

**Cryptic species complexes in the Cladocera (Crustacea)  
and the investigation of environmental factors affecting  
microcrustacean communities in small-sized temporary  
waters**

THESES OF THE PH.D. DISSERTATION

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## ***Background***

Temporary waters are special habitat types due to the periodic and unpredictable alteration of the dry and aquatic phases and because the waterbody is mostly hidrologically isolated. Species occurring in temporary waters present peculiar adaptation strategies to be able to cope with the extremes of the water table change, therefore this habitat type deserves protection to conserve the special fauna and flora elements.

The number and area of the small-sized temporary waters – pools and puddles on floodplains or pastures, parts of the ploughed agricultural fields covered with rainwater, wheeltracks on dirtroads – has been severely decreasing due to the worldwide expansion of agricultural activity, building of drainage systems and river regulation.

Aquatic microcrustaceans, mainly cladocerans and copepods, are the most characteristic animals and especially important elements of the food web in temporary waters. *Moina brachiata* (Jurine, 1820), *Daphnia atkinsoni* Baird, 1859 and *Daphnia bolivari* (Richard, 1888) are typically occurring in small temporary waters in Hungary.

*Daphnia bolivari* (Richard, 1888) differs markedly from *Daphnia atkinsoni*, since it presents a 'crown of thorns' around the headshield. Also the Fauna Europea taxon database consideres these two as different species.

Hydroperiod (the length of the aquatic phase), salinity and depth were mentioned among the most important local factors affecting the microcrustacean communities. The effect of agricultural landuse on the permanent and temporary waters was investigated at the regional scale; the effect of local landuse, namely the ploughing of the bottom of the waterbody, was not investigated before.

In my Ph.D. dissertation I investigated three topics related to the microcrustacean fauna of small-sized temporary waters in Hungary:

**1) Investigation of the *Moina brachiata* species:**

- a) Can genetic divergence be detected within *Moina brachiata* in the Hungarian Great Plain, and if so, to what extent?
- b) Is the occurring genetic divergence related to any abiotic condition of the habitats?
- c) Can we detect geographic separation in the occurrence of the genetically separated groups within *Moina brachiata*?

**2) Investigation of *Daphnia atkinsoni* and *Daphnia bolivari*:**

- a) Are *Daphnia atkinsoni* and *Daphnia bolivari* different species based on allozyme markers?
- b) Are there different cryptic lineages within *Daphnia atkinsoni* found in the Hungarian Great Plain?

**3) Investigation of the factors affecting the species composition of microcrustacean communities in small-sized temporary waters:**

- a) Which biotic and abiotic factors influence the species richness and composition of microcrustacean communities occurring in small-sized temporary waters?
- b) Does the ploughing of the bottom of the waterbody disadvantageously affect the species richness of the microcrustacean communities?

## ***Methods***

1. Investigated populations of *Moina brachiata* were collected using a 85µm mesh-sized plankton net from small-sized temporary waters in three clusters in the Hungarian Great Plain, namely from the Kiskunság (8 locations), Körös-Maros (4 locations) and the Hortobágy (8 locations) National Parks.

Parthenogenetic egg-bearing female individuals were used one by one for DNA isolation followed by the sequencing of the COI and the 16S mitochondrial regions. Both regions were sequenced from both directions and the final, later analysed and into GenBank also uploaded sequences were obtained by the alignment of the two-sided sequences.

**2.** Phylogenetic analyses were performed on the concatenated COI+16S regions (1080 basepairs, maximum likelihood and Bayesian reconstruction, 22 different *Moina brachiata* haplotypes, *Moina macrocopa* and *Polyphemus pediculus* as outgroups).

**3.** Phylogenetic analyses were performed on the COI region (604 basepairs, maximum likelihood and Bayesian reconstruction, four *Moina brachiata* haplotypes representing four presumably cryptic lineages based on method **2.**, in addition *D. longispina*, *D. lacustris*, *D. dentifera*, *D. mendotae*, *D. galeata* and *D. cucullata* from the *Daphnia longispina* group and *Ceriodaphnia dubia* as outgroup).

**4.** We calculated the K2p pairwise COI sequence divergence for the haplotypes analysed in method **3.**

**5.** We performed allozyme polymorphism investigations by cellulose acetate gelelectrophoresis for the AAT, PGI, MDH, MPI and PGM loci on 698 *Moina brachiata* individuals collected from 19 locations.

**6.** In 21 cases we used the same individual to obtain data for the mitochondrial (COI, 16S) and the nuclear (AAT, PGI, MDH, MPI and PGM) markers.

**7.** Links between genetic and abiotic characteristics were addressed by multivariate multiple regression analyses with forward selection of explanatory environmental variables that best fit the observed genetic structure. The distance matrix of the response variable for the analysis consisted of Nei's unbiased genetic distances calculated based on the data gained by method **5.** Tests were run in four settings: 1. for the total dataset, 2. excluding the presumable lineage 'C', 3. excluding the presumable lineage 'B' and 4. only within the presumable lineage 'A'.

Explanatory environmental variables were depth, salinity, surface area of the habitat and its distance to the nearest pool.

**8.** A minimum spanning network at 99% connection limit was generated based on the statistical parsimony cladogram estimation method. This haplotype network for COI+16S gained from 53 *Moina brachiata* individuals was compared to the geographical distribution of the haplotypes.

