UNIVERSIDADE DE ÉVORA

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INSTITUTO SUPERIOR DE AGRONOMIA

Genetic diversity and population structure of the sea lamprey (*Petromyzon marinus* L.) across its distributional range

Catarina Sofia Pereira Mateus

Orientação: Professor Doutor Pedro Raposo de Almeida

Mestrado em Gestão e Conservação de Recursos Naturais

Dissertação

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GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE SEA LAMPREY (*PETROMYZON MARINUS* L.) ACROSS ITS DISTRIBUTIONAL RANGE

Abstract

Lampreys are a group of ancient vertebrates with 360 million years of existence. Throughout their evolution, they have acquired local adaptations to the colonized habitats, showing high plasticity and adaptive capacities. The sea lamprey (*Petromyzon marinus* L.) is a parasitic and anadromous species that occurs in both sides of the North Atlantic. The aims of this study were to analyse, using microsatellite markers, the genetic diversity and population structure of sea lamprey throughout its distributional range. Analyses demonstrated consistent signs of high population differentiation between European and North American samples (two-groups structure), most probably due to isolation by distance, but low differentiation among populations from the same coast. The apparent lack of homing in this species is in line with its high evolutive success, as homing may bring adults back to natal habitats that have changed, or that are intermittently unfavourable. Analyses also demonstrated higher levels of genetic diversity in North American samples.

Keywords: Anadromy, fisheries management, homing, microsatellite loci, population structure, sea lamprey

DIVERSIDADE GENÉTICA E ESTRUTURA POPULACIONAL DA LAMPREIA-MARINHA (*PETROMYZON MARINUS* L.) AO LONGO DA SUA ÁREA DE DISTRIBUIÇÃO

Resumo

As lampreias são organismos ancestrais com cerca de 360 milhões de anos de existência. No decorrer da longa escala evolutiva têm vindo a adquirir adaptações aos locais que colonizaram, tendo uma forte capacidade evolutiva e adaptativa. A lampreia-marinha (*Petromyzon marinus* L.) é uma espécie parasita e anádroma que ocorre em ambas as costas do Atlântico Norte. Este estudo teve como principal objetivo estudar a diversidade genética e a estrutura populacional desta espécie ao longo da sua área de distribuição, através do uso de microssatélites. Os resultados demonstraram forte divergência entre populações das costas Este e Oeste do Atlântico Norte, provavelmente devido à elevada distância entre populações, mas pouca diferenciação entre populações da mesma costa. A ausência de *homing* nesta espécie terá contribuído para o seu sucesso evolutivo, uma vez que o *homing* pode levar indivíduos a reproduzirem-se em habitats que se tornaram desfavoráveis ou intermitentemente inapropriados. Os resultados demonstraram também uma maior variabilidade genética nas populações americanas.

Palavras-chave: Anadromia, estrutura populacional, gestão pesqueira, *homing*, lampreia-marinha, microssatélites

1. Introduction

1.1. The sea lamprey

The sea lamprey (*Petromyzon marinus* L., 1758) is a parasitic and anadromous species that occur at both sides of the North Atlantic. As anadromous, they migrate to freshwater for spawning, and the life cycle is divided in two distinct phases: an adult marine phase of parasitic feeding and a freshwater larval phase (Fig. 1). The larval stage is spent entirely in fresh water and is the longest period, lasting for 2-8 years (Hardisty & Huggins 1970; Beamish & Potter 1975; Morkert *et al*. 1998; Quintella *et al*. 2003), depending on the location and the environmental conditions. During this period, the lamprey larvae (usually called ammocoetes) live burrowed in fine sediment deposits of rivers and streams, and are filter feeders, feeding on organic detritus and microorganisms, especially diatoms (Hardisty & Potter 1971a; Moore & Mallatt 1980). After this period, larvae undergo a metamorphosis, with drastic remodelling of the cephalic region and of the digestive apparatus. In the majority of Northern Hemisphere lamprey species, the main external changes associated with metamorphosis are initiated from mid-July to September (Hardisty & Potter 1971b). After metamorphosis, juveniles initiate a downstream migration to salt water, where they feed parasitically for 1.5 to 2.5 years, especially on bony fish (e.g. Silva *et al*. 2013). Adults return to rivers for reproduction, where they become sexually mature, and build nests, a depression in the bed of the stream which construction is initiated by the males, with later involvement of the females. Lampreys are semelparous, dying after spawning.

A landlocked form of the sea lamprey, considered a pest, can be found in the Laurentian Great Lakes region, in North America. The sea lamprey was firmly established in all of the Great Lakes by the late 1940's and causes extraordinary damage to the fish stocks, posing serious threats to fisheries (Pearce *et al*. 1980; Smith & Tibbles 1980). In this sense, studies on sea lamprey in each side of the Atlantic are generally quite distinct, with European studies directed to conservation and American studies to eradication methods. No landlocked form has been reported for Europe (Kottelat & Freyhof 2007).

Though still widely distributed, the sea lamprey is now considered an endangered or rare species in some parts of its range, being the subject of important commercial

fisheries during their upstream spawning migration in parts of Spain, Portugal and France. In Portugal, it can be found in all the main river basins, being more abundant in the North and Central regions of the country (Mateus *et al*. 2012). Fishing activity concentrates in these regions, mainly Minho and Mondego, but also Lima, Cávado, Vouga and Tagus, as well as a more reduced fishery in the river Guadiana in the south (Almeida *et al*. 2002; Stratoudakis *et al*. 2016). Fishing takes place during the anadromous movement of pre-spawners from January to April, when licensed fishing for this resource constitutes one of the main activities of many hundreds of artisanal fishers (Stratoudakis *et al*. 2016). The sea lamprey is classified as *Vulnerable* according to the Portuguese Red List of Threatened Vertebrates (Cabral *et al*. 2005).

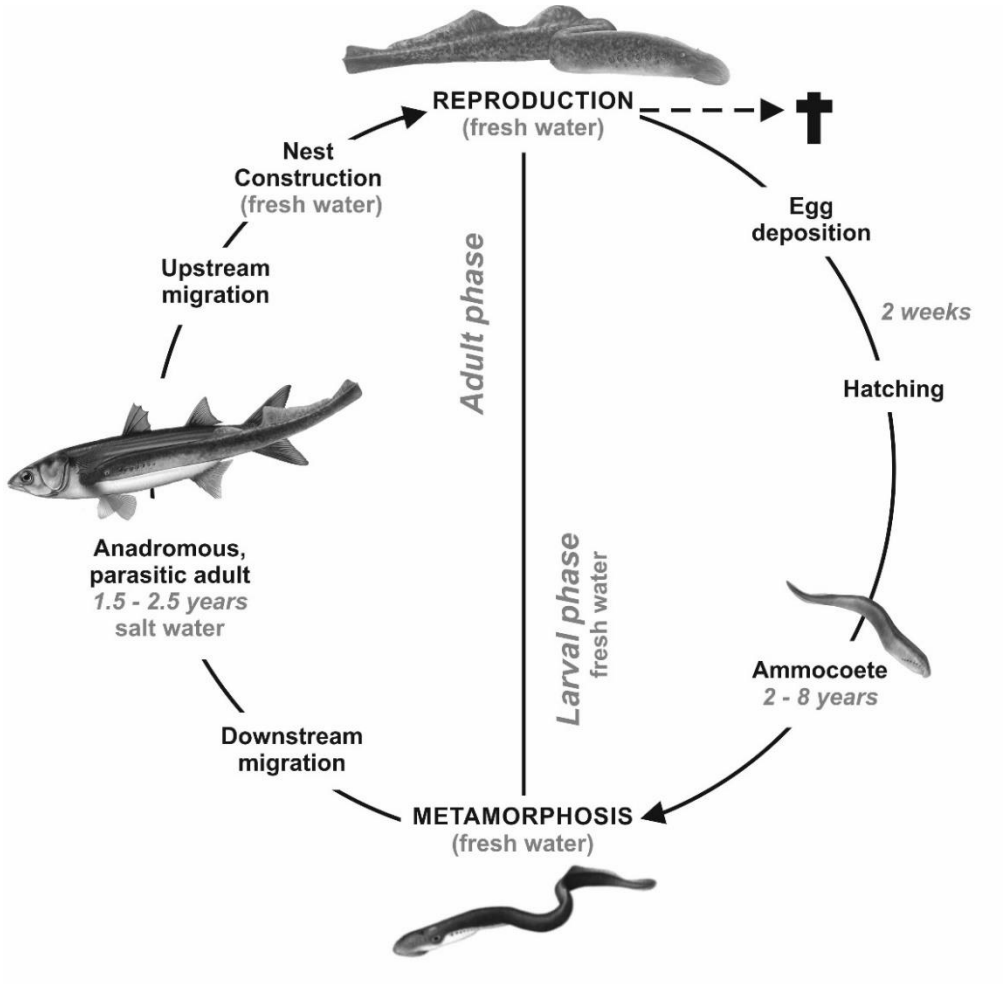

Figure 1. The anadromous life cycle of the sea lamprey, *Petromyzon marinus*.
Lamprey drawings with courtesy of Fernando Correia, Lab. de Ilustração Científica - dbio/UA.

