

Comparing the population structure of two sympatric caddisflies in eastern Europe: First results

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[†] This study is dedicated to the memory of Krassimir KUMANSKI with whom I experienced an incredible field trip through the Bulgarian highlands.

Abstract

In our study to examine and compare the intraspecific population structure of two sympatric caddisfly species in the Carpathians and Bulgarian highlands and address how population genetic structure varies between sympatric species that occupy the same ecological niche. Study species are *Drusus discolor* and *D. romanicus*. 99 specimens of *D. discolor* and 42 *D. romanicus* (Total = 141) were sequenced. A 498bp long region of mitochondrial DNA (mtCOI) was analysed. Median-Joining networks show that in both species there is clear population structure within the Carpathian Mts. This supports previous findings of intraspecific differentiation between individual ranges in the Carpathians. Lineage divergence within *D. romanicus* is twice as high as within *D. discolor*. This would suggest that populations of *D. romanicus* have been isolated from each other much longer than those of *D. discolor*. A more in depth study comparing both species phylogeography is needed to further investigate this interesting first results.

Keywords: median-joining networks, lineage divergence, genetic structure, species diversification

Introduction

The Carpathians and highlands of the Balkan peninsula are well known as Pleistocene refugia and as centres for species diversification (HEWITT 2004, SCHMITT 2007). This is also true for caddisflies, where numerous studies show a high degree of diversity in the region based on intra- and interspecific morphological variation (BOTOȘĂNEANU 1975, MEY & BOTOȘĂNEANU 1985, KUMANSKI 2005). However, studies of molecular diversity are still lacking. In our study we wish to examine and compare the intraspecific population structure of two sympatric caddisfly species in the Carpathians and Bulgarian highlands and address how population genetic structure varies between sympatric species that occupy the same ecological niche. Here we present first results based on a preliminary sampling.

Study species

Drusus discolor and *D. romanicus* are highland species that inhabit high gradient headwater streams in montane regions. *D. discolor* occurs in all higher mountain ranges from the Iberian Peninsula to Northern Greece, while *D. romanicus* has a restricted range in the Southern and Western Carpathians and Bulgarian highlands. The closely re-

lated species are very distinct based on adult genital morphology (Fig. 4). The larvae and females, however are indistinguishable. Larvae of both species are filter-feeding carnivores.

Material and Methods

In 2003, we collected adult and larval specimens from 26 sites in the Carpathians and Bulgarian highlands using hand nets and light traps (Fig. 1). From this collection we sampled 99 specimens of *Drusus discolor* and 42 *Drusus romanicus* (Total = 141). We extracted whole genomic DNA using DNeasy Tissue Kits (Qiagen) and amplified a 498bp long region of mitochondrial DNA (mtCOI) via Polymerase chain reaction (PCR). Methods are described in detail in Pauls et al. (2006). Sequences were generated using the PCR primers. ABI traces were assembled and manually checked using the program SEQMAN (Lasergene). Sequences were aligned using CLUSTAL W (Thompson et al. 1994) as implemented in BIOEDIT (Hall 1999). Haplotype files were generated in DNASP. Median-Joining Networks (MJ, BANDELTA et al. 1999) were calculated in Network (FLUXUS-TECHNOLOGY).

Table 1

Specimens used in this study and haplotypes found.

<i>D. discolor</i>	Populations	Individuals	No. haplotypes	Haplotypes (N)
Northern Carpathians	2	8	4	d-A (5) d-B (1) d-C (1) d-D (1)
Ukraine	1	1	1	d-E
Rodna Mts	3	15	5	d-A (1) d-F (10) d-G (2) d-H (1) d-I (1)
Rarau Mts	1	1	1	d-J (1)
Tarcau Mts	1	1	1	d-I (1)
Caliman Mts	1	1	1	d-A (1)
Bucegi Mts	1	16	4	d-K (13) d-L (1) d-M (1) d-N (1)
Fagaras Mts	1	7	2	d-F (6) d-O (1)
Retezat Mts	2	14	2	d-F (11) d-I (3)
Central Balkan	1	1	1	d-P (1)
Pirin Mts	2	18	5	d-Q (10) d-R (2) d-S (1) d-T (3) d-U (2)
Rila Mts	2	10	4	d-T (7) d-V (1) d-W (1) d-X (1)
Shar Mts (Macedonia)	3	6	2	d-F (1) d-Q (5)
Total _{D. discolor}	22	99		
<i>D. romanicus</i>	Populations	Individuals	No. haplotypes	Haplotypes (N)
Apuseni Mts	2	9	4	r-E (4) r-G (1) r-H (3) r-I (1)
Bucegi Mts	1	3	1	r-J
Fagaras Mts	1	6	1	r-D
Retezat Mts	3	11	3	r-D (9) r-E (1) r-F (1)
Pirin Mts	1	13	3	r-A (8) r-B (1) r-C (4)
Total _{D. romanicus}	8	42		

