divergence between the *M brachiata* lineages found in Hungary is comparable to that between *M. micrura* species '1', '2' and '3' in Mexico.

Direct evidence through DNA sequence and allozyme electrophoresis data on the same individuals was not provided for the separation of the 'C' lineage, but the detected pattern (i.e. mitochondrial lineage 'C' and the AAT1 allele at a high frequency found in the same pool and only in that pool, HNP8) is unlikely to be caused by chance. The fact that the AAT1 allele occurred both in homozygous and heterozygous form raises the possibility of hybridisation between the dif-

ferent *M brachiata* lineages, but elucidating this pattern was beyond the scope of the present study. Our study was restricted to the Pannonian Plain, a relatively small geographical area, but at the global scale *Moina brachiata* might represent a cryptic species complex with more members than currently detected.

Moina micrura was first considered as a cryptic species complex by Petrusek et al. (2004). Elías-Gutiérrez et al. (2008) provided further support, but *Moina macrocopa* was still known to represent a well defined species. The comparison of the Mexican and the Hungarian *M. macrocopa* haplotypes showed deep divergence in our study. Decades passed since Goulden's (1968) work was published on the Moinidae and a thorough revision applying molecular tools is needed.

Environmental factors structuring genetic variation in *Moina brachiata*

Salinity and depth are important drivers of the distribution of cryptic taxa in the *Moina brachiata* species complex. Salinity differences are well known for their direct impact on physiology affecting species composition of zooplankton communities (Boronat et al. 2001, Frisch et al. 2006, Waterkeyn et al. 2008). Depth itself may be important in permanent waters (as a refugium against UV or visual predators), but in temporary waters it is most likely a proxy for hydroperiod, as these two variables are strongly related (Boven et al. 2008).

The abundant occurrence of the 'B' individuals in the bomb craters (KNP5 and KNP8) illustrates the clearcut preference of lineage 'B' for deeper, semipermanent habitats. Our observation that cryptic taxa may be associated with differences in depth and habitat permanence is in line with other work. Schwenk et al. (2004) showed that depth associated with other factors was an important ecological variable to explain the separation of *Daphnia umbra* and *D. longispina*, later considered as *Daphnia lacustris* (Nilssen et al. 2007), with the former species occurring in larger and deeper waterbodies and the latter occurring in shallow ponds.

Depth is an important driver of the well-studied freshwater habitat gradient (Wellborn et al. 1996) and habitat predictability associated with depth has been repeatedly reported as an important variable explaining variation in community composition in temporary water bodies such as floodplain ponds and Mediterranean temporary wetlands (Medley & Havel 2007, Waterkeyn et al. 2008). Our study adds to this by showing that depth is also important in structuring genetic variation across strongly related, cryptic taxa.

Several studies have provided evidence of adaptations in life history traits to hydroperiod in both cladocerans (*Daphnia obtusa* Kurz, 1875) (Nix & Jenkins 2000) and copepods (*Diaptomus leptopus* Forbes, 1882) (Piercey & Maly 2000). In addition, hydroperiod also selects for differences in the timing of sexual reproduction: species that are adapted to ephemeral habitats may be much more sensitive to switch to sexual reproduction when the volume of the habitat is decreasing (Roulin et al. 2013). While we picked up depth and salinity as important factors influencing the occurrence of different *M. brachiata* clades, part of this impact may be mediated by biotic interactions like predation, which are associated with hydroperiod (Wellborn et al. 1996).

The importance of the conservation of temporary habitats with long hydroperiod has been stressed on the grounds that invertebrate communities of the short-lived wetlands tend to be nested within assemblages found in habitats with long hydroperiod (Boven & Brendonck 2009, Waterkeyn et al. 2008). Yet, our results indicate that for conservation purposes of temporary ponds one should not be biased towards long hydroperiod, instead the selection should cover a number of habitats that differ in hydroperiod (Heino et al. 2009, Pyke 2004), providing suitable conditions both to species occupying short lived pools and to good competitors abundant in the species rich communities of longer lived temporary waters.

Multimodel ensemble means of ten regional climate models tested for 2071–2100 (Hagemann & Jacob 2007, Hagemann et al. 2009) predict a significant drying of the catchment area of the Danube, due to a combination of higher temperatures in summer, decreased amounts of rainfall in the summer and a significant increase in evapotranspiration throughout the year. Habitats that currently are characterised by short hydroperiod under such a scenario might develop hydroperiods that are too short even for species with special adaptations, while suitable habitats for species that are adapted to longer hydroperiod might completely disappear from the area. The future occurrence of the differently adapted *Moina brachiata* lineages will be affected by the combined effect of habitat loss, dispersal and range shifts.

