



A new mtDNA control region haplotype from sea lamprey (*Petromyzon marinus* Linnaeus, 1758: Petromyzontiformes) collected off the Italian Adriatic coast

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Abstract

Given the lack of genetic information about Mediterranean sea lamprey, taking advantage of recent capture of two individuals in the western Adriatic Sea, molecular analysis of a partial sequence of the mitochondrial DNA control region has been performed on two Mediterranean specimens for the first time. A new haplotype is reported for this species. Furthermore, the finding of both juvenile and adult individuals suggests the presence of suitable rivers for reproduction in the Adriatic region.

Keywords D-loop sequencing · Genetic diversity · Relic populations · Endangered species

Introduction

The sea lamprey, *Petromyzon marinus* (Linnaeus, 1758), is a parasitic and anadromous species, widespread in both American and European coastal and estuarine waters of North Atlantic, between 70°N and 30°N (Maitland 1980; Renaud 2011). In the Great Lakes of North America, *P. marinus* formed invasive landlocked populations that represent a serious problem (Smith and Tibbles 1980; Bryan et al. 2005), whereas in Europe, this species is listed in Annex II of the EU Habitats Directive (92/43/EEC) and Annex III of the Bern Convention (82/72/CEE) as species of conservation concern. The main drivers of decline of the European sea lamprey populations are (1) habitat loss (dam construction, dredging, gravel extraction, channelization) that have rendered the

nesting areas inaccessible or unsuitable, (2) water pollution, due to the low quality of sewage treatments, and (3) in western European countries, unsustainable fisheries (Kelly and King 2001; Mateus et al. 2012). This species is also potentially susceptible to projected climate. According to the future scenario proposed by Lassalle et al. (2008), sea lamprey could disappear in the southern part of its distribution range, by the end of the twenty-first century.

At present, the most substantial European populations are recorded from large Atlantic flowing rivers, while in the Mediterranean region, the species is very rare. Its stable presence is documented in rivers and coastal waters of Spain (Perea et al. 2011), France (Inventaire National du Patrimoine Naturel 2017, available at https://inpn.mnhn.fr/espece/cd_nom/66315?lg=en), and in the Ligurian Sea close to Magra river mouth (Ciuffardi et al. 2007). Sporadic records are reported throughout the Mediterranean basin (Economidis et al. 1999; Holčík et al. 2004; Mizzan and Vianello 2007; Çevik et al. 2010; Thessalou-Legaki et al. 2012; Clavero et al. 2014; Karachle and Machias 2014; Rafrafi-Nouira et al. 2015). In the mid-twentieth century, the sea lamprey was widely distributed in Italian waters, in both Tyrrhenian and in Adriatic sides (Zanandrea 1957, 1961, 1962), but this species is currently regarded as extremely rare or even extinct in some areas of the Italian peninsula (Zerunian 2004). For these reasons, the sea lamprey is listed in the IUCN Italian Red list of threatened vertebrate species as critically endangered (Rondinini et al. 2013).

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With these premises and given the lack of information regarding Mediterranean sea lamprey in the field of population genetics, the aim of the present study is to perform for the first-time molecular analysis of a partial sequence of the mitochondrial DNA control region on two Mediterranean specimens.

Materials and methods

We sequenced a fragment of mtDNA control region from two specimens caught during commercial trawling activities in central Adriatic Sea GSA 17 (GFCM Geographic Subarea), both ca 20–25 miles off Ancona (Italy). Morphological measurements, myomere counts, color patterning, and oral disc structure identified them as *P. marinus*, according to Vladykov (1986) and Kottelat and Freyhof (2007) (Fig. 1). The former caught on July 2017 had a total length (TL) of 524 mm and a weight (W) of 321 g; based on Kelly and King (2001) and Hansen et al. (2016), the animal is under the range of a European spawning adult (600–1100 mm). The latter caught on March 2018 has been classified as a juvenile according to Ciuffardi et al. (2007) and Hansen et al. (2016) (215 mm TL, 18 g W; Fig. 1). Both individuals were stored in ethanol 70% and deposited with the Life and Environmental Science department collection of Polytechnic University of Marche (UNIVPM), Italy.

