The occurrence of two opecoeliid digeneans in *Mullus barbatus* and *M. surmuletus* from the Spanish south-eastern Mediterranean

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Abstract

The infection by *Opecoeloides furcatus* and *Poracanthium furcatum* (Opecoeliidae) was studied in 121 *Mullus barbatus* and 113 *M. surmuletus* collected from the Spanish south-eastern Mediterranean. The prevalence of infection was most frequent in *M. surmuletus* with values of 81.42% for *O. furcatus* and 38.05% for *P. furcatum*. In *M. barbatus* the prevalences of *O. furcatus* and *P. furcatum* were 54.54% and 14.88% respectively. Statistically significant differences were found between the infection of the two hosts with *P. furcatum*. No significant differences in worm burdens could be attributable to host size or to seasonal changes, although a lower infection of *M. barbatus* by *O. furcatus* occurred in the autumn. Furthermore, the electrophoretic mobility of the enzyme malic dehydrogenase (MDH) was also studied and both digeneans presented different patterns, corresponding in both cases to homozygotic genotypes.

Introduction

The taxonomy of *Opecoeloides furcatus* (Lühe 1900) Odhner 1928 and *Poracanthium furcatum* (Stossich 1883) Dollfus 1948 has been the subject of much controversy as the two species were described as a single species under the name of *Distoma furcatum* Brenser, until Dollfus (1948), faced with discrepancies between the descriptions given by various authors, considered the existence of two species. This author designated the name *O. furcatum* to the species with an accessory sucker and *P. furcatum* to the species with a spinous genital pore. The new description given by López Román & Guevara Pozo (1977) provided new differentiating anatomical data relating to the two species, i.e. the shape, size and position of the testis and the ovary, and location of the vitelline follicles. Bartoli & Gibson (1991) carried out a detailed study of *P. furcatum*, pointing out other morphological differences with respect to *O. furcatus*, i.e. the union of the caeca to form an anus and the shape of the excretory vesicle. The aim of the present study is to contribute further information on these two poorly known digeneans from two highly exploited fish species, *Mullus barbatus* and *M. surmuletus*, collected from the Spanish south-eastern Mediterranean. At the same time, as isoenzymatic patterns may reflect genetic differences between closely related species (Mattiucci *et al*., 1997; Martín-Sánchez *et al*., 1998), an electrophoretic study was also undertaken to obtain biochemical data for differenting the two species.

Material and methods

Collection of fish and parasites

A total of 121 striped mullet, *Mullus barbatus*, and 113

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striped red mullet, *M. surmuletus*, with sizes ranging from 12 to 23 cm were collected from the Spanish south-eastern Mediterranean during 1996. Each fish was measured to determine its length, dissected and the alimentary tract removed for examination of digenean parasites. These were washed in a solution of NaCl at 0.9%, fixed in 70% alcohol, stained with Semichon's carmine, dehydrated, cleared in xylene and mounted in Canada balsam. Specimens intended for the isoenzyme electrophoretic study were preserved at –80°C. To determine prevalence, mean abundance and mean intensity of infection of the striped mullet and striped red mullet with *O. furcatus* and *P. furcatum*, the criteria of Margolis *et al*. (1982) were followed. For the statistical study, independence tests based on the distribution χ^2 were used.

Isoenzymatic study

The isoenzyme electrophoretic technique using thick starch gel as previously described by Martín-Sánchez *et al*. (1994) was used. Protein extracts from individual specimens were obtained by cellular rupture employing physical methods including the use of a potter and freezing–thawing processes in liquid nitrogen. To complete the process of cellular rupture 40 μl of Triton X–100 at 5% in distilled water were added for each specimen. The enzymatic loci analysed were: glucose phosphate isomerase (GPI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1), phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), malic dehydrogenase (MDH, EC 1.1.1.37) and malic enzyme (ME, EC 1.1.1.40).

Results

Infection parameters

A total of 1403 specimens of *O. furcatus* and 497 of *P. furcatum* were isolated in the 121 striped mullet and the 113 striped red mullet examined. Table 1 shows the prevalence, mean abundance and mean intensity values for *O. furcatus* and *P. furcatum* in the two fish hosts studied. The highest prevalence values (81.42%) and mean abundance values (6.89) were found for *O. furcatus* in *M. surmuletus*. However, the greatest mean intensity was for *P. furcatum* (9.60) followed by *O. furcatus* (9.43) recovered from *M. surmuletus* and *M. barbatus* respectively. On the other hand, the lowest prevalence, mean abundance and mean intensity of infection corresponded to *P. furcatum* in *M. barbatus*, with statistically significant differences with respect to the infection of *M. surmuletus* by *P. furcatum* with a value for χ^2 of 5.78049 (critical level of *P* being 0.0162). Differences between infections by both parasite species in the two hosts were significant, *O. furcatus* being the most abundant species ($\chi^2 = 42.3856$, critical level $P = 7.9491 \times 10^{-11}$, for *M. surmuletus* and $\chi^2 = 1.0726 \times 10^{-4}$, critical level $P = 2.2020 \times 10^{-10}$, for *M. barbatus*).

