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Short communication

ANALYSIS OF MTDNA SEQUENCES OF EUROPEAN GRAYLING, *THYMALLUS THYMALLUS*, FROM SOUTHWESTERN POLAND

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ABSTRACT. Nucleotide sequences of the partial mitochondrial DNA (mtDNA) control region and flanking $tRNA^{Phe}$ gene (a total of 150bp) were determined in eight specimens of European grayling, *Thymallus thymallus*, representing five rivers in southwestern Poland. Two haplotypes, A and B, were identified which differed from each other by two nucleotide substitutions. Haplotype A was detected in fish from two rivers, the Zadrna and Bóbr, whereas haplotype B was detected in fish from three others, the Widna, Biala Gluchołazka and Metuja. The different phylogenetic position of the mtDNA haplotypes observed in samples from rivers which are in close proximity may suggest genetic structuring of grayling populations in the region, but further sampling is required to test this hypothesis.

Key words: D-LOOP, mtDNA VARIATION, EUROPEAN GRAYLING (*THYMALLUS THYMALLUS*)

European grayling *Thymallus thymallus* is widely distributed across the continent. It occurs in two main regions; the majority of this species is found in northern Europe and a much smaller part is distributed throughout the western area, including England and Denmark. In Poland, the grayling is distributed mainly in the southern upland part of the country throughout the mountain regions in the basins of the Oder and Vistula rivers. A few other populations occur in northern Poland mainly due to stocking (Witkowski et al. 1984).

Recent molecular-based phylogeographic studies on European grayling have shown rich and complex distribution of mtDNA variation which is difficult to explain in the light of simple zoogeographical generalizations (Koskinen et al. 2000, Weiss et al. 2002). However, there is growing appreciation of the fact that a significant amount of genetic variation remains to be uncovered among other populations, including those of *T. thymallus* in Poland. This communication reports the first observations of mtDNA variation among the grayling individuals inhabiting rivers in southwestern Poland.

TABLE 1

Sample distribution at various locations including major river drainage systems, ocean basin and the number of individuals sequenced (N). Haplotype denotes mtDNA type observed in a sample

	Population: river (site)	Drainage system	Basin	N	haplotype
1.	River Bóbr (next to Wleń)	Oder	Baltic Sea	1	**A**
2.	River Zadrna	Bóbr →Oder	Baltic Sea	2	**A**
3.	River Widna	Nysa Kłodzka →Oder	Baltic Sea	1	**B**
4.	River Biala Glucholaska	Nysa Kłodzka →Oder	Baltic Sea	2	**B**
5.	River Metuja	Elbe	North Sea	2	**B**

Eight European graylings were sampled (Fig. 1, Table 1). The fish were caught by electrofishing and with gillnets between 1998 and 2000, and small fin clips were preserved in 96% ethanol.

Whole genomic DNA was extracted from the alcohol-preserved tissue and diluted with nuclease-free water. The part of mtDNA that included the D-loop region and flanking $tRNA^{Phe}$ was amplified in all specimens using the following pair of primers L5 (5′-ACAACTTTGGCACCGACAATCCTA-3′) (Brzuzan, unpublished data) and H (5′-ACTTTCTAGGGTCCATC-3′) (Bernatchez et al. 1992).

PCR amplifications were performed in a reaction volume of 50 μl containing 2.5U of *Taq* DNA polymerase, 5 μl of reaction buffer (500 mM KCl, 100 mM Tris-HCl pH

