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Genetic characterization of Belgian noble crayfish populations *(Astacus astacus)*

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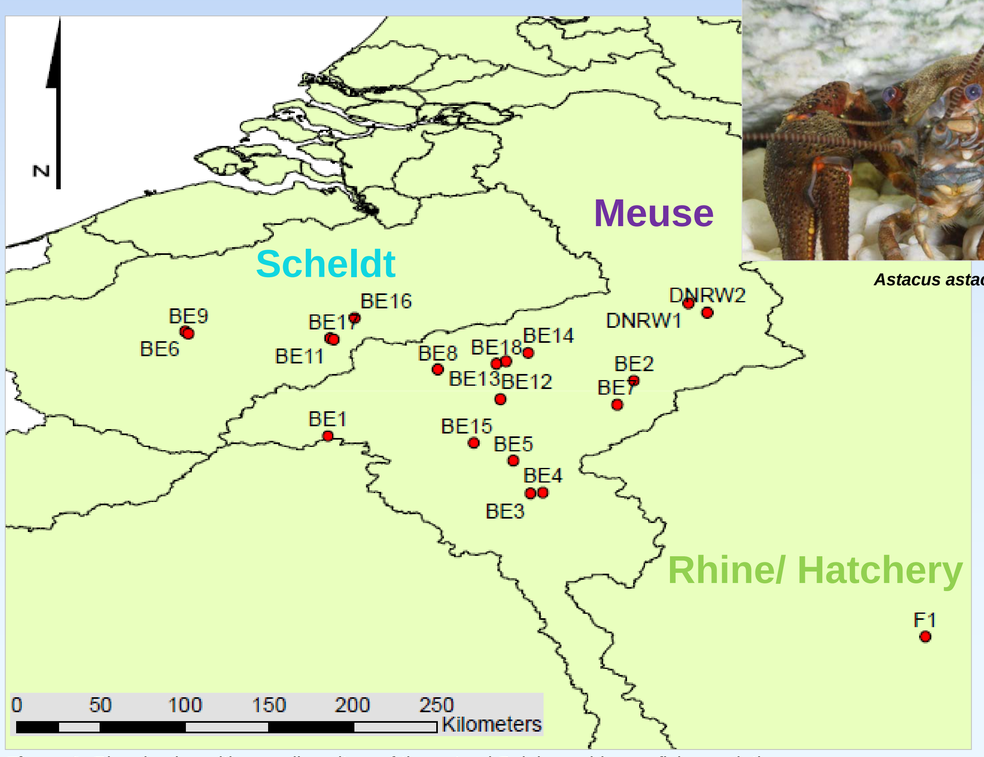
Introduction

One goal in conservation biology is to conserve the genetic diversity of endangered species to protect their adaptative potential. Therefore, it is essential to explore the genetic composition of natural populations to define evolutionary significant units (ESUs) as basis for modern conservation management plans. In cases where no genetic data is available conventional management plans treat different catchments as separate ESUs for restocking (*1*). The aim of this study was to resolve the genetic structure of natural Belgian noble crayfish populations for future conservation strategies in Belgium. We analyzed five populations present in the Scheldt basin and 14 populations present in the Meuse basin as well as a Belgian hatchery with crayfish originating from the Rhine basin in France (F1; Fig. 1), and compared the data with a European-wide dataset. In particular, we addressed the following questions:

1. **Do Belgian noble crayfish differ from European noble crayfish in their genetic composition?**
2. **Do the populations present in the river basins Scheldt and Meuse form two independent ESUs?**
3. **Is it advisable to (re)stock natural waters with individuals from the Belgian hatchery?**



Astacus astacus (Foto: D. Herman)

Figure 1. River basins with sampling sites of the natural Belgian noble crayfish populations.

Table 1. Overview of sample sites and genetic data by river basins. Number of the most common Haplotype (Hap01), number of all other haplotypes and Information about the expected (He) and observed (Ho) heterozygosity are shown.