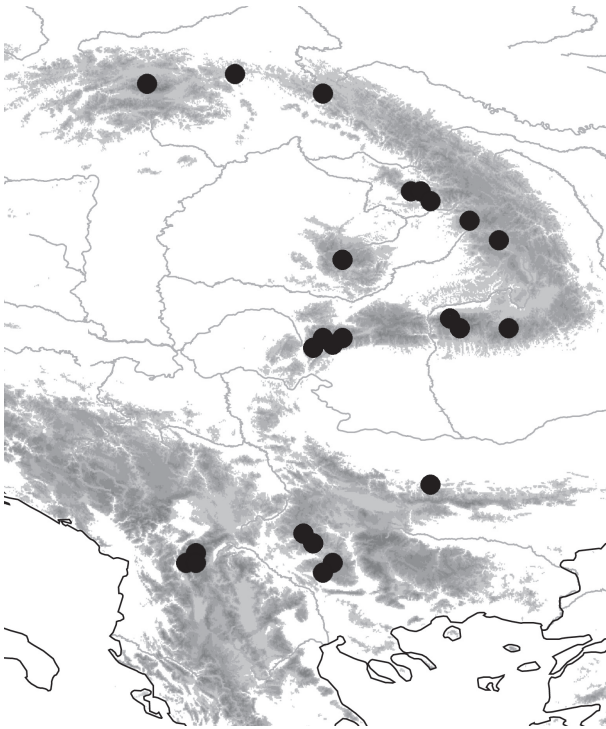


Fig. 1. Locations where specimens for this study were sampled.

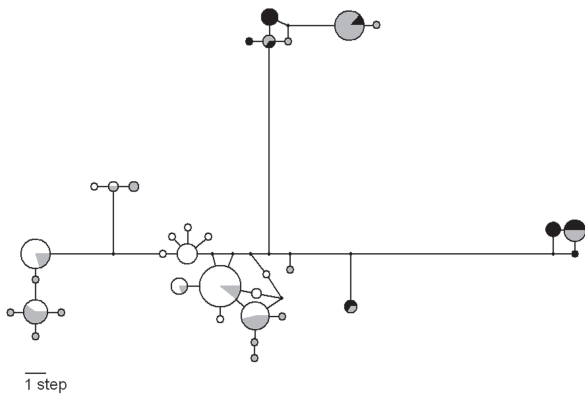


Fig. 2. Median-Joining Network of *D. discolor* (white) and *D. romanicus* (black) haplotypes. Grey shading of haplotypes indicates uncertain larvae or females. Lines between haplotypes correspond to genetic distances between haplotypes. Median vectors are shown as black nodes.

Results and Discussion

The MJ networks for the combined data set is shown in Fig. 2. We can see three clearly diverged lineages of *D. romanicus* (black and grey haplotypes) and several lineages of *D. discolor* (white and grey haplotypes). Uncertain individuals, i.e. larvae and females are shaded in grey and were assigned to either *D. discolor* or *D. romanicus* based on their haplotype association with adult males. This seems valid, as no haplotypes were shared between the two species. The MJ networks for individual species are shown in Fig. 3. In *D. discolor* (Fig. 3A) there are

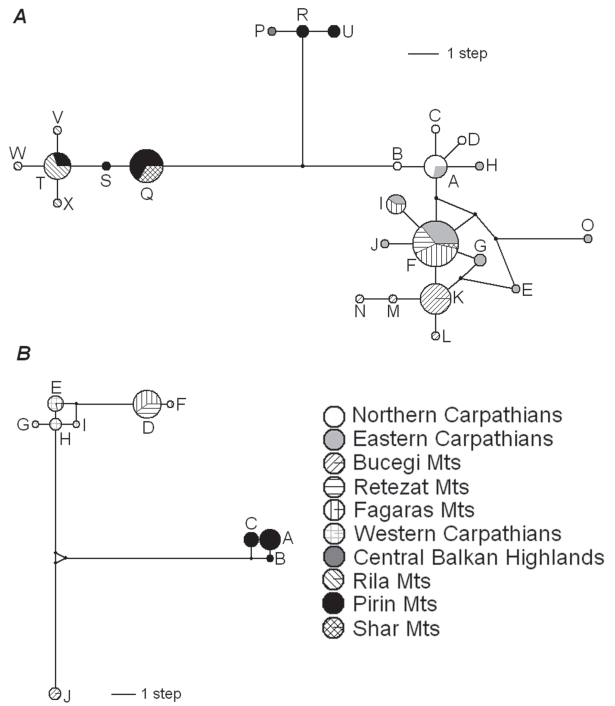


Fig. 3. Median-Joining Networks for A) *D. discolor* and B) *D. romanicus*. Shading and hatching of haplotypes indicates origin of specimens carrying these haplotypes. Haplotype names same as in Table 1. Otherwise as Fig. 2.

three differentiated and clearly diverged haplotype groups ($p = 2.4\%$ to 2.8%). One group comprises individuals from the Carpathians, the other two groups represent two distinct lineages with partial geographic overlap in the Bulgarian and Macedonian highlands ($p = 2.8\%$). Within the Carpathian clade there is genetic structure from north to south along the Carpathian range: one subclade dominates the northern range, another the southern range and a third the Bucegi Mts with only endemic haplotypes. The eastern populations are transitional.