Spatial distribution

Population genetic analyses carried out for lineage 'A', the most widespread and abundant taxon in the study

area, revealed strong population subdivision resembling genetic differentiation typically found in other cyclical parthenogens. Wright's F statistics showed no separation between regions indicating the absence of geographic structure in lineage 'A' across the Great Plain. The absence of regional structure is supported also by the haplotype network analysis in which the 'A' lineage was found in all of the three studied regions. *M. brachiata* 'A' likely represents a taxon adapted to highly ephemeral habitats (wheeltracks, puddles) that are small in size but relatively abundant in the field.

While lineage 'A' was widespread throughout the Great Plain, the remaining *M. brachiata* lineages 'B', 'C' and 'D' occurred at low densities and were restricted to only one of the studied regions, indicating reduced colonisation capacities or strong ecological preferences limiting their distribution.

Considering the age of the bomb craters (approx. 60 years) in the Kiskunság National Park, one possible scenario explaining the distribution of *M. brachiata* 'B', which is adapted to longer hydroperiods, is that its high frequency in the bomb craters is the result of recent colonisation events that were followed by competition against 'A' and finally population expansion. KNP3 is a shallow waterlogged area used as pasture, and grazing cattle may serve as abundant dispersal vectors (JN, personal observations) between KNP5, KNP8 and KNP3.

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Supplementary Table 1. Complete list of the sequences obtained in this study. Ind: individual identifier. Site: code of the sampling site, KMNP: Körös-Maros National Park, KNP: Kiskunság National Park, HNP: Hortobágy National Park. HID: haplotype ID that corresponds to Figure 2 and 4. Letters in the HID indicate the lineage, numbers are the haplotype identifiers. Lineage: indication of the different lineages based on the DNA information. Mmac = Moina macrocopa. MLG: multilocus genotype of AAT, PGI, MPI, PGM, MDH for the animals analysed in parallel for nuclear and mitochondrial variability, '00' – missing data. COI and 16 S: GenBank accession numbers. Geographical coordinates of the sites are listed in Table 1, except for K (EOVX768614, EOVY126538) and B (EOVX139114, EOVY526829).

Ind	Site	HID	Lineage	MLG	COI	16S
1	KMNP1	A14	A		JN641808	JN651422
2		D1	D		JN641809	JN651423
3	KMNP2	A15	A		JN641810	JN651424
4	KMNP3	A1	A		JN641811	JN651425
5	KMNP4	A16	A		JN641812	JN651426
6	KNP1	A5	A		JN641813	JN651427
7		A5	A	22,22,44,24,22	JN641814	JN651428
8		A5	A	22,22,44,34,22	JN641815	JN651429
9		A12	A	22,22,24,44,22	JN641816	JN651430
10		A12	A	22,22,24,44,22	JN641817	JN651431
11		A12	A	22,12,24,44,22	JN641818	JN651432
12		A12	A	22,22,44,44,22	JN641819	JN651433
13		A12	A	22,22,44,44,22	JN641820	JN651434
14		A11	A		JN641821	JN651435
15		A11	A	22,22,24,24,22	JN641822	JN651436

Supplementary Table 1. Continued.

