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SHORT REPORT

Genetic relationships of brook lamprey of the genus *Lampetra* in a Pyrenean stream in Spain

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Abstract The genetic relationships of a population of brook lamprey *Lampetra planeri* from Spain were analysed using a fragment of the mitochondrial non-coding region in order to describe its relationships with other European *Lampetra* populations. DNA sequences were obtained from ten larvae, all corresponding to a single private haplotype. Its closest haplotype differed by five mutations and was found in several samples of *Lampetra fluviatlis*, both from the North Sea and the Baltic. These results argue for the great conservation value of this brook lamprey population, likely originated from *L. fluviatilis* ancestors.

Keywords *Lampetra* · Phylogeography · Spain · Conservation unit · Multiple origins of West European brook lampreys

Introduction

Lampreys and hagfishes are the only surviving agnathans, thus representing the more basal vertebrate lineages still alive. Their origin predates the formation of jaws and paired fins (Takezaki et al. 2003).

Typically, lampreys spawn in freshwater and have a larval phase that lasts for several years, during which the larvae (ammocoetes) live buried in the sediment, filter-feeding. After this period, individuals undergo an extensive metamorphosis that leads to the formation of functional eyes, restructuring of the mouth and digestive tracts, and changes in coloration and osmoregulatory capacity. Some species then migrate downstream to lakes or to the sea. At this stage they adopt a parasitic or predatory feeding mode, using their sucker-like mouth to attach to other fish or sea mammals. With their tongue, they open a wound, from which they suck blood, other fluids and even scratches of tissue. These parasitic/predatory lampreys growth massively, and after 1 or more years, they migrate back to rivers where they spawn and die (Hardisty 2006).

Alternatively, in some species of lampreys, when the individuals reach metamorphosis, their sexual maturation is so advanced that they do not migrate to the sea and initiate spawning soon after emerging from the sediment, at a much more reduced size, upon which they die. This alternative life history means that there is neither feeding nor seaward migration after metamorphosis (Hardisty 2006).

Many studies support the hypothesis that the non-migratory lampreys derived from parasitic ancestors through a suppression of the migratory parasitic life stage. This event occurred independently in several lamprey lineages and gave rise to several freshwater species that are composed only of non-migratory individuals (Zanandrea 1959; Hardisty 2006).

Traditionally, in the genus *Lampetra*, two species have been recognised in western Europe: the migratory and predatory river lamprey *Lampetra fluviatilis* and the non-migratory, strictly freshwater brook lamprey, *Lampetra*

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planeri. The two species display similar distributions from the Iberian Peninsula to Scandinavia, sometimes co-occurring in the same river (Kottelat and Freyhof 2007).

Two studies on the phylogeography of *L. planeri* in West Europe have been made (Espanhol et al. 2007; Pereira et al. 2010). Espanhol et al. (2007), using ATPase and cytochrome *b* genes of mitochondrial DNA (mtDNA), provided unequivocal evidence that the genealogy of the European *Lampetra* species does not conform to a model of two separate species, one migratory and parasitic and the other including all brook lampreys. On the contrary, brook lamprey haplotypes appeared in different branches of the phylogenetic tree amongst haplotypes of *L. fluviatilis*. Pereira et al. (2010), using another molecular marker (non-coding region I), also found a lack of monophyly in west European *L. planeri*.

These studies have also shown that the southern populations of *L. planeri* are clearly differentiated (Espanhol et al. 2007; Pereira et al. 2010). Using mtDNA non-coding region I, Pereira et al. (2010) proposed that some of the Portuguese populations of this species, namely the ones of the rivers Sado, Nabão, São Pedro and Inha, correspond to different conservation units (see Fig. 1 for geographic position). Their conclusions were supported by the isolation of these populations from other populations of *L. planeri* and *L. fluviatilis*, by the exclusive presence of private haplotypes in these populations and by their confinement to small water bodies.

