

Macroparasites of allis shad (*Alosa alosa*) and twaite shad (*Alosa fallax*) of the Western Iberian Peninsula Rivers: ecological, phylogenetic and zoonotic insights

M. Bao^{1,2,3} · A. Roura^{1,4} · M. Mota^{5,6,7} · D.J. Nachón^{8,9} · C. Antunes^{6,7} · F. Cobo^{8,9} · K. MacKenzie¹⁰ · S. Pascual¹

Received: 8 June 2015 / Accepted: 18 June 2015 / Published online: 5 July 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Samples of anadromous *Alosa alosa* (Clupeidae) ($n=163$) and *Alosa fallax* (Clupeidae) ($n=223$), caught in Western Iberian Peninsula Rivers from 2008 to 2013, were examined for buccal, branchial and internal macroparasites, which were identified using morphological and molecular methods. *Alosa alosa* were infected with *Anisakis simplex* s.s., *Anisakis pegreffii*, *Hysterothylacium aduncum*, *Rhadinorhynchus pristis*, *Mazocraes alosae*, *Hemiusurus appendiculatus*, *Ceratothoa italica* and an unidentified ergasilid copepod. *Ceratothoa italica* represents a new host record for *A. alosa*. *Alosa fallax* were infected with *A. simplex* s.s., *A. pegreffii*, *H. aduncum*, *H. appendiculatus*, *Clavellisa emarginata* and an unidentified cymothoid isopod. This is the first report of *C. italica*, *C. emarginata* and *M. alosae* in the Iberian Peninsula. The phylogenetic positions of *M. alosae*, *H. appendiculatus* and *C. emarginata* were assessed using 18S and 28S ribosomal RNA (rRNA); our contributions pro-

vide a better understanding of the phylogenetic relationships within their groups. Qualitative and quantitative differences in the parasite faunas of these two shad species are consistent with different feeding strategies. The results provide information about host migration behaviour and transmission pathways through diet during the marine trophic phase of the shad's life cycle and their roles as paratenic or final hosts and transporters of parasites between seawater and freshwater environments. The zoonotic parasites *A. simplex* s.s. and *A. pegreffii* pose a risk for consumers or riverine mammals (e.g. European otter). The use of parasites as biological tags for shad stocks in Western Iberian Rivers could be a useful approach in multidisciplinary studies concerning fish stock delimitation and characterization.

Keywords *Alosa* spp · Macroparasites · *Anisakis* spp · Phylogeny · Freshwater · Iberian Rivers

✉ M. Bao
mbao@iim.csic.es

¹ ECOBIOMAR, Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

² OCEANLAB, University of Aberdeen, Main Street, Newburgh, Aberdeenshire AB41 6AA, UK

³ College of Physical Science, School of Natural and Computing Sciences, University of Aberdeen, St. Machar Drive, Cruickshank Bld., Aberdeen AB24 3UU, UK

⁴ Department of Ecology, Environment and Evolution, La Trobe University, Kingsbury Drive, Bundoora, Melbourne 3086, Australia

⁵ ICBAS–Institute of Biomedical Sciences Abel Salazar, University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

⁶ Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

⁷ Aquamuseum of Minho River, Parque do Castelinho, 4920-290 Vila Nova de Cerveira, Portugal

⁸ Department of Zoology and Physical Anthropology, Faculty of Biology, University of Santiago de Compostela, Campus Vida s/n, 15782 Santiago de Compostela, Spain

⁹ Station of Hydrobiology 'Encoro do Con', Castroagudín s/n, 36617 Vilagarcía de Arousa, Pontevedra, Spain

¹⁰ School of Biological Sciences (Zoology), University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK

Introduction

The European shad, allis shad, *Alosa alosa* (Linnaeus, 1758), and twaite shad, *Alosa fallax* (Lacépède, 1803), are anadromous members of the family Clupeidae that have a pelagic behaviour at sea, especially in areas close to the shore, migrating into rivers to reproduce (Aprahamian et al. 2002; Baglinière et al. 2003; Acolas et al. 2004). Their original distribution extends from Iceland and Norway in the North to Morocco in the South (Aprahamian et al. 2002). *Alosa alosa* once occurred in the Mediterranean Sea but is thought to have disappeared from these waters. *Alosa fallax*, however, still inhabits the Mediterranean, being especially abundant in the Aegean Sea; its occurrence is scarce in the Marmara and Black Seas (Baglinière 2000; Aprahamian et al. 2002; Ceyhan et al. 2012; La Mesa et al. 2015). Due to direct and indirect anthropogenic impacts (such as construction of dams on rivers that prevent spawning migrations, habitat loss, water pollution and overfishing) (Costa et al. 2001; Aprahamian et al. 2002; Baglinière et al. 2003; Doadrio et al. 2011; MIGRANET 2012), European shad have suffered significant reductions in their distribution and abundance in river basins, which led to their inclusion in Appendix III of the Bern Convention; Annexes II and V of the EU Habitats Directive. Moreover, in the case of *A. alosa*, as a result of its greater degree of anadromy and lower ecological plasticity (Baglinière 2000), marine catches and genetic loss appear to be accelerating population decline (OSPAR 2009; Jolly et al. 2012; Rougier et al. 2012). This decline was also observed in populations from Northwestern Iberian Peninsula (Galicia and North of Portugal) rivers (e.g. Minho and Ulla), so that both species are considered as endangered by some authors (Solórzano 2004; Cabral et al. 2006). Despite the fact that these populations are currently scarce, they still have great local importance from economic, recreational, cultural and ecological points of view in both Galician and north Portuguese river basin counties (XUNTA 2008; Mota and Antunes 2011; Pereira et al. 2013). Stable populations of both shad coexist in the River Minho (Mota et al. 2015). Formerly, *A. alosa* was reported in the River Ulla, but this was probably a case of misidentification, since a monospecific population of *A. fallax* was recently discovered in this river (Cobo et al. 2010; Nachón et al. 2013; Silva et al. 2013). On the other hand, shad still migrate upstream in the River Mondego, despite the construction of a dam that restricted their spawning migration to the last 35 km of the river (Costa et al. 2001).

The spawning migration of adults usually starts in the late winter and continues throughout the spring. Shad upstream reproductive migration roughly occurs from March to July in the rivers referred to above. In addition, spawning takes place in fresh water during the

night in both the tidal and non-tidal parts of the river (Aprahamian et al. 2002). The larvae and juveniles grow in fresh water and migrate to the ocean in their first year of life (Taverny et al. 2000; Aprahamian et al. 2002; Mota and Antunes 2012). After several years on marine feeding grounds (Taverny and Elie 2001a), they return to estuaries and begin their upstream spawning migration. During this upstream migration, most *A. alosa* do not feed (Mota et al. 2015), whereas at least part of the population of *A. fallax* may feed actively (Nachón et al. 2013).

There is a considerable lack of knowledge on the adult marine life stage of both *Alosa* species (Taverny et al. 2000; OSPAR 2009). Previous studies reported that both shad are coastal in their habit with different diet and depth preferences (Taverny et al. 2000; Taverny and Elie 2001a, b; Trancart et al. 2014; La Mesa et al. 2015). Whilst *A. alosa* is mainly a zooplanktophagous species with small fish forming a less important part of the diet, *A. fallax* is predominantly ichthyophagous with small crustaceans as secondary prey (Assis et al. 1992; Taverny and Elie 2001b; Maitland and Lyle 2005; Ceyhan et al. 2012; Skóra et al. 2012). In the Bay of Biscay, both species remain in a range of about 30 mi. away from the coast and above a depth of 115 m (Taverny and Elie 2001a). A recent study corroborated the presence of both species of shad at depths between 15 and 115 m along the French coast (Trancart et al. 2014). In NW Iberian waters, this depth distribution might be even deeper for both species, with reports of their occurrence between 9 and 390 m (Bao et al. 2015).

By feeding on zooplankton during their marine phase or even in the estuaries during their upstream migration, anadromous fish acquire marine parasites, e.g. hemiurid digeneans, acanthocephalans and nematodes, which they accumulate and transport to freshwater ecosystems in their returning migration (MacKenzie 1987). Parasites can be used as indicators of trophic relationships and fish population structure (Williams et al. 1992). Thus, any information concerning the parasite ecology of both anadromous shad populations will be important to understand their natural life cycles.

The present paper provides new information about the macroparasite communities of spawning individuals' *A. alosa* and *A. fallax* caught in Western Iberian Peninsula Rivers; the specific aims were to (1) identify the parasite species present, (2) obtain and compare parasite burdens between shad species and years, (3) assess the phylogenetic position of those parasites not present in the genetic database, (4) discuss the ecological implication of these findings within the life cycle of both parasites and shad species and (5) stress the zoonotic relevance due to the presence of *Anisakis* spp. in anadromous shad.

Materials and methods

Sampling

Two studies in relation to the ecology of *A. alosa* and *A. fallax* in the Rivers Minho and Ulla provided the opportunity to study the community richness and diversity of the macroparasite fauna (platyhelminthes, nematodes, acanthocephalans and crustaceans) of both shad species caught in estuaries and freshwater environments. In addition, individuals of *A. alosa*, caught by professional fishing in the River Mondego, made possible to broaden the study of the macroparasite fauna of shad of the Western Iberian Peninsula Rivers.

Shad were caught during their upstream spawning migration from 2008 to 2013 in the Rivers Minho, Ulla and Mondego by experimental, professional (i.e. trammel net) or sport fishing (i.e. rod and reel) (Table 1) (for more detailed information on study area and sampling sites, see Bao et al. (2015) and references therein).

Collection of parasites

Total length (measured in mm) was registered for each fish. Due to some fish having been previously processed, whole specimens were not always available for the location and identification of the parasites. Information and results from accessible shad samples are shown in Tables 1 and 2. When whole fish were available (Table 2), visual observation of the buccal cavity and gills for macroparasites was carried out. The head and branchial region was separated from the rest of the body, which was frozen for later visual inspection. The fish body was mostly available for internal parasites (Table 2). The stomach and internal organs were removed and conserved either frozen or in 70 % ethanol. The branchial region was dissected, and the gill arches were extracted and examined

under a stereomicroscope for macroparasites. The contents of the stomach were examined under a stereomicroscope, and the surfaces of the other visceral organs were macroscopically examined for macroparasites. Later, the entire viscera and flesh of some shad were digested following artificial digestive methods in order to separate the remaining nematode larvae (Bao et al. 2015). All macroparasites found were removed and placed in 70 % ethanol for further morphological and molecular diagnosis.

Parasite identification

Morphological identification

Parasites were identified morphologically under stereomicroscope using the following publications: Berland (1989) for nematodes, Horton (2000) for isopods, Kabata (1979, 1992) for copepods, Yamaguti (1968), Dawes (1968) and Akmirza (2013) for monogeneans, Dawes (1968) and Gibson and Bray (1979, 1986) for digeneans and Yamaguti (1963) and Gregori et al. (2013) for acanthocephalans.

Molecular identification

The following groups were selected for molecular analysis: 72 *Anisakis* spp.: larvae 46 from *A. alosa* and 26 from *A. fallax*; 19 non-anisakid nematodes (*Hysterothylacium aduncum*): 15 from *A. alosa* and 4 from *A. fallax*; a single copepod (*Clavellisa emarginata*) from *A. fallax*; 18 digeneans (*Hemiurus appendiculatus*): 5 from *A. alosa* and 13 from *A. fallax*; 3 monogeneans (*Mazocraes alosae*) from *A. alosa*; and 13 acanthocephalans (*Rhadinorhynchus pristis*) from *A. alosa*.