Ind	Site	HID	Lineage	MLG	COI	16S
16			A	22,22,24,44,22	JN641823	
17			A			JN651437
18			A			JN651438
19			A			JN651439
20			A			JN651440
21			A			JN651441
22	KNP2	A1	A		JN641824	JN651442
23		A5	A		JN641825	JN651443
24		A12	A		JN641826	JN651444
25		A7	A		JN641827	JN651445
26		A8	A		JN641828	JN651446
27			A			JN651447
28			B			JN651448
29	KNP3	A1	A		JN641829	JN651449
30		A1	A		JN651421	JN651450
31		A13	A		JN641830	JN651451
32	KNP4	A10	A		JN641831	JN651452
33	KNP5	A1	A	22,12,24,00,22	JN641832	JN651453
34		B1	B		JN641833	JN651454
35		B1	B	22,22,55,11,11	JN641834	JN651455
36		B1	B	22,22,45,11,11	JN641835	JN651456
37		B2	B	22,22,55,11,11	JN641836	JN651457
38		B2	B	22,22,55,11,11	JN641837	JN651458
39		A9	A	22,12,24,00,22	JN641838	JN651459
40			A	22,12,24,00,22	JN641839	
41			B	22,22,55,11,11	JN641840	
42			A	22,12,44,00,22	JN641841	
43			B	22,22,55,11,11		JN651460
44			B	22,22,55,11,11		JN651461
45			A	22,12,24,00,22		JN651462
46	KNP6	B2	B		JN641842	JN651463
47	KNP7	A1	A		JN641843	JN651464
48		A11	A		JN641844	JN651465
49	KNP8	A1	A		JN641845	JN651466
50		B2	B		JN641846	JN651467
51		B3	B		JN641847	JN651468
52		B3	B		JN641848	JN651469
53		B3	B		JN641849	JN651470
54			B			JN651471
55			B			JN651472
56			B			JN651473
57			B			JN651474
58			B			JN651475
59	HNP1	A1	A		JN641850	JN651476
60	HNP2	A1	A		JN641851	JN651477
61		A1	A		JN641852	JN651478
62	HNP3	A6	A		JN641853	JN651479
63	HNP4	A2	A		JN641854	JN651480
64		A4	A		JN641855	JN651481
65	HNP5	A1	A		JN641856	JN651482
66		A1	A		JN641857	JN651483
67	HNP6		A			JN651484
68	HNP7	A1	A		JN641858	JN651485
69		A1	A		JN641859	JN651486
70		A1	A		JN641860	JN651487
71			A			JN651488
72	HNP8	C1	C		JN641861	JN651489
73		C2	C		JN641862	JN651490
74		A3	A		JN641863	JN651491
75	KNP4		Mmac1		JN657688	JN657692
76	KNP2		Mmac1		JN657691	JN657695
77	B		Mmac1		JN657690	JN657694
78	K		Mmac2		JN657689	JN657693

Supplementary Table 2. Allele frequencies and observed heterozygosity (H) in the studied populations. Numbers in brackets under the allele identifier (1–6): absolute mobility (mm) of the allele. Sampling site identifiers in the first column. Number of studied individuals per population is given in Table 1.