**9.** We performed allozyme polymorphism investigations by cellulose acetate gelelectrophoresis for the AAT, PGI, MDH and PGM loci on five *Daphnia atkinsoni* and two *Daphnia bolivari* populations. Afterwards the UPGMA clustering of the pairwise Nei's unbiased genetic distances between populations was done.

**10.** We collected zooplankton samples from 55 sites during spring and 47 sites during summer and after the identification of the species we analysed the presence-absence matrices per location. We also measured the pH, conductivity,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  content. The habitats could be categorized based on their basin type (ploughed land or natural), presence of vegetation (no plants, presence of non-aquatic taxa, presence of aquatic or semi-aquatic taxa) and connection to ditches.

**11.** We performed NMS analyses (Sørensen index) on the matrices gained by method **10**. The axes obtained by NMS explained most part of the variance in the data and were nearly orthogonal, therefore we could perform linear regression and ANOVA for the coordinates with the environmental variables and additive combinations of the environmental variables. Finally we calculated the AICc values of the models.

**12.** Using 5000 random permutations we performed a nestedness analysis to see whether species poor communities are nested within species rich communities.

## **Results**

### **Investigation of *Moina brachiata*:**

1. We found four ('A', 'B', 'C' and 'D'), genetically divergent lineages within *Moina brachiata* (Jurine, 1820) based on the phylogenetic analyses of the COI+16S regions from samples collected in the Hungarian Great Plain.
2. Pairwise COI sequence divergence between *Daphnia cucullata* and *Daphnia galeata* – widely accepted as different species – was found to be equal to that between *Moina brachiata* 'B' and 'C'. Pairwise sequence divergence between the *Moina brachiata* 'A' and 'B', 'A' and 'C', and 'C' and 'D' lineages was found to be even higher than that between *Daphnia cucullata* and *galeata*.
3. Based on 698 individuals from 19 locations from three areas of the Hungarian Great Plain we revealed complete linkage disequilibrium for the 'MDH1' (we detected altogether two alleles at this locus) and the 'PGM1' (we detected altogether four alleles at this locus) alleles.
4. Individuals in linkage disequilibrium for the MDH1-PGM1 loci belonged to the *Moina brachiata* 'B' lineage (7 out of 21 individuals). Individuals presenting different alleles at these loci belonged to the *Moina brachiata* 'A' lineage (14 out of 21 individuals).
5. There were two alleles present at the AAT locus in the entire dataset. The 'AAT1' allele was detected in three locations, all of them from the Hortobágy National Park region. Frequencies of the 'AAT1' allele in these populations were: 0.024; 0.103 and 0.556 (the latter is the HNP8 habitat).
6. We detected the mitochondrial lineage 'C' based on only two individuals that were both collected from the HNP8 habitat.
7. Based on the multivariate multiple regression there is a link between the genetic divergence between lineages and the salinity and depth of their habitats.

8. '*Moina brachiata* 'B' prefers deeper (120-130 cm) small-sized temporary waters.
9. The occurrence pattern of the four cryptic *Moina brachiata* lineages in the Hungarian Great Plain is different: 'A' was found everywhere, 'B' only in the Kiskunság National Park and 'C' only in the Hortobágy National Park. 'D' was detected based on only one individual collected from the Körös-Maros National Park.

#### **Investigation of *Daphnia atkinsoni* and *Daphnia bolivari*:**

10. In the UPGMA clustering of the pairwise Nei's genetic distances the morphologically *Daphnia bolivari* populations did not form one group but were nested between the *Daphnia atkinsoni* populations.
11. There are two distinct groups within *Daphnia atkinsoni* based on the UPGMA clustering of the pairwise Nei's genetic distances of 7 populations.

#### **Investigation of the factors affecting the species composition of microcrustacean communities in small-sized temporary waters:**

12. The basin type and the presence of aquatic or semi-aquatic vegetation has a prominent role in shaping the species richness of microcrustacean communities.
13. Species poor communities of habitats disturbed by ploughing are nested within species rich communities of undisturbed habitats.

## ***Conclusions***

The former *Moina brachiata* (Jurine, 1820) is actually a cryptic species complex in which the level of divergence differs between the cryptic lineages.

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

Salinity plays a role in shaping the occurrence and divergence of different *Moina brachiata* species probably through its direct effect on physiology, while depth might be the indicator of a directly unknown ecological factor – most probably this is hydroperiod.

Based on our allozyme studies *Daphnia bolivari* (Richard, 1888), the bearer of a 'crown of thorns', is an identical species category to *Daphnia atkinsoni* Baird, 1859.

At the same time *Daphnia atkinsoni* is a species complex represented by two lineages in the Hungarian Great Plain.

Ploughing of the bottom of temporary waters affects badly the microcrustacean community: habitats with a ploughed basin have a species poorer community compared to the undisturbed habitats.

The investigation on *Moina brachiata* points out that the selection of wetlands for conservation purposes should cover larger areas with habitats with different hydroperiods (some weeks to several months) and salinities, while the survey on the microcrustacean communities pointed out the importance of waterbodies with undisturbed bottom and aquatic or semi-aquatic vegetation.



## ***Publications in the subject of the dissertation***

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