Table 1. Infection parameters of *Opecoeloides furcatus* and *Poracanthium furcatum* in *Mullus barbatus* and *M. surmuletus*.

Host (N)	Parasite	n	P	A	I
M. barbatus (121)	*O. furcatus*	624	54.54	5.16	9.43
	P. furcatum	84	14.88	0.69	4.67
M. surmuletus (113)	*O. furcatus*	779	81.42	6.89	8.47
	P. furcatum	413	38.05	3.65	9.60

N, number of hosts; n, number of parasites; P, prevalence (%); A, mean abundance; I, mean intensity.

Table 2. Infection parameters of *Opecoeloides furcatus* and *Poracanthium furcatum* in *Mullus barbatus* and *M. surmuletus* relative to host size.

Host	Host length (N)	Parasite	n	P	A	I
M. barbatus	<16 cm (67)	*O. furcatus*	155	46.25	2.31	5.00
		P. furcatum	37	11.94	0.55	4.62
	>16 cm (54)	*O. furcatus*	469	64.81	8.64	13.4
		P. furcatum	47	18.52	0.87	4.70
M. surmuletus	<16 cm (50)	*O. furcatus*	353	80.00	7.06	8.82
		P. furcatum	166	38.00	3.32	8.74
	>16 cm (63)	*O. furcatus*	426	82.54	6.76	8.19
		P. furcatum	247	38.09	3.92	10.29

N, number of hosts; n, number of parasites; P, prevalence (%); A, mean abundance; I, mean intensity.

The infection parameters, relative to host size are presented in table 2. There are no significant differences in worm burden that could be attributed to host size (length greater or lesser than 16 cm) with values of $\chi^2 = 3.4338$ (critical level $P = 0.0638$) and $\chi^2 = 0.0102$ (critical level $P = 0.9193$) for *O. furcatus* in *M. barbatus* and *M. surmuletus* respectively, and values of $\chi^2 = 0.5683$ (critical level $P = 0.4509$) and $\chi^2 = 1.0726 \times 10^{-4}$ (critical level $P = 0.9917$) for *P. furcatum* in the respective hosts, *M. barbatus* and *M. surmuletus*.

The relationship between the various infection parameters and the seasons of the year are presented in table 3. There appears to be only a significant seasonal influence in the infection of *M. barbatus* by *O. furcatus* ($\chi^2 = 12.6541$, critical level $P = 5.4475 \times 10^{-3}$). The lowest values of prevalence (29.41%) and mean intensity (0.53) occur in the autumn.

Isoenzymatic study

In a preliminary analysis, the isoenzymatic activity and electrophoretic mobility of five enzymes (GPI, PGM, PGD, MDH and ME) were studied in various specimens of *O. furcatus* and *P. furcatum*. Among the loci analysed, malic dehydrogenase enzyme (MDH) enables us to distinguish between specimens of *O. furcatus* and *P. furcatum* because they present a different electrophoretic pattern. The 35 specimens of *O. furcatus* studied showed a monomorphic phenotypical pattern of the enzyme MDH, revealing the existence of two loci: MDH–1 and MDH–2; in both cases, and these are homozygotic genotypes. Similarly, the 25 specimens of *P. furcatum* studied also showed a monomorphic phenotypical pattern for the MDH enzyme, corresponding, in this case, to a single enzymatic locus in the homozygotic state: MDH–1.

The relative electrophoretic mobility of the locus MDH–1 in both species is 100 and 160 for *P. furcatum*

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Table 3. Infection parameters of *Opecoeloides furcatus* and *Poracanthium furcatum* relative to season.

Host	Parasite		Winter	Spring	Summer	Autumn
M. barbatus	*O. furcatus*	N	24	33	30	34
		P	62.50	60.61	70.00	29.41
		A	5.46	8.24	6.77	0.53
		I	8.73	13.60	9.67	0.53
	P. furcatum	N	24	33	30	34
		P	20.83	12.12	23.33	5.88
		A	0.58	0.67	1.53	0.06
		I	2.80	5.50	6.57	1.00
M. surmuletus	*O. furcatus*	N	33	22	34	24
		P	72.73	86.36	88.23	79.17
		A	6.51	5.27	8.32	6.87
		I	8.96	6.10	9.43	8.68
	P. furcatum	N	33	22	34	24
		P	30.30	50.00	29.41	50.00
		A	2.61	2.91	4.71	4.29
		I	8.60	5.82	16.00	8.58

N, number of hosts; P, prevalence (%); A, mean abundance; I, mean intensity.