Genomic DNA was isolated using Qiagen DNeasy™ Tissue Kit according to the manufacturer's instructions. The identification of the parasites was performed amplifying

Table 1 Sampling details obtained by *Alosa* species, river, year (period), method, number of fish analyzed (*n*) and total length mean and standard deviation (TL±SD (min-max) (cm))

Host species	River	Sampling dates	<i>n</i>	TL±SD (min-max)
<i>Alosa alosa</i>	Minho	2009 (23 Mar–21 Jul)	41	62.7±4.30 (55.5–74.0)
		2010 (21 Mar–2 Aug)	90	65.0±3.09 (57.0–73.0)
		2011 (3 Apr–9 Jul)	13	66.0±5.88 (56.0–73.0)
		2013 (6 May)	9	63.9±5.92 (53.5–71.6)
	Mondego	2012 (14 May)	5	56.6±2.38 (54.5–60.0)
		2013 (15 May)	5	54.0±2.57 (49.5–56.0)
<i>Alosa fallax</i>	Ulla	2008 (01 May–08 Jun) ^a	6	39.6±5.39 (31.0–44.7)
		2011 (15 Apr–06 Jun)	89	45.4±5.72 (33.7–56.2)
		2012 (30 Mar–27 Jul)	59	44.8±4.45 (32.8–55.0)
	Minho	2011 (29 Apr)	27	38.2±3.37 (34.7–50.0)
		2012 (14 May)	42	36.7±2.58 (28.0–42.6)

^a Samples obtained by sport fishing

Table 2 Quantitative descriptors of parasitic infections in both shad species examined by visual methods, all samples per *Alosa* species combined

Parasite	Organ	<i>n</i>	<i>I</i>	<i>P</i> (%)	<i>mA</i> ± <i>SD</i>	<i>mI</i> ± <i>SD</i> (range)
<i>Alosa alosa</i>						
<i>M. alosae</i>	Gill	9	8	88.9	7.3±15.0	8.2±15.7 (1–47)
<i>H. appendiculatus</i>	Stomach and viscera	163	118	72.4	24.1±47.9	33.3±53.6 (1–314)
<i>H. aduncum</i>	Stomach and viscera	162	101	62.3	10.6±15.3	17.0±16.3 (1–99)
<i>Anisakis</i> spp.	Stomach and viscera	162	156	96.3	171.8±205.5	178.5±206.6 (1–1206)
<i>R. pristis</i>	Stomach and viscera	162	24	14.8	0.4±1.3	2.92±2.19 (1–8)
<i>C. italica</i>	Buccal cavity	9	1	11.1	0.1±0.3	1
Unidentified copepod	Gill	9	1	11.1	0.1±0.3	1
<i>Alosa fallax</i>						
<i>H. appendiculatus</i>	Stomach and viscera	220	123	55.9	6.5±14.8	11.6±18.2 (1–100)
<i>H. aduncum</i>	Stomach and viscera	74	36	48.6	1.6±3.3	3.3±4.1 (1–25)
<i>Anisakis</i> spp.	Stomach and viscera	214	25	11.7	1.8±8.8	15.7±21.9 (1–89)
<i>C. emarginata</i>	Gill	49	14	28.6	1.1±3.0	4.0±4.7 (1–17)
Unidentified isopod	Buccal cavity	49	1	2.0	0.02±0.1	1

Area inspected for macroparasites (organ)

n number of fish examined; *I* number of fish infected; *P* prevalence; *mA*±*SD* mean abundance and standard deviation; *mI*±*SD* (range) mean intensity, standard deviation and range

different genes, depending on the group. For nematodes, the entire ITS (ITS1, 5.8S ribosomal DNA (rDNA) gene and ITS2) was amplified using the forward primer NC5 (5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3') and reverse primer NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') (Zhu et al. 2000). Partial small subunit ribosomal RNA gene (18S ribosomal RNA (rRNA) or small subunit (SSU)) was selected to identify monogeneans, digeneans, acanthocephalans and the siphonostomatoid copepod, using the universal primers 18SU467F (5'-ATC CAA GGA AGG CAG CAG GC-3') and 18SL1310R (5'-CTC CAC CAA CTA AGA ACG GC-3') (Suzuki et al. 2008). Moreover, the large subunit ribosomal RNA gene (28S rRNA or large subunit (LSU)) was partially amplified on the digeneans with the primers LSU-5 (5'-TAG GTC GAC CCG CTG AAY TTA AGC A-3') and 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') (Olson et al. 2003).

PCR reactions were performed in a total volume of 25 µl, containing 1 µl of genomic DNA (20–40 ng), PCR buffer at 1× concentration, 1.5 mM MgCl₂, 0.2 mM nucleotides (Roche Applied Science), 0.3 µM primers and 0.025 U/µl Taq DNA polymerase (Roche Applied Science). The cycling protocol used to amplify the ITS1, 5.8S and ITS2 genes from anisakids was as follows: 2 min at 94 °C, then 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 75 s at 72 °C, followed by a final elongation of 7 min at 72 °C. The cycling protocol for 18S and 28S rRNA genes was 3 min at 94 °C, 40 cycles of 30 s at 94 °C, 45 s at 56 °C and 2 min at 72 °C, followed by 7-min extension hold at 72 °C. All PCRs were carried out in a Tgradient thermocycler (Biometra), and a negative control (no DNA) was included for each set of PCR reactions. Positive

PCR products were purified for sequencing using ExoSAP-IT[®] (USB corporation). Sequencing was performed in a specialized service (SecuGen, Madrid). All sequences were subjected to an identity search using Basic Local Alignment Search Tool (BLASTn) through web servers of the National Center for Biotechnology Information (NCBI) database. Sequences obtained in this study were deposited in GenBank under accession numbers (Table 3).

Phylogenetic analysis

Since the sequences of the digenean *H. appendiculatus*, the monogenean *M. alosae* and the copepod *C. emarginata* were not present in the genetic database, phylogenetic trees were built to assess their phylogenetic position. Sequences that displayed highest matches on BLASTn were downloaded to infer the phylogeny of the monogenean polyopisthocotyleans (*n*=37 for SSU), the higher digenean plagiorchiiideans (*n*=46 for SSU and *n*=49 for LSU) and the siphonostomatoid copepods (*n*=41 for SSU). The digenean *Petasiger phalacrocoracis* (accession number AY245709) was used as an outgroup for the monogenean tree, the diplostomid *Schistosoma haematobium* (accession number Z46521) was used as an outgroup for the digenean trees, and the calanoid copepod *Calanus finmarchicus* (accession number AF367719) was used as the outgroup for the siphonostomatoids.

The different subsets of sequences were aligned using Mafft implemented in Geneious (version 7.1.7). GBLOCKS (Castresana 2000) was then used to identify and remove highly divergent regions and poorly aligned positions, which are common features in these ribosomal genes. Afterwards, the

Table 3 Accession numbers and related information of parasite sequences available at GenBank

Fish host	Parasite species	River	Year	Location	Accession number
<i>A. alosa</i>	<i>H. aduncum</i>	Minho	2013	Stomach	KR349114
		Mondego	2013	Stomach	KR349115
	<i>R. pristis</i>	Minho	2009	Visceral cavity	KR349116
		Mondego	2013	Visceral cavity	KR349117
	<i>H. appendiculatus</i>	Minho	2013	Stomach	KR349118
	<i>M. alosae</i>	Minho	2013	Gill	KR349119
<i>A. fallax</i>	<i>H. aduncum</i>	Minho	2012	Stomach	KR349120
	<i>H. appendiculatus</i>	Ulla	2012	Stomach	KR349121
		Minho	2009	Stomach	KR349123
	<i>C. emarginata</i>	Minho	2012	Gill	KR349122

best model of nucleotide substitution was selected under the corrected Akaike information criterion (Akaike 1974) in ModelTest2 (Darriba et al. 2012). In all the subsets, the general time reversible model with estimates of invariant sites and gamma-distributed model (GTR+I+G) was chosen. For the digenean *H. appendiculatus*, datasets of partial SSU sequences ($n=46$), partial LSU sequences ($n=53$) and concatenated SSU and LSU sequences ($n=31$) were analyzed. Maximum likelihood (ML) and Bayesian Inference (BI) analysis were run in Geneious 7.1.7 implemented with PhyML and MrBayes. For ML phylogenetic trees, nodal support was estimated with a bootstrap procedure with 1000 replicates (Felsenstein 1985). Bayesian analysis was run twice, and log likelihoods were estimated over 1,100,000 generations using four Markov Chain Monte Carlo (MCMC) chains, with every 200th tree saved. Nodal support for BI trees was given by posterior probabilities.

Quantitative descriptors

The quantitative descriptors of parasite infections, such as prevalence, mean abundance and mean intensity, were calculated as described in Bush et al. (1997). Prevalences of infection were compared using the chi-squared test or Fisher's exact test and abundances using the non-parametric Mann-Whitney *U* test or the Kruskal-Wallis test. Statistical analyses were carried out using GraphPad Prism 6. The level of statistical significance was set at 95 % ($p<0.05$).

Results

Parasite identification

Ten parasite taxa were found, eight of which were identified to species using morphology and genetics (Figs. 1 and 2). Five of these had perfect matches against the genetic database (100 % homology), and three were not present in the genetic database

(see “Molecular phylogeny” section and Table 3 for accession numbers). Two parasites—the unidentified copepod from *A. alosa* and the unidentified isopod from *A. fallax*—failed to amplify the selected genes, and thus, morphological identification was not confirmed.

The digenean *H. appendiculatus* was common to both species from all sampling rivers, as were the three nematode species. The larval anisakid nematodes were found to represent two species: *Anisakis simplex* s.s. and *Anisakis pegreffii*. In addition, the acanthocephalan *R. pristis* was identified in *A. alosa* from the Rivers Minho and Mondego. The monogenean *M. alosae*, the cymothoid isopod *Ceratothoa italica* and an unidentified ergasilid copepod (genus *Ergasilus*) were found in *A. alosa* from the River Minho. The lernaeopodid copepod *C. emarginata* and an unidentified mouth-dwelling isopod (family Cymothoidae) were found in *A. fallax* samples from the River Minho.

Comparative infection data

The quantitative descriptors of parasitic infections in the two shad species (all samples combined) using visual inspection methods are shown in Table 2. *Anisakis* spp. larvae and *M. alosae* were the most prevalent parasites of *A. alosa*, whereas *H. appendiculatus* and *H. aduncum* were the most common species infecting *A. fallax*. A single specimen, each of the isopod *C. italica*, and an unidentified parasitic copepod were found on *A. alosa*, and a single specimen of an unidentified isopod was found on *A. fallax*.

A comparative analysis of the three common parasites of *A. alosa* and *A. fallax* (all samples combined) was performed (Table 4). Mean abundance was significantly higher for *H. appendiculatus*, *H. aduncum* and *Anisakis* spp. in *A. alosa*, while prevalence was significantly higher for *H. appendiculatus* and *Anisakis* spp. in *A. alosa*, but the difference was not statistically significant for *H. aduncum*.

A similar comparison was carried out with infection values of the same parasite species found in both shad species from the

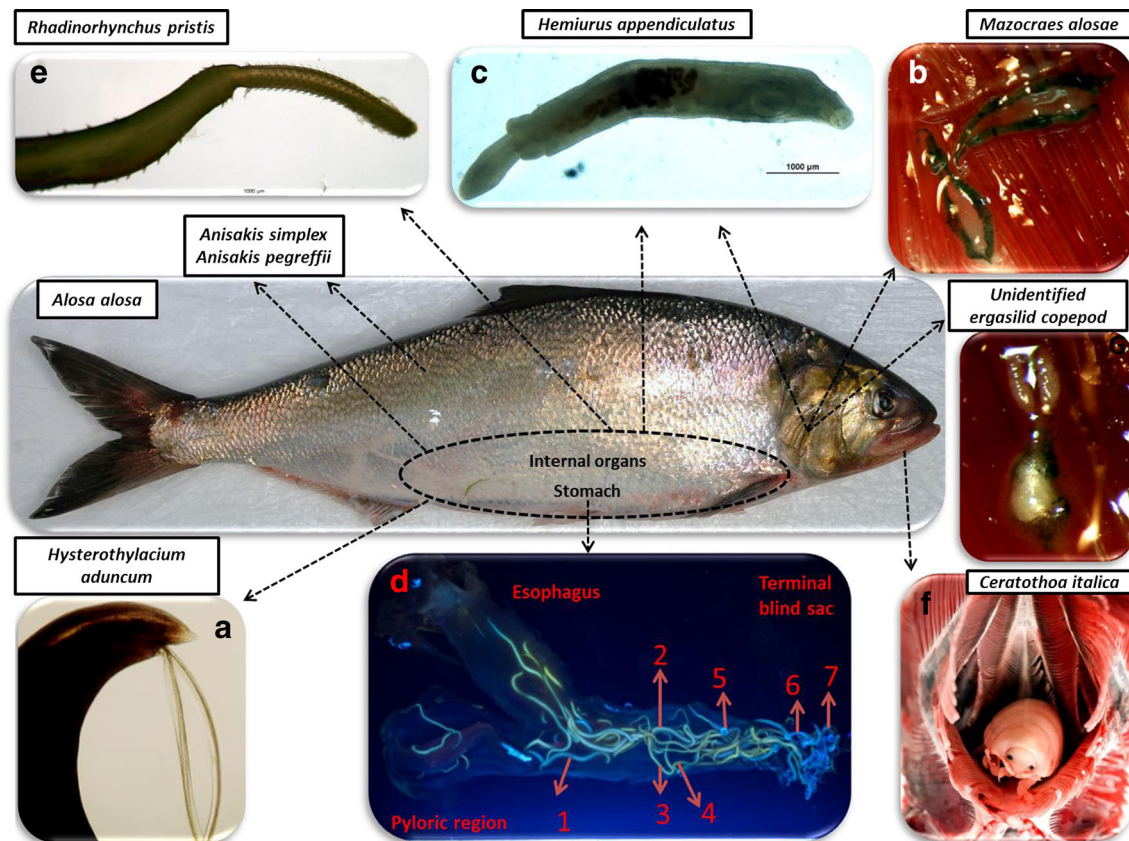


Fig. 1 Parasite taxa recorded, with sites of infection in *Alosa alosa*. **a** The posterior (tail) region of an adult male of the rhabdiascarid nematode *Hysterothylacium aduncum*. **b** Two specimens of the monogenean *Mazocraes alosae* in situ on the gill filaments. **c** The digenean *Hemiurus appendiculatus* with eviscerated body. **d** Ultraviolet photograph of an opened *A. alosa* stomach showing the intensity of the infection and the different colours and fluorescence brightness of the

anisakid and rhabdiascarid nematode larvae. Several parasite larvae were molecularly identified; thus, larvae nematode numbers 1, 2, 3 and 4 belong to adult *H. aduncum*, numbers 5 and 7 to *A. simplex* s.s. and 6 to *A. pegreffii* third larval stage. **e** The acanthocephalan *Rhadinorhynchus pristi* showing the body spination and proboscis. **f** The mouth-dwelling isopod *Ceratothoa italica* in situ. **g** Female unidentified ergasilid copepod (probably *Ergasilus* spp.) attached to the gill filaments

River Minho in 2011 (Table 5). Prevalence and mean abundance were significantly higher for *H. appendiculatus* and significantly lower for *Anisakis* spp. in *A. fallax* than in *A. alosa*. Differences in prevalence and mean abundance of *H. aduncum* between host species were not statistically significant.