	AAT			PGI				MPI							PGM					MDH		
	1 (29)	2 (32)	H	1 (32)	2 (34)	3 (36)	H	1 (25)	2 (26)	3 (27)	4 (28)	5 (29)	6 (30)	H	1 (32)	2 (39)	3 (41)	4 (42)	H	1 (22,5)	2 (24,5)	H
HNP1	0.103	0.897	0.138	0.190	0.138	0.672	0.448	0.000	0.431	0.000	0.569	0.000	0.000	0.172	0.000	0.155	0.310	0.534	0.655	0.000	1.000	0.000
HNP2	0.000	1.000	0.000	0.619	0.012	0.369	0.571	0.000	0.202	0.131	0.667	0.000	0.000	0.548	0.000	0.405	0.286	0.310	0.762	0.000	1.000	0.000
HNP3	0.000	1.000	0.000	0.244	0.423	0.333	0.923	0.000	0.115	0.064	0.821	0.000	0.000	0.205	0.000	0.231	0.397	0.372	0.769	0.000	1.000	0.000
HNP4	0.024	0.976	0.000	0.280	0.134	0.585	0.561	0.000	0.402	0.037	0.561	0.000	0.000	0.439	0.000	0.402	0.049	0.549	0.366	0.000	1.000	0.000
HNP5	0.000	1.000	0.000	0.000	0.500	0.500	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
HNP6	0.000	1.000	0.000	0.067	0.883	0.050	0.233	0.000	0.417	0.417	0.167	0.000	0.000	0.800	0.000	0.367	0.550	0.083	0.667	0.000	1.000	0.000
HNP7	0.000	1.000	0.000	0.014	0.541	0.446	0.351	0.000	0.189	0.176	0.635	0.000	0.000	0.622	0.000	0.378	0.054	0.568	0.486	0.000	1.000	0.000
HNP8	0.556	0.444	0.556	0.222	0.125	0.653	0.556	0.000	0.153	0.208	0.611	0.028	0.000	0.333	0.000	0.222	0.500	0.278	0.639	0.000	1.000	0.000
KNP1	0.000	1.000	0.000	0.073	0.927	0.000	0.145	0.000	0.245	0.000	0.745	0.009	0.000	0.491	0.000	0.045	0.009	0.945	0.109	0.000	1.000	0.000
KNP2	0.000	1.000	0.000	0.463	0.500	0.037	0.634	0.000	0.244	0.098	0.598	0.061	0.000	0.463	0.000	0.122	0.402	0.476	0.610	0.000	1.000	0.000
KNP3	0.000	1.000	0.000	0.355	0.581	0.065	0.452	0.000	0.306	0.629	0.048	0.016	0.000	0.516	0.097	0.177	0.597	0.129	0.355	0.097	0.903	0.000
KNP4	0.000	1.000	0.000	0.372	0.606	0.021	0.489	0.053	0.106	0.766	0.064	0.011	0.000	0.277	0.000	0.245	0.000	0.755	0.362	0.000	1.000	0.000
KNP5	0.000	1.000	0.000	0.273	0.727	0.000	0.364	0.000	0.197	0.000	0.273	0.530	0.000	0.485	0.576	0.061	0.000	0.364	0.061	0.576	0.424	0.000
KNP6	0.000	1.000	0.000	0.214	0.655	0.131	0.476	0.000	0.024	0.000	0.929	0.048	0.000	0.048	0.048	0.024	0.071	0.857	0.190	0.048	0.952	0.000
KNP7	0.000	1.000	0.000	0.438	0.453	0.109	0.875	0.000	0.078	0.000	0.922	0.000	0.000	0.156	0.000	0.094	0.047	0.859	0.281	0.000	1.000	0.000
KNP8	0.000	1.000	0.000	0.023	0.977	0.000	0.045	0.000	0.068	0.114	0.352	0.443	0.023	0.295	0.955	0.023	0.000	0.023	0.045	0.955	0.045	0.000
KMNP1	0.000	1.000	0.000	0.325	0.300	0.375	0.600	0.025	0.163	0.288	0.500	0.025	0.000	0.475	0.000	0.388	0.138	0.475	0.575	0.000	1.000	0.000
KMNP2	0.000	1.000	0.000	0.438	0.219	0.344	0.625	0.000	0.281	0.688	0.031	0.000	0.000	0.563	0.000	0.125	0.344	0.531	0.563	0.000	1.000	0.000
KMNP3	0.000	1.000	0.000	0.188	0.250	0.563	0.475	0.000	0.038	0.038	0.925	0.000	0.000	0.150	0.000	0.313	0.100	0.588	0.525	0.000	1.000	0.000

Supplementary Table 3. Pairwise divergence for COI calculated under the K2 p model for the haplotypes corresponding to the taxa on Fig. 3.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	*Ceriodaphnia dubia*															
2	*M. brachiata* 'A'	0.229														
3	*M. brachiata* 'B'	0.204	0.121													
4	*M. brachiata* 'C'	0.190	0.132	0.116												
5	*M. brachiata* 'D'	0.211	0.038	0.107	0.118											
6	*M. macrocopa* (HU)	0.223	0.195	0.178	0.163	0.173										
7	*M. macrocopa* (ME)	0.218	0.189	0.170	0.163	0.165	0.128									
8	*M. micrura* '1'	0.216	0.175	0.169	0.163	0.160	0.164	0.163								
9	*M. micrura* '2'	0.232	0.198	0.157	0.155	0.182	0.164	0.142	0.124							
10	*M. micrura* '3'	0.223	0.190	0.169	0.154	0.171	0.166	0.165	0.119	0.115						
11	*Daphnia longispina*	0.243	0.301	0.287	0.285	0.278	0.286	0.290	0.272	0.264	0.277					
12	*Daphnia dentifera*	0.241	0.300	0.289	0.285	0.274	0.282	0.290	0.295	0.254	0.277	0.088				
13	*Daphnia mendotae*	0.270	0.309	0.319	0.280	0.309	0.283	0.295	0.312	0.286	0.301	0.163	0.184			
14	*Daphnia galeata*	0.250	0.296	0.303	0.270	0.291	0.268	0.278	0.299	0.281	0.293	0.155	0.180	0.022		
15	*Daphnia cucullata*	0.285	0.282	0.277	0.282	0.277	0.273	0.288	0.301	0.286	0.303	0.163	0.144	0.113	0.116	
16	*Daphnia lacustris*	0.259	0.289	0.286	0.269	0.272	0.278	0.257	0.285	0.259	0.291	0.205	0.211	0.242	0.222	0.222