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To cite this article: E. Mancini, G. Furfaro, M. Cervelli, A. Di Giulio, M. Oliverio, D. Salvi & P. Mariottini (2018) Molecular detection of parasites (Trematoda, Digenea: Bucephalidae and Monorchidae) in the European flat oyster *Ostrea edulis* (Mollusca: Bivalvia), *The European Zoological Journal*, 85:1, 8-16, DOI: [10.1080/24750263.2017.1420829](https://doi.org/10.1080/24750263.2017.1420829)

To link to this article: <https://doi.org/10.1080/24750263.2017.1420829>



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Published online: 15 Jan 2018.



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Molecular detection of parasites (Trematoda, Digenea: Bucephalidae and Monorchidae) in the European flat oyster *Ostrea edulis* (Mollusca: Bivalvia)

E. MANCINI^{1*}, G. FURFARO¹, M. CERVELLI¹, A. DI GIULIO¹, M. OLIVERIO ²,
D. SALVI^{3,4}, & P. MARIOTTINI¹

¹Department of Sciences, Roma Tre University, Rome, Italy, ²Department of Biology and Biotechnologies “C. Darwin”, Sapienza University of Rome, Rome, Italy, ³Department of Health, Life and Environmental Sciences, University of L’Aquila, L’Aquila, Italy, and ⁴CIBIO-InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, Vairão, Portugal

(Received 29 September 2017; accepted 16 December 2017)

Abstract

Members of the globally distributed bivalve family Ostreidae (oysters) have a significant role in marine ecosystems and include species of high economic importance. In this work, we report the occurrence of digenean parasites of the families Bucephalidae (*Proisorhynchoides* sp.) and Monorchidae (*Postmonorchis* sp.) in Mediterranean native populations of *Ostrea edulis* (but not in the introduced *Magallana gigas*). Molecular detection was based on DNA sequencing of the ribosomal intergenic spacer 2 (ITS2) marker. The importance of detecting the presence of overlooked digenean parasites in Mediterranean oysters is discussed.

Keywords: *Ostrea edulis*, *Magallana gigas*, *Trematoda* parasites, *Mediterranean Sea*, *ITS2*

Introduction

Oysters (family Ostreidae) are cosmopolitan sessile filter-feeder bivalves that play a remarkable ecological role in estuarine, intertidal and shallow-water ecosystems, through the mitigation of the excess of nutrients, algae and sediment. Among the *c.* 75 currently known species, many are of economic importance, being voluminously produced by the aquaculture industry. In Europe, oyster fisheries were historically based entirely on the autochthonous flat oyster *Ostrea edulis* Linnaeus, 1758, which occurs on hard substrata in estuarine and shallow coastal waters of the eastern Atlantic, from Scandinavia to North Africa, and into the Mediterranean Sea as far as the Black Sea (Yonge 1960). Oyster cultivation, based on the management of natural stocks, dates to the 17th century in Japan and earlier in China, and to Roman times in Europe, over

2000 years ago (Yonge 1960, 1970). A widespread decline occurred at the end of the 19th century (Gosling 2003) because of overfishing, habitat destruction and pollution (Orton 1937; Gosling 2003; Kirby 2004). Furthermore, the spread of a parasitic disease due to the haplosporidian protozoan *Bonamia ostreae* (Pichot et al. 1980), included in the International Aquatic Animal Health Code by the World Organisation for Animal Health (<http://www.oie.int>), led to massive mortality of European flat oysters in the last century (Renault et al. 1995). This has caused a shift to the rearing in Europe of the Pacific cupped oyster *Magallana gigas* Thunberg, 1793 (formerly *Crassostrea gigas*; nomenclature after Salvi and Mariottini 2017; see also Bouchet and Marshall 2016), native to the Pacific coast of Asia, but introduced into North America, Australia, Europe and

*Correspondence: E. Mancini, Department of Sciences, Roma Tre University, V. G. Marconi 446, I-00146, Rome, Italy. Tel: +339 06 57336359. Fax: +339 06 57336321. Email: emiliano.mancini@uniroma3.it