Infection levels of the three most common parasites were compared for *A. alosa* of the River Minho for the years 2009, 2010 and 2011 (Table 6). Mean abundance decreased significantly during the sampling years for all parasites, but the only significant decrease in prevalence was for *H. aduncum*. Differences in mean abundance were statistically significant for all three parasites.

Molecular phylogeny

A total of 500 bp were aligned for partial SSU gene (37 species) for the monogenean *M. alosae*. Of these, 396 were unambiguously alignable (77 %). Phylogenetic trees constructed with ML and BI displayed similar topologies for the basal groups but differed in the resolution of the most divergent order

Mazocraeidea (Fig. 3). The monogenean identified on the gills of *A. alosa* belonged to the Mazocraeidae family with high ML bootstrap values 84.8 and BI posterior probability of 0.99. The topology obtained is in agreement with previous works, despite only a small region of the SSU gene being analyzed. Polystomatids (parasites of tetrapods) are the sister group of the oligonchoinea (parasites of fishes). Among the latter group, *Pseudohexabothrium taeniurae* (parasite of sharks) displays a basal position in relation to the parasites of teleost fishes (Order Mazocraeidea). Within this group the family Mazocraeidae, where the parasite found in the shad *A. alosa* is located, is the sister group of all the others.

For the digenean *H. appendiculatus*, a total of 349 sites were aligned for partial SSU gene (46 species), though only 229 were unambiguously aligned and informative (65 %), while 450 sites were aligned for partial LSU gene (53 species), but only 233 were unambiguous (51 %). The interrelationships found with the SSU sequences were better resolved than in the monogenean tree, showing that this region is more variable for digeneans. The phylogenetic trees constructed showed similar topologies for

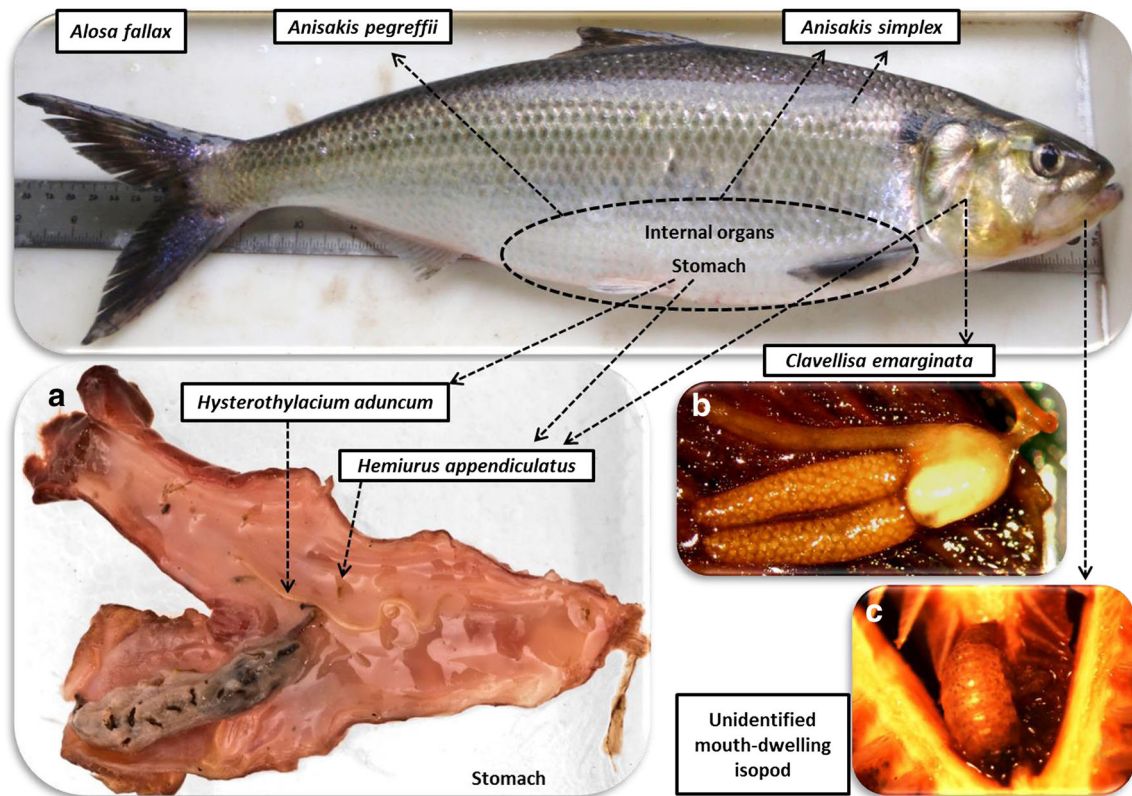


Fig. 2 Parasite taxa recorded, with sites of infection in *Alosa fallax*. *Anisakis* spp. usually appeared in the visceral cavity, but one larva was observed in the stomach of one fish. **a** The nematode *Hysterothylacium*

aduncum and the digenean *Hemiurus appendiculatus*. **b** The parasitic copepod *Clavellisa emarginata* in situ on the gill filaments. **c** An unidentified mouth-dwelling isopod in situ in the buccal cavity

both genes when analyzed individually (Figs. 4 and 5), although deeper nodes were less resolved on LSU tree (Fig. 5). The combined analysis of LSU and SSU (Fig. 6) shows a robust phylogeny where most of the relationships are well supported. The basal Plagiorchiida (named after Olson et al. 2003) are grouped together, with the monophyletic suborders Bivesiculata and Transversotremata being the basal lineages and the monophyletic suborder Hemiurata as the sister clade. Within it, the superfamily Azygioidea (represented by *Otodistomum cestoides*) is basal to the superfamily Hemiuroidea. The relationships found within the latter lineage show two main clades, as shown on the individual LSU and SSU trees: the first clade comprises the families Derogenidae (polyphyletic), Hirudinellidae, Syncoelidae,

Accacoelidae, Sclerodistomidae and the monophyletic Didymozoidae, while the second comprises the polyphyletic families Lecithasteridae and Hemiuridae. Independently of the gene analyzed, the digenean found in the digestive tract of *Alosa* falls within a supported Hemiuroidea lineage constituted by the subfamilies Lecithochirinae, which is monophyletic and sister clade to the following: Plerurinae (polyphyletic), Dinurinae (polyphyletic), Elytrophallinae (monophyletic) and Hemiurinae (monophyletic). Within the latter hemiurid clade, *H. appendiculatus* consistently appears basal to the subfamilies Dinurinae and Elytrophallinae. In the SSU tree, this digenean is basally located in the Hemiurinae subfamily with *Hemiurus communis* (95 % bootstrap, Fig. 4) in a supported clade

Table 4 Comparative prevalences and mean abundances of parasite taxa common to the two shad species, all samples combined, with results of tests of statistical significance

Parasite	Prevalence (%)			Mean abundance		
	<i>A. alosa</i>	<i>A. fallax</i>	<i>P</i> value	<i>A. alosa</i>	<i>A. fallax</i>	<i>P</i> value
<i>H. appendiculatus</i>	72.4	55.9	0.0013**	24.12	6.48	<0.0001***
<i>H. aduncum</i>	62.3	48.6	0.064 NS	10.59	1.61	<0.0001***
<i>Anisakis</i> spp.	96.3	11.7	<0.0001***	171.84	1.83	<0.0001***

NS not significant
*Significant; **,***highly significant

Table 5 Comparative prevalences and mean abundances of parasite taxa common to the two shad species caught in the river Minho in the same year (2011), with results of tests of statistical significance

Parasite	Prevalence (%)			Mean abundance		
	<i>A. alosa</i>	<i>A. fallax</i>	<i>P</i> value	<i>A. alosa</i>	<i>A. fallax</i>	<i>P</i> value
<i>H. appendiculatus</i>	69.2	100	<0.0001***	15.8	32.9	0.002*
<i>H. aduncum</i>	38.5	61.5	0.196 NS	5.2	2.4	0.692 NS
<i>Anisakis</i> spp.	76.9	23.1	0.002*	23.3	1.3	0.0014**

NS not significant

*Significant; **,***highly significant

(65.6 % and 0.91 posterior probability) that included other Hemiuridae subfamilies (Dinurinae and Elytrophallinae).

For the copepod *C. emarginata*, a total of 804 bp were aligned (41 species) and 788 bp were unambiguously aligned (98 %) (Fig. 7). The topologies obtained by ML and BI were equivalent, and therefore, we added the Bayesian posterior probabilities to the ML tree. The phylogenetic relationships obtained show a low bootstrap support and posterior probabilities on the deeper nodes. Nonetheless, the more divergent relationships are well resolved and locate the copepod *C. emarginata* within a highly supported clade (100 % bootstrap and posterior probability of 1) that includes the family Sphyriidae (represented by *Paeon elongatus*) basal to the members of the family Lernaepodidae. *C. emarginata* falls within the Lernaepodidae family with high bootstrap value (93.6 %) and posterior probabilities (1).

Discussion

Metazoan parasite fauna

To date, parasitological studies on European shad have been done mainly on adult specimens from rivers or estuaries flowing into the Bay of Biscay, Celtic Sea, North Sea and

Baltic Sea for *A. alosa* and from the same geographical areas, plus Mediterranean Sea, for *A. fallax* (Arahamian et al. 2002 and references therein; Doherty et al. 2004; Rokicki et al. 2009). Recently, Mota et al. (2015) cited the presence of *A. pegreffii*, *H. aduncum* and *R. pristis* in *A. alosa* from the River Minho. In addition, Bao et al. (2015) reported the presence of mixed infections of *A. simplex* s.s. and *A. pegreffii* for the first time in both shad species from Western Iberian Peninsula Rivers. Notwithstanding the foregoing, this is the first report of the quantitative descriptors and ecology aspects of the metazoan parasite fauna of both anadromous shad species in Western Iberian Peninsula Rivers. In addition, to the best of our knowledge, this is the first time that *C. italica* has been reported on *A. alosa* and, additionally, in a clupeid host. Moreover, the presence of *C. italica*, *C. emarginata* and *M. alosae* is reported for the first time in the Iberian Peninsula. In general, the parasite fauna of *A. alosa* and *A. fallax* was characterized by anisakid and rhabdiascarid nematodes and hemiurid digeneans, whilst acanthocephalans, monogeneans, copepods and isopods were less important.

Mazocraes alosae (Hermann, 1782)

The mazocraeid monogenean *M. alosae* is a specific parasite of the gills of *Alosa* spp. It was reported from

Table 6 Comparative prevalences and mean abundances of the most common parasite taxa in samples of *A. alosa* caught in River Minho in different sampling years

Parasite	Year	Prevalence (%)	<i>P</i> value	Mean abundance	<i>P</i> value
<i>H. appendiculatus</i>	2009	88	0.072 NS	58.0	<0.0001***
	2010	69		14.7	
	2011	69		15.8	
<i>H. aduncum</i>	2009	78	<0.017*	12.6	<0.0001***
	2010	57		7.6	
	2011	38		5.2	
<i>Anisakis</i> spp.	2009	100	NV	313.2	<0.0001***
	2010	97		84.1	
	2011	77		23.3	

The 2013 sample was excluded because of the small number of fish examined

NS not significant, NV test not valid because of zeros and numbers <5 in some cells