1.2. Site selection, population structure and stock identification

Anadromy occurs in *c.* 1 per cent of the known species of fish, but subsists as a life-history strategy quite widely across the diversity of fishes, and is widely accepted as a trait with adaptive and selective advantages, that has evolved multiple times (McDowall 2001a,b). The evolution of anadromy has provided fish with the opportunity for more rapid growth, larger size, and higher fecundity through access to richer food resources, but may result in greater mortalities resulting from predation during migration and when at sea, involves more costs related to osmoregulatory demands when shifting between fresh and salt waters, and has the tendency to disperse stocks very widely (reviewed in McDowall 2001a). Anadromy and homing are often suggested to have coevolved. Homing in anadromous fishes allows the development of local stocks adapted to local conditions (McDowall 2001a,b). Where there is precise homing of the returning fish, gene flow among populations will be much reduced, enhancing genetic differentiation among populations, and thus within-species diversity (McDowall 2001b). Homing, however, may bring adults back to natal habitats that have changed, or that are intermittently unfavourable, condemning most of their progeny (McDowall 2001a; Cury 1994).

Lampreys apparently do not show homing behaviour (Bergstedt & Seelye 1995; Waldman *et al*. 2008). Most molecular studies that have been developed with European and North American populations of sea lamprey are based on mitochondrial markers (e.g. Rodríguez-Muñoz *et al*. 2004; Waldman *et al*. 2008), and all demonstrate a lack of fixed differences among populations of the same coast (suggesting lack of homing), but an absence of shared haplotypes between coasts.

Lança *et al*. (2014) used morphological characters and heart tissue fatty acid signature to analyse the existence of a stock structure on sea lamprey populations sampled in the major Portuguese river basins. The authors suggest the existence of three different sea lamprey stocks in Portugal, namely North/Central group, Tagus group, and Guadiana group, possibly promoted by seabed topography isolation during the oceanic phase of the life cycle (Fig. 2). According to the authors, detected differences are probably related with environmental variables to which lampreys may have been exposed. A stock can be defined as a population or portion of a population of which all

members are characterized by similarities which are not heritable, but are induced by the environment, and which include members of several different subpopulations (Marr 1957). The identification of stocks is fundamental for both fisheries and endangered species management, as individuals from a given stock are adapted to the environment where they live, and therefore must be managed according to the specific characteristics of the stock.

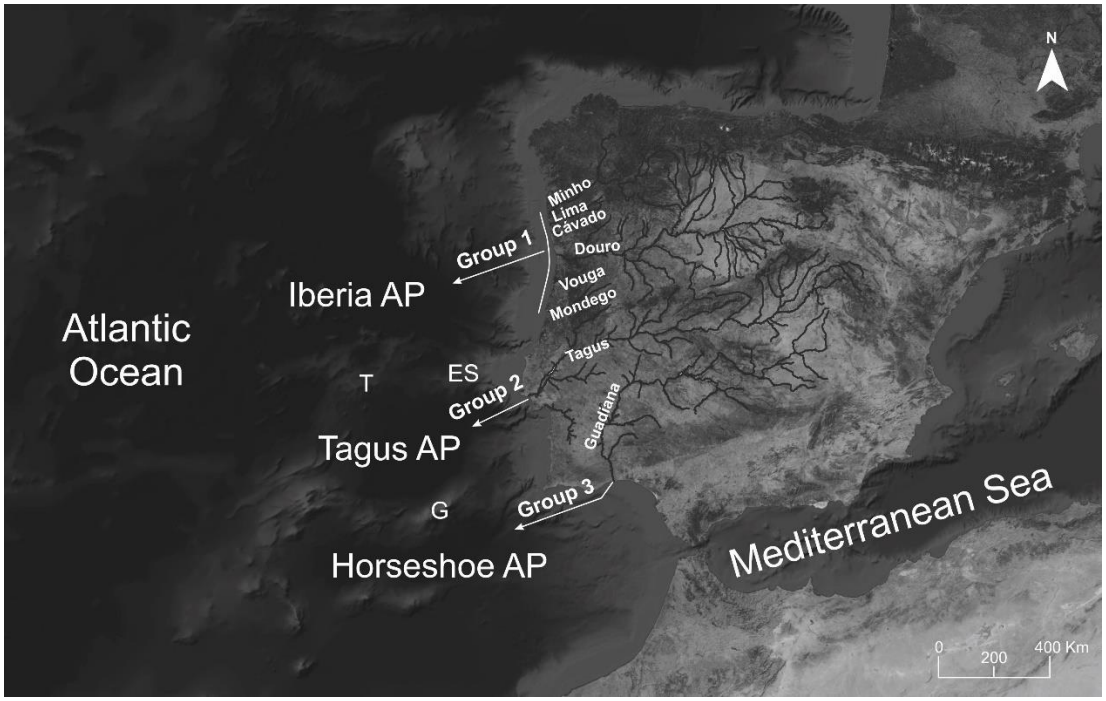

Figure 2. Sea lamprey groups suggested by Lança *et al.* (2014), probably associated to the three isolated abyssal plains (and/or nearby continental slopes) off western Iberian Peninsula, detected by analysis of morphological characters and heart tissue fatty acid signature. Physiographic features of the west Iberia Margin are presented, as well as the seamounts and canyons that contour the three abyssal plains. Iberia AP - Iberia Abyssal Plain; Tagus AP - Tagus Abyssal Plain; Horseshoe AP - Horseshoe Abyssal Plain. T – Tore Seamount; ES – Estremadura Spur; G – Gorringe Bank. Adapted from Lança *et al*. (2014).

For the sea lamprey, the absence of genetic differentiation along the European Atlantic coast and the existence of distinct stocks, would imply that the oceanic phase of the life cycle is composed by a dispersion period during the juvenile migration, followed by a much less mobile adult stage, which would restrict the mixture of adult lampreys from different geographical groups. However, genetic differentiation of European populations of sea lampreys has been accessed only with mitochondrial DNA, which is especially useful to investigate historic patterns of reproductive isolation and colonization. Markers such as microsatellites, which are highly polymorphic and

have high mutation rates, reveal more contemporary patterns of interactions among populations, making them especially useful for the study of fine-scale population structure and capable of detecting differences among closely related populations, not revealed by the mitochondrial DNA (O'Connell & Wright 1997).

1.3. Objectives

The aims of this study are to analyse, using microsatellite markers, the genetic diversity and population structure of sea lamprey from both sides of North Atlantic, and to give new insights on the stock structure identified in Portugal, most likely promoted by geographical segregation during the oceanic parasitic phase of the life cycle.

To accomplish these objectives, the following specific tasks were established:

a) Measure and compare the genetic diversity of *P. marinus* populations across its distributional range;

b) Analyze the genetic differentiation among populations of *P. marinus*, to infer the dispersal patterns and site fidelity of the species;

c) Analyze if the geographical groups previously identified through morphological characters and heart tissue fatty acid signature are genetically distinct.

Ultimately, this study is intended to contribute for an informed management of fisheries and application of conservation measures, especially in areas where the species is considered endangered or rare.

2. Material and Methods

2.1. Sampling and DNA extraction

For the majority of sites, samples were collected from commercial lamprey catches or during monitoring studies. In two sites (rivers Eo and Sella, Asturias, Spain) specimens were collected by electro fishing. Lampreys collected by electro fishing were anaesthetized by immersion in 2-phenoxyethanol (0.3 ml L^{-1}), a piece of tissue was removed from the dorsal fin, and after recovery individuals were released near the capturing sites. All tissue samples were preserved in alcohol pro-analyses. A total of 20 sites were sampled, two from Sweden, one from the Netherlands, eight from Portugal, three from Spain, one from France and five from the west Atlantic North American coast (Fig. 3; Table 1).