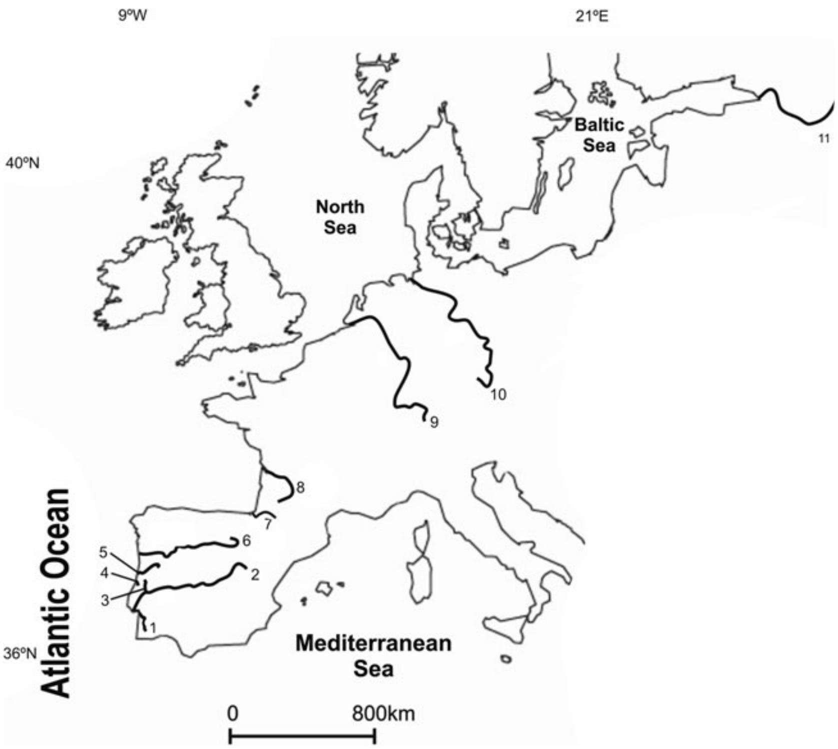
In Spain, one population ascribed to this species is present in the Olabidea-Ugarana River. This river is close to the Pyrenees and flows into the Atlantic, Bay of Biscay, in Saint Jean de Luz (France), where it is called the Nivelle (Alvarez and Doadrio 1986). This population may be in the same situation as those in the Portuguese rivers Sado, Nabão, São Pedro and Inha, because it is confined to a small water body, where *L. fluviatilis* is absent.

In this paper, a fragment of the mitochondrial non-coding region I was used to evaluate the genetic relationships of this population with other European populations.

Materials and methods

DNA sequences were obtained from ten ammocoetes from the Olabidea-Ugarana River (for collection site location, see Fig. 1), collected by electrofishing. Ammocoetes were used because there was no risk of confusion with *Lampetra fluviatilis*, as only brook lampreys have been reported in the area (Alvarez and Doadrio 1986). Care was taken to collect only a small fin clip in order to avoid harming the larvae, which were subsequently returned to the river. Total genomic DNA was extracted from fin clips preserved in ethanol by an SDS/proteinase-K based protocol (Sambrook et al. 1989, with modifications). A total of 644 base pairs (bp) including a partial sequence of the ND6 gene as well as the control region (non-coding region I according to Lee and Kocher 1995) was amplified (GenBank accession nos. from HM212773 to HM212782) using the primers Lamp-For 5′-ACA CCC AGA AAC AGC AAC AAA-3′ and

Fig. 1 Map with collection site locations. *1* Sado, *2* Tagus, *3* Nabão, *4* São Pedro, *5* Mondego, *6* Inha, *7* Olabidea-Ugarana, *8* Garonne, *9* Elbe, *10* Rhine, *11* Neva



LampRev 5′-GCT GGT TTA CAA GAC CAG TGC-3′ (Almada et al. 2008). PCR conditions followed Almada et al. (2008). Sequencing reactions were performed in StabVida (Lisbon) on a 3700 ABI DNA sequencer (Applied Biosystems) using the ABI PRISM BigDye Terminator v1.1 Cycle-sequencing kit (Applied Biosystems) (http://www.stabvida.com). Sequences were aligned with Clustal X (Thompson et al. 1997). An indel of 39 bp described by Pereira et al. (2010) in many *Lampetra* haplotypes was coded following these authors. Relationships among haplotypes were analysed with a parsimony network estimated by the software TCS version 1.18 (Clement et al. 2000). ARLEQUIN software package version 3.01 (Excoffier and Schneider 2005) was used to estimate population differentiation and genetic diversity. To access the relationships between the Spanish samples and other European samples, all available sequences of the genus *Lampetra* in Europe for the same fragment were included in the analysis [see Pereira et al. (2010) for GenBank accession numbers].

