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Petromyzon marinus (Petromyzontidae), an unusual host for helminth parasites in western Europe

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Abstract :

The sea lamprey *Petromyzon marinus*, which is among the most phylogenetically ancient vertebrates, is a hematophagous ectoparasite that feeds on vertebrates and is considered vulnerable in Europe but is a pest in the North American Great Lakes. We conducted a literature review of helminth parasites of *P. marinus* and investigated postmetamorphic lampreys sampled in rivers and northeast Atlantic coastal waters (western France) during spawning migration. Based on the literature review, 16 helminth taxa have been recorded in *P. marinus*, among them 14 in North America but only 2 in Europe, with no species in common between these areas. Specific parasites are lacking, and helminth parasites recorded in *P. marinus* are mostly opportunistic and are trophically transmitted to fish hosts with both extremely low prevalence and mean intensity. Thus, *P. marinus* seems an unusual host that is probably infected through accidental ingestion of parasites by microphagous larvae (ammocoetes) and/or hematophagous postmetamorphs. Our field study supports this hypothesis, since only a single third-stage larva of *Anisakis simplex* sensu stricto was found in 2 postmetamorphic *P. marinus* among the 115 individuals dissected. This opportunistic, trophically transmitted, and cosmopolitan nematode species has never been recorded in North American sea lampreys and only once in Galician rivers (southern Europe). Infestation pathways of *P. marinus* by *A. simplex* are proposed vis-à-vis the feeding strategy of postmetamorphs and fish host species which potentially harbor anisakid larvae in their musculature. More generally, the complexity of biotic interactions is discussed considering *P. marinus* both as a host for helminth parasites and as a parasite for hosts such as fish and mammals, which are also potential predators of sea lamprey.

Keywords : Sea lamprey, Helminth parasites, Unusual host, Accidental ingestion, Anisakis simplex sensu strict, Nematode

1. Introduction

The anadromous sea lamprey *Petromyzon marinus* is a fascinating biological model as it is one of the most phylogenetically ancient vertebrates and a survivor of the jawless group, whose life style and body structure have been highly conserved for at least 350 million years (Hardisty 2006, Taverny & Elie 2010 for reviews). This species is also uncommon among vertebrates being a parasitic blood feeder after metamorphosis, using various aquatic vertebrates (at least 36 fish species, and some marine mammals such as whales and dolphins) and involving a prolonged attachment (up to 83 days) to the body surface of its hosts (Farmer 1980, Hardisty 2006, Taverny & Elie 2010). Because of its feeding strategy, *P. marinus* becomes widely dispersed through transport by its diverse hosts, and does not home to its native river but rather exhibits regional panmixia (Waldman et al. 2008).

Native to the North Atlantic, *P. marinus* is distributed within the Atlantic drainages of North America from the coast of Labrador as far south as Florida, and throughout Western Europe from Varanger Fjord in Norway to the Western Mediterranean (Beamish 1980, Hardisty 2006, Taverny & Elie 2010). Despite their high migratory capacity which may extend over several hundred kilometers, North American and European populations of *P. marinus* are isolated, as shown by their genetic differences; the separation presumably occurred during the Cretaceous period, some 70 million years ago (Beamish 1980, Hardisty 2006, Taverny & Elie 2010).

In Europe, *P. marinus* is considered a vulnerable species (listed in Annex II of the EU Habitats Directive and Annex III of the Bern Convention), classified as of „Least Concern" by IUCN since 2008 (Taverny & Elie 2010). In contrast, *P. marinus* is considered a pest in the Great Lakes of North America, which it invaded in the 1930s (Hardisty 2006). In these lakes, the sea lamprey is landlocked in inland waters and the hematophagous postmetamorphics cause tremendous damage to fish stocks with a consequent yearly expenditure of millions of dollars on their control (e.g., Bence et al. 2003, Neeson et al. 2007).