	Samplings sites	Town	N (Mic.)/Seq.	Hap01	combined Haplotypes	He	Ho*
Meuse						**0.180**	**0.140**
	BE1	Momignies	10/10	10		0.043	0.050
	BE2	Lierneux	20/10	10		0.159	0.166
	BE3	Sainte-Cécile	20/10	9	Hap35(1)	0.248	0.271
	BE4	Florenville	20/10	10		0.178	0.150
	BE5	Paliseul	20/10	10		0.181	0.079
	BE7	La Roche	15/10	10		0.130	0.101
	BE8	Fosses-la-Ville	16/10	10		0.309	0.261
	BE12	Ciney	20/10	10		0.129	0.141
	BE13	Ohey	20/10	0	Hap40(10)	0.397	0.257
	BE14	Marchin	19/10	10		0.167	0.095
	BE15	Gedinne	20/10	10		0.135	0.086
	BE18	Gesves	16/10	10		0.037	0
	DNRW1	Aachen	14/10	10		0.411	0.207
	DNRW2	Aachen	20/10	10		0.065	0.034
Scheldt						**0.225**	**0.180**
	BE6	Tournai	19/10	10		0.263	0.177
	BE9	Tournai	19/10	9	Hap35(1)	0.206	0.210
	BE11	Ecaussinnes	20/10	3	Hap35(3), Hap47(2), Hap48(2)	0.245	0.207
	BE16	Braine-le-Château	20/10	10		0.171	0.088
	BE17	Ecaussinnes	20/10	8	Hap35(2)	0.239	0.218
Rhine/ Hatchery							
	F1	Bitche	19/10	8	Hap41(2)	0.601	0.564

*No significant deviation from Hardy Weinberg equilibrium in any population

Results and Discussion

Among 200 sequenced individuals we detected six distinct haplotypes (Fig. 2). Overall haplotype diversity was 0.214. 88.5% of the samples exhibited the most common European haplotype Hap01. However, we found two private haplotypes in population BE11 (Hap47 + 48), which differed from Hap01 by only one or two mutations and could be the result of homoplasy. Other Belgian populations bear Black Sea (Hap40) and North Sea (Hap35) haplotypes. In the Belgian hatchery we detected the haplotype Hap01, but also a distinct Croatian haplotype Hap41.

Based on microsatellites, the FCA showed that Belgian populations clustered within European samples (Fig. 3A). Populations present in Meuse and Scheldt basins showed high genetic similarity (Fig. 3B). Moreover, no distinct differentiation between Meuse and Scheldt populations was detected based on pairwise F_{ST}/R_{ST} (data not shown). Genetic differentiation between the hatchery and all natural populations present in the Meuse and Scheldt basins was high (Fig. 3A). The hatchery exhibited the highest heterozygosity and the highest number of private alleles (Tab. 1).

Methods

A total of 362 crayfish specimens from 20 sampling locations (Fig.1, Tab.1) were collected. We sequenced a 350 base pair (bp) fragment of the mitochondrial cytochrome oxidase subunit I (COI) and a 500 bp fragment of 16s rRNA (16S) for ten individuals per population. Haplotypes were identified and compared with a median joining (MJ) network (NETWORK 4.510) consisting of 46 haplotypes from 540 specimens (data set from the University of Koblenz-Landau).

A microsatellite analysis was performed based on six polymorphic loci (*2, 3*) for up to 20 individuals per population. The values of expected (He) and observed heterozygosity (Ho) and a factorial component analysis (FCA) were computed with the software GENETIX v. 4.05. The number of private alleles was estimated with the software GDA v.1.0. Pairwise F_{ST}/R_{ST} for all sites were calculated with Genepop v.4.0.10.