New Zealand. In 2004 *M. gigas* represented the bulk of farmed oyster world production (96.2%), whereas the production of the European flat oyster represented less than 0.11% of the total global production of all farmed oyster species (Svåsand et al. 2007). Today *M. gigas* is still among the most cultivated oysters, with a global annual production estimated at 583,464 tons, as compared to 2872 tons of *O. edulis* in 2015 (<http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en>). Monitoring of *O. edulis* and *M. gigas* populations for the presence (and putative overlapping) of parasite communities is necessary to set optimal aquaculture rearing practices and, thus, prevent or limit risks of cross-contamination (Mineur et al. 2014), as also specified by the World Organization for Animal Health – International Aquatic Animal Health Code (Alday-Sanz 2009). It is noteworthy that the co-presence in both oyster species of parasites, especially flatworms, is known in the natural environment (Aguirre-Macedo & Kennedy 1999). During laboratory experiments for a molecular phylogenetic study of Ostreidae (Salvi et al. 2014), the co-occurrence of Ostreidae-specific and non-target Polymerase Chain Reaction (PCR) products of the rRNA intergenic-spacer 2 (ITS2) was occasionally assessed as multiple bands in gel electrophoresis. These results suggested that the slightly degenerated ITS2 universal primers employed, originally designed by Oliverio and Mariottini (2001) and herein named ITS2-3d* and ITS2-4r*, successfully amplified the target gene fragment both in oysters and in organisms associated with the analysed oyster tissues. Hence, we aimed to

taxonomically identify the source of these ITS2-extra bands on a larger oyster sample by analysing a total of 36 specimens of *O. edulis* and *M. gigas* collected at 18 sites (Figure 1) over a period of 3 years (2014–2016; Table I).

Materials and methods

Genomic DNA was extracted using the “DNeasy Blood and Tissue Kit” (Qiagen, Hilden, Germany) from mantle tissues of each alcohol-preserved specimen of *O. edulis* and *M. gigas*. Before PCR amplification, quality and quantity of the extracted DNA were verified by 1% agarose gel electrophoresis runs. ITS2 amplifications were performed on all collected specimens of *O. edulis* (n = 18) and *M. gigas* (n = 18) using slightly degenerated ITS2 universal primers derived from the ones described by Oliverio and Mariottini (2001) and named ITS2-3d* (5'-GCATCGRTGAAGARCGCAG-3') and ITS2-4r* (5'-AGTTTTYTTYTCCTCCGCTTA-3'), targeting the entire ITS2 region with the following thermal conditions: 3 min at 94°C, 35 cycles of 30 sec at 94°C, 30 sec at 50–55°C, 90 sec at 72°C, 6 min at 72°C for final extension. Oyster-specific ITS2 products were isolated, purified with the “Sure Clean” kit (Bioline) and sequenced by MacroGen Europe (Amsterdam, The Netherlands) (Genbank accession no. MF374290-MF374317). Co-amplified ITS2 extra bands (Figure 2(a,b)) having different molecular weights from those expected for *O. edulis* or *M. gigas* (i.e. ~600 and ~550 bp,