Up to now, the parasitofauna of *P. marinus* has been mainly studied in the North American Great Lakes in the context of pest control research (Applegate 1950, McLain 1952, Guilford 1954, Bangham 1955, Wilson & Ronald 1967, Muzzal & Whelan 2011), whereas parasitological data are scarce in Europe, originating only from countries of the former USSR (Sobecka et al. 2009), or focusing on one parasite taxon (Bao et al., 2013).

In this study, helminth parasites of *P. marinus* were reviewed in its entire geographical distribution and investigated in postmetamorphics sampled in French rivers and North East Atlantic coastal waters (Bay of Biscay, Europe). Based on this literature review and our field investigation, *P. marinus* seems an unusual host whose infestation probably occurs through accidental ingestion of helminth parasites. The occurrence of these opportunist and trophically-transmitted helminths is discussed in terms of the complexity of the biotic interactions (parasitism, predation) involving the sea lamprey as a host for helminth parasites and a parasite for vertebrate hosts (fish and mammals), which are also potential predators of the sea lamprey.

2. Materials and methods

The European "Natura 2000" networking program, which aims to protect and preserve the biodiversity of lampreys, provided a total of 115 *P. marinus* postmetamorphics. These were caught by professional fishermen in three French rivers [the Dordogne (17), Garonne (12) and Loire (24)] and in the North East Atlantic coastal waters [South Brittany in the Bay of Biscay (44)] in May 2010 and February-March 2011.

All the sea lampreys were frozen before the search for helminth parasites in various organs (skin, gills, heart, body cavity, digestive system, liver, and gonad), dissected using a binocular microscope. When found, the parasites were morphologically identified and preserved in alcohol (70%) for molecular identification via DNA sequencing (Cytochrome Oxidase sub-unit 2, cox2).

Total DNA of parasites (i.e., nematodes Anisakidae) was extracted from all the individual nematode tissues, using the ChargeSwitch® Forensic DNA Purification Kit (Invitrogen). A 530 bp fragment of the cox2 gene was amplified using the primers F-univ-nem 5‟ GGTGTTCTTTCTTTTGTTTCTG and R-univ-nem 5‟ GTGGGGCTAACCATAGTTTTAT specifically designed in the framework of the ANR Fish-Parasites project (data not yet published) to amplify Anisakidae. PCR amplification was carried out in a total of 50 µL with the following composition: 0.5 to 2.5 µL DNA template was added to a PCR mix consisting of 0.2 mM of each dNTP (Interchim), 2 mM $MgCl_2$, 10 µL 5X buffer, 1.25 units of GoTaq® polymerase (Promega), 0.2 µM of each primer and molecular biology grade water (Interchim) to adjust to the final volume. Conditions of cycling were as follows: an initial denaturing step at 95 °C for 5 min, followed by 35 cycles of denaturing at 95°C for 30 sec, annealing at 48°C for 30 sec and extension at 72°C for 40 sec, and a final extension step at 72°C for 7 min. Automated DNA sequencing was performed by Genoscreen (Lille, France) using the primer R-univ-nem. Sequences were analyzed using BioEdit software (Hall, 1999) and compared with nucleotide sequences from GenBank (National Center for Biotechnology Information, NCBI) with the program BLAST (Basic Local Alignment Search Tool).

The parasite populations were described by prevalence (number of hosts infected with a particular parasite species / number of examined hosts) and mean intensity (total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite) (Bush et al. 1997).

Mean values of data are reported as means ± standard error (SE).

3. Results

3.1. Literature review of helminth parasites in Petromyzon marinus

Helminth parasites have rarely been studied in *P. marinus*, particularly in Europe (two references vs five in North America; Table 1). A total of 16 helminth parasite taxa were recorded in *P. marinus*, among them 14 in North America but only 2 in Europe, without a common species in these two areas (Table 1). All the helminth parasites recorded in *P. marinus* are heteroxenous and, except for *Cystidicola stigmatura* (adult in salmonid swimbladder), they become adults in the digestive tract of a piscivorous vertebrate.