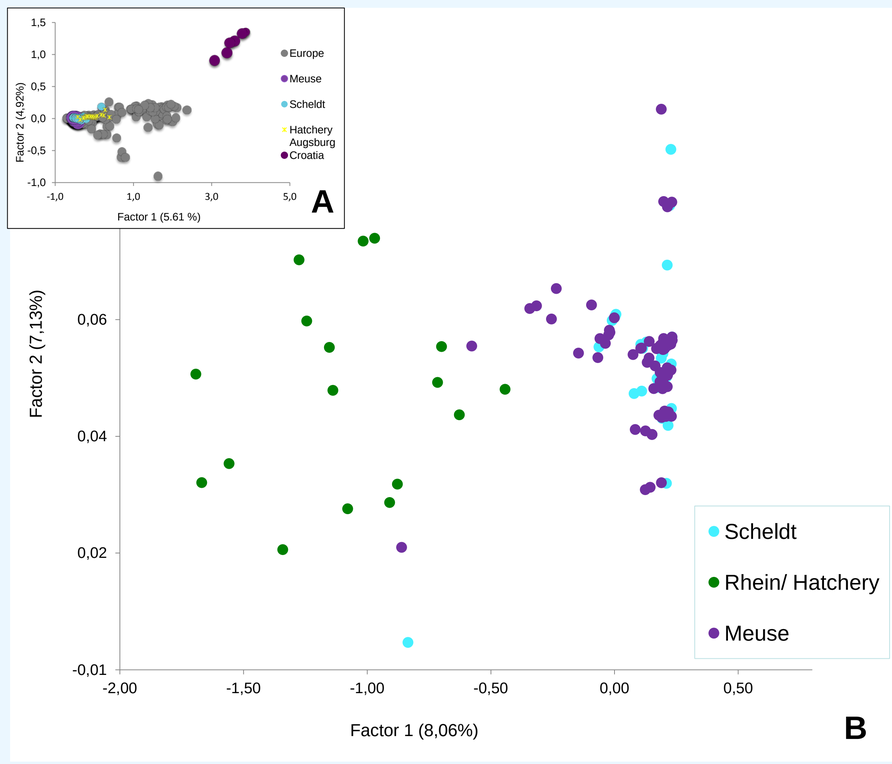


Figure 3. Factorial Correspondence Analysis (FCA) over six micro-satellite loci. Each point represent one individual. The distance between points reflects the degree of genetic differentiation among individuals. **A:** Belgian natural populations were compared with a European data set. **B:** Belgian natural populations were compared with samples from the Belgian hatchery.

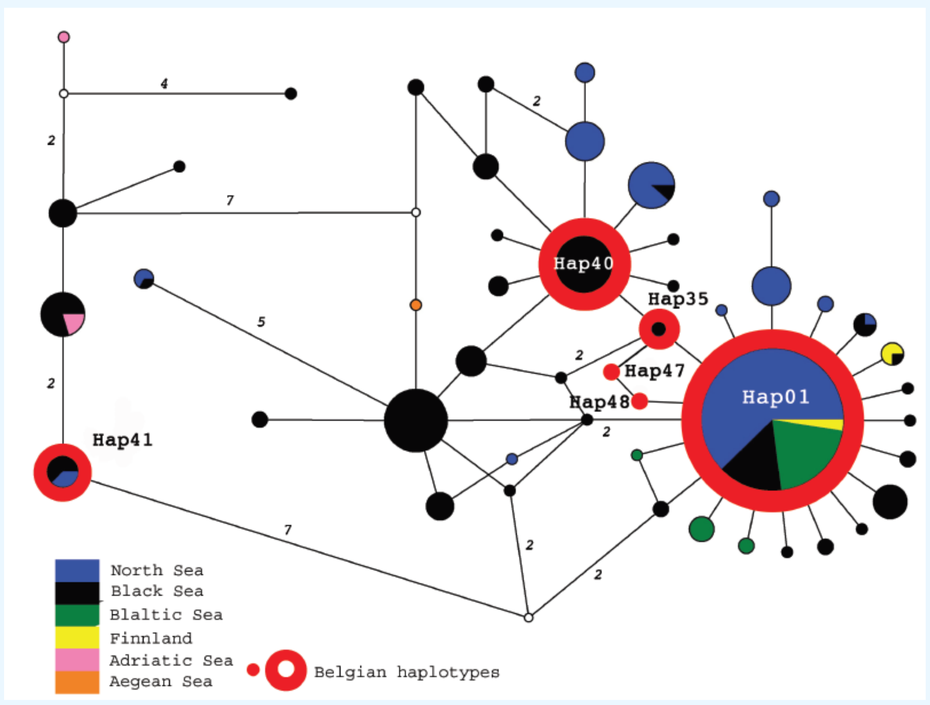


Figure 2. Median joining network of combined COI and 16S haplotypes from 540 individuals of *Astacus astacus*. The size of the circles is proportional to the frequency of the haplotypes. Median vectors are indicated as white dots. The number of base pair (bp) changes is given; no number = 1 bp change. Haplotypes from this study are highlighted in red.

Conclusions

1. **The Belgian populations did not show an exceptional genetic structure within the European dataset.**
2. **The Meuse and Scheldt river catchments did not exhibit any significant differentiation and can be treated as one ESU. However, population BE11 (Scheldt) needs special management to protect the endemic Belgian haplotypes.**
3. **The hatchery was highly differentiated compared to natural Belgian populations. Although crayfish from this hatchery were already stocked in some Belgian waters, we advice against such a practice if we want to protect the Belgian natural heritage.**

References

1 Taugbøl T, Peay S, 2004. Roundtable session 3, Reintroduction of native crayfish and habitat restoration. Bull Fr Pêche Piscic. 372-373(1-2): 465-471.
2 Koiv, K, Gross, R, Paver, T, Kuehn, R, 2008. Isolation and characterization of first microsatellite markers for the noble crayfish, *Astacus astacus*, Conserv Genet. 9, 1703-1706.
3 Koiv, K, Gross, R, Paver, T, Kuehn, R, 2009. Isolation and characterization of 11 novel microsatellite DNA markers in the noble crayfish, *Astacus astacus*. Anim Genet. 40, 124.

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