In *D. romanicus* there are three distinct lineages diverged by 5.6 to 6.2% (Fig. 3B). These correspond to the two known subspecies *D. r. romanicus* and *D. r. meridionalis*, plus a third distinct lineage from the Bucegi Mts. The first lineage is represented in the southern and western Carpathians. Within this clade there is also clear differentiation and divergence (1.2%). Haplotype overlap is limited to a single specimen. The lineage of *D. r. meridionalis* is very distinct, and was only found in the Rila Mts. Genetically, the Bucegi lineage is relatively close to *D. discolor*, but morphologically clearly *D. romanicus* (Fig. 4). It seems that the Bucegi Mts. lineage is a previously unrecognised, independent evolution-

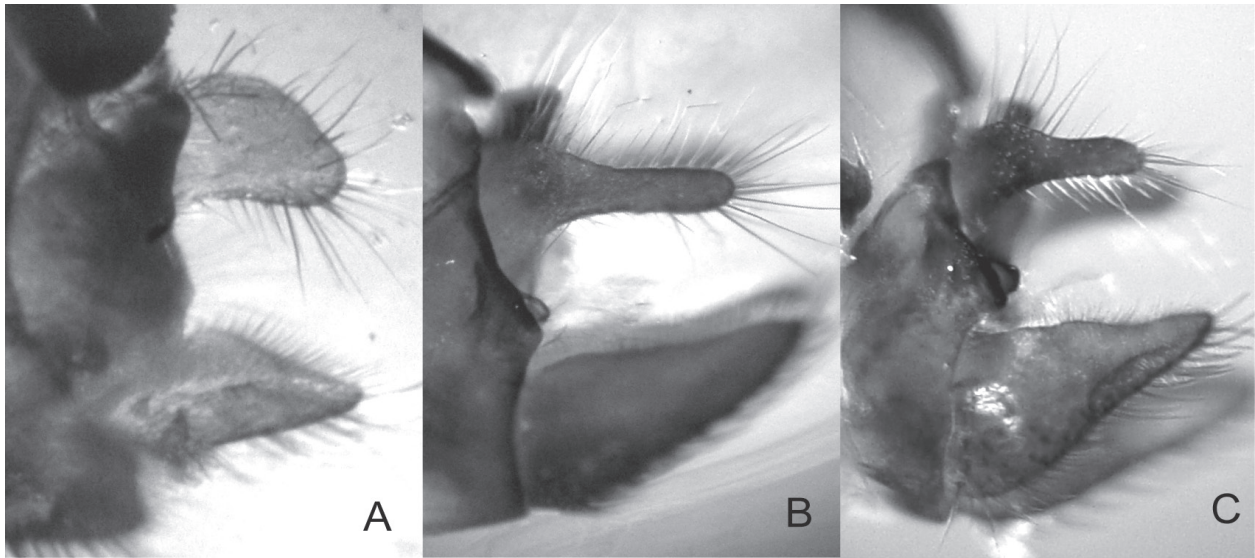


Fig. 4. Adult genitalia of A) *D. discolor* from Bucegi Mts, B) *D. romanicus romanicus* from Apuseni Mts, and C) *D. romanicus* from Bucegi Mts.

any entity. Further morphological studies may reveal characters suitable for subspecies description.

First conclusions

In both species there is clear population structure within the Carpathian Mts. This supports previous findings of intraspecific differentiation between individual ranges in the Carpathians (e.g. MEY & BOTOȘĂNEANU 1985). The multiple lineages observed in the Balkan Peninsula in *D. discolor* support existence of several Balkan refugia, or at least considerable structure within Balkan refugia (SCHMITT et al. 2006).

The comparison shows that the Bucegi Mts support endemic lineages in both species. While the “Bucegi lineage” of *D. romanicus* is more strongly diverged than that of *D. discolor*, both are clearly distinct from the other populations of the respective species. This confirms that the Bucegi Mts. represent an isolated section of the Carpathian range, which played or plays an important role in species diversification.

Lineage divergence within *D. romanicus* is twice than high, as within *D. discolor*. This would suggest that populations of *D. romanicus* have been isolated from each other much longer than those of *D. discolor*. Considering the close relationship between the two species, their similar ecology and their sympatric distribution in the region, this result is surprising. A more in depth study comparing both species phylogeography may give sufficient insight to explain this result.

Acknowledgements

We would like to thank M. BALINT, B. JOZEWOFIC, B. SZCZESNY for providing us with some of the material

used in this study. This study was financially supported by a Research Grant for Doctoral Candidates from the German Academic Exchange Service (DAAD) to SUP.

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Received: 24.11.2007
Accepted: 14.12.2007
Printed: 28.10.2008