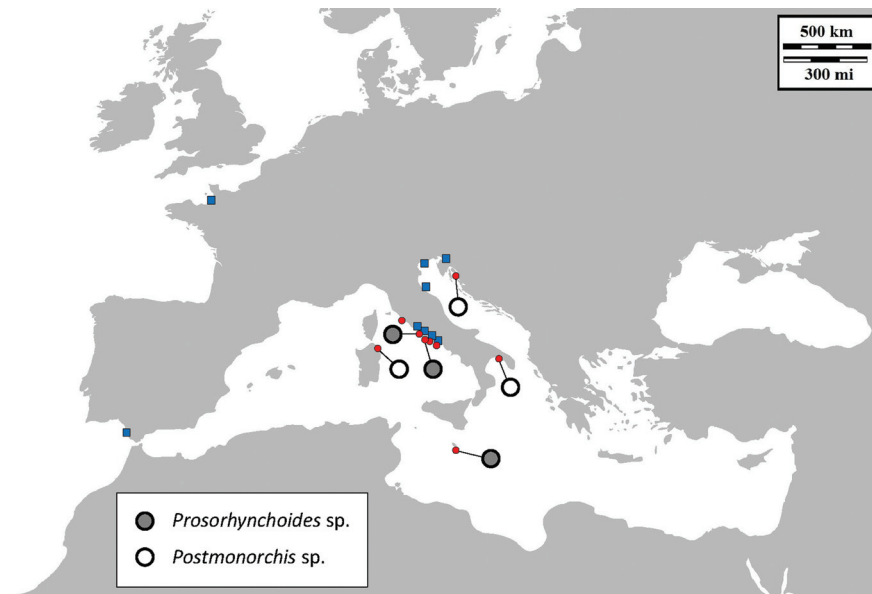


Figure 1. Map of the sampling localities of *Ostrea edulis* (small red circles) and *Magallana gigas* (blue squares). The presence of *Prosorhynchoides* sp. (grey circles) and *Postmonorchis* sp. (empty circles) is reported.

Table I. Geographic information of collection sites for all oyster specimens analysed in this study. Habitat/date of collection sites is reported (* indicates specimens collected in the Atlantic; all other samples were collected in the Mediterranean). The presence/absence of digenean parasites was confirmed by screening samples with the ITS2-DIG1/DIG2 PCR diagnostic tool. Voucher and ITS2 sequence accession numbers for both oysters and parasites are provided (for each locality one parasite specimen was vouchered and its sequence submitted to GenBank).

Species	Specimen voucher	Oyster ITS2 (accession no.)	Sampling locality	Geographic coordinates	Habitat	Parasite taxon	Parasite voucher	Parasite ITS2 (accession no.)
<i>Ostrea edulis</i>	BAU01714	LM993872	Cape Circeo/April 2012	41°13'30"N, 13°05'44"E	12 m depth, rocky substrate/June 2015	Absent	-	-
<i>Ostrea edulis</i>	BAU01715	LM993873	Cape Circeo/April 2012	41°13'30"N, 13°05'44"E	12 m depth, rocky substrate	Absent	-	-
<i>Ostrea edulis</i>	BAU01716	LM993874	Ginosa, Italy/August 2013	40°25'34"N, 16°53'41"E	3 m depth, epibiont on hermit-crabbed shells of <i>Tritia mutabilis</i> /August 2014	<i>Postmonorchis</i> sp.	BAU03025	MF374321
<i>Ostrea edulis</i>	BAU01717	LM993875	Ginosa, Italy/August 2013	40°25'34"N, 16°53'41"E	3 m depth, epibiont on hermit-crabbed shells of <i>Tritia mutabilis</i>	<i>Postmonorchis</i> sp.	-	-
<i>Ostrea edulis</i>	BAU02996	MF374290	Secche di Tor Paterno, Italy/September 2012	41°36'18"N, 12°20'20"E	6 m depth, cemented on a buoy	<i>Prosorhynchoides</i> sp.	BAU03022	MF374318
<i>Ostrea edulis</i>	BAU02997	MF374291	Secche di Tor Paterno, Italy/September 2012	41°36'18"N, 12°20'20"E	6 m depth, cemented on a buoy	<i>Prosorhynchoides</i> sp.	-	-
<i>Ostrea edulis</i>	BAU02998	MF374292	Cape Mount Argentario, Italy/July 2015	42°26'42"N, 11°06'51"E	5 m depth, rocky substrate	Absent	-	-
<i>Ostrea edulis</i>	BAU02999	MF374293	Cape Mount Argentario, Italy/July 2015	42°26'42"N, 11°06'51"E	5 m depth, rocky substrate	Absent	-	-
<i>Ostrea edulis</i>	BAU03000	MF374294	San Nicola, Italy/February 2012	41°55'57"N, 12°06'27"E	2 m depth, rocky substrate	<i>Prosorhynchoides</i> sp.	BAU03023	MF374319
<i>Ostrea edulis</i>	BAU03001	MF374295	San Nicola, Italy/February 2012	41°55'57"N, 12°06'27"E	2 m depth, rocky substrate	<i>Prosorhynchoides</i> sp.	-	-
<i>Ostrea edulis</i>	BAU03002	MF374296	Sliema Harbor, Malta/March 2014	35°54'04"N, 14°30'20"E	5 m depth, rocky substrate	<i>Prosorhynchoides</i> sp.	BAU03024	MF374320
<i>Ostrea edulis</i>	BAU03003	MF374297	Sliema Harbor, Malta/March 2014	35°54'04"N, 14°30'20"E	5 m depth, rocky substrate	<i>Prosorhynchoides</i> sp.	-	-
<i>Ostrea edulis</i>	BAU03004	MF374298	Torvaianica, Italy/February 2015	41°38'11"N, 12°26'31"E	Shored, epibiont on a buoy rope	Absent	-	-
<i>Ostrea edulis</i>	BAU03005	MF374299	Torvaianica, Italy/February 2015	41°38'11"N, 12°26'31"E	Shored, epibiont on a buoy rope	Absent	-	-
<i>Ostrea edulis</i>	BAU03006	MF374300	Rab Island, Croatia/March 2016	44°47'29"N, 14°42'19"E	Cemented on the marina rocky berth, intertidal	<i>Postmonorchis</i> sp.	BAU03029	MF374322
<i>Ostrea edulis</i>	BAU03007	MF374301	Rab Island, Croatia/March 2016	44°47'29"N, 14°42'19"E	Cemented on the marina rocky berth, intertidal	<i>Postmonorchis</i> sp.	-	-
<i>Ostrea edulis</i>	BAU03027	MF374302	Lido del Sole-Olbia, Sardinia/December 2015	40°54'54"N, 09°34'06"E	Epibiont on <i>Pinna nobilis</i> , 2 m depth	<i>Postmonorchis</i> sp.	BAU03030	MF374323
<i>Ostrea edulis</i>	BAU03028	MF374303	Lido del Sole-Olbia, Sardinia/December 2015	40°54'54"N, 09°34'06"E	Epibiont on <i>Pinna nobilis</i> , 2 m depth	<i>Postmonorchis</i> sp.	-	-