*Significant; **,***highly significant

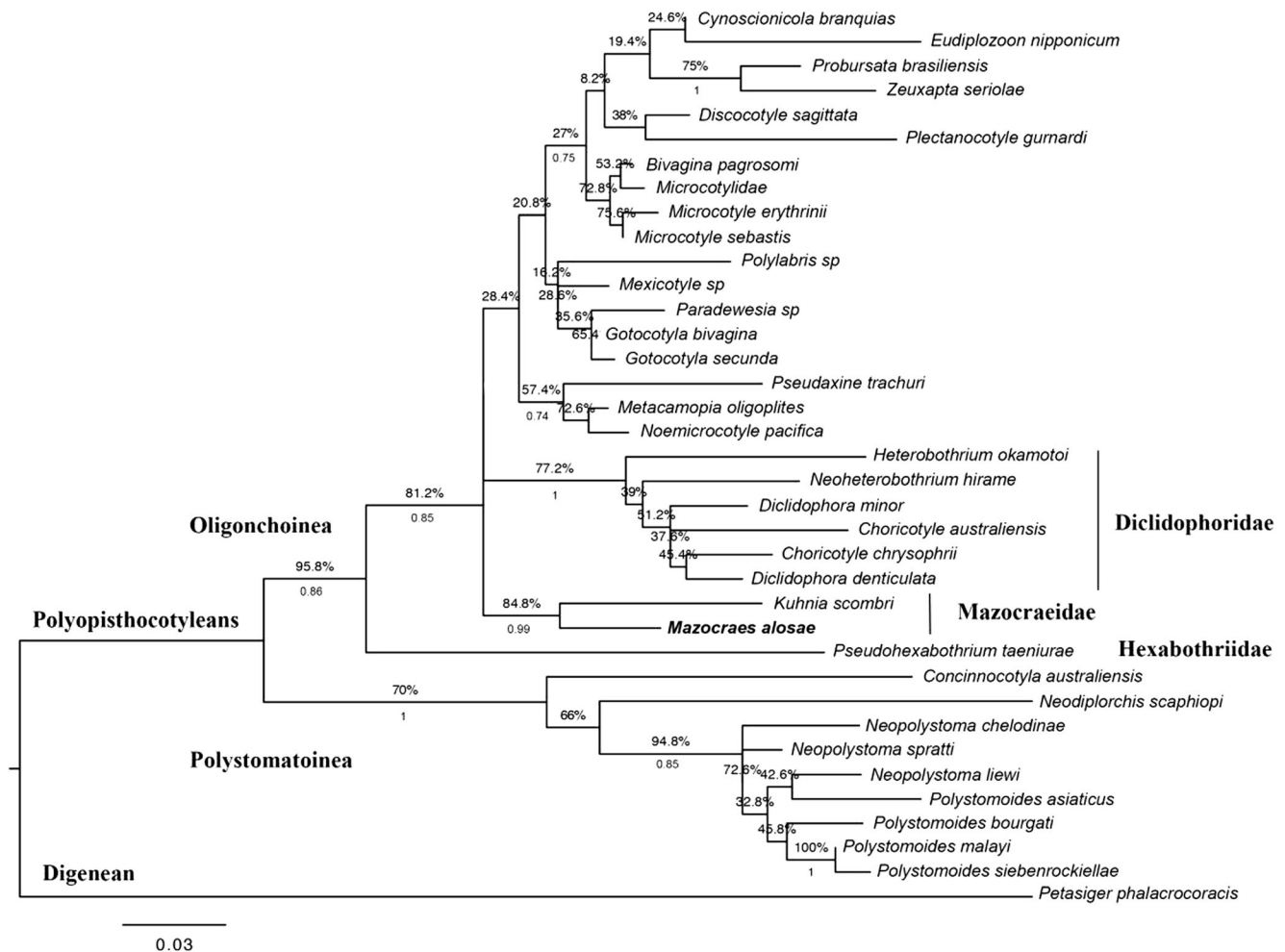


Fig. 3 Maximum likelihood phylogenetic tree using GTR+I+G model based on partial small subunit ribosomal RNA gene (18S rRNA or SSU) to infer the phylogenetic position of the monogenean platyhelminth

morphologically identified as *Mazocraes alosae*. Nodal support is given by bootstrap percentages after 1000 replicates above the node and Bayesian posterior probability values ≥ 0.7 below the node

A. alosa and *A. fallax* by Dawes (1968). Its occurrence in European shad was recorded in the Gironde system (France), River Rhine (Germany and Switzerland), River Barrow and Waterford estuary (Ireland) and in the Irish Sea, River Severn and estuary and at Plymouth and Aberdeen (Britain) (Dawes 1968; Aprahamian et al. 2002 and references therein), Caspian, Black and Azov Seas (Mamaev 1982; Özer et al. 2013) and the NE Aegean Sea (Akmirza 2013). Monogeneans are parasites which may cause serious damage in both wild and farmed fish (Dezfuli et al. 2007; Lia et al. 2007; Akmirza 2013 and references therein).

Hemiurus appendiculatus (Rudolphi, 1802)

Digenetic trematodes of the family Hemiuridae usually occur in the stomachs of marine and freshwater teleosts and the lungs of piscivorous sea snakes. They have a

unique organ—a “tail” or *ecsoma* at the posterior region of the body—which has the ability to be retracted within the body or *soma*. This structure is thought to be a feeding organ extruded only when conditions in the stomach are favourable (Gibson and Bray 1979, 1986). According to Gibson and Bray (1986), *H. appendiculatus* appears to be restricted to *A. alosa* and *A. fallax* (especially in the latter) in Mediterranean and European Atlantic waters as far north as southern Norway (Moravec 2004). In addition, it was previously reported in *A. fallax* of the Atlantic coast of Africa and the Portuguese Coast (Rodrigues et al. 1972 cited in Aprahamian et al. 2002).

Hemiurus appendiculatus showed higher values of prevalence and abundance in *A. alosa* (Table 4), even though it was found to be the most prevalent and abundant parasite species of *A. fallax* following visual methodologies (Table 2).

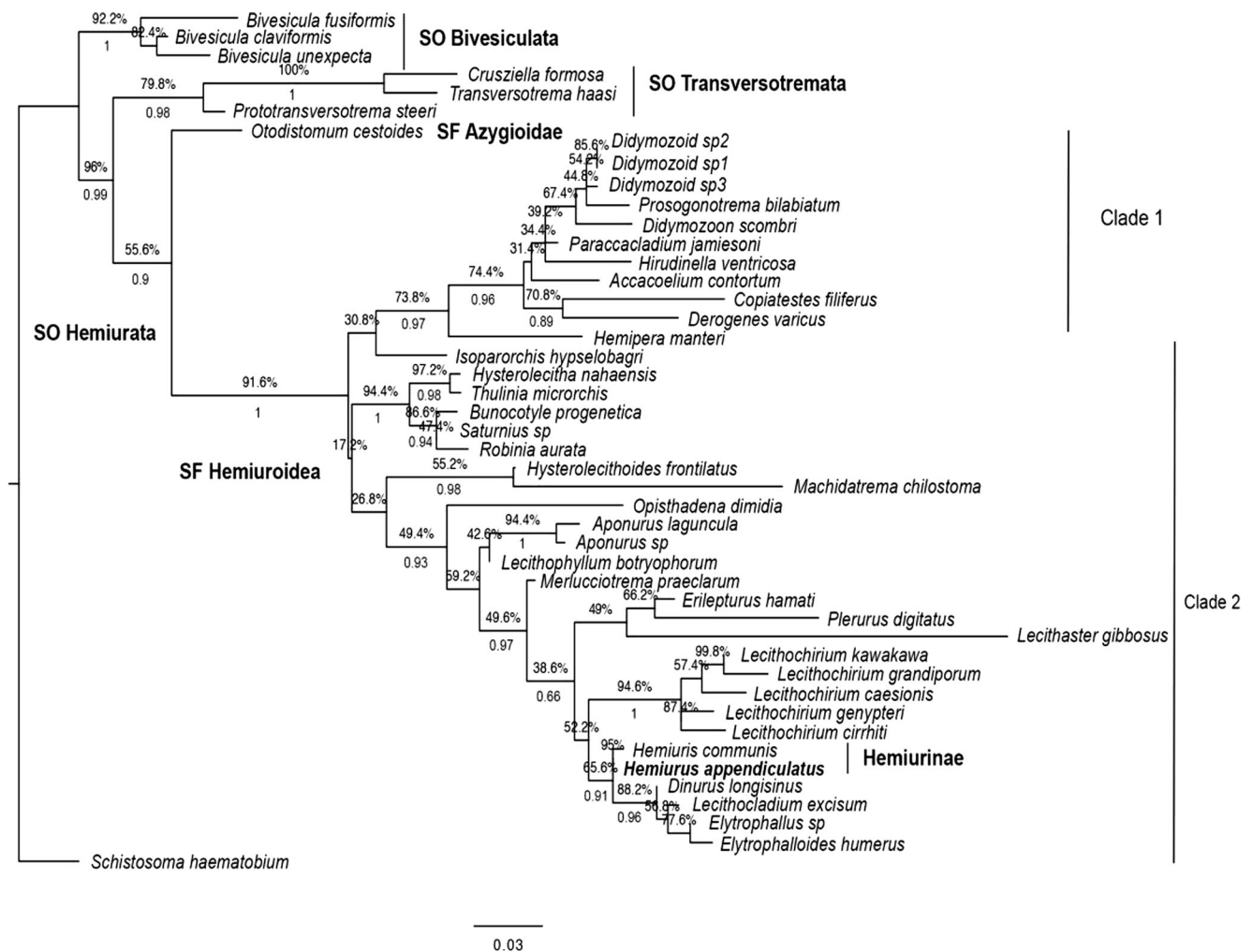


Fig. 4 Maximum likelihood phylogenetic tree using GTR+I+G model based on partial SSU gene to infer the phylogenetic position of the digenean platyhelminth morphologically identified as *Hemiuirus*

appendiculatus. Nodal support is given by bootstrap percentages after 1000 replicates above the node and Bayesian posterior probability values ≥ 0.7 below the node

Anisakis spp. (*Anisakis simplex sensu stricto* (Rudolphi, 1809); *Anisakis pegreffii* (Campana-Rouget & Biocca, 1955))

The presence of mixed infections of *A. simplex* s.s. and *A. pegreffii* in both *Alosa* spp. was previously reported and discussed in Bao et al. (2015). Herein, we report the quantitative descriptors of *Anisakis* spp. infection using visual methodologies in order to compare infection values with other parasite data obtained using visual methods. Thus, *Anisakis* sp. was clearly the most common and abundant parasite of *A. alosa* (Table 2) (Bao et al. 2015), whilst this predominance was not so evident for *A. fallax*, which showed low prevalence and abundance values following visual methods (Table 2), but can reach values of up to 83 % prevalence and

44.17 mean abundance using a combination of visual and digestive methods (see Bao et al. 2015).

Hysterothylacium aduncum (Rudolphi, 1802)

Many teleost fish species have been shown to be definitive hosts of *H. aduncum*, while crustaceans (copepods, amphipods, isopods, euphausiids and mysids) act as obligate intermediate hosts. In addition, non-crustacean invertebrates (ctenophores, chaetognaths, polychaetes and ophiuroids), as well as fish, may act as obligate second intermediate or transport hosts (Smith 1983; Koie 1993; Shih and Jeng 2002). The presence of both the fourth larval stage and adults of *H. aduncum* in both *Alosa* species confirms their role as definitive hosts in the life

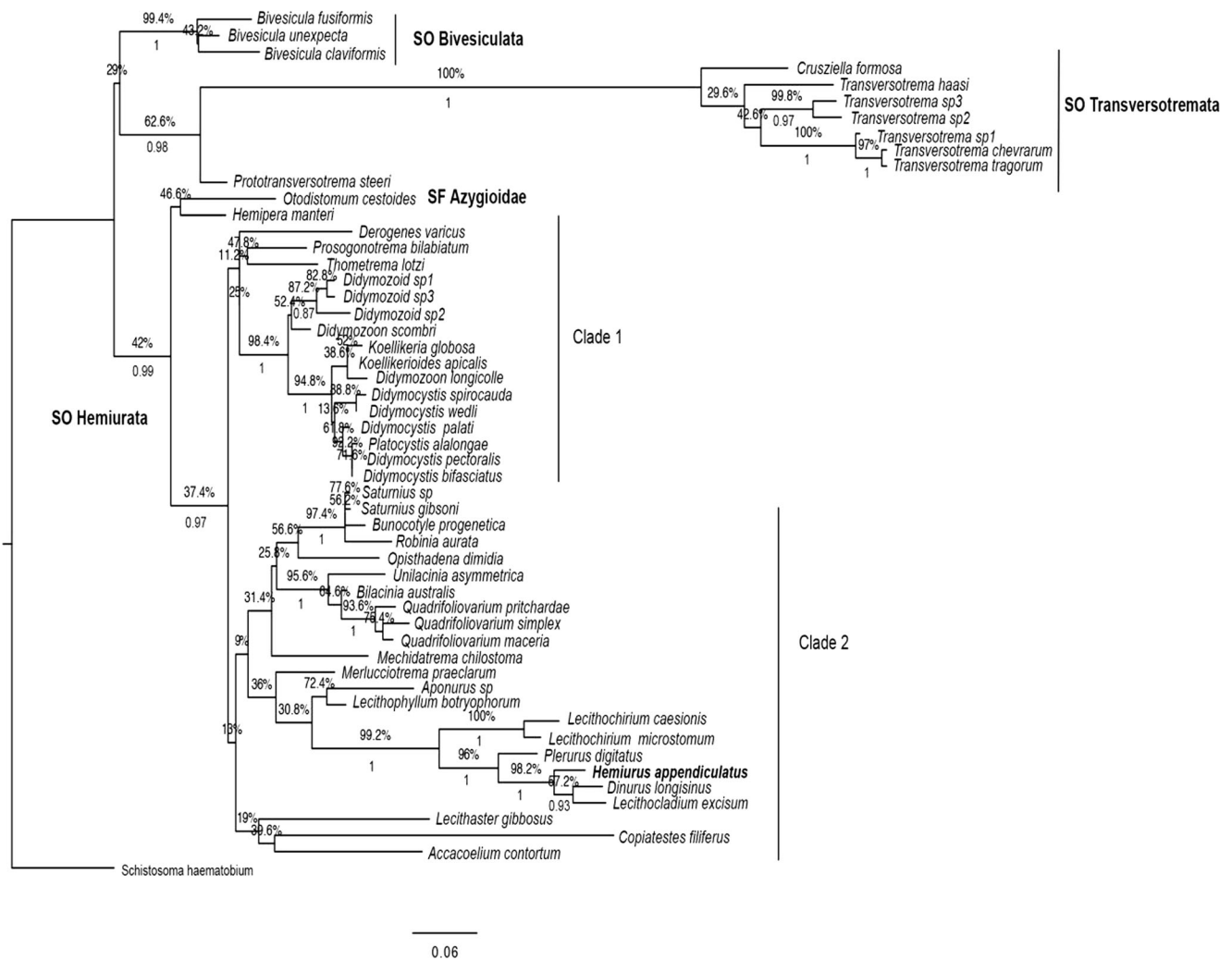


Fig. 5 Maximum likelihood phylogenetic tree using GTR+I+G model based on partial large subunit ribosomal RNA gene (28S rRNA or LSU) to infer the phylogenetic position of the digenean platyhelminth

morphologically identified as *Hemiurus appendiculatus*. Nodal support is given by bootstrap percentages after 1000 replicates above the node and Bayesian posterior probability values ≥ 0.7 below the node

cycle of this rhabdiascarid nematode. Additionally, *H. aduncum* was shown as a component parasite of both shad species but was more abundant in *A. alosa* (Table 4).