3.2. Helminth parasites in postmetamorphics from French rivers and coastal waters

Only two individuals (from the Dordogne in February 2011 and from the Garonne in May 2010, respectively) among the 115 dissected *P. marinus* were parasitized (prevalence = 1.74 ± 0.01%), and only by one single nematode (mean intensity = 1.00 ± 0.00) at the third larval stage located on the gonadal mesorchium. According to the DNA sequence (cox2), the parasite was unambiguously identified as *Anisakis simplex* sensu stricto (Anisakidae). In fact, based on 460 DNA base pairs, the query sequence was 100% covered and had 100% identity with four *A. simplex* sequences (KC810002, KC480042, GQ338430, and GQ338433) present in the GenBank nucleotide database.

4. Discussion

The helminth parasitofauna of *P. marinus* lacks specific forms that have co-evolved with its host and is mainly composed of opportunistic fish parasites (Table 1). Most of them are cosmopolitan and described in other host species both in North America and in Europe (Khalil et al. 1994, Moravec 1994, Chappell et al. 1994; Mattiucci & Nascetti 2008). Despite this, no common helminth parasites are found between *P. marinus* from North America and Europe, thus reinforcing the idea of anciently separated North American and European populations (Beamish 1980, Hardisty 2006, Taverny & Elie 2010).

The occurrence of helminths in *P. marinus* is often sporadic or even accidental, with a prevalence ≤ 5% (Table 1) and only one or two specimens found per host (McLain 1952, Sobecka et al. 2009). In some circumstances, the prevalence of some helminths can reach significant values, such as for *Proteocephalus* sp. (61.5%) and *Proteocephalus exiguus* (45.5%) in North America, or *A. simplex* sensu stricto (56.3%) in Europe (Table 1). Except for *Diplostomum* spp., most of the parasites listed in Table 1 are probably trophically-transmitted to the sea lamprey. First, the filter-feeding ammocoetes may accidentally ingest infective helminths in diverse forms (eggs, free larvae or parasitized intermediate hosts, such as crustaceans for *Proteocephalus* spp.) as suggested for *Truttaedacnitis stelmioides* by Moravec (1980). Second, hematophagous postmetamorphics can ingest helminth parasites encysted in fish flesh [e.g., plerocercoids of *Triaenophorous crassus* or third-stage larvae of *A. simplex* (e.g., McLain 1952, Angelucci et al. 2011)] because the anticoagulant lamphredin secreted by their buccal glands has cytolytic effects on host tissues (Farmer 1980).

Concerning *A. simplex* sensu stricto, the single helminth species recorded in our field study, its definitive hosts are marine cetaceans, such as dolphins, which are usually infected by eating intermediate hosts (zooplankton) or paratenic hosts (fish, cephalopods) containing third-stage larvae (Mattiucci & Nascetti 2008 for a review). The first record of these third-stage larvae in *P. marinus* occurred in 2012 in two Galician rivers (Southern Europe) with a much higher prevalence (60.0 ± 0.5% and 42.9 ± 1.9%) than in our study, but a comparable intensity (2.4 ± 1.7 and 1.0 ± 0.0) (Bao et al. 2013). *P. marinus* is therefore a potential paratenic host, which has probably acquired anisakid larvae by feeding on infected fish acting as paratenic hosts for the nematode (Table 2). *A. simplex* larvae are never found in the blood of their fish hosts, but generally in the body cavity, and sometimes in the flesh, as shown for cod, herring or mackerel (Petrie et al. 2005, Mattiucci & Nascetti 2008, Angelucci et al. 2011). Thus, when postmetamorphic sea lampreys feed on blood and, to a much lesser extent, on tissue cytolysis products, they can ingest anisakid larvae potentially encapsulated in fish muscle. Up to now, no studies have mentioned marine cetaceans among the fish, reptiles, birds and mammals known to be predators of *P. marinus* (see Cochran 2009 for a review).