Total genomic DNA was extracted following a standard SDS-proteinase K/phenol–chloroform protocol and stored at -20ºC. DNA concentration was measured using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer and standardized to 50 ng µl-1 per sample.

Figure 3. Sampling sites from the west Atlantic North American coast and the east Atlantic coast assayed in this study. Sampled countries in dark gray. For details about sampling sites see Table 1.

Table 1. Sampled localities, including site label as in Figure 3 and number of samples.

Site	Country	River	N	Provided by
ROL	Sweden	Rolfsan	26	Micael Söderman
ATR	Sweden	Atran	26	Jonas Andersson
ROE	The Netherlands	Roer	8	Rob Gubbels
GIR	France	Gironde	19	Mario Lepage
SEL	Spain	Sella	50	This study
EO	Spain	Eo	49	This study
ULL	Spain	Ulla	50	Maria C. Rodicio
MIN	Portugal	Minho	49	This study
LIM	Portugal	Lima	50	This study
CAV	Portugal	Cávado	44	This study
DOU	Portugal	Douro	50	This study
VOU	Portugal	Vouga	28	This study
MON	Portugal	Mondego	38	This study
TEJ	Portugal	Tagus	44	This study
GUA	Portugal	Guadiana	28	This study
LaH	USA	LaHave	40	Weiming Li
COCH	USA	Cocheco	28	Weiming Li
MER	USA	Merrimack	35	Weiming Li
WES	USA	Westfield	22	Weiming Li
CON	USA	Connecticut	18	Weiming Li

2.2. Microsatellite amplification, genotyping and fragment size determination

A total of 702 specimens of *P. marinus* from 20 sites were used in the analysis (Fig. 3 and Table 1). Initially, the following 19 microsatellite primer sets developed for *P. marinus* and other lamprey species were screened using the described protocols and further optimized: Pmaμ 2, Pmaμ 3, Pmaμ 4, Pmaμ 5, Pmaμ 7, Pmaμ 8 and Pmaμ 9 developed for *P. marinus* (Bryan *et al*. 2003; Filcek *et al*. 2005); Lspn 005, Lspn 013, Lspn 021b, Lspn 044, Lspn 050, Lspn 094, developed for *Lethenteron* sp. N (Takeshima *et al*. 2005); Iun 2, Iun 4, Iun 5, Iun 6, Iun 7 and Iun 13 developed for *Ichthyomyzon unicuspis* and *I. fossor* (McFarlane & Docker 2009). Twelve primer sets produced unambiguously determined bands and were polymorphic: Pmaμ 2, Pmaμ 3, Pmaμ 4, Pmaμ 5, Pmaμ 7, Pmaμ 8, Iun 2, Iun 5, Iun 6, Lspn 044, Lspn 050 and Lspn 094. These 12 loci were used for analysis and all others were rejected. The reverse primers were 5'-labelled with 6-FAM, NED, PET or VIC (Applied Biosystems®) fluorescent dyes. Microsatellite loci were multiplex amplified by polymerase chain reactions (PCR) set up in 12 μl volumes

containing 2 µL of 50 ng μl^{-1} genomic DNA, 1.0 to 3.0 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 µM for each primer, 1 unit of DreamTaq™ DNA Polymerase (Fermentas) and 1× DreamTaq™ Buffer. PCR conditions were as follows: initial denaturation at 94 °C for 1 min, followed by 23 to 25 cycles of 30 sec at 94 °C, annealing for 30 sec at temperatures ranging from 57 to 60 °C and 30 sec at 72 °C, and a final extension of 7 min at 72 °C. For some loci of difficult amplification, a Multiplex PCR Kit (Qiagen®) was used, with 5 µl Qiagen Multiplex PCR master Mix, 3 µl RNase-free water, 1 µl Primer Mix (2 µM each primer) and 1 µl of 50 ng μl^{-1} of genomic DNA, using the following protocol: initial activation step at 95 °C for 15 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 57 °C for 90 sec and extension at 72 °C for 60 sec, and a final extension of 30 min at 60 °C. The PCR reactions were conducted on a Bio-Rad® thermal cycler.

Samples were genotyped in an ABI PRISM® 310 Genetic Analyzer and fragments were sized with GeneScan™-500 LIZ™ Size Standard. Allele sizes were visually determined using the software GeneMapper® 3.7 (Applied Biosystems®).

2.3. Data analysis

Microsatellite loci were tested for null alleles, large allele dropout and stuttering using MICROCHECKER 2.2.3 (van Oosterhout *et al*. 2004), and visually examined for correction. Genetic diversity was measured through observed heterozygosity (Ho), and unbiased expected heterozygosity (He, *sensu* Nei 1978), inferred using GENETIX 4.05.2 (Belkhir *et al*. 1996), and the mean allelic richness (AR), which was calculated and corrected for sample dimension by rarefaction using HP-Rare (Kalinowski *et al*. 2005).

Differentiation among populations was determined using the software GENETIX through pairwise F_{ST}, using the Weir & Cockerham's estimator (Weir & Cockerham 1984). Significance was assessed with 10,000 permutations.

The distribution of genetic variation was accessed through locus-by-locus analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992). This analysis accomplishes three components of genetic variation: among groups (F_{CT}), among populations within each group (F_{SC}), and within populations (F_{ST}). These analyses were

performed in ARLEQUIN 3.5.2.2 (Excoffier *et al*. 2005), using the allelic frequencies as the genetic distance and 20,000 permutations.

Patterns of differentiation were visualized by principal coordinates analysis (PCoA). This analysis was computed using GenAlEx 6.5 (Peakall & Smouse 2006,2012).

Population clustering was analyzed using the Bayesian model-based clustering approach implemented in STRUCTURE 2.3.4 (Pritchard *et al*. 2000; Falush *et al*. 2003). Runs were performed under the admixture model, with correlated allelic frequencies. STRUCTURE runs were performed for a number of groups (K) set between 1 and 20, with 10 replicates of each K, with an initial burn-in of 100,000 MCMC (Markov Chain Monte Carlo) generations, followed by 1,000,000 MCMC steps.

The most likely number of clusters in each dataset was inferred using a combination of two metrics: changes in LnP(D), the probability of the data given K, for consecutive K (when several values of K give similar estimates of LnP(D), the smallest of these is often the correct K, i.e., when values plateau), as suggested by Pritchard *et al*. (2010), and the protocol developed by Evanno *et al*. (2005), both obtained using STRUCTURE HARVESTER 0.6.94 (Earl & VonHoldt 2012).

3. Results

The total number of alleles per locus across populations varied from 1 to 15. Eight private alleles (alleles found in a single population) were found: one in E0, one in MIN, two in LaH, two in COCH, one in MER and one in WES, and the mean allelic richness (AR) per locus ranged from 1.00 to 7.4109. The summary statistics of the genetic diversity indices for each locus and sample are provided in Table 2.

F_{ST} values between sites ranged from -0,0109 (between VOUG, Portugal and GIR, France) to 0,28717 (between SELL, Spain and WES, USA; Table 3), with a global F_{ST} of 0.10508. A total of 120 of the 190 F_{ST} values (63%) were statistically significant, however, many values were low, with the exception of comparisons between European and North American samples, which accounted for 62.5% of the total significant pairwise comparisons, and all being significant at the 0.1% level ($P<0.001$).

Genetic structure analyses were consistent with these findings, as STRUCTURE analyses demonstrated consistent signs of high population differentiation between European and North American samples (two-groups structure), but low differentiation among populations from the same coast (Fig. 4). The most likely number of clusters in each dataset was computed using a combination of estimated LnP(D) values and ΔK of the Evanno method, and indicate that the most consistent structure attained for the entire dataset is a group in each side of the North Atlantic (Fig. 4A). Pritchard *et al*. (2010), suggested that when one have a situation with two clear populations, and is trying to decide whether one (or both) of these is further subdivided, then one can run STRUCTURE using subsets of populations that might be subdivided. Following this, three additional runs of STRUCTURE were performed: one including the 15 European populations (Fig. 4B); another including the five North American populations (Fig. 4C); and another (Fig. 4D) with the eight populations from Portugal, to test the hypothesis of regional differentiation suggested by Lança *et al*. (2014). This allows detection of further structure in these populations, if present, that otherwise would be hidden due to the high differentiation between the European and North American samples. Results from these analyses revealed, however, the same groups, with further differentiation only

within samples, i.e, there is no further structure among populations from the same coast, or among samples from Portugal.