Rhadinorhynchus pristis (Rudolphi, 1802)

The presence of *R. pristis* in *A. alosa* was previously recorded in one specimen caught in the River Rhine by Golvan (1969) and also by Mota et al. (2015) in the River Minho. This acanthocephalan has a complex life cycle, using euphausiids (e.g. *Nyctiphanes couchii*) as intermediate hosts and marine fish as definitive hosts in the NW Atlantic area (Gregori et al. 2013). This parasite was only found in *A. alosa*, which is consistent with its feeding habits.

Ceratothoa italica (Schioedte & Meinert, 1883)

The mouth-dwelling isopod *C. italica* has a direct life cycle which involves fish of the family Sparidae as final hosts. It was previously reported in Mediterranean and North-West African waters (Horton 2000; Sala-Bozano et al. 2012). European shad can migrate long distances from the rivers where they born and reproduce to their feeding grounds at sea (Sabatié 1993). Our results might suggest a similar migrating behaviour; a movement from the Western Iberian Rivers to southern productive sea areas of the Coast of Portugal, which is the northern limit of distribution of this parasite and where shad may have better chances of infection. This hypothesis was previously suggested by Bao et al. (2015).

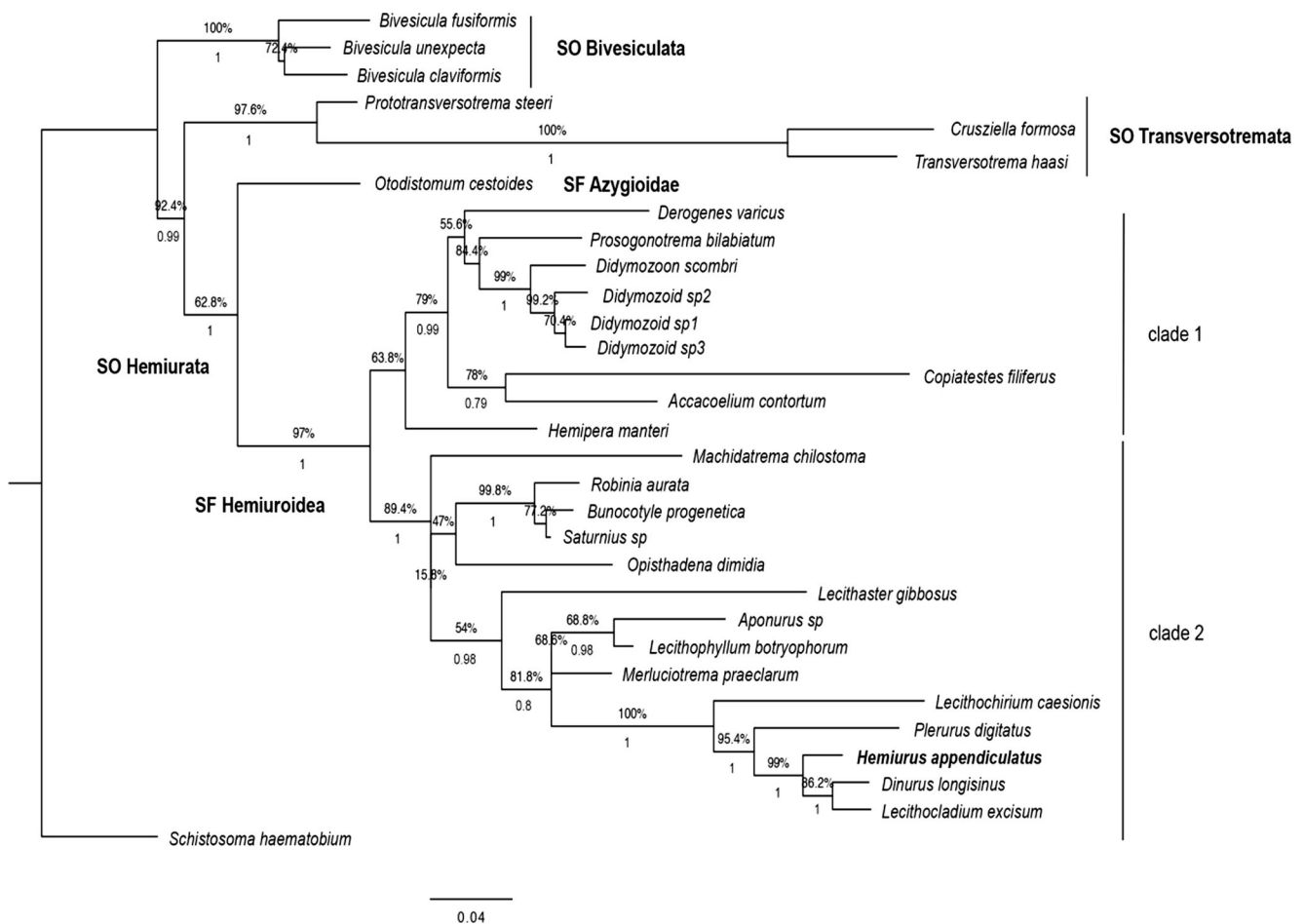


Fig. 6 Maximum likelihood phylogenetic tree using GTR+I+G model based on combined partial SSU and LSU to explore the phylogenetic position of the digenean plathelminth morphologically identified as

Hemiuurus appendiculatus. Nodal support is given by bootstrap percentages after 1000 replicates above the node and Bayesian posterior probability values ≥ 0.7 below the node

Clavellisa emarginata (Krøyer, 1837)

The lernaeopodid copepod *C. emarginata* is a specialist parasite of the gills of clupeid fish belonging to the genera *Alosa*, *Caspialosa* and *Chupeonella* (Kabata 1992). Its occurrence includes the North, Irish, Mediterranean, Black and Azov Seas, the lower reaches of the Danube, Southern Bug, Dnieper and Don and also in the Gironde system (France), River Rhine (Germany), River Barrow and Waterford estuary (Ireland), River Severn and estuary and at Plymouth (Britain) and is probably even more widespread (Kabata 1992; Aprahamian and references therein, 2002).

Phylogenetic position of the parasites

Mazocraes alosae (Hermann, 1782)

Monogeneans are primarily ectoparasites of fishes, with few exceptions, and their mode of feeding splits the two major clades: the Monopisthocotylea (that erode the epidermis) and

the Polyopisthocotylea (specialized to feed on blood) (Olson and Tkach 2005). Large and small rRNA (LSU and SSU) data shows that polyopisthocotylean parasites (also known as Heteronchoinea) are monophyletic with a basal split between radiations in tetrapods (Polystomatoinea) and fishes (Oligonchoinea) (Boeger and Kritsky 1997; Littlewood et al. 1999; Mollaret et al. 2000; Jovelin and Justine 2001; Olson and Littlewood 2002; Olson and Tkach 2005). The latter clade shows a split of lineages with the oligonchoinean parasites of chondrichthyans (chimaeras: Chimaerocolidae, sharks: Hexabothriidae) as a sister group of the oligonchoinean parasites of teleosts (Mazocraeidea) (Jovelin and Justine 2001). The more derived oligonchoineans, the mazocraeids, are a monophyletic clade with poorly supported interrelationships, as observed herein, using either morphology or phylogeny (Jovelin and Justine 2001; Olson and Littlewood 2002). Nonetheless, within this group of parasites, the basal position of the Mazocraeidae is consistent, being mainly parasites of clupeid fishes, as well as scombrids (Mollaret et al. 2000; Jovelin and Justine 2001).

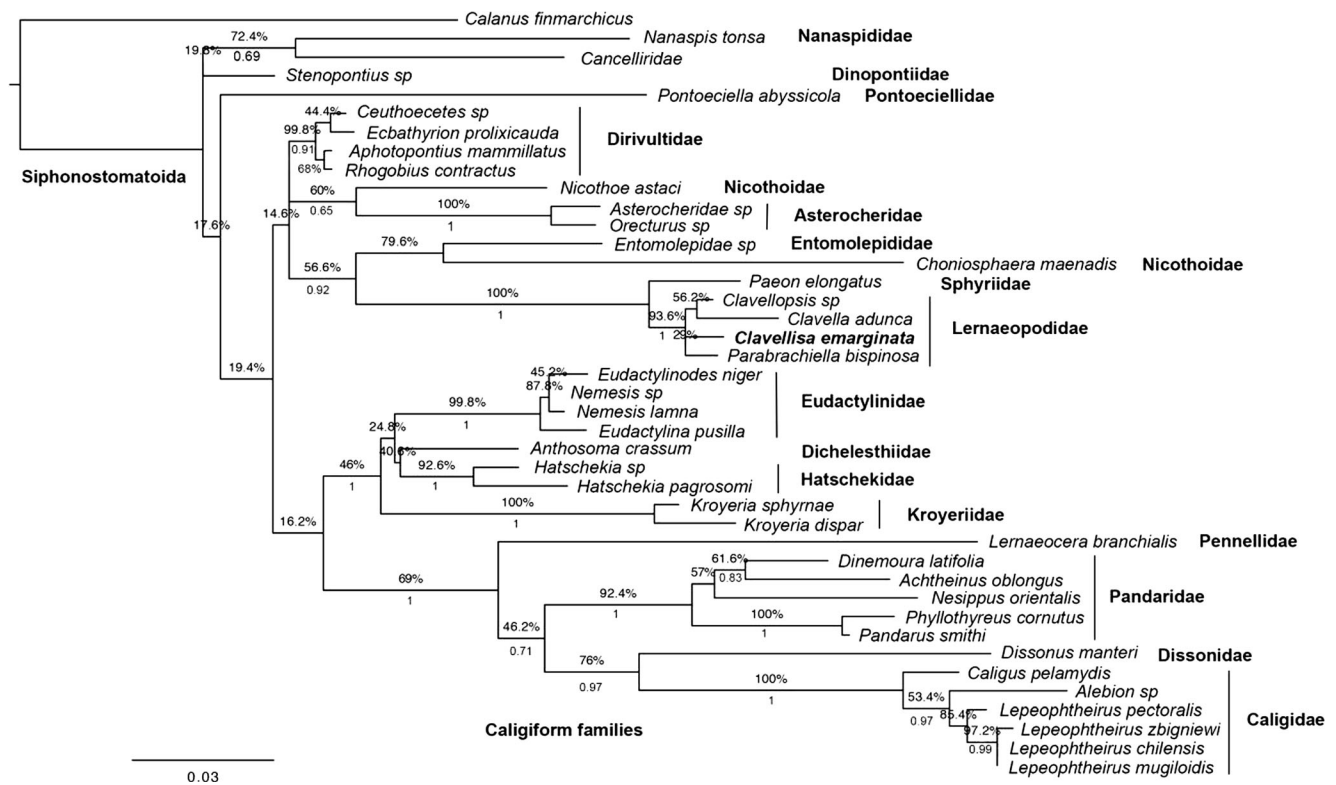


Fig. 7 Maximum likelihood phylogenetic tree using GTR+I+G model based on partial SSU gene to infer the phylogenetic position of the siphonostomatoid copepod morphologically identified as *Clavellisa*

emarginata. Nodal support is given by bootstrap percentages after 1000 replicates above the node and Bayesian posterior probability values ≥ 0.7 below the node

Even though only a small region of the SSU gene was analyzed herein, the phylogeny obtained is in agreement with previous studies (Mollaret et al. 2000; Jovelín and Justine 2001; Olson and Littlewood 2002), placing *M. alosae* consistently as a polyopisthocotylean monogenean (Order Mazocraeidea, Family Mazocraeidae). The low bootstrap values or posterior probabilities as well as the small length of the terminal branches in the sister clade of Mazocraeidae (Fig. 3) are noteworthy. These features are the result of low divergent rates in this lineage—four times lower than those measured for the Polyonchoinea lineage in analyses of the complete SSU sequences (Olson and Littlewood 2002)—and the main reason for the lack of support for higher interrelationships in mazocraeids. Fortunately, the basal relationships of the mazocraeids are well resolved and the monogenean found in the gills of *A. alosa* undoubtedly belongs to the basal Mazocraeidae.