(Continued)

Table I. (Continued).

Species	Specimen voucher	Oyster ITS2 (accession no.)	Sampling locality	Geographic coordinates	Habitat	Parasite taxon	Parasite voucher	Parasite ITS2 (accession no.)
<i>Magallana gigas</i>	BAU01706	LM993864	Touristic Harbour San Felice Circeo, Italy/ February 2012	41°13'34"N, 13°05'38"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU01707	LM993865	Touristic Harbour San Felice Circeo, Italy/ February 2012	41°13'34"N, 13°05'38"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU01708	LM993866	Rimini, Italy/August 2015	44°04'43"N, 12°34'17"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU01709	LM993867	Rimini, Italy/August 2015	44°04'43"N, 12°34'17"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03008	MF374304	San Nicola, Italy/January 2015	41°55'57"N, 12°06'29"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03009	MF374305	San Nicola, Italy/January 2015	41°55'57"N, 12°06'29"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03010	MF374306	Canale dei Pescatori-Ostia, Italy/June 2013	41°43'09"N, 12°18'11"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03011	MF374307	Canale dei Pescatori-Ostia, Italy/June 2013	41°43'09"N, 12°18'11"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03012	MF374308	Goro, Italy/August 2013	44°84'07"N, 12°30'26"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03013	MF374309	Goro, Italy/August 2013	44°84'07"N, 12°30'26"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03014	MF374310	Cancalle, France/August 2013	48°04'04"N, 01°51'25"E	Intertidal rocky substrate*	Absent	-	-
<i>Magallana gigas</i>	BAU03015	MF374311	Cancalle, France/August 2013	48°04'04"N, 01°51'25"E	Intertidal rocky substrate*	Absent	-	-
<i>Magallana gigas</i>	BAU03016	MF374312	Cadiz, Spain/April 2014	36°28'05"N, 06°15'10"E	Intertidal rocky substrate*	Absent	-	-
<i>Magallana gigas</i>	BAU03017	MF374313	Cadiz, Spain/April 2014	36°28'05"N, 06°15'10"E	Intertidal rocky substrate*	Absent	-	-
<i>Magallana gigas</i>	BAU03018	MF374314	Touristic Harbour Riva di Traiano, Italy/February 2016	42°03'45"N, 11°48'49"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03019	MF374315	Touristic Harbour Riva di Traiano, Italy February 2016	42°03'45"N, 11°48'49"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03020	MF374316	Harbour of Trieste, Italy/April 2017	45°38'54"N, 13°49'47"E	Intertidal rocky substrate	Absent	-	-
<i>Magallana gigas</i>	BAU03021	MF374317	Harbour of Trieste, Italy/April 2017	45°38'54"N, 13°49'47"E	Intertidal rocky substrate	Absent	-	-