The principal coordinates analysis (PCoA), also revealed the existence of mainly two groups, the same detected by STRUCTURE (European and North American samples; Fig. 5).

These results were in agreement with the analysis of molecular variance (AMOVA), which revealed low genetic variation among sea lamprey sites (10.7%; AMOVA I; Table 4) with variation within populations accounting for 89.3% of the total variation. When comparing the European and American samples, according to the results attained with PCoA and STRUCTURE (AMOVA II), variation between coast accounts for 23.7% of the total variation, and within populations variation is 75.3% of the total. In AMOVA III, where Portuguese populations were grouped according to the 3-stock structure suggested by Lança *et al*. (2014), virtually all variation, almost 100%, occurred within populations (99.6%; Table 4).

Table 2. Measures of genetic diversity assayed at twelve microsatellite DNA loci for each sampled location. Sample acronyms correspond to locations as in Table 1. Sample size (n), number of alleles per locus (Na) with number of private alleles in parentheses, mean allelic richness (AR), unbiased expected heterozygosity (He), observed heterozygosity (Ho).

	ROL	ATR	ROE	GIR	SEL	EO	ULL	MIN	LIM	CAV	DOU	VOU	MON	TEJ	GUA	LaH	COCH	MER	WES	CON
	n=26	n=26	n=8	n=19	n=50	n=49	n=50	n=49	n=50	n=44	n=50	n=28	n=38	n=44	n=28	n=40	n=28	n=35	n=22	n=18
Locus																				
Pma*μ* 2																				
AlRan	96-100	98-100	100	96-100	98-100	98-100	94-100	94-100	98-100	98-100	98-100	94-100	98-100	96-100	94-100	94-100	94-100	94-100	94-100	94-100
Na	3	2	1	3	2	2	3	3	2	2	2	4	2	3	3	4	4	4	4	4
AR	2.1925	1.9257	1	2.3528	1.9705	1.973	2.1317	2.1124	1.9878	1.9915	1.9987	2.4366	1.9955	2.1336	2.1945	2.7159	3.3925	2.8791	3.2504	2.6661
He	0.3703	0.2919	-	0.5401	0.3685	0.3737	0.4941	0.4198	0.4160	0.4303	0.4848	0.4759	0.4507	0.4825	0.4084	0.5753	0.6526	0.5975	0.6311	0.5540
Ho	0.1154	0.0385	-	0.2353	0.0000	0.0000	0.3778	0.0638	0.2200	0.0682	0.1600	0.3333	0.1111	0.1364	0.0714	0.3750	0.4643	0.3235	0.3636	0.2222
Iun 2																				
AlRan	114-117	111-117	114-117	114-117	111-117	111-117	111-117	114-117	114-117	114-117	111-117	114-120	111-117	114-120	114-120	105-117	114-117	105-120	114-117	114-117
Na	2	3	2	2	3	3	3	2	2	2	3	3	3	3	3	3	2	4	2	2
AR	1.9847	2.1925	2	1.9995	2.1054	2.1989	2.1295	1.9921	1.9931	1.9608	2.1143	2.2128	2.1868	2.1367	2.3809	2.3041	1.9971	2.3869	2	1.9998
He	0.3927	0.3703	0.5250	0.4908	0.4216	0.3936	0.4543	0.4342	0.4396	0.3483	0.4600	0.4994	0.3130	0.4813	0.4909	0.4538	0.4580	0.5394	0.5127	0.4966
Ho	0.1200	0.3077	0.6250	0.5789	0.4800	0.3469	0.4773	0.2500	0.4000	0.3023	0.3600	0.3929	0.2632	0.4419	0.4286	0.1143	0.2800	0.2903	0.2778	0.1333
Iun 5																				
AlRan	250-274	250-277	250-274	250-274	250-277	250-286	250-286	250-274	250-286	250-277	250-277	250-286	250-277	250-286	250-274	253-298	250-289	250-289	247-289	253-292
Na	4	6	3	4	5	6	5	4	6	5	5	6	5	7	4	15(1)	13	14	10(1)	10
AR	3.3837	3.569	2.8571	3.3545	3.2542	3.571	3.0558	3.3627	3.3625	3.3989	3.3107	3.5874	3.1439	3.3768	3.0914	7.054	7.4109	6.6272	5.8878	7.0938
He	0.5407	0.5566	0.6044	0.5619	0.5824	0.6181	0.4932	0.5998	0.5574	0.6053	0.5354	0.4987	0.5747	0.5909	0.5721	0.8761	0.8994	0.8644	0.8150	0.8966
Ho	0.5769	0.4615	1.0000	0.6316	0.6000	0.6531	0.4318	0.5625	0.4800	0.5909	0.6400	0.5000	0.7105	0.4773	0.5714	0.8684	0.7500	0.8667	0.7727	1.0000
Iun 6																				
AlRan	124-130	124-130	127	124-127	121-130	121-130	124-130	124-130	124-127	124-130	121-130	124-127	124-130	124-130	124-130	121-133	124-136	121-133	124-130	121-130
Na	3	3	1	2	4	4	3	3	2	3	4	2	3	3	3	5	5(1)	5	3	4

Table 2. (*Continued)* Measures of genetic diversity assayed at twelve microsatellite DNA loci for each sampled location. Sample acronyms correspond to locations as in Table 1. Sample size (n), number of alleles per locus (Na) with number of private alleles in parentheses, mean allelic richness (AR), unbiased expected heterozygosity (He), observed heterozygosity (Ho).

AR	2.425	2.2306	1	1.9999	2.2389	2.6358	2.3197	2.1313	1.9989	2.2552	2.3446	1.9999	2.4007	2.1295	2.2141	3.6033	3.372	3.730	2.9344	3.2891
He	0.5282	0.5271	0.0000	0.5121	0.5119	0.5157	0.5158	0.4891	0.4887	0.5277	0.5121	0.5084	0.4895	0.4543	0.5266	0.7043	0.6701	0.7002	0.5973	0.5793
Ho	0.3600	0.5769	0.0000	0.5263	0.5000	0.5306	0.5400	0.5111	0.4200	0.5455	0.5000	0.7500	0.5000	0.4318	0.4643	0.6286	0.3929	0.6061	0.6364	0.4667
Pma*μ* 5																				
AlRan	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	131-137	125-139	125-137	131-137	125-139	125-139
Na	2	2	2	2	2	3	2	2	2	2	2	2	2	2	2	5	4	3	5	6
AR	1.9996	1.9997	2	1.9999	1.9997	2.2293	1.9994	1.9997	1.9997	1.9998	1.9997	1.9998	1.9993	1.9998	1.9996	3.7415	3.1567	2.9236	4.0623	4.6604
He	0.4977	0.5030	0.5333	0.5078	0.5042	0.5041	0.4978	0.5045	0.5032	0.5055	0.5042	0.5065	0.4940	0.5055	0.4987	0.7060	0.6344	0.5967	0.7433	0.7504
Ho	0.5385	0.5769	1.0000	0.4737	0.6400	0.4490	0.4800	0.6087	0.6200	0.4318	0.5200	0.3571	0.4737	0.5682	0.7143	0.4872	0.6786	0.5625	0.6190	0.4706
Lspn050																				
AlRan	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-152	134-154	134-154	134-156	134-154	134-152
Na	2	2	2	2	2	3(1)	2	2	2	2	2	2	2	2	2	4	4	5(1)	4	3
AR	1.9997	1.9999	2	1.9998	1.9996	2.2283	1.9997	1.9982	1.9989	1.9998	1.9962	1.9999	1.9961	1.9998	1.9996	2.8039	2.7337	2.9796	2.7224	2.8398
He	0.5029	0.5098	0.5000	0.5007	0.5018	0.4948	0.5042	0.4787	0.4887	0.5055	0.4596	0.5084	0.4561	0.5055	0.4987	0.5344	0.4494	0.4693	0.4598	0.5865
Ho	0.4800	0.5385	0.7500	0.5263	0.4800	0.4082	0.5200	0.3542	0.4200	0.5227	0.5400	0.4643	0.4211	0.3864	0.5714	0.4737	0.3929	0.5000	0.4091	0.5882
Pma*μ* 7																				
AlRan	113	113	113	111-113	113	113	113	113	113	113	113	113	111-113	111-113	113	111-113	111-113	111-113	111-113	111-113
Na	1	1	1	2	1	1	1	1	1	1	1	1	2	2	1	2	2	2	2	2
AR	1	1	1	1.3158	1	1	1	1	1	1	1	1	1.2926	1.7506	1	1.9998	1.9952	1.9886	1.9998	1.9923
He	0.0000	0.0000	0.0000	0.0526	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0519	0.1857	0.0000	0.5051	0.4442	0.4141	0.5021	0.4127
Ho	0.0000	0.0000	0.0000	0.0526	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0526	0.2045	0.0000	0.2000	0.2143	0.1143	0.1364	0.2222
Lspn094																				
AlRan	156	156	156	156	156	156	156	156	156	154-156	156	156	156	156	156	154-156	154-156	154-156	154-156	154-156
Na	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	2	2	2	2
AR	1	1	1	1	1	1	1	1	1	1.2555	1	1	1	1	1	1.9991	1.9622	1.9851	1.9998	1.8873
He	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0449	0.0000	0.0000	0.0000	0.0000	0.0000	0.4902	0.3429	0.4012	0.5017	0.2460
Ho	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0286	0.0000	0.0556