This is the first time that a sequence of 18S rRNA from a mazocraeid obtained from its clupeid host has been deposited on GenBank, since the other mazocraeid available on GenBank for this region, *Kuhnna scomбри*, was isolated from the scombrid *Scomber scombrus* (see Olson and Littlewood 2002). This genetic information is valuable since it allows testing of the hypothesis suggested by Bychowsky (1961) and reassessed with molecular data by Mollaret et al. (2000).

These authors suggested that the family Mazocraeidae is parasitic in the relatively early divergent teleost fish family Clupeidae, with later host switching to the scombrids, represented by the species *Kuhnna* and *Grubea* (Mollaret et al. 2000; Jovelín and Justine 2001). Our results support this hypothesis, since the mazocraeids *M. alosae* and *K. scomбри* constitute a monophyletic group basal to all other polyopisthocotyleans, suggesting that they diverged earlier. Moreover, it is also in agreement with the systematic work of Boeger and Kritsky (1997) on the coevolution of the monogeneans with their fish hosts. Within the mazocraeids, *M. alosae* is basal to *K. scomбри*, confirming that the parasitic relationship first evolved in clupeids, with a later host switching to the most derived scombrids that share the coastal pelagic ecosystems with clupeids (Rosen 1982). There is another study on the mazocraeid *Mazocraeoides gonialosae* found in the gizzard shad, *Konosirus punctatus*, but it is focused on the variability in the mitochondrial cytochrome *c* oxidase subunit I gene (COI) to study the phylogeographical patterns along the coast of China (Li et al. 2011). Although these authors did not ask phylogenetic questions about the position of *M. gonialosae*, according to our results, we hypothesize that this species will be placed basal to the other mazocraeids *Kuhnna* or *Grubea*, close to *M. alosae*, since both share a clupeid host. Unfortunately, since we were not able to

amplify the 28S region of *M. alosae*, we cannot test this hypothesis with molecular data.

Hemiurus appendiculatus (Rudolphi, 1802)

The digenean phylogenies of the basal Plagiorchiida obtained analyzing either individual regions of SSU and LSU and combined show similar topologies to previous studies in the basal relationships with minor modifications on the most divergent groups. The relationships found within the superfamily Hemiuroidea, with two main clades separating the (Accacoelidae + Derogenidae + Syncoeliidae + Sclerodistomidae + Didymozoidae + Isoparorchidae) and the (Hemiuridae + Lecithasteridae), are in agreement with previous studies (Blair et al. 1998; Cribb et al. 2003; Olson et al. 2003; Pankov et al. 2006). Nonetheless, our results differ slightly from those found by Blair et al. (1998), since we found the Hemiuridae and Lecithasteridae to be polyphyletic, since more genetic data was included in the present analysis. The combined analysis of both ribosomal regions showed a topology very similar to that obtained by Pankov et al. (2006), but in our analysis, the basal topology of the clade that includes the families Hemiuridae and Lecithasteridae was better resolved. The lecithasterid *Machidatrema chilostoma*, the hemiurid *Opisthadenia dimidia* and the monophyletic hemiurid subfamily Bunocotylinae constitute a consistent clade that is basal in the hemiurid/lecithasterid clade. The basal position of the monophyletic subfamily Bunocotylinae in the hemiurid/lecithasterid clade does not change according to the region studied, but their closest relatives do when the different regions are analyzed separately. The SSU region shows the bunocotylinids strongly supported with the lecithasterids *Hysterolecitha* and *Thulinia* (as in Pankov et al. 2006, Fig. 5), while the LSU region shows a poorly resolved relationship with the lecithasterid *M. chilostoma*, the hemiurid *Opisthadenia dimidia* and the monophyletic hemiurid subfamily Quadrifolioviriinae as a sister clade. When analyzing the LSU region considering the gaps, the latter relationship is strongly supported both by bootstrap 97% and posterior probabilities 1 (data not shown). These inconsistencies may be the result of multiple factors: (i) the phylogenetic signal found in both regions is not very strong, (ii) a consequence of including different taxa to the different regions, like the hemiurid subfamily Quadrifolioviriinae that is represented in the database with LSU data but is absent on SSU, and also (iii) the inclusion of an outgroup, which is not present in the combined analysis of partial LSU and complete SSU carried out by Pankov et al. (2006).

Clavellisa emarginata (Krøyer, 1837)

The recovered phylogeny of the available sequences of the order Siphonostomatoidea shows the same family groupings

as those obtained in previous works (Huys et al. 2006, 2007), but the relative position of these families is slightly different. This difference may rely on the phylogenetic signal of the SSU rDNA, the species included in the analysis and the analysis used (ML versus maximum parsimony). Previous works used the whole sequence of the SSU rDNA from 16 (Huys et al. 2006) and 20 siphonostomatoid taxa (Huys et al. 2007), while we used less than half to build our alignment file (804 bp) with up to 40 siphonostomatoid taxa. Accordingly, they obtained a better resolution on the deeper nodes (indicated by the higher bootstrap values and posterior probabilities), since they have more informative positions. Nonetheless, as more taxa are added into our phylogenetic tree, the relative position of the families may change and new relationships appear. Apart from that, we have enough signal in our database to assign consistently the parasitic copepod *C. emarginata* within the family Lernaeopodidae, which includes parasites of marine and anadromous fishes. Our analysis reveals a consistent sister taxa relationship between the families Lernaeopodidae and Sphyrriidae (parasites of sharks). These two families are the sister group of a clade that includes the families Entomolepidae, parasites of sponges (as shown in Huys et al. 2006) and Nicothoidae (represented by *Choniosphaera maenadis*, a parasite of crabs). It is important to note that the relative position of *C. maenadis* differed between ML and BI analysis, with the BI representation placing it within the Dirivultidae family as in Huys et al. (2007).

It is interesting to point out the parallelism found between host evolution in monogeneans and siphonostomatoid copepods. With the available data, the phylogenetic trees suggest that the parasites present in clupeiform fishes (monogenean and copepods) evolved from those present in elasmobranchs. This is clearly shown in the monogeneans, where the family Hexabothriidae is basal to the Mazocraeidae (Olson et al. 2003), and supported by the systematic work carried out by Boeger and Kritsky (1997). Surprisingly, this host switching seems to have occurred in a similar way in the siphonostomatoid copepods where the family Lernaeopodidae (parasites of clupeiforms and other marine fishes) evolved from the Sphyrriidae (parasites of elasmobranchs and deep sea fishes). This parallelism suggests a coevolution of two different groups of parasites in their hosts and deserves further study.

Feeding and transmission pathways

According to Williams et al. (1992) and Arthur (1997), the parasite fauna of a host species reflects its diet and characterizes the feeding ecology of the host.

Alosa alosa is mainly zooplanktophagous, with euphausiids (e.g. *N. couchii*), copepods (*Calanus* spp.) and mysids as favourite preys and small clupeids (e.g. *Engraulis encrasicolus* and *Sprattus sprattus*) as less important parts of the diet at sea (Taverny and Elie 2001b; Maitland and Lyle 2005). *Alosa*

fallax is mainly ichthyophagous, with small pelagic fish (e.g. *E. encrasicolus*, *S. sprattus*, *Atherina boyeri*, *Sardina pilchardus*, *Pomatoschistus minutus*, *Pomatoschistus microps*) as preferred preys, followed in importance by euphausiids (e.g. *N. couchii*) and mysids (*Neomysis* spp.). Other crustaceans, such as decapods (*Crangon crangon*), amphipods, isopods, ostracods and insects, are of less importance (Assis et al. 1992; Taverny and Elie 2001b; Maitland and Lyle 2005; Ceyhan et al. 2012; Skóra et al. 2012). Thus, copepods do not seem to be an important part of the diet of adults at sea, even though calanoid and harpacticoid copepods may be important prey for 0+ juveniles during their estuarine phase (Aprahamian 1989; Nunn et al. 2008). Moreover, *A. fallax* feed on fish and crustaceans (Decapoda and Mysidacea) at the sea bottom (160 m) during the winter months and on fish (*S. sprattus*, *S. pilchardus*, *E. encrasicolus*, *Atherina* spp.) close to the surface during the summer months (Ceyhan et al. 2012 and references therein). Furthermore, *A. alosa* do not feed during their spawning migration (Mota et al. 2015), whereas *A. fallax* might feed actively, especially not only on aquatic and terrestrial invertebrates, but also on fish (*A. boyeri*, *Pseudochondrostoma duriense*) (Nachón et al. 2013).

The euphausiid *N. couchii* was found to be an intermediate host of the acanthocephalans *Bolbosoma balaenae* and *Rhadinorhynchus* sp. and of the *A. simplex* complex (*A. simplex* s.s. and *A. pegreffii*) in NW Iberian Peninsula waters (Gregori et al. 2012, 2013, 2015). It was additionally reported as intermediate host of *A. simplex* on the Scottish East Coast and of *Hysterothylacium* sp. on the Scottish East Coast and Portuguese Coast (Smith 1983). Furthermore, it is the main component of the marine diet of *A. alosa* and the secondary diet of *A. fallax* (Taverny and Elie 2001a). It is also the main euphausiid in the European continental shelf, with high concentrations present near the Spanish coast (Roura et al. 2013). Hence, *N. couchii* may represent an important transport host for *A. simplex*, *A. pegreffii*, *H. aduncum* and *R. pristis* to both *Alosa* spp. off Western Iberian Marine waters.

The high infection values of these anisakid and rhabdiascarid nematodes found in *A. alosa* and the comparatively low ones found in *A. fallax* (Table 4) appear to be consistent with their feeding habits, linking zooplankton as intermediate hosts and main transmission vectors through shad (especially *A. alosa*) and small pelagic fish as paratenic hosts and secondary transmission vectors through shad (especially *A. fallax*). In relation to this, small pelagic fish usually carry low anisakid burdens, or at least lower than bigger specimens of the same species, since accumulation of parasites during the host lifetime has been previously reported in numerous studies (Mladineo and Poljak 2014 and references therein), which may explain the relatively low anisakid infection values of *A. fallax*. When shad samples from the same river (Minho) and sampling year (2011) were

compared, *Anisakis* spp. also showed higher infection values in *A. alosa* than *A. fallax* (Table 5). Moreover, the presence of *R. pristis* in *A. alosa* but not in *A. fallax* also supports the previously suggested “parasite-host” transmission pathway by feeding routes.

The life cycle of *H. appendiculatus* is not known but may be assumed to follow a similar pattern to those of other hemiurids, having a marine mollusc as first intermediate host, metacercariae in second intermediate hosts (crustaceans, especially copepods and chaetognaths) and adults in the stomach of *Alosa* spp. (Gibson and Bray 1986; Moravec 2004). In relation to this, cercariae of the hemiurid digenean *H. communis* were found in the opisthobranch snail *Retusa truncatula* and calanoid copepods were found to be the second intermediate hosts (Køie 1995). Likewise, cercariae of *Hemiurus luehei* were found in the opisthobranch *Philine denticulata*, while the chaetognath *Sagitta* sp. was found to be naturally infected by metacercariae, probably by feeding on its second intermediate hosts, calanoid copepods (*Temora longicornis*, *Acartia tonsa*, unidentified copepod), which were shown to be susceptible to infection by metacercaria of *H. luehei* (Køie 1990). Thus, the high hemiurid infection found in *A. alosa* (Tables 2 and 4) is in accordance with their zooplanktophagous diet. However, the high values of *H. appendiculatus* found in *A. fallax* (Tables 2 and 4) suggest that zooplankton (especially calanoid copepods) could also be an important part of their diet in the marine environment, which may have been underestimated previously. Moreover, comparison of hemiurid infection values of both shad in the River Minho in 2011 showed even higher values of *H. appendiculatus* in *A. fallax* than in *A. alosa* which is consistent with this hypothesis (Table 5).

Mazocraes alosae, *C. italica* and *C. emarginata* are ectoparasites with direct life cycles, so reinfection or parasite transmission from infected to uninfected specimens will occur directly.

Shad parasites as biological tags

Parasites can be used effectively as biological tags or indicators in population studies of their hosts. This method is particularly useful for anadromous fish species which acquire different parasites during their stay in freshwater, brackish and marine environments in the course of their migrations (MacKenzie and Hemmingsen 2014). Bao et al. (2015) found that levels of infection with *A. pegreffii* were higher than those of *A. simplex* in all Western Iberian shad samples. *Anisakis pegreffii* is more common further south off the coast of Portugal, which suggests that these shad became infected during feeding migrations to these southern areas. This hypothesis is supported by the occurrence in our samples of the isopod *C. italica*, which was previously reported as a parasite of sparid fishes in the Mediterranean and off the northwest coast of Africa (Horton 2000). The marine cestode *Eubothrium fragile*

is a specific parasite of shad (*Alosa* spp.) but appears to be restricted to northern Europe (Kennedy 1981; Aprahamian et al. 2002; Kuchta et al. 2005) and has not been reported from shad south of the Bay of Biscay. We did not find *E. fragile* in our samples, which suggests that Western Iberian shad do not migrate to more northern feeding grounds. In this regard, Martin et al. (2015), based on otolith microchemistry and microsatellite genetic analyses, reported that migrations of *A. alosa* of hundreds of kilometres might occur, either south or northward from natal to spawning rivers, even though such long distance straying was not frequent. Therefore, migration of some Western Iberian shad to northern feeding grounds cannot be completely discounted.