Based on the parasitism patterns of the 16 helminth taxa found in *P. marinus*, we can conclude that helminth parasites are neither a threat to vulnerable European populations nor a potential tool to control pest populations in the North American Great Lakes. Further research on the ecological foraging of *P. marinus* and trophic interactions is needed to determine if *P. marinus* constitutes a dead end for these helminth parasites or a benefit as an intermediate or paratenic host preyed on by the definitive host. Further investigations are also needed to better understand the lack of specific parasites in *P. marinus*, particulary on the immunological capabilities of *P. marinus* against parasites.

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Table 1. List of the helminth parasites (16 taxa) recorded in *Petromyzon marinus* in its geographical range [Europe (Eu), North American Great Lakes (Am)], with information on parasite development stages (L: larval, I: immature, A: adult), infected organs and prevalence. NA: not available.

Parasite taxa	**Stage**	**Infected organ**	**Range**	**References**
Trematoda				
Diplostomum sp.	L	eye lens	Am	Bangham 1955 (7.7%)
Diplostomum huronensis	L	eye lens	Am	Wilson and Ronald 1967 (10.6%)
Podocotyle lepomis	A?	digestive tract	Am	Wilson and Ronald 1967 (0.1%)
Cestoda				
Eubothrium sp.	I	digestive tract	Am	Applegate 1950 (3.0%); McLain 1952 (1.0%); Guilford 1954 (2.6%)
Proteocephalus exiguus	I	digestive tract	Am	Guilford 1954 (45.5%)
Proteocephalus laruei	A	digestive tract	Am	Bangham 1955 (15.4%)
Proteocephalus sp.	I or A	digestive tract	Am	Bangham 1955 (61.5%); Wilson and Ronald 1967 (0.7%)
Triaenophorous crassus	L or I	digestive tract	Am	Applegate 1950 (3.0%); McLain 1952 (1.0%); Guilford 1954 (2.6%); Bangham 1955 (15.4%); Wilson and Ronald 1967 (0.4%)
Acanthocephala				
Echinorhynchus leidyi	A	digestive tract	Am	McLain 1952 (0.2%); Guilford 1954 (5.2%)
Echinorhynchus salmonis	A	digestive tract	Am	Applegate 1950 (17.0%); McLain 1952 (9.5%); Guilford 1954 (9.1%); Bangham 1955 (7.7%); Wilson and Ronald 1967 (8.3%)
Neoechinorhynchus cylindratus	A?	digestive tract	Am	Guilford 1954 (1.3%)
Nematoda				
Anisakis simplex sensu stricto	L	body cavity	Eu	Bao et al. 2013 (56.3%); this study (1.7%)
Camallanus sp.	A	liver	Am	McLain 1952 (1.0%)
Cystidicola stigmatura	I	digestive tract	Am	Guilford 1954 (1.3%)
Hysterothylacium aduncum	NA	NA	Eu	Polyanskii 1955 (prevalence not given)
Truttaedacnitis stelmioides	L	various organs	Am	Wilson and Ronald 1967 (1.8%)

Table 2. List of the fish species recorded as hosts of both *Petromyzon marinus* (Beamish 1980, Farmer 1980, Tavernie & Elie 2010, Silva et al. 2012) and *Anisakis* third-stage larvae (Berland 1961, Petrie et al. 2005, Angelucci et al. 2011, Mladineo et al. 2011).

**Salmo trutta* is also known to prey upon the sea lamprey (Applegate 1950).

Order	**Host species (vernacular name)**
Clupeiformes	*Clupea harengus* (herring)
Gadiformes	*Gadus morhua* (Atlantic cod)
	Merluccius spp. (European hake)
	Pollachius virens (pollock)
Perciformes	*Scomber scombrus* (Atlantic mackerel)
	Thunnus thynnus (Atlantic bluefin tuna)
Salmoniformes	*Salmo trutta** (brown trout and sea trout)