Total genomic DNA was extracted from muscle tissue using specific cartridge 401 in the MagCore® automated

Nucleic Acid extractor (MagCore®, Genomic DNA Tissue Kit, n° 401). A 624 bp, portion of the mtDNA control region was amplified following Almada et al. (2008). Sequencing reactions were performed on 3700 ABI DNA sequencer (Applied Biosystems) using both forward and reverse primers. After checking chromatograms by eye, the derived sequences (623 bp long) were compared with all available sequences for sea lampreys from European area (Almada et al. 2008; Genner et al. 2012). Sequences were aligned with ClustalX (Thompson et al. 1997). Relationships among haplotypes were analyzed with a parsimony network estimated by the software TCS Version 1.18 (Clement et al. 2005).

Results and discussion

Two haplotypes were observed. From the juvenile individual, the resultant haplotype corresponds to PMVG8, the principal and widely distributed European haplotype, detected in rivers from Portugal to Germany (Almada et al. 2008). It was also the only haplotype found in Icelandic marine and river waters (Pereira et al. 2012). The adult specimen showed a new haplotype (PMmed1, GenBank Accession No. MK531166) that differed from PMVG8 for a single mutation, deletion of C at position 152.

The relationship between haplotypes is represented in the TCS network (Fig. 2). The observed haplotypes are included in the star-like structure grouping the European sea lampreys.

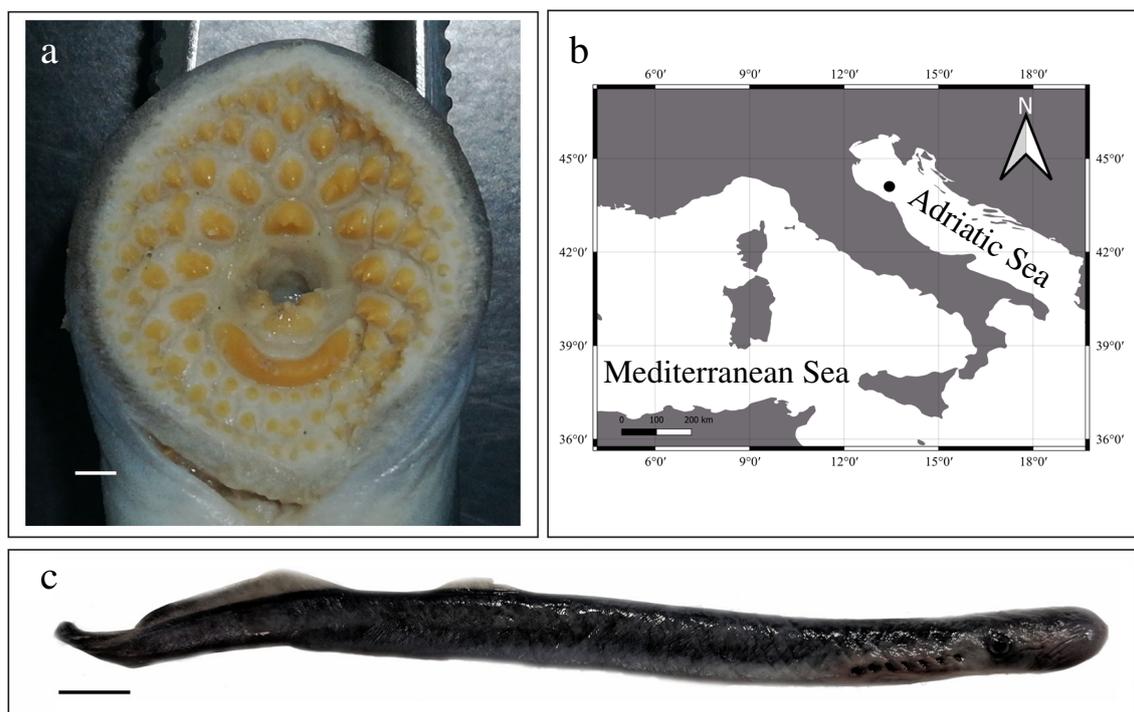


Fig. 1 a,c photographs of the juvenile specimen of *Petromyzon marinus* caught on March 2018 and whole specimen. Scale bar = 10 mm (c) and particular of the oral disc scale bar = 1 mm; **b** Map showing the sampling site