Results and discussion

From the 644 bp, 19 sites were polymorphic. The ten Spanish samples correspond only to one haplotype. The genetic diversity of this population is therefore equal to zero for all indices. A haplotype network (Fig. 2) with all

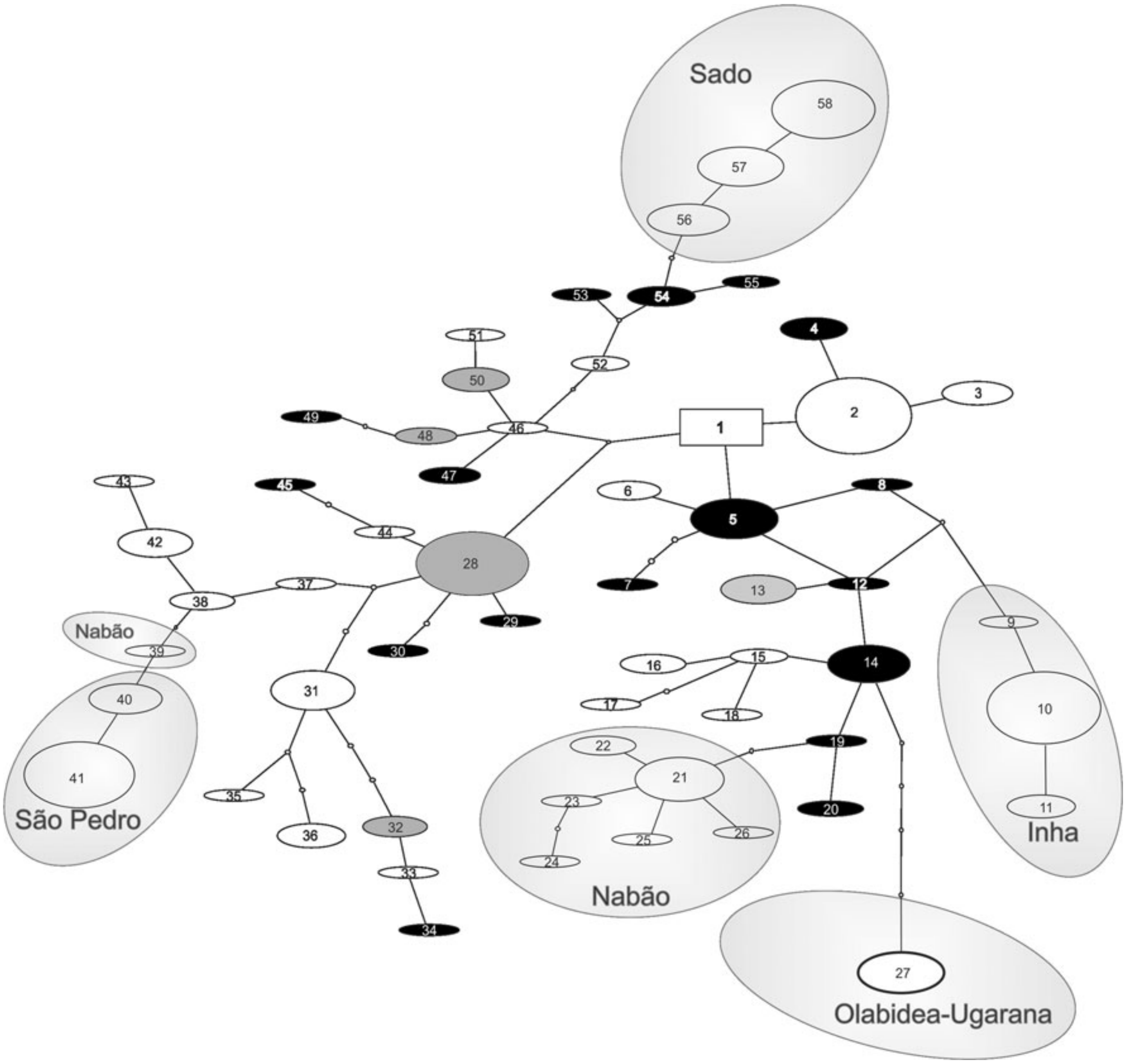


Fig. 2 Network of the non-coding region I haplotypes of European *Lampetra*. *Ellipses* represent haplotypes found in Pereira et al. (2010) and in this study, and *small open circles* missing intermediate haplotypes. Size of ellipses is proportional to the number of individuals where the haplotypes is found. *Black ellipses* represent haplotypes found exclusively in *L. fluviatilis* samples, *grey ellipses* haplotypes found in *L. fluviatilis* and in *L. planeri*, and *white ellipses* haplotypes present in *L. planeri* samples. Haplotype 1 is the most probable ancestral haplotype. Conservation units defined by Pereira et al. (2010) are marked, as well as the haplotype found in Olabidea-Ugarana River (*27*). For complete information about haplotypes, see ESM Table S1