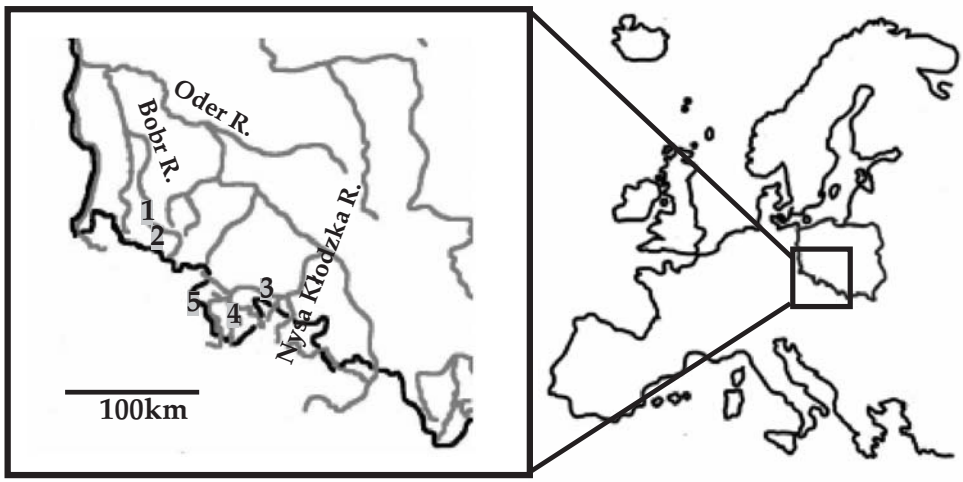

Fig. 1. Map of the study area showing the location of major river drainage systems in southwestern Poland and sampling points (numbered).

8.8, 25 mM $MgCl_2$, 1% Triton X-100), 2.5 mM of $MgCl_2$, 20 pmol of each primer, 500 μM of dNTPs and 2 μl of DNA extract.

The amplifications were performed using a 2400 thermal cycler (Applied Biosystems) with a 1 min predenaturation at 95°C, 30 cycles of 92°C for 1 min, 50°C for 1 min and 72°C for 1.5 min, followed by a final extension at 72°C for 5 min. Before sequencing, the products of amplification were purified with a QIAquick DNA Purification Kit (Qiagen). Sequencing was conducted at the Institute of Biochemistry and Biophysics, Warsaw, Poland.

To assess the general relation of mtDNA lineages formed in the studied region, an inferred phylogenetic tree was generated from the aligned sequences using the PAUP* program package (Swofford 2000). Neighbour-joining (NJ) search criteria were used (Saitou and Nei 1987). The NJ-phylogram included some sequences that were used in Weiss et al. (2002) (Table 2). The K2P (Kimura-2-parameter) DNA substitution model was chosen for phylogenetic reconstructing. The tree was rooted with the mtDNA sequence of *Thymallus arcticus baicalensis brevipinnis* as determined previously by Jurczyk (2001).

Two mtDNA haplotypes were detected in the studied sample of graylings from southwestern Poland. The nucleotide variation defining these haplotypes is shown in Table 2. Haplotype A differed from haplotype B by two substitutions at positions 19 and 23 (Table 2). Haplotype A was observed in specimens from the Bóbr and Zadrna populations (Bóbr drainage system), whereas haplotype B was detected in individuals from the Biała Głuchołazka and Widna rivers (Nysa Kłodzka drainage system) and the Metuja River (Elbe drainage system).

The NJ tree of haplotypes revealed two groups (Fig. 2). The first group of haplotypes represented the Danube basin (northern and southern Alps). The other group contained haplotypes that were enigmatic in their geographical distribution and have been observed in central Europe, Scandinavia and Slovenia. The mtDNA haplotypes A and B derived from specimens inhabiting rivers in southwestern Poland were included in this cluster. The apparent inconsistency of an arrangement of grayling mtDNA haplotypes in Europe has been reported previously and may be explained by multiple-refugial origin during the post-glacial expansion of populations of *T. thymallus* (Koskinen et al. 2000, Weiss et al. 2002).

The observation that grayling populations which inhabit rivers in close proximity in southwestern Poland carry two distinct mtDNA haplotypes may be of interest to

TABLE 2

Variable nucleotide positions for *Thymallus thymallus* haplotypes defined in the current study as A and B. Dashes (–) indicate a matching base and asterisks (*) indicate a deletion or insertion event. Shaded area indicates variation for the grayling mtDNA sequences that were obtained previously by Weiss et al. (2002). The root sequence of *Thymallus acticus baicalensis brevipinnis* is also included