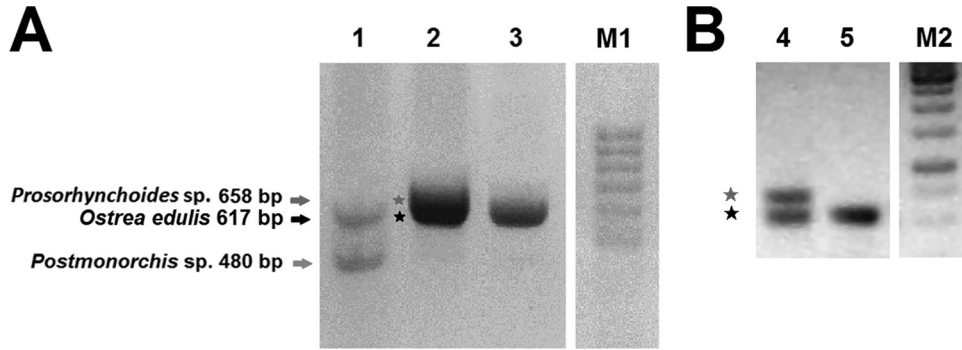


Figure 2. (a) Electrophoresis analyses of double PCR products amplified with ITS2-3d*/4r* in *Ostrea edulis* from Ginosa (lane 1) and from Tor Paterno (lane 2). Specific oyster-product of *Ostrea edulis* from Tor Paterno obtained with ITS2-OED1/OED2 is shown (lane 3). M1 = marker (Hyperladder II, Bioline™). (b) Longer (+ 30 min at 80 V compared to the gel in Figure 2(a)) electrophoretic run of samples of lanes 2 (4) and 3 (5) of panel A samples. M2 = marker GeneRuler 1 kb DNA ladder (MBI Fermentas).

respectively; see Salvi et al. 2014) were observed in some cases. These extra bands were isolated, purified with the “PCR Clean up Gel Extraction” kit (Macherey-Nagel) and sequenced by MacroGen Europe. Non-oyster organisms whose DNA was co-amplified in ITS2 PCRs were taxonomically identified as belonging to two different digenean genera (*Postmonorchis* Hopkins, 1841 and *Proisorhynchoides* Dollfus, 1929; see Results) through the National Center for Biotechnology Information (NCBI) BLASTn tool using default search parameter settings. GenBank matching sequences returning the highest values of “Max Score” (= highest alignment score or bit-score between the query sequence and the database sequence segment), “Query Coverage” (= percentage of the query length included in the aligned segments calculated over all segments) and “Identity”

(percentage of similarity between the query and subject sequences over the length of the coverage area) served to outline the most plausible genera parasitising the collected oysters. The ITS2 sequences of these digeneans were used to design a novel primer pair to target more specifically both parasitic ITS2 sources: ITS2-DIG1 (5'-AATGTGAACTGCGTACTG-3') and ITS2-DIG2 (5'-AAGTTCAGCGGGTATTCA-3') (Figure 3). These novel primers were used as a diagnostic PCR tool to screen the whole oyster sample (n = 36) of *O. edulis* and *M. gigas* to assess (or confirm) the presence of DNA from the two identified digeneans in the oysters' mantle tissues used in DNA extractions [*O. edulis* ITS2 was then specifically amplified using ITS2-OED1 (5'-AATGTGAATTGCAGGACA-3') and ITS2-OED2 (5'-AAGTTCAGGGCGGTAGTCT-3')