General comments

Finally, we report the first presence of *M. alosae* and *C. emarginata* in NW Iberia. This finding is not unexpected since both are specific parasites of *Alosa* spp. and this is the first time that the macroparasite community of shad has been described in this area. In this regard, *M. alosae* and *C. emarginata* were found only in *A. alosa* and *A. fallax* respectively, from River Minho, whereas infections of both gill parasites in both *Alosa* spp. might be expected. Nonetheless, further assumptions cannot be made since a comparison is not possible because gill samples of *A. alosa* were only available in 2013, but no *A. fallax* samples were available that year.

Significantly decreasing abundances were found for *H. appendiculatus*, *H. aduncum* and *Anisakis* spp. in *A. alosa* during 2009, 2010 and 2011 (Table 6), but this decrease in infection was not always confirmed for prevalence of infection. This variation of annual infection values is not easily explained since parasite burdens can be influenced by many biotic and abiotic variables (Kleinertz et al. 2012; Mladineo and Poljak 2014). Further research will be needed to confirm or refute this trend.

To conclude, both shad species are shown to represent suitable final hosts for a number of ecto- and endo-parasite species. They also form strong connections between intermediate hosts (zooplankton) and larger transport or definitive hosts (larger fish and marine mammals), in both freshwater and marine environments of Western Iberia. Monitoring of parasite diversity introduced from the marine environment to the freshwater ecosystem is thus desirable to confirm these connections and also to control parasite and/or allergen risk to human consumers and riverine mammals due to zoonotic *A. pegreffii* and *A. simplex* s.s. (Bao et al. 2015). In relation to this, it is noteworthy to mention that an Inuk woman was previously diagnosed with gastric anisakiasis after the ingestion of raw anadromous fish (arctic char, *Salvelinus alpinus alpinus*) caught in a local river of northern Quebec (Canada) (Bhat and Cleland 2010). Our results provide new information regarding the life cycle and ecology of these macroparasites

and also suggest host feeding habits during the marine trophic phase and migration patterns of *A. alosa* and *A. fallax* in the Western Iberian Peninsula. Overall, this study contributes to a better understanding of the phylogenetic relationships within monogenean and digenean platyhelminthes as well as to the diverse order of the Siphonostomatoid copepods. Further research integrating the use of parasites as biological tags in a multidisciplinary approach (i.e. molecular genetics, biometrics, life histories, modelling, otolith microchemistry, artificial tags) (Catalano et al. 2014) with appropriate statistical methods would be desirable to confirm feeding behaviour and migration routes and also to determine recruitment and aggregation patterns at sea and to differentiate stocks of these vulnerable shad species in Western Iberia.

Acknowledgments The authors sincerely thank M.N. Cueto, J.M. Antonio and M.E. Garci of the ECOBIOMAR group at IIM-CSIC for molecular analysis, technical support and quality images of some parasites. M. Bao is supported by a PhD grant from the University of Aberdeen and also by financial support of the contract from the EU Project PARASITE (grant number 312068). A. Roura is supported by “Fundación Barrié de la Maza” postdoctoral fellowship and a Securing Food, Water and the Environment Research Focus Area grant (La Trobe University). This study was partially supported by a PhD grant from the Portuguese Foundation for Science and Technology (FCT) (SFRH/BD/44892/2008) and partially supported by the European Regional Development Fund (ERDF) through the COMPETE—Operational Competitiveness Programme and national funds through FCT—Foundation for Science and Technology, under the project “PEst-C/MAR/LA0015/2013. The authors thank the staff of the Station of Hydrobiology of the USC “Encoro do Con” due their participation in the surveys, with special mention to J. Sánchez for separating digenean fauna existing in the stomachs of *A. fallax*. This work has been partially supported by the project 10PXIB2111059PR of the Xunta de Galicia and the project MIGRANET of the Interreg IV B SUDOE (South-West Europe) Territorial Cooperation Programme (SOE2/P2/E288). D.J. Nachón is supported by a PhD grant from the Xunta de Galicia (PRE/2011/198).

Conflict of interests The authors declare that they have no competing interests.

Authors’ contributions MB and SP conceived and designed the study; MB, MM, DJN, CA and FC collected the data and collaborated in designing the study; MB performed the morphological identification studies; MB and AR performed the molecular genetic and phylogenetic studies; MB and KM performed the statistical analysis; MB, AR and KM analyzed the data and drafted the manuscript. All authors critically reviewed the manuscript and gave the final approval of the version to be published.

References

- Acolas ML, Bégout-Anras ML, Véron V, Jourdan H, Sabatié MR, Baglinière JL (2004) An assessment of upstream migration and reproductive behaviour of allis shad (*Alosa alosa* (L.)) using acoustic tracking. ICES J Mar Sci 61(8):1291–1304
- Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19:716–722

- Akmirza A (2013) Monogeneans of fish near Gökçeada, Turkey. *Turk Zool Derg* 37:441–448
- Aprahamian MW (1989) The diet of juvenile and adult twaite shad *Alosa fallax fallax* (Lacépède) from the rivers Severn and Wye (Britain). *Hydrobiologia* 179:173–182
- Aprahamian MW, Baglinière JL, Sabatié MR, Alexandrino P, Aprahamian CD (2002) Synopsis of biological data on *Alosa alosa* and *Alosa fallax* spp. R&D Technical Report W1-014. Environment Agency R&D Dissemination Center, WRc, Frankland Road, Swindon, Wilts. SN5 8YF, pp. 314
- Arthur JR (1997) Recent advances in the use of parasites as biological tags for marine fish. In: Flegel TW, MacRae IH (eds) Diseases in Asian aquaculture III. Fish Health Section, Asian Fisheries Society, Manila, pp 141–154
- Assis CA, Almeida PR, Moreira F, Costa JL, Costa MJ (1992) Diet of twaite shad *Alosa fallax* (Lacépède) (Clupeidae) in the River Tagus Estuary, Portugal. *J Fish Biol* 41:1049–1050
- Baglinière JL (2000) Le genre *Alosa* sp. In: Baglinière JL, Elie P (eds) Les aloses (*Alosa alosa* et *Alosa fallax* spp.). Écobiologie et variabilité des populations. CEMAGREF-INRA Editions, Paris, pp 3–30
- Baglinière JL, Sabatié MR, Rochard E, Alexandrino P, Aprahamian MW (2003) The Allis shad *Alosa alosa*: biology, ecology, range, and status of populations. *Am Fish Soc Symp* 35:85–102
- Bao M, Mota M, Nachón DJ, Antunes C, Cobo F, Garcí ME, Pierce GJ, Pascual S (2015) *Anisakis* infection in allis shad, *Alosa alosa* (Linnaeus, 1758), and twaite shad, *Alosa fallax* (Lacépède, 1803) from Western Iberian Peninsula Rivers: zoonotic and ecological implications. *Parasitol Res* 114(6):2143–2154
- Berland B (1989) Identification of larval nematodes from fish. In: Möller H (ed) Nematode problems in North Atlantic Fish. Workshop Report, Kiel, Germany, 3–4 April 1989. Int Councl Explor Sea CM/F:6, pp 16–22
- Bern Convention (1979) Convention on the Conservation of European Wildlife and Natural Habitats. Council of Europe: Bern, Switzerland. European Treaty Series number 104
- Bhat M, Cleland P (2010) Gastric anisakiasis. *Clin Gastroenterol Hepatol* 8(8):A20
- Blair D, Bray RA, Barker SC (1998) Molecules and morphology in phylogenetic studies of the Hemiuroidea (Digenea: Trematoda: Platyhelminthes). *Mol Phylogenet Evol* 9(1):15–25
- Boeger WA, Kritsky DC (1997) Coevolution of the Monogeneoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *Int J Parasitol* 27(12):1495–1511
- Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 83(4): 575–583
- Bychowsky BE (1961) Monogenetic trematodes their systematics and phylogeny. English translation edited by Hargis WJ Jr. Washington: American Institute of Biological Sciences
- Cabral MJ, Almeida J, Almeida PR, Dellinger T, Ferrand de Almeida N, Oliveira ME, Palmeirim JM, Queiroz AL, Rogado L, Santos-Reis M (eds) (2006) Red book of the Portuguese vertebrates. 2nd ed. Instituto da Conservação da Natureza Assírio & Alvim Lisboa
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–552
- Catalano SR, Whittington ID, Donnellan SC, Gillanders BM (2014) Parasites as biological tags to assess host population structure: guidelines, recent genetic advances and comments on a holistic approach. *Int J Parasitol Parasites Wildl* 3:220–226
- Ceyhan T, Akyol O, Sever TM, Kara A (2012) Diet composition of adult twaite shad (*Alosa fallax*) in the Aegean Sea (Izmir Bay, Turkey). *J Mar Biol Assoc UK* 92(3):601–604
- Cobo F, Nachón DJ, Vieira-Lanero R, Barca S, Sánchez-Hernández J, Rivas S, Couto MT, Gómez P, Silva S, Morquecho C, Lago L, Servia MJ (2010) Seguimento da poboación de saboga ou zaborca (*Alosa fallax* Lacépède, 1803) no río Ulla. Estación de Hidrobioloxía de “Encoro do Con”-Universidade de Santiago de Compostela. Xunta de Galicia
- Costa MJ, Almeida PR, Domingos IM, Costa JL, Correia MJ, Chaves ML, Teixeira CM (2001) Present status of the main shads’ populations in Portugal. *Bull Fr Peche Piscic* 362(363):1109–1116
- Cribb TH, Bray RA, Olson PD, Timothy D, Littlewood J (2003) Life cycle evolution in the Digenea: a new perspective from phylogeny. *Adv Parasitol* 54:197–254
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772
- Dawes B (1968) The trematoda. Cambridge University Press, London
- Dezfuli BS, Giari L, Simoni E, Menegatti R, Shinn AP, Manera M (2007) Gill histopathology of cultured European sea bass, *Dicentrarchus labrax* (L.), infected with *Diplectanum aequans* (Wagener 1857) Diesing 1958 (Diplectanidae: Monogenea). *Parasitol Res* 100:707–713
- Doadrio I, Perea S, Garzón-Heydt P, González JL (2011) Ictiofauna continental española. Bases para su seguimiento. DG Medio Natural y Política Forestal. MARM, Madrid
- Doherty D, O’Maoiléidigh N, McCarthy TK (2004) The biology, ecology and future conservation of twaite shad (*Alosa fallax* Lacépède), allis shad (*Alosa alosa* L.) and Killarney shad (*Alosa fallax killarneyensis* Tate Regan) in Ireland. *Biol Environ Proc R Ir Acad* 104B:93–102
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Gibson DI, Bray RA (1979) The Hemiuroidea: terminology, systematics and evolution. *Bull Br Mus Nat Hist Zool* 36:35–146
- Gibson DI, Bray RA (1986) The Hemiuridae (Digenea) of fishes from the north-east Atlantic. *Bull Br Mus Nat Hist Zool* 51:1–125
- Golvan YJ (1969) Systématique des acanthocéphales (Acanthocephala Rudolphi 1801). Première partie l’ordre des Palaeacanthocephala Meyer 1931, première fascicule la super-famille des Echinorhynchoidea (Cobbold 1896) Golvan et Houin 1963. *Mém Mus Natn Hist Nat* 57:1–373
- Gregori M, Aznar FJ, Abollo E, Roura A, González AF, Pascual S (2012) *Nyctiphanes couchii* as intermediate host for the acanthocephalan *Bolbosoma balaenae* in temperate waters of the NE Atlantic. *Dis Aquat Organ* 99:37–47
- Gregori M, Aznar FJ, Abollo E, Roura A, González AF, Pascual S (2013) *Nyctiphanes couchii* as intermediate host for *Rhadinorhynchus* sp. (Acanthocephala, Echinorhynchidae) from NW Iberian Peninsula waters. *Dis Aquat Organ* 105:9–20
- Gregori M, Roura A, Abollo E, González AF, Pascual S (2015) *Anisakis simplex* complex (Nematoda: Anisakidae) in zooplankton communities from temperate NE Atlantic waters. *J Nat Hist* 49:755–773
- Habitats Directive (1992) Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora. *Off J L206:7–50*
- Horton T (2000) *Ceratothoa steindachneri* (Isopoda: Cymothoidea) new to British waters with a key to north-east Atlantic and Mediterranean *Ceratothoa*. *J Mar Biol Assoc UK* 80(6):1041–1052
- Huys R, Llewellyn-Hughes J, Olson PD, Nagasawa K (2006) Small subunit rDNA and Bayesian inference reveal *Pectenophilus ornatus* support assignment of Chondracanthidae and Xarifiidae to Lichomolgoidea (Cyclopoida). *Biol J Linn Soc* 87:403–425
- Huys R, Llewellyn-Hughes J, Conroy-Dalton S, Olson PD, Spinks JN, Johnston DA (2007) Extraordinary host switching in siphonostomatoid copepods and the demise of the Monstrilloidea: integrating molecular data, ontogeny and antennular morphology. *Mol Phylogenet Evol* 43:368–378
- Jolly MT, Aprahamian MW, Hawkins SJ, Henderson PA, Henderson PA, Hillman R, O’Maoiléidigh N, Maitland PS, Piper R, Genner MJ (2012) Population genetic structure of protected allis shad (*Alosa alosa*) and twaite shad (*Alosa fallax*). *Mar Biol* 159:675–687