Table 2. (*Continued)* Measures of genetic diversity assayed at twelve microsatellite DNA loci for each sampled location. Sample acronyms correspond to locations as in Table 1. Sample size (n), number of alleles per locus (Na) with number of private alleles in parentheses, mean allelic richness (AR), unbiased expected heterozygosity (He), observed heterozygosity (Ho).

Pmaμ 4																				
AlRan	**157**	**157**	**157**	**155-157**	**157**	**157**	**155-157**	**157**	**155-157**	**157**	**155-157**	**155-157**	**155-157**	**157**	**157**	**157-163**	**155-161**	**155-163**	**157-161**	**157-161**
Na	**1**	**1**	**1**	**2**	**1**	**1**	**2**	**1**	**2**	**1**	**2**	**2**	**2**	**1**	**1**	**4**	**4**	**5**	**3**	**3**
AR	**1**	**1**	**1**	**1.5377**	**1**	**1**	**1.4053**	**1**	**1.12**	**1**	**1.12**	**1.2143**	**1.4073**	**1**	**1**	**3.0144**	**2.8646**	**3.332**	**2.9012**	**2.5597**
He	**0.0000**	**0.0000**	**0.0000**	**0.1024**	**0.0000**	**0.0000**	**0.0776**	**0.0000**	**0.0200**	**0.0000**	**0.0200**	**0.0357**	**0.0768**	**0.0000**	**0.0000**	**0.6297**	**0.5639**	**0.6457**	**0.6308**	**0.5270**
Ho	**0.0000**	**0.0000**	**0.0000**	**0.1053**	**0.0000**	**0.0000**	**0.0800**	**0.0000**	**0.0200**	**0.0000**	**0.0200**	**0.0357**	**0.0789**	**0.0000**	**0.0000**	**0.4615**	**0.3704**	**0.4545**	**0.4000**	**0.3333**
Pmaμ 8																				
AlRan	**164**	**164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**162-164**	**160-164**	**156-164**	**156-164**	**160-164**	**160-164**
Na	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**5**	**4**	**3**	**3**
AR	**1**	**1**	**1,75**	**1.9808**	**1.2267**	**1.9618**	**1.9998**	**1.2355**	**1.6546**	**1.8728**	**1.6546**	**1.993**	**1.7653**	**1.9105**	**1.8752**	**2.865**	**3.5476**	**3.1299**	**2.6461**	**2.865**
He	**0.0000**	**0.0000**	**0.1250**	**0.3713**	**0.0396**	**0.3518**	**0.5048**	**0.0412**	**0.1487**	**0.2547**	**0.1487**	**0.4305**	**0.1909**	**0.2861**	**0.2494**	**0.6018**	**0.6674**	**0.6480**	**0.5749**	**0.6190**
Ho	**0.0000**	**0.0000**	**0.1250**	**0.3684**	**0.0400**	**0.4490**	**0.9800**	**0.0417**	**0.1200**	**0.2955**	**0.1600**	**0.5357**	**0.1053**	**0.3409**	**0.2857**	**0.6579**	**0.5556**	**0.6000**	**0.9048**	**0.6875**
Lspn044																				
AlRan	**207-209**	**207-209**	**207-209**	**207-209**	**207-209**	**207-209**	**205-209**	**207-209**	**205-209**	**207-209**	**207-209**	**207-209**	**207-209**	**207-209**	**207-209**	**205-217**	**205-217**	**205-217**	**205-217**	**205-217**
Na	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**2**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**6**	**7(1)**	**6**	**6**	**6**
AR	**1,9999**	**1.9989**	**2**	**1.9999**	**1.9975**	**1.9997**	**2.1191**	**1.9998**	**2.1196**	**1.9973**	**1.9998**	**1.999**	**1.9998**	**1.9983**	**1.9998**	**4.4584**	**4.6942**	**4.4585**	**4.0013**	**4.8884**
He	**0.5098**	**0.4827**	**0.5250**	**0.5121**	**0.4709**	**0.5043**	**0.5036**	**0.5049**	**0.5125**	**0.4681**	**0.5048**	**0.4857**	**0.5063**	**0.4796**	**0.5065**	**0.7649**	**0.7736**	**0.7650**	**0.7357**	**0.8085**
Ho	**0.6154**	**0.4615**	**0.6250**	**0.5263**	**0.5400**	**0.4694**	**0.5400**	**0.5306**	**0.5000**	**0.4545**	**0.5800**	**0.3571**	**0.5000**	**0.4545**	**0.6429**	**0.7297**	**0.8519**	**0.8182**	**0.8636**	**0.9375**
Pmaμ 3																				
AlRan	**216-226**	**216-226**	**216-224**	**216-226**	**216-226**	**216-226**	**216-226**	**216-234**	**216-224**	**216-226**	**216-226**	**216-226**	**216-226**	**216-226**	**216-226**	**216-228**	**218-228**	**218-228**	**218-228**	**216-228**
Na	**3**	**3**	**2**	**3**	**4**	**4**	**3**	**5(1)**	**3**	**3**	**4**	**4**	**4**	**4**	**3**	**6(1)**	**5**	**4**	**4**	**4**
AR	**2.6617**	**2.4091**	**2**	**2.3157**	**2.6309**	**2.2445**	**2.4115**	**2.5797**	**2.1197**	**2.1351**	**2.4409**	**2.9522**	**2.8707**	**2.5097**	**2.3854**	**3.7971**	**3.3274**	**3.0203**	**2.8104**	**2.8709**
He	**0.5724**	**0.5098**	**0.5250**	**0.5391**	**0.5545**	**0.5235**	**0.5405**	**0.5263**	**0.5145**	**0.4982**	**0.5428**	**0.6010**	**0.5818**	**0.5368**	**0.5409**	**0.6089**	**0.4922**	**0.4791**	**0.4165**	**0.4778**
Ho	**0.5769**	**0.5769**	**0.8750**	**0.6842**	**0.5200**	**0.5918**	**0.4490**	**0.5208**	**0.5400**	**0.5000**	**0.5200**	**0.5556**	**0.5789**	**0.4318**	**0.4643**	**0.5750**	**0.5000**	**0.5143**	**0.5000**	**0.6111**
All loci																				
AR	**1.89**	**1.86**	**1.63**	**1.99**	**1.87**	**2.00**	**1.96**	**1.87**	**1.86**	**1.91**	**1.91**	**2.03**	**2.00**	**2.00**	**1.93**	**3.36**	**3.37**	**3.29**	**3.10**	**3.30**

Table 3. Pairwise estimates of genetic differentiation (FST) among sites (above diagonal) and corresponding P values (below diagonal). For populations' acronyms, please check Table 1.