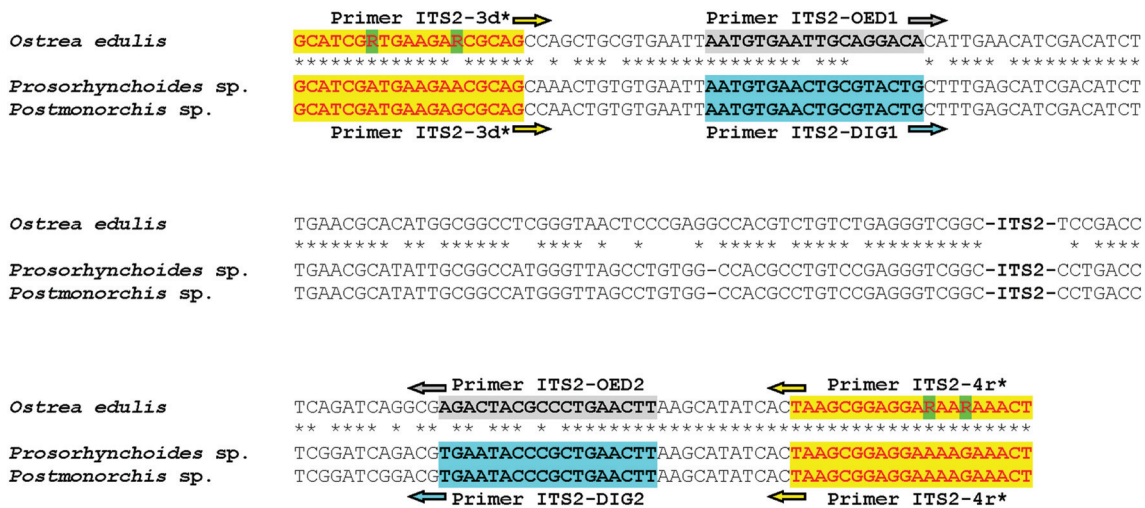


Figure 3. 5.8S and 28S rDNA sequence alignment of *Ostrea edulis*, *Proisorhynchoides* sp. and *Postmonorchis* sp. Specific primers designed and used in this work are boxed and labelled. Primers ITS2-3d*/ITS2-4r* are boxed in yellow; degenerated positions are highlighted in green.

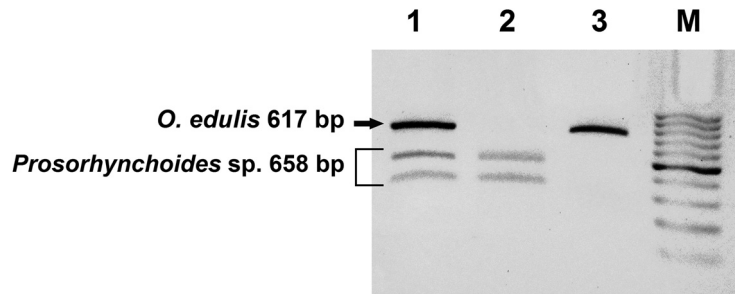


Figure 4. Gel electrophoresis analysis of PCR products from a sample of *Ostrea edulis* parasitised by *Proserhynchooides* sp. from Tor Paterno amplified with ITS2-3d*/4r* (lane 1), ITS2-DIG1/DIG2 (lane 2) and ITS2-OED1/OED2 (lane 3) and digested with *Bgl*III. M = marker (Hyperladder II, Bioline™).