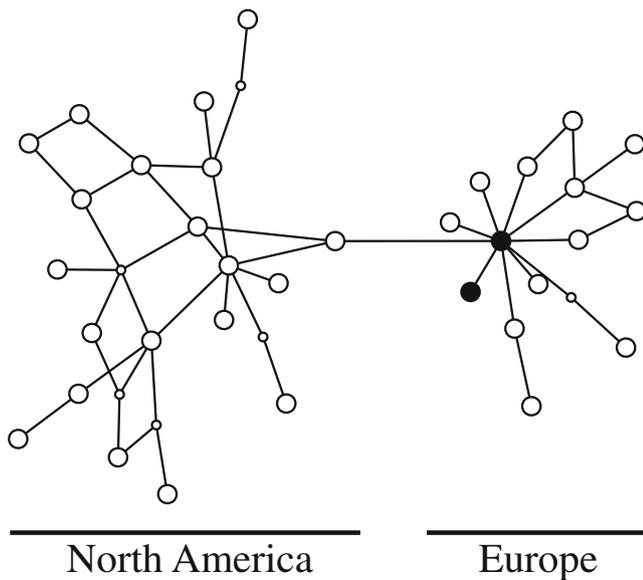


Fig. 2 TCS Haplotype network including North American (Genner et al. 2012) and European (Almada et al. 2008) specimens. Black points indicate the observed haplotypes

Based on the available data alone, we are unable to test for genetic segregation between Mediterranean and European Atlantic populations, but we find it plausible that there is gene flow between these regions. According to Almada et al. (2008), the genetic homogeneity observed in the European Atlantic sea lamprey is due to a lack of natal homing combined to parasitic adult feeding stage, allowing the species to spread, thanks to passive transport on hosts. For instance, sea lampreys often parasitize highly migratory fish like sharks, tunas, swordfish, and cetaceans (Silva et al. 2014) which are able to cross the Strait of Gibraltar, and thus enabling gene flow through the Atlantic and Mediterranean basins. The observed genetic diversity in European rivers may result from recent recolonization events (Rodríguez-Muñoz et al. 2004; Almada et al. 2008) and in particular from a population expansion dated around 125,000 years ago (Genner et al. 2012). If so, then genetic diversity will have persisted through European glacial periods. The Quaternary glaciations, in fact, have caused cyclical extinction phenomena during the glacial periods, to the north of the Pyrenees and the Alps. Conversely, in Southern Europe, particularly Mediterranean region, the weaker effect of glaciations has played a key role on genetic divergence among populations of several fish species by providing refugia (e.g., Hewitt 2000). Endemic Mediterranean haplotypes have been described for *Lampetra planeri* by Bianco et al. (2011). Isolated Mediterranean and northern European resident populations of *L. planeri* showed closely related haplotypes (single indel). Therefore, we cannot exclude that the new haplotype, not described for the Atlantic,

could represent Mediterranean endemism conserved in relict populations inhabiting Mediterranean glacial refugia.

We hypothesize that sea lamprey spawning sites are present along the northern Italian Adriatic coast. Based on the small post-metamorphosis size of the juvenile specimen analyzed, the growth rate estimate for this species (Silva et al. 2016), and the catch period that corresponds to the peak of downstream migrations of juveniles in Atlantic (Silva et al. 2013), we might suppose the presence of spawning sites in some rivers that flow in the Adriatic Sea. Both mature adults (Balkan coast: Holčík et al. 2004) and feeding juveniles (Venice Lagoon: Mizzan and Vianello 2007; waters off Ancona: Lucchetti, personal observation) have been recently reported for the Adriatic Sea. In the last century, sea lamprey was widely distributed in rivers along the eastern Italian side such as Adige, Po, Reno, and Pescara rivers (Zanandrea 1962; Tagliavini et al. 1994). The latter river is close to the site where the specimens here studied were collected. Therefore, it is possible to assume that some watercourses of the Italian Adriatic side are still accessible and exploited by sea lamprey, as observed in the Magra river (Ligurian Sea: Ciuffardi et al. 2007).

Despite of the low number of individuals, these are the only specimens analyzed for the Mediterranean Sea. Implementation of genetic studies on more specimens is necessary to draw robust inferences on genetic population of sea lamprey in the Mediterranean Sea. Detection and protection of sea lamprey populations and spawning sites would promote the conservation of this species in the Mediterranean Sea.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities.

Data availability The datasets generated during and/or analyzed during the current study are available in the GenBank repository (Accession No. MK531166).

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