Table 1 Pairwise population F_{ST} (distance method: pairwise differences) between all pairs of European *Lampetra* populations analysed in Pereira et al. (2010) and in this study

	Tagus	Elbe	Inha	Mondego	Nabão	Neva^{LF}	Rhine^{LF}	Rhine	São Pedro	Sado	Garonne
Elbe	0.5617										
Inha	0.5167	0.9339									
Mondego	0.4030	0.8939	0.9082								
Nabão	0.5748	0.7827	0.8909	0.7792							
Neva^{LF}	0.2740	0.4951	0.6335	0.4069	0.5053						
Rhine^{LF}	0.3316	0.4666	0.7143	0.4811	0.5141	0.0835					
Rhine	0.2484	0.2982	0.5980	0.3039	0.5123	0.2314	0.2767				
São Pedro	0.6216	0.9456	0.8830	0.9530	0.8865	0.6934	0.7515	0.6584			
Sado	0.7680	0.9131	0.9404	0.9090	0.8687	0.6647	0.7730	0.6841	0.9411		
Garonne	0.3838	0.6221	0.8434	0.6625	0.5413	0.2091	0.1700	0.3273	0.8356	0.8194	
Olabidea-Ugarana	0.9942	0.9993	0.9993	0.9998	0.9971	0.9929	0.9950	0.9913	0.9993	0.9984	0.9957

Populations of *Lampetra fluviatilis* are marked with^{LF}

All values were significant ($P < 0.01$) after Bonferroni corrections for multiple comparisons, except for the Garonne–Rhine^{LF} comparison ($P = 0.099$)

the haplotypes found by Pereira et al. (2010), with the conservation unit defined in that paper, and the Spanish haplotype are represented [for complete information about the haplotypes, see Electronic supplementary material (ESM) Table S1].

The haplotype of the Olabidea-Ugarana River (haplotype 27) is exclusively found in that river. Inspection of Fig. 2 shows that it is separated by five mutations from the nearest neighbour (haplotype 14), which is a haplotype found in *Lampetra fluviatilis*, both from the North Sea and Baltic. While in many populations there is a polymorphism for the 39 bp indel mentioned above, the samples from the Olabidea-Ugarana River were homogenous, suggesting the ancestor of this population displays this insertion.

Pereira et al. (2010), based on a population differentiation analysis, in a multidimensional scaling analysis and in the analysis of the haplotype network (which differs from the present one only in the presence of the Spanish haplotype), concluded that the populations of the Portuguese rivers Sado, Nabão, São Pedro and Inha constitute different conservation units. This conclusion was supported by the following criteria: their low genetic diversity (gene diversity less than 0.6608 ± 0.1143 in all cases), by the fact that the haplotypes found in each of those rivers occur only in that river and that they are derived one from each other. These results suggest a considerable history of local independent evolution and argue for the great conservation value of those *Lampetra planeri* populations (Pereira et al. 2010). The population of the Olabidea-Ugarana River has the same characteristics described for the rivers Sado, Nabão, São Pedro and Inha. It forms a distinct phylogroup composed of a private haplotype, and it is the population that is at the greatest distance both from of the two populations of *L. fluviatilis* previously studied and from all the populations of *L. planeri* described (see F_{ST} matrix in Table 1). This distinctiveness and considerable separation (for *Lampetra* patterns) suggest that this population should be considered an additional conservation unit, but this issue must remain open until a more complete survey is conducted. Pereira et al. (2010) presented evidence that several Portuguese brook lamprey populations originated independently from an ancestral *L. fluviatilis* stock and predicted that similar situations were likely to be found in other European countries. The brook lampreys of the Olabidea-Ugarana River conform to that prediction, bringing into question the monophyly of the brook lamprey species *L. planeri*.

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