	ROL	ATR	ROE	GIR	SEL	EO	ULL	MIN	LIM	CAV	DOU	VOU	MON	TEJ	GUA	LaH	COCH	MER	WES	CON
ROL	-	-0.01049	0.05647	0.01403	-0.00577	0.01872	0.05233	-0.00967	-0.00721	0.00630	-0.00052	0.01350	0.00491	0.01135	-0.00547	0.24156	0.20797	0.20984	0.26780	0.21627
ATR	NS	-	0.08699	0.02497	-0.00873	0.01861	0.06100	-0.00129	0.00092	-0.00322	0.01710	0.01781	0.01347	0.01476	0.00244	0.25190	0.21786	0.21888	0.27797	0.23164
ROE	*	***	-	0.09220	0.06715	0.07128	0.10975	0.05382	0.06046	0.09935	0.07153	0.07978	0.07752	0.05269	0.07575	0.22603	0.19675	0.21113	0.24821	0.21126
GIR	NS	*	**	-	0.01680	0.00970	0.00242	0.01535	0.00388	0.00352	0.00371	-0.01090	0.02368	-0.00348	-0.00196	0.17864	0.14052	0.14472	0.19598	0.14791
SEL	NS	NS	**	*	-	0.01498	0.05178	-0.00397	-0.00442	-0.00158	0.00739	0.01479	0.00724	0.00470	0.00037	0.26109	0.22563	0.23055	0.28717	0.23516
EO	*	*	***	NS	**	-	0.02619	0.02046	0.01355	0.00800	0.02631	0.01477	0.01525	0.00719	0.01245	0.24077	0.20118	0.20596	0.25105	0.20003
ULL	***	***	***	NS	***	***	-	0.05445	0.03516	0.03083	0.03717	0.00192	0.04628	0.02270	0.02734	0.22521	0.18468	0.18750	0.23466	0.18111
MIN	NS	NS	**	NS	NS	**	***	-	-0.00625	0.00650	-0.00256	0.01714	-0.00019	0.00488	-0.00374	0.25047	0.21446	0.22012	0.27519	0.21937
LIM	NS	NS	**	NS	NS	*	***	NS	-	0.00228	-0.00529	0.00603	0.00328	0.00137	-0.00825	0.25219	0.21681	0.22228	0.27738	0.22169
CAV	NS	NS	***	NS	NS	NS	***	NS	NS	-	0.01324	0.00392	0.00987	0.00454	-0.00027	0.23857	0.20129	0.20260	0.25617	0.20571
DOU	NS	*	**	NS	NS	***	***	NS	NS	*	-	0.01039	0.00336	0.00560	-0.00130	0.24402	0.20989	0.21574	0.26992	0.20997
VOU	NS	*	**	NS	*	*	NS	*	NS	NS	NS	-	0.02321	0.00289	-0.00023	0.20643	0.16926	0.17471	0.22410	0.17790
MON	NS	NS	**	*	NS	*	***	NS	NS	NS	NS	**	-	0.00724	0.00896	0.24486	0.21383	0.21659	0.26226	0.20358
TEJ	NS	*	**	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	-	0.00543	0.21041	0.17150	0.18314	0.22636	0.17295
GUA	NS	NS	**	NS	NS	*	**	NS	NS	NS	NS	NS	NS	NS	-	0.22569	0.19125	0.19408	0.25050	0.20082
LaH	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	-	0.00107	0.00492	-0.00532	0.02182
COCH	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	NS	-	-0.00604	0.00711	0.00033
MER	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	NS	NS	-	0.00167	0.00349
WES	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	NS	NS	NS	-	0.00884
CON	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*	NS	NS	NS	-

*, P<0.05; **, P<0.01; ***, P<0.001; NS, not significant (P > 0.05)

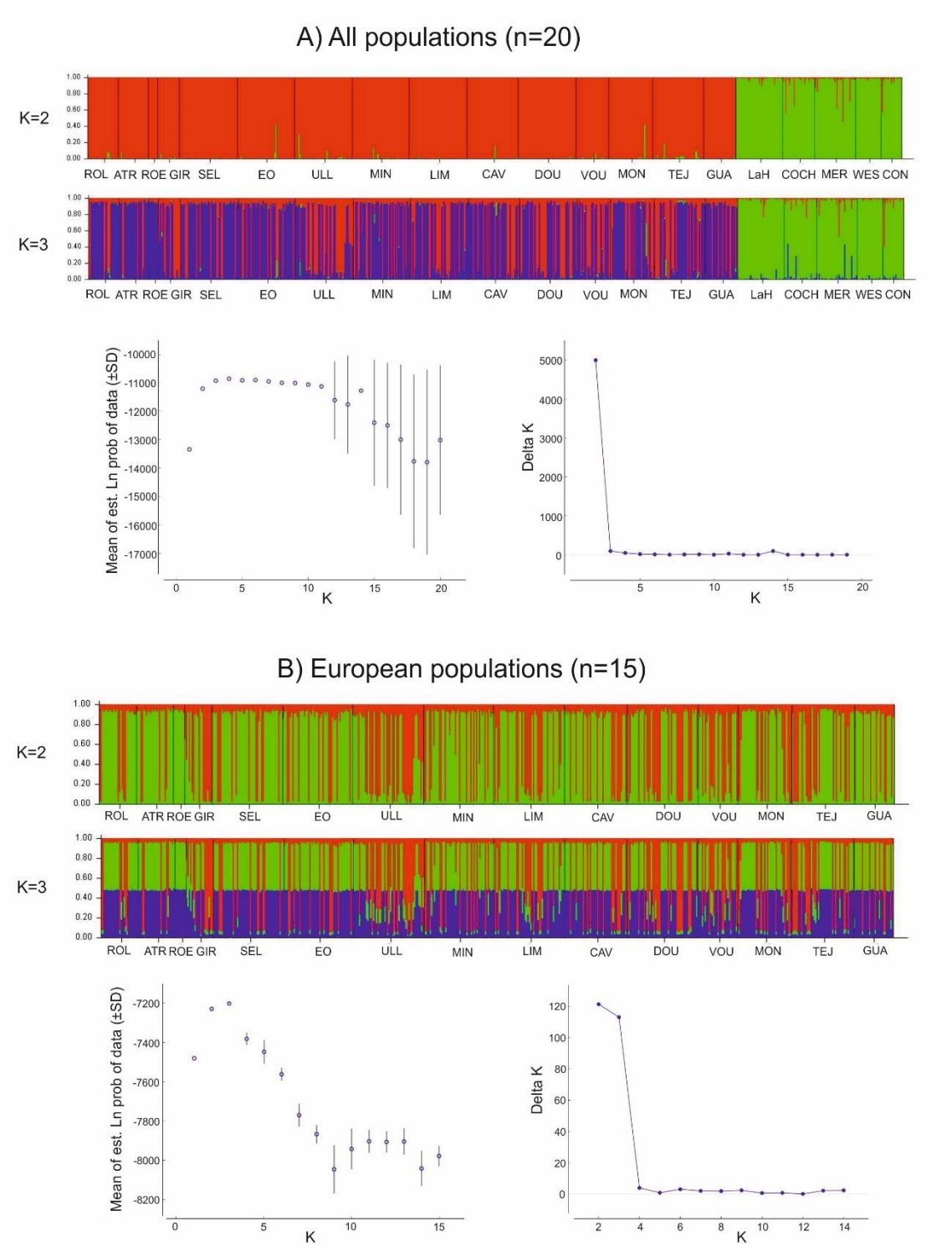

Figure 4. STRUCTURE bar plots generated from 12 microsatellite loci for A) the entire dataset of 20 populations from both sides of North Atlantic; B) European populations, composed of 15 sites; C) Five North American populations; and D) Eight populations from Portugal, to test the hypothesis of regional differentiation, following Lança *et al*. 2014. The most likely number of clusters in each dataset was computed using a combination of estimated LnP(D) values and ΔK of the Evanno method, as represented by the charts. These analyses indicate that the most consistent structure attained is a group in each side of the North Atlantic, as additional structuring in subgroups composed by samples from the same coast results in the same number of groups, with further differentiation within samples. Each individual is represented by a vertical bar, and sampled locations are indicated below plot.