(Figure 3) (see below and Figure 4)]. Restriction enzyme digestion with *Bgl*III was carried on PCR products amplified with ITS2-3r*/4d*, ITS2-DIG1/DIG2 and ITS2-OED1/OED2 to confirm the presence of two distinct (but slightly overlapping) electrophoretic bands in the case of *O. edulis* parasitised by *Proserhynchooides* sp (Figure 2(a,b)). A PCR-amplified sample with ITS2-3d/4r was cut with *Bgl*III restriction enzyme and produced three bands, as shown in Figure 4 (lane 1), due to the restriction site occurring exclusively in the *Proserhynchooides* sp. DNA sequence (see Results and Discussion). The *Proserhynchooides* sp. PCR product amplified with the specific primers ITS2-DIG1/DIG2 was also cut with the *Bgl*III restriction enzyme to produce the two corresponding gel bands (Figure 4, lane 2). Digenean PCR products were obtained using the same thermal cycling protocol as reported above, then purified (using the “SureClean” Bioline Kit), sequenced and deposited in GenBank (Genbank Accession no. MF374318-MF374323).

Results and discussion

We detected the presence of DNA from two distinct species of Digenea in mantle tissues of *O. edulis*, but not in those of *M. gigas*. In fact, electrophoresis of ITS2 products obtained with the primers ITS2-3d*/4r* (Figure 2(a)) revealed the co-amplification in *O. edulis* of a 480-bp extra band in six specimens and of a 658-bp extra band in six other individuals (Table I). BLASTn results indicated that the best match with the 480-bp extra-PCR product was the ITS2 sequence of an unidentified trematode ascribed to the genus *Postmonorchis* (Digenea: Monorchidae; Accession no. KC603478; max score = 856, query cover = 98%, identity 99%), whereas the 658-bp PCR band matched best with

the ITS2 sequence of *Proserhynchooides paralichthydis* (Digenea: Buchephalidae; Accession no. KT273398; max score = 1068, query cover = 99%, identity 96%). Flatworm infection in the analysed oysters was then assessed by screening the whole sample with the novel primer pair (Figure 3) designed to target more specifically the ITS2 from the two identified digeneans.

Parasitism by digeneans has been already recorded in different oyster species (Millar 1963; Lee et al. 1996; Princep et al. 1996; Aguirre-Macedo & Kennedy 1999). Bucephalid trematodes are known to be common parasites of commercially important molluscs (Cheng 1967) and cause parasitic castration in several bivalves (Lauckner 1983), including oysters (Cheng & Burton 1965; Feng & Canzonier 1970; Tripp 1973; Chun 1974; Mohan 1978). Infection by *Bucephalus haimeanus* was formerly reported in Mediterranean *O. edulis* (from the delta of the Ebro River in Spain), in which sporocysts were observed to invade the interstitial conjunctive tissues and to obstruct digestive gland tubes, affecting food absorption and ultimately causing host death (Princep et al. 1996). Since the bucephaline genera *Proserhynchooides*, *Rhipidocotyle* and *Bucephalus* as currently conceived are polyphyletic – as revealed by morphological and molecular data from ITS2 (D1–D3 region) and 28S rDNA markers (Nolan et al. 2015) – no conclusion can be drawn about the actual systematics of the trematode species associated with *O. edulis* in our study. However, our data revealed a bucephalid infection in *O. edulis* in the Mediterranean basin, as this occurred in the Italian and Maltese stocks (Figure 1). Bucephalidae are a large cosmopolitan family with a life cycle including sporocysts and cercariae developing in bivalves (intermediate host), metacercariae encysting within the tissues of fishes (second intermediate host), and sexual adult stages inhabiting the digestive tract, and rarely other sites, in piscivorous teleosts (definitive

host) (Overstreet & Curran 2002). The closest bucephalid to our targets retrieved in Genbank, i.e. *Proisorhynchoides paralichthydis*, parasitises the southern flounder *Paralichthys lethostigma*. It would be interesting to identify the Mediterranean definitive fish host – arguably a benthic species such as flat fishes – parasitised by the *Proisorhynchoides* species found in *O. edulis*, to improve our knowledge of the parasite life cycle and its potential impact on the health of oysters and fishes.