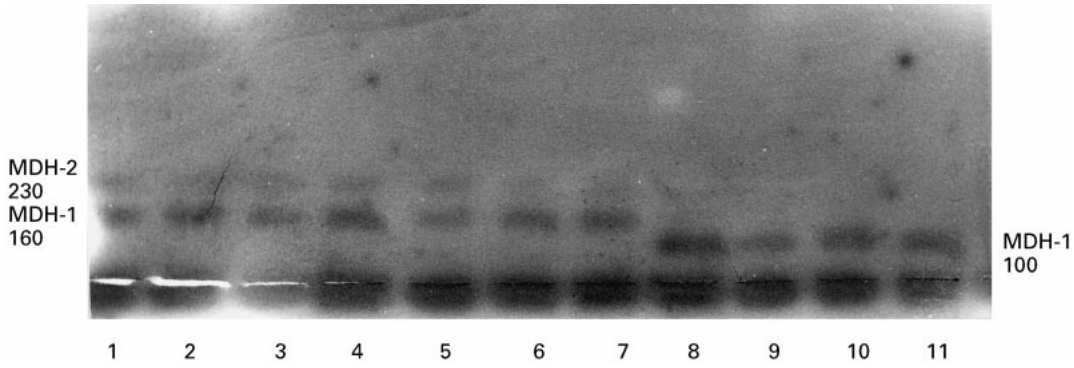

Fig. 1. MDH isoenzymatic patterns of *Opecoeloides furcatus* (lanes 1–7) and *Poracanthium furcatum* (lanes 8–11).

and *O. furcatus* respectively. The relative electrophoretic mobility of the locus MDH–2 of *O. furcatus* is 230.

Discussion

Infection parameters

Opecoeloides furcatus is the species most frequently recovered from both *M. surmuletus* and *M. barbatus*, there being significant differences with respect to the presence of *P. furcatum* in both hosts. Several authors have studied the parasites of *Mullus* spp. in the Mediterranean (Janiszewska, 1953; Fischthal, 1980, 1982; Orecchia *et al*., 1988; Arculeo *et al*., 1997; Pommelet *et al*., 1997 among others). However, only Lopez-Román & Guevara-Pozo (1977), Bartoli & Gibson (1991) and Pommelet *et al*. (1997) have indicated the presence of *P. furcatum*. On the other hand, *O. furcatus* has frequently been found at various points of the Mediterranean. Bartoli & Gibson (1991), consider that *P. furcatum* presents a lower prevalence and a more restricted distribution compared with *O. furcatus* which occurs in *Mullus* spp. and a range of other fish hosts in and outside of the Mediterranean. *Poracanthium furcatum* appears to be restricted to *Mullus* spp. in the Western Mediterranean Basin and is an abundant species in the Nature Reserve at Scandola (Corsica, France). At this point of the Mediterranean, these authors isolated *P. furcatum* from *M. surmuletus*, reporting prevalence and mean intensity of infection values of 70.8% and 28.7 respectively. The values found in the present study are much lower, with a prevalence of 38.05 and a mean intensity value of 9.60, although *P. furcatum* presented a mean intensity of 16.00 during the summer months. The infection rate of *P. furcatum* in *M. barbatus* was lower than in *M. surmuletus*, with statistically significant differences, which could indicate a greater preference by this parasite for striped red mullet as a host. Alternatively, it could reflect the existence of differences in feeding habits and food preferences between the two fish hosts (Arculeo *et al*., 1997). It has been suggested that the diet of striped red mullet consists of at least 59 different prey species belonging to five major groups. Crustaceans (decapods

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and amphipods) are the most important prey group, with polychaetes, mysids and fish less important components (Labropoulou *et al*., 1997). Although both fish species scoop up the substrate when feeding and use barbels to detect their prey, *M. barbatus* digs deeper and takes a broader range of polychaete species, which comprise the dominant prey (Labropoulou & Plaitis, 1995; Labropoulou *et al*., 1997). These differences in feeding habits could be responsible for the lower levels of parasitization by both digeneans in *M. barbatus* compared with *M. surmuletus*. With reference to *O. furcatus*, several authors have located this digenean at various points of the Mediterranean, but only Arculeo *et al*. (1997) quoted a prevalence (0.3%) in *M. surmuletus* sampled in a restricted coastal area of the Gulf of Palermo (Sicily, Italy). On the other hand, *O. furcatus* does not appear to show any preference for either host, as, although in absolute terms it is recovered more frequently from *M. surmuletus*, the differences are not statistically significant.

There are no significant differences in infection levels that could be attributed to host size or to seasonal variations, except for the lower levels of infection of *O. furcatus* in *M. barbatus* during the autumn and despite the fact that the feeding habits of *M. surmuletus* vary significantly with fish size (specimens larger and smaller than 161 mm) and with respect to season (Labropoulou *et al*., 1997; Machias *et al*., 1998)

Isoenzymatic study

Digenean species often show a low level of host range and no distinct host preference and host specificity patterns are often not easy to interpret. However, isoenzymatic analyses are of much assistance in studying related species with complicated patterns of host–specificity (Gibson & Bray, 1994; Martín-Sánchez *et al*., 1998).

In the present case, it has enabled us to demonstrate the existence of genetic differences between two closely related digenean species which share the same host range and about which controversy exists from a taxonomic point of view.

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