	1	2	2	2	2	3	3	4	6	7	7	9	9	9	9	9	1 0	1 0	1 0	1 0	1 0	1 1	1 2	1 2	1 3
	9	1	3	8	9	5	8	8	5	5	9	0	3	6	7	8	1	2	3	4	5	0	2	8	4
Consensus	C	A	C	G	G	A	T	G	T	T	C	A	T	C	C	A	A	C	G	G	C	T	T	A	G
A AY219912	A	–	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	A
B AY219913	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	A
AF522409	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	*	–	–	–	–	–	G	–	–
AF522399	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–	–	–	G	–	–
AF522406	–	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	*	–	–	–	–	–	G	–	–
AF522396	–	–	–	–	–	–	–	–	–	–	–	–	–	–	T	–	*	–	–	–	–	–	–	–	–
AF522400	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–	–	–	–	G	–
AF522408	–	–	–	–	–	–	–	–	A	–	–	–	–	–	–	–	*	–	–	–	–	–	–	–	–
AF522402	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	C	*	–	–	–	–
AF522397	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	–	–	–	*	–	–	–	–
AF522398	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	*	A	–	–	–	–	–	–	–
AF522419	–	G	–	–	–	–	C	–	–	–	–	–	C	–	–	–	–	–	–	A	–	–	–	–	–
AF522412	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AF522411	–	–	–	–	–	C	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AF522442	A	–	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	A
AF522431	G	–	T	T	–	–	–	–	–	–	–	–	–	–	–	G	–	–	A	–	–	C	–	–	A
AF522426	G	–	T	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	A
AF522418	G	–	T	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	A
AF522439	–	–	–	–	–	–	–	–	–	–	–	G	–	–	–	–	–	–	A	–	–	C	–	–	A
AF522433	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	–
AF522435	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	–
AF522436	–	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	*	–	A	–	–	C	–	–	–
AF522434	–	–	–	–	–	–	–	T	–	–	–	–	–	–	–	–	–	–	A	–	–	C	–	–	–
T.a.b.brevipinnis	–	–	–	–	A	–	–	–	–	C	T	–	C	T	A	–	–	–	–	–	–	–	–	–	–

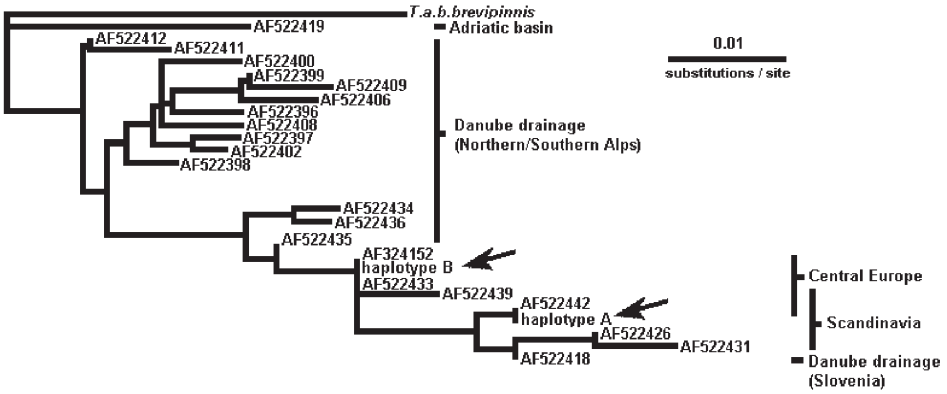

Fig. 2. NJ phylogram based on the K2P model of substitution. Positions of revealed haplotypes are marked with arrows. Names of the regions followed those proposed by Weiss et al. (2002).

fishery managers. As their management efforts should focus on riverine fish conservation, it seems reasonable that they avoid translocations of stocking material derived from neighboring grayling populations. This is especially important, as, to date, information about native and restocked *T. thymallus* in southwestern Poland is incomplete (Witkowski, personal communication). Sampling is currently being continued, as it is required in order to test rigorously the genetic structuring of the European grayling populations in southwestern Poland.

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STRESZCZENIE

ANALIZA SEKWENCJI mtDNA LIPIENIA *THYMALLUS THYMALLUS* Z POŁUDNIOWO-ZACHODNIEJ POLSKI

Analizowano sekwencje nukleotydowe o długości 150 par zasad, obejmujące fragment regionu regulacyjnego mtDNA 8 osobników lipienia europejskiego, *Thymallus thymallus*, reprezentujących stada z pięciu rzek południowo-zachodniej Polski (rys. 1). Wykryto obecność dwu typów (haplotypów) mtDNA („A" i „B"; tab. 2), różniących się od siebie dwoma podstawieniami w pozycjach 19 i 23 analizowanej sekwencji. Uzyskane wstępne wyniki badań wskazują na zróżnicowanie genetyczne lipieni w tym regionie Polski.

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