- Jovelin R, Justine JL (2001) Phylogenetic relationships within the polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. *Int J Parasitol* 31:393–401
- Kabata Z (1979) Parasitic copepoda of British fishes. Ray Society, London, p 468
- Kabata Z (1992) Copepods parasitic on fishes: keys and notes for identification of the species. *Synopses Br Fauna New Ser* 47:1–264
- Kennedy CR (1981) The occurrence of *Eubothrium fragile* (Cestoda: Pseudophyllidae) in twaite shad, *Alosa fallax* (Lacépède) in the River Severn. *J Fish Biol* 19:171–177
- Kleinertz S, Klimpel S, Palm HW (2012) Parasite communities and feeding ecology of the European sprat (*Sprattus sprattus* L.) over its range of distribution. *Parasitol Res* 110:1147–1157
- Koie M (1990) On the morphology and life-history of *Hemiurus luehei* Odhner, 1905 (Digenea: Hemiuridae). *J Helminthol* 64:193–202
- Koie M (1993) Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). *Can J Zool* 71(7):1289–1296
- Koie M (1995) The life-cycle and biology of *Hemiurus communis* Odhner, 1905 (Digenea, Hemiuridae). *Parasite* 2(2):195–202
- Kuchta R, Hanzelová V, Shinn AP, Poddubnaya LG, Scholz T (2005) Redescription of *Eubothrium fragile* (Rudolphi, 1802) and *E. rugosum* (Batsch, 1786) (Cestoda: Pseudophyllidae), parasites of fish in the Holarctic region. *Folia Parasitol* 52:251–260
- La Mesa G, Annunziatellis A, Jr E-F, Fortuna CM (2015) Modeling environmental, temporal and spatial effects on twaite shad (*Alosa fallax*) by-catches in the central Mediterranean Sea. *Fish Oceanogr* 24(2):107–117
- Li M, Shi S-F, Brown CL, Yang T-B (2011) Phylogeographical pattern of *Mazocraeoides gonialosae* (Monogenea, Mazocraeidae) on the dotted gizzard shad, *Konosirus punctatus*, along the coast of China. *Int J Parasitol* 41(12):1263–1272
- Lia RP, Zizzo N, Tinelli A, Lionetti A, Cantacessi C, Otranto D (2007) Mass mortality in wild greater amberjack (*Seriola dumerili*) infected by *Zeuxapta seriolae* (Monogenea: Heteraxinidae) in the Ionian Sea. *Bull Eur Assoc Fish Pathol* 27(3):108–111
- Littlewood DTJ, Rohde K, Clough KA (1999) The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biol J Linn Soc* 66:75–114
- MacKenzie K (1987) Parasites as indicators of host populations. *Int J Parasitol* 17(2):345–352
- MacKenzie K, Hemmingsen W (2014) Parasites as biological tags in marine fisheries research: European Atlantic waters. *Parasitology* 142(01):54–67
- Maitland PS, Lyle AA (2005) Ecology of Allis shad *Alosa alosa* and Twaite shad *Alosa fallax* in the Solway Firth, Scotland. *Hydrobiologia* 534:205–221
- Mamaev YL (1982) Notes on the systematics of mazocraeid monogeneans with a redescription of some poorly studied taxa. *Helminthologia* 19:25–39
- Martin J, Rougemont Q, Drouineau H, Launey S, Jatteau P, Bareille G, Beraïl S, Pécheyran C, Feunteun E, Roques S, Clavé D, Nachón DJ, Antunes C, Mota M, Réveillac E, Daverat F (2015) Dispersal capacities of anadromous Allis shad population inferred from a coupled genetic and otolith approach. *Can J Fish Aquat Sci*. doi:10.1139/cjfas-2014-0510
- MIGRANET (2012) Observatorio de las Poblaciones de Peces Migradores en el Espacio SUDOE. Informe General del Estado de Conservación y Amenazas de las Especies de Peces Migradores: Ríos Ulla y Umia de Galicia (España); Río Nivelle de Aquitaine (Francia) y Tramo Internacional del Río Miño (Portugal-Norte)
- Mladineo I, Poljak V (2014) Ecology and genetic structure of zoonotic *Anisakis* spp. from Adriatic commercial fish species. *Appl Environ Microbiol* 80(4):1281–1290
- Mollaret I, Jamieson BGM, Justine JL (2000) Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *Int J Parasitol* 30:171–185
- Moravec F (2004) Metazoan parasites of salmonid fishes of Europe. Academia (Praha). pp 510
- Mota M, Antunes C (2011) First report on the status of Allis shad (*Alosa alosa*) in the Minho River (Northwestern Iberian Peninsula). *J Appl Ichthyol* 27(3):56–59
- Mota M, Antunes C (2012) A preliminary characterization of the habitat use and feeding of Allis shad (*Alosa alosa*) juveniles in the Minho river tidal freshwater wetlands. *Limnetica* 31(1):165–172
- Mota M, Bio A, Bao M, Pascual S, Rochard E, Antunes C (2015) New insights into biology and ecology of the Minho River Allis shad *Alosa alosa* L.—contribution to the conservation of one of the last European shad populations. *Rev Fish Biol Fish* 25(2):395–412
- Nachón DJ, Sánchez-Hernández J, Vieira-Lanero R, Cobo F (2013) Feeding of twaite shad, *Alosa fallax* (Lacépède, 1803), during the upstream spawning migration in the River Ulla (NW Spain). *Mar Freshw Res* 64:233–236
- Nunn AD, Noble RAA, Harvey JP (2008) The diets and parasites of larval and 0+ juvenile twaite shad in the lower reaches and estuaries of rivers Wye, Usk, and Towy, UK. *Hydrobiologia* 614:209–218
- Olson PD, Littlewood DTJ (2002) Phylogenetics of the Monogenea—evidence from a medley of molecules. *Int J Parasitol* 32:233–244
- Olson PD, Tkach VV (2005) Advances and trends in the molecular systematics of the parasitic platyhelminthes. *Adv Parasitol* 60:165–243
- Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *Int J Parasitol* 33:733–755
- OSPAR (2009) Background document for Allis shad *Alosa alosa*. Biodiversity series. Publication Number: 418/2009
- Özer A, Öztürk T, Komyychuk Y (2013) First report of *Mazocraes alosae* (Herman, 1782), *Pronoprymna ventricosa* (Rudolphi, 1891) and *Lecithaster confusus* Odhner, 1905 in pontic shad *Alosa immaculata* Bennet, 1835 near Turkish coast of the Black Sea. *Lucrări Științifice Ser Zootehnie* 59:311–314
- Pankov P, Webster BL, Blasco-Costa I, Gibson DI, Littlewood DTJ, Balbuena JA, Kostadinova A (2006) *Robinia aurata* n.g., n. sp. (Digenea: Hemiuridae) from the mugilid *Liza aurata* with a molecular confirmation of its position within the Hemiuroidea. *Parasitology* 133(2):217–227
- Pereira TG, Batista I, Bandarra NM, Ferreira J, Fradinho N, Afonso F (2013) Chemical composition and nutritional value of raw and fried allis shad (*Alosa alosa*). *Int J Food Sci Technol* 48:1303–1308
- Rodrigues HO, Carvalho-Varela M, Rodrigues e Rigoletto SS (1972) Alguns trematódeos digenéticos de peixes do Oceano Atlântico—Costa Continental Portuguesa Costa Continental de África [Some digenic trematodes of fish from the Atlantic Ocean—the continental Portuguese coast and the continental African coast]. *Atlas da Sociedade De Biologia do Rio De Janeiro* 15:87–93
- Rokicki J, Rolbiecki L, Skóra A (2009) Helminth parasites of twaite shad, *Alosa fallax* (Actinopterygii: Clupeiformes: Clupeidae), from the Southern Baltic Sea. *Acta Ichthyol Piscat* 39(1):7–10
- Rosen DE (1982) Teleostean interrelationships, morphological function and evolutionary inference. *Am Zool* 22:261–273
- Rougier T, Lambert P, Drouineau H, Girardin M, Castelnaud G, Carry L, Arahamian M, Ribot E, Rochard R (2012) Collapse of Allis shad, *Alosa alosa*, in the Gironde system (southwest France): environmental change, fishing mortality, or Allee effect? *ICES J Mar Sci* 69(10):1802–1811
- Roura A, Álvarez-Salgado XA, González AF, Gregori M, Rosón G, Guerra A (2013) Short-term meso-scale variability of mesozooplankton communities in a coastal upwelling system (NW Spain). *Prog Oceanogr* 109:18–32
- Sabatié MR (1993) Recherches sur l'écologie et la biologie des aloses au Maroc (*Alosa alosa* Linné, 1758 et *Alosa fallax* Lacépède, 1803):

- exploitation et taxonomie des populations atlantiques, bioécologie des aloses de l'Ouest Sebou. Ph.D. thesis. University of Bretagne Occidentale, Brest
- Sala-Bozano M, Van-Oosterhout C, Mariani S (2012) Impact of a mouth parasite in a marine fish differs between geographical areas. *Biol J Linn Soc* 105:842–852
- Shih HH, Jeng MS (2002) *Hysterothylacium aduncum* (Nematoda: Anisakidae) infecting a herbivorous fish, *Siganus fluorens*, off the Taiwanese coast of the Northwest Pacific. *Zool Stud* 41(2):208–215
- Silva S, Servia MJ, Vieira-Lanero R, Cobo F (2013) Downstream migration and hematophagous feeding of newly metamorphosed sea lampreys (*Petromyzon marinus* Linnaeus, 1758). *Hydrobiologia* 700(1): 277–286
- Skóra ME, Sapota MR, Skóra KE, Pawelec A (2012) Diet of twaite shad *Alosa fallax* (Lacépède, 1803) (Clupeidae) in the Gulf of Gdansk, the Baltic Sea. *Oceanol Hydrobiol Stud* 41(3):24–32
- Smith JW (1983) Larval *Anisakis simplex* (Rudolphi, 1809, det. Krabbe, 1878) and larval *Hysterothylacium* sp. (Nematoda: Ascaridoidea) in euphausiids (Crustacea: Malacostraca) in the North-East Atlantic and northern North Sea. *J Helminthol* 57:167–177
- Solórzano M (2004) Peixes. In: Viéitez E, Rey JM (eds) A natureza ameazada. Consello da Cultura Galega. Sección de Patrimonio Cultural
- Suzuki N, Hoshino K, Murakami K, Takeyama H, Chow S (2008) Molecular diet analysis of Phyllosoma larvae of the Japanese spiny lobster *Palinurus japonicus* (Decapoda: Crustacea). *Mar Biotechnol* 10:49–55
- Taverny C, Elie P (2001a) Répartition spatio-temporelle de la grande alose *Alosa alosa* (Linné, 1766) et de L'alose feinte *Alosa fallax* (Lacépède, 1803) dans le golfe de Gascogne. *Bull Fr Peche Piscic* 362(363):803–821
- Taverny C, Elie P (2001b) Régime alimentaire de la grande alose *Alosa alosa* (Linné, 1766) et de L'alose feinte *Alosa fallax* (Lacépède, 1803) dans le golfe de Gascogne. *Bull Fr Peche Piscic* 362(363): 837–852
- Taverny C, Cassou-Leins JJ, Cassou-Leins F, Elie P (2000) De l'oeuf à l'adulte en mer. In: Baglinière JL, Elie P (eds) Les aloses (*Alosa alosa* et *Alosa fallax* spp.). Écobiologie et variabilité des populations. INRACEMAGREF, Paris, pp 93–124
- Trancart T, Rochette S, Acou A, Lasne E, Feunteun E (2014) Modeling marine shad distribution using data from French bycatch fishery surveys. *Mar Ecol Prog Ser* 511:181–192
- Williams HH, MacKenzie K, McCarthy AM (1992) Parasites as biological indicators of the population biology, migrations, diet and phylogenetics of fish. *Rev Fish Biol Fish* 2:144–176
- XUNTA (2008) Plan Galego de Ordenación dos recursos piscícolas e ecosistemas acuáticos continentais. Xunta de Galicia
- Yamaguti S (1963) *Systema helminthum*, vol V, Acanthocephala. Wiley Interscience, New York
- Yamaguti S (1968) *Systema helminthum*, vol IV, Monogenea and Aspidogotylea. Wiley Interscience, New York
- Zhu X, Gasser RB, Jacobs DE, Hung GC, Chilton NB (2000) Relationship among some ascaridoid nematodes based on ribosomal DNA sequence data. *Parasitol Res* 86:738–744