Larval stages of digeneans of the family Monorchidae have been described mostly in bivalves of the Atlantic and Pacific oceans (Carella et al. 2013). Only recently, metacercariae of *Postmonorchis* were detected in tissues of the Mediterranean wedge clam *Donax trunculus* Linnaeus, 1758 collected along the Italian Tyrrhenian coast (Carella et al. 2013). This pathogen invades several molluscan tissues such as gills, labial palps, mantle, gut, kidney epithelium, and foot, triggering a strong inflammatory response in the host (Carella et al. 2013). The adult stages of *Postmonorchis* may occur in Sciaenidae (presumably *Umbrina* sp. in Mediterranean waters), but further data are required to identify the definitive host species (Carella et al. 2013). In our study, a DNA source of a parasite plausibly referred to *Postmonorchis* sp. was detected for the first time in oysters (Figure 1; Table I). This result would suggest the co-occurrence of this trematode in both *Ostrea* and *Donax*, consistent with previous observations indicating that digeneans parasitising oysters are commonly shared also with distantly related bivalve families (Lauckner 1983). On the other hand, no parasites were detected in any of our 18 specimens of *M. gigas* collected over a wide geographic range (Figure 1; Table I), although a further sampling effort would be needed to confirm the absence of bucephalids and monorchids in non-autochthonous populations of *M. gigas*. In this regard, it is relevant to remark that digeneans are known to parasitise the native Indo-Pacific *M. gigas*, and particularly the human intestinal trematode *Gymnophalloides seoi* (Digenea: Gymnophallidae) is commonly transmitted by this oyster species in Korea (Lee et al. 1996; Pyo et al. 2013). It is possible that the successful thriving of the invasive *M. gigas* is related to a lack of parasites in the invaded areas, as expected under the enemy release hypothesis (ERH: Torchin et al. 2001; Keane & Crawley 2002; Mitchell & Power 2003). However, it is reasonable to assume that with time host-shifts by bucephalids and *Postmonorchis* sp.

trematodes can potentially occur, so a risk of cross-infection between *O. edulis* and *M. gigas* cannot be excluded in the Mediterranean waters.

Conclusions

The growth of aquaculture – including oyster farming – has been accompanied by the emergence of new and transboundary diseases, stimulating epidemiological studies of aquatic animal pathogens. However, studies evaluating the occurrence and the impact of pathogens in wild aquatic animal populations are still sparse compared to those considering farmed species (Peeler & Taylor 2011). As a by-product of a molecular phylogenetic study on Ostreidae through a universal barcode marker (ITS2) (Salvi et al. 2014), it was possible to detect overlooked digenean parasites in wild Mediterranean populations of *O. edulis*. Notably, putative co-amplification of host/parasite bands were also occasionally observed during molecular phylogenetic studies on Mactridae and Donacidae (e.g. *Mactra* and *Donax* Salvi D., personal observation), suggesting that an ITS2 host/parasite DNA barcode approach may contribute in uncovering a still-hidden digenean diversity in benthic communities.

The identification of Bucephalidae and Monorchidae in the Mediterranean populations of flat oyster encourages future efforts in exploring their epidemiological consequences on such economically important molluscan species. Histopathological, taxonomical and ecological analyses on a larger collection of samples are certainly required to better characterise these digenean infections and to link the infection prevalence with environmental parameters and seasonality. These will allow evaluating the impact of these parasites on oyster health and fitness, the potential risks of cross-contamination to other oyster species (e.g. *M. gigas*) and, ultimately, the level of biosecurity and surveillance necessary to avoid the putative emergence of food-borne diseases.

Acknowledgements

We thank Dr Marialetizia Fioravanti (Università di Bologna) for suggestions on the first draft of the manuscript. The authors are greatly indebted to Mrs Rosetta Ponzio-Leonetti for the revision of the English text.

Funding

ADG, GF, EM, MC and PM wish to thank the “Università di Roma Tre” for financial support (contribution to the laboratory CAL/2016). DS is

currently supported by the programme “Rita Levi Montalcini” for the recruitment of Young Researchers at the University of L’Aquila.

Conflict of interest statement

The authors declare that there are no competing interests.

ORCID

M. Oliverio  <http://orcid.org/0000-0002-0316-4364>

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