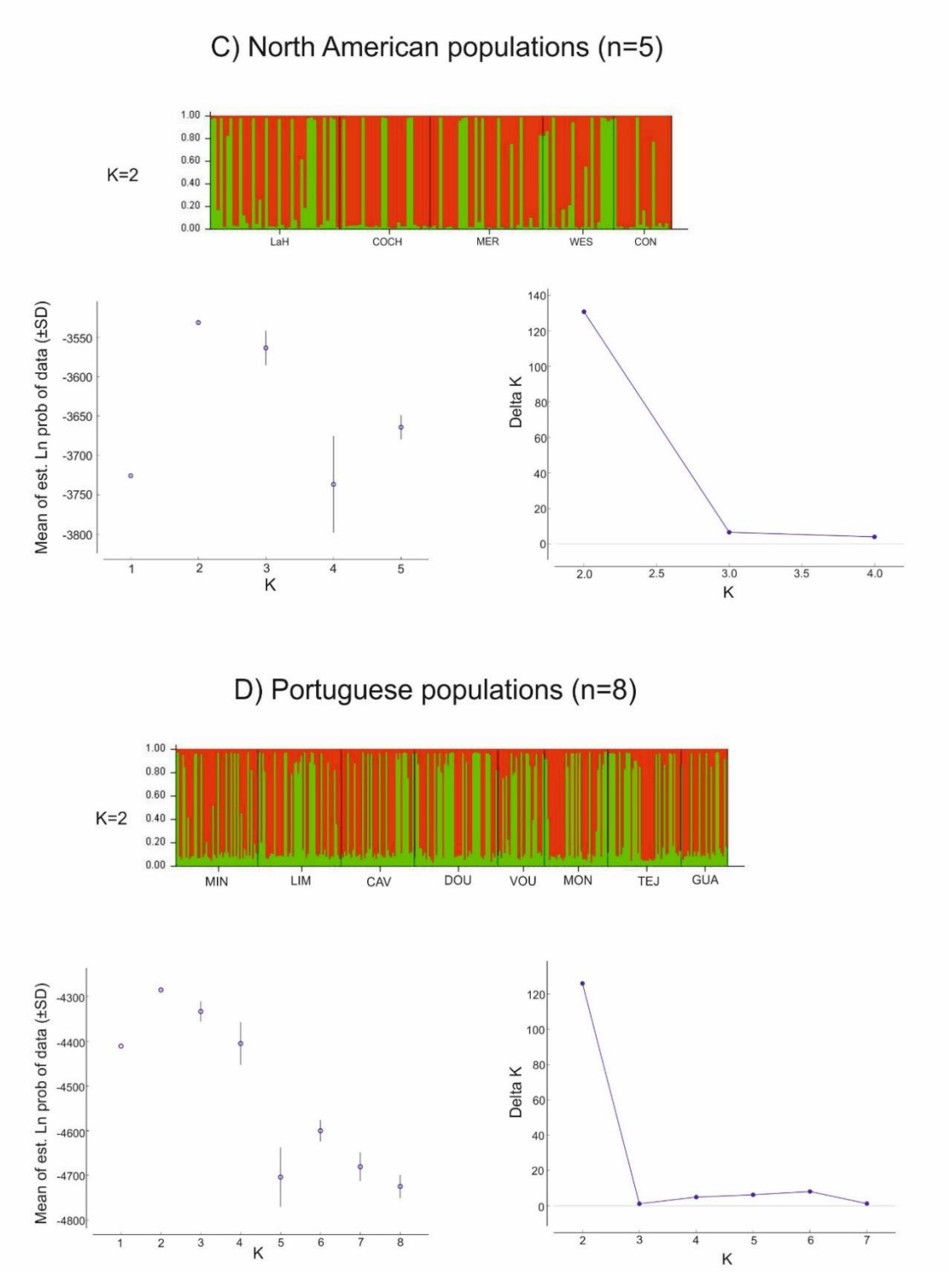

Figure 4. (*Continued)* STRUCTURE bar plots generated from 12 microsatellite loci for A) the entire dataset of 20 populations from both sides of North Atlantic; B) European populations, composed of 15 sites; C) Five North American populations; and D) Eight populations from Portugal, to test the hypothesis of regional differentiation, following Lança *et al*. 2014. The most likely number of clusters in each dataset was computed using a combination of estimated LnP(D) values and ΔK of the Evanno method, as represented by the charts. These analyses indicate that the most consistent structure attained is a group in each side of the North Atlantic, as additional structuring in subgroups composed by samples from the same coast results in the same number of groups, with further differentiation within samples. Each individual is represented by a vertical bar, and sampled locations are indicated below plot.

Table 4. Locus-by-locus analysis of molecular variance (AMOVA).

Source of variation	Sum of squares	Variance components	Percentage of variation	*P*	Fixation Indices
AMOVA I					
Among populations	417.461	0.28551	10.65		
Within populations	3257.205	2.39494	89.35	<0.001	F_{ST}: 0.10651
Total	3674.666	2.68045			
AMOVA II					
Among groups	336.441	0.75470	23.73	<0.001	F_{CT}: 0.23730
Among populations within groups	81.020	0.03068	0.96	<0.001	F_{SC}: 0.01265
Within populations	3257.205	2.39494	75.31	<0.001	F_{ST}: 0.24695
Total	3674.666	3.18033			
AMOVA III					
Among groups	5.195	-0.00286	-0.14	>0.05	F_{CT}: -0.00135
Among populations within groups	15.381	0.01129	0.53	>0.05	F_{SC}: 0.00532
Within populations	1372.135	2.11244	99.61	>0.05	F_{ST}: 0.00397
Total	1392.711	2.12087			

In AMOVA I all populations were included (n=20), in AMOVA II the same populations were grouped into the two clusters suggested by the STRUCTURE analyses (i.e., European and North American populations), and in AMOVA III individuals from Portuguese populations (n=8) were assembled into the three groups suggested by Lança *et al.* (2014).

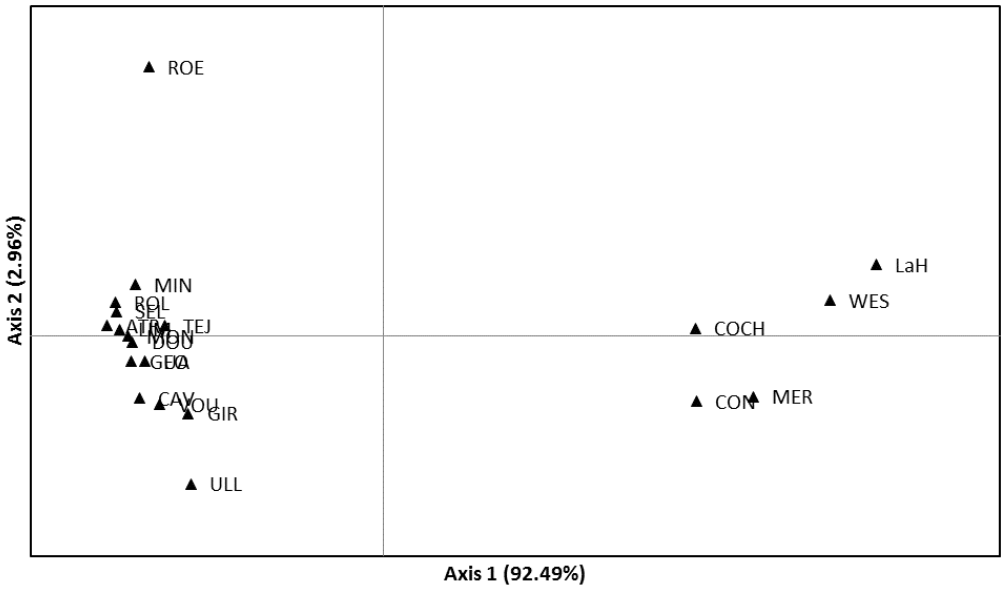

Figure 5. Principal coordinates analysis plot (PCoA) computed by GenAlEx. The percentage of variation explained by each axis is shown, with most variation explained by axis 1. Samples' acronyms as in Table 1.

4. Discussion

4.1. Dispersal at sea, site selection and evolutionary implications

Anadromous sea lamprey populations from the west and east coast of the North Atlantic show significant genetic differentiation and a strong geographic clustering, i.e., a two-groups structure, with restricted dispersal between, and fidelity to, the east and west coast of the Atlantic. This suggests that European and North American populations are isolated and there is an absence of gene flow between both sides of the Atlantic, probably due to the long distance between coasts. These results are in accordance with a previous study using mitochondrial DNA (Rodríguez-Muñoz *et al*. 2004), that showed an absence of genetic exchange among sea lamprey populations spawning in the west and east Atlantic coasts.

Analysis were performed to clarify if there is further differentiation within the American and European populations, but at this scale the results revealed low genetic differentiation among locations of the same coast (although some significant pairwise comparisons), and no geographic clustering. This suggests a lack of natal homing, in agreement with the results of Waldman *et al*. (2008), where no structure was revealed between sea lampreys collected from 11 North American east coast rivers (some of them included in this analysis). Using a portion of the mitochondrial DNA control region, these authors found no significant differences in haplotype frequencies among them, with almost 99 per cent of haplotypic diversity occurring within populations. Bryan *et al*. (2005) also found no significant genetic differences among anadromous populations of sea lamprey along the North American Atlantic coast, and Rodríguez-Muñoz *et al*. (2004) found the same lack of structure along the European Atlantic coast.

The apparent lack of natal homing in sea lamprey is observed in other anadromous lamprey species, like for instance the Pacific lamprey (*Entosphenus tridentatus*), where no geographic structure was detected among 20 locations from west coast of North America, using nine microsatellite loci (Spice *et al*. 2012). These authors, however, suggest limits to dispersal at sea, which precludes panmixia in this species. This assumption was based in the somewhat higher and often significant F_{ST} values found among Pacific lamprey locations (Spice *et al*. 2012). In fact, the extent to which

lampreys are panmictic will be dependent on their dispersal capabilities at sea. The sea lamprey is apparently another case of restricted dispersal at sea in some areas of its distribution, namely in the western Iberian Peninsula, where significant morphological and physiological differences were found between adults from different geographical groups, segregated by seabed topography (Lança *et al*. 2014). This differentiation is most likely the result from the influence of environmental factors to which lampreys may have been exposed during the oceanic trophic phase of the life cycle. Indeed, in the present study no genetic structure was attained for the exact same populations, meaning that the oceanic phase of the sea lamprey life cycle is most likely composed by a dispersion period during the juvenile migration, followed by a much less mobile adult stage, which will restrict the mixture of adult lampreys from different geographical groups. Adaptation in the larval stage also seems to occur to some extent. Almeida *et al*. (2008) analysed the morphological variability of sea lamprey larvae from the main Portuguese river basins, and found morphometric segregation of populations (the total classification rate estimated from cross-validation procedure was 54.8%), meaning that during the long larval phase individuals also adapt to the environmental conditions encountered in the natal stream.

These results indicate local adaptation of i) sea lamprey populations inhabiting the east and west coast of the North Atlantic, with genetic differentiation detected both at mitochondrial DNA (Rodríguez-Muñoz *et al*. 2004) and microsatellite loci (this study), and ii) groups of populations from western Iberian Peninsula, differentiated at the morphological and physiological levels, as result of ecological factors (Almeida *et al*. 2008; Lança *et al*. 2014), rather than derived from a genetic basis (this study).

The apparent lack of natal homing in anadromous lampreys contrasts to strong natal homing in other anadromous fish, like salmonids. McDowall (2001a) suggested that homing raises adaptation of stocks to favourable local spawning conditions, allowing the evolution of local adaptations. Even though homing is generally regarded as adaptive advantageous, it seems that lampreys have evolved in the direction of regional adaptations, instead of natal site fidelity. Indeed, even though homing makes fish return to habitats of known spawning success, it may become disadvantageous. Cury (1994), in a review about reproductive behaviours, such as natal homing, referring

to marine turtles and salmon, species that return very accurately to their natal sites, explains that individuals cannot respond, in terms of adaptability, to changes in their spawning habitat. If, for natural or anthropogenic reasons, the spawning site becomes unsuitable, the individuals do not attempt to breed at alternative areas, and spawn at sites that are unfavourable for successful production of progeny. In this sense, the author considers that "strays are essential for long-term dynamics by exploring and fixing new environmental solutions that later may become possible for the species" (Cury 1994). In the study of Lança *et al*. (2014), classification results revealed a few of such individuals, similar to the "strays" of species exhibiting homing, i.e., individuals that were classified in other geographical groups, which, for instance if their hosts have carried them far from their natal rivers, can explore other areas nearby. According to the exposed above, lampreys seem to present regional panmixia, with local adaptations at the morphological and physiological levels, but apparently no genetic segregation, which seem to provide adaptive advantage for the species survival in the long-term. Also, it is known that parasitic lampreys may be displaced over hundreds of kilometres by host fishes (Johnson *et al*. 2015), and thus returning to the natal stream would imply high energetic costs, which lampreys, not homing, can invest in reproduction (such as gonadal production, upstream migration and nest construction).

In the absence of homing, Waldman *et al*. (2008) suggested that lampreys use a strategy referred to as 'suitable river', to complete its life cycle. According to this strategy, and because many rivers are unsuitable for sea lamprey reproduction, instead of returning to natal streams, sea lamprey use chemical cues to locate spawning habitat that is suitable for larvae. The perception that there are populations of conspecifics in upstream catchments ("kin recognition") through body odours or pheromones released incidentally or deliberately by populations upstream was also recognized as a strategy to relocate and exploit favourable spawning habitats by McDowall (2001a). Lamprey larvae release unique bile acids that function as migratory pheromones detectable by adults in marine waters (e.g. Li *et al*. 1995; Bjerselius *et al*. 2000; Polkinghorne *et al*. 2001) and then reproductively mature males release a bile acid that acts as a potent sex pheromone, inducing preference and searching behaviour in ovulated female lampreys (Li *et al*. 2002). This strategy allows ammocoetes to "advise" the former generations to

spawn where they occur, because it means that, at that moment, the habitat conditions are favourable, and allows adult lampreys to locate suitable spawning and rearing habitat. Another advantageous characteristic of this strategy is that the attraction is not species specific, as pheromones emitted by larvae are conserved among lamprey species (e.g. Fine *et al*. 2004; Robinson *et al*. 2009).

The great capacity of adaptation, both in the larval and adult phases, seems to bring lampreys adaptive advantages, as it enhances the plasticity of the species to adapt to inconstant environments. Also, the absence of homing, but instead the kin recognition allows the selection of watersheds with suitable spawning and rearing habitat. The cues for initiating upstream migration are another issue of great interest among the lamprey community, and temperature and flow appear to be the key triggers for upstream migration (Moser *et al*. 2015). Studies have demonstrated that migratory activity increases with increased stream discharge, which may be a mechanism to ensure that lamprey passage is facilitated through difficult areas (reviewed in Moser *et al*. 2015). In basins where the available water is reduced in the months with higher temperatures, like the Guadiana basin, in southern Portugal, river flow reduction in drought years can reduce the watershed attractiveness of the basin to migratory adults. This southern basin constitutes a geographical group with unique morphological and physiological characteristics as adults (Lança *et al*. 2014), but the absence of homing allows some individuals to enter nearby watersheds. This plasticity allows lampreys to respond positively to, for instance, potential effects of climate change, moving northwards and shifting the species distribution.

4.2. Implications for conservation

Populations from the west Atlantic coast revealed higher levels of genetic diversity than European samples (see Table 2). This is in agreement with findings from Bryan *et al*. (2005), where the authors found evidence for a genetic bottleneck in River Mondego, using eight microsatellite loci, and significant differences in allele frequencies between Mondego and North American anadromous populations.

This scenario may be due to the distinct threats faced by populations from both sides of the Atlantic. *P. marinus* is considered threatened in the European countries holding the main populations (i.e. France, Spain and Portugal), where it has been fished for centuries during their upstream spawning migration, and is considered a gastronomic delicacy with high socioeconomic value (Almeida *et al*. 2002; Mateus *et al*. 2012; Stratoudakis *et al*. 2016). Overfishing, together with habitat loss, are the main threats to this species in the Iberian Peninsula (Almeida *et al*. 2002; Mateus *et al*. 2012), and both have led to a large reduction in population size, and consequently, the populations are more prone to genetic bottlenecks due to the loss of variation. Therefore, these populations require special conservation and management actions, especially in what concerns fishing regulations and habitat restoration. In Portugal, actions directed to the conservation of diadromous fish are being conducted, both intended to recover stretches of habitat that became unavailable after construction of impassable barriers (Pereira *et al*. 2016), and directed to sustainable fisheries (Stratoudakis *et al*. 2016), to guarantee the long-term persistence of the species.

The identification of stocks, and whether they have a genetic basis, or rather are derived from environmental factors, is key for the management of fisheries. Conservation priorities for sea lamprey were defined as the effective articulation between fisheries management and habitat recovery, to guarantee cost-effective monitoring and sustainable long-term exploitation (Stratoudakis *et al*. 2016).

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