RESEARCH NOTE

ON THE PRESENCE OF *LECITHOCHIRIUM MUSCULUS* (DIGENEA: HEMIURIDAE) IN *CONGER CONGER*

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Lecithochirium musculus (Looss, 1907) is a hemiurid trematode common in *Anguilla anguilla* (Linnaeus, 1758) in the Mediterranean and Black Seas and the north-east Atlantic Ocean. This worm is also a parasite of a wide range of fish predators of gobiids. However, the presence of this fluke in species of the genus *Conger* (Cuvier, in Oken, 1817) is uncertain. Although there are reports of immature specimens identified as *L. musculus* in *Conger myriaster* and *Conger japonicus* from Japan (Yamaguti S. 1934: Jpn. J. Zool. 5: 249–541; Ichihara A. 1974: Proc. Third Int. Congress Parasitol. 3: 1614–1615), these records require confirmation because there are several morphologically similar species with which they may be confused (Gibson D.I., Bray R.A. 1986: Bull. Brit. Mus. Nat. Hist. Zool. 51: 83–89). These authors confirm that *L. musculus* has not been identified accurately as a parasite of species of the genus *Conger*, therefore the few recordings of this worm in conger eels may actually correspond to other species. Although *L. musculus* has been reported as a parasite of *C. conger* (Artedi, in Linnaeus, 1758) in the Mediterranean Sea (cited by Radujković B.M., Orecchia P., Paggi L. 1989: Acta Adriat. 30: 137–187), reported as *Sterrhurus musculus* in the same host in the Canary Islands (Gijón-Botella H., López-Román R. 1989: Rev. Ibér. Parasitol. 49: 137–138), and in the north-east Atlantic (Sproston N.G. 1939: Trav. Stn. Biol. Roscoff 16: 33–58), there are no definite reports of this hemiurid fluke occurring in conger eels nor are there data on the degree of parasitism in fish of the genus *Conger* (but see Paniagua E., Vilas R. 2001: Acta Parasitól. Port. 8: 166).

In this study, we examined 156 conger eels, from the spring of 1999 to the summer of 2001. The fish were caught in the Ría de Arousa (a coastal embayment in Galicia, north-western Spain). During this time we detected only 15 individuals obtained from six conger eels that could have been *L. musculus*, and of these helminths, 5 were subjected to morphometric analysis. The trematodes were stained with iron acetocarmine and mounted in Canada balsam. Comparison of the measurements made on these helminths and those made on a sample taken from *A. anguilla* caught in the same area are

Table 1. Comparison of the measurements of *Lecithochirium musculus* from *Conger conger* (n = 5) and *L. musculus* from *Anguilla anguilla* (n = 20).

	Lecithochirium musculus (*Conger*)				*Lecithochirium musculus* (*Anguilla*)*			
	Mean	± SD	Range	CV	Mean	± SD	Range	CV
Body length	1880	99.5	1625–2175	5.13	1947	112.6	1125–2975	11.40
Body width	462	24.8	410–520	2.58	431	22.6	270–660	4.86
Oral sucker – maximum diameter	146	9.1	125–170	1.69	124	4	90–150	1.62
Acetabulum – maximum diameter	282	10.7	250–310	1.42	273	9.1	200–325	2.45
Sucker-ratio	0.25	0.03	0.21–0.37	0.51	0.19	0	0.17–0.27	0.05
Left testis – length	124	19.9	80–180	3.39	194	12.5	90–315	4
Left testis – width	108	8	90–130	1.72	148	8.7	80–210	3.19
Right testis – length	131	18.7	95–190	3.66	192	11	85–270	3.55
Right testis – width	107	3	100–115	0.65	155	8.6	85–210	3.09
Ovary – length	143	12.4	120–190	2.32	168	8	95–215	2.77
Ovary – width	169	10.3	135–200	1.77	169	8.3	105–240	2.85
Distance between suckers	298	5.8	280–310	0.75	301	13.4	180–420	3.46
Forebody length	450	16.4	390–490	1.73	438	17.2	270–580	3.67
Distance ovary – anterior extremity	933	113.1	510–1175	8.27	1130	53	680–1450	7.04
Distance left testis – anterior extremity	817	53.1	660–975	4.15	760	32.3	450–1025	5.23
Distance right testis– posterior extremity	1021	87.1	750–1260	6.10	1049	77.5	520–1850	10.70

* Modified from Vilas R., Paniagua E., Sanmartín M.L. 2002: Parasitol. Res. 88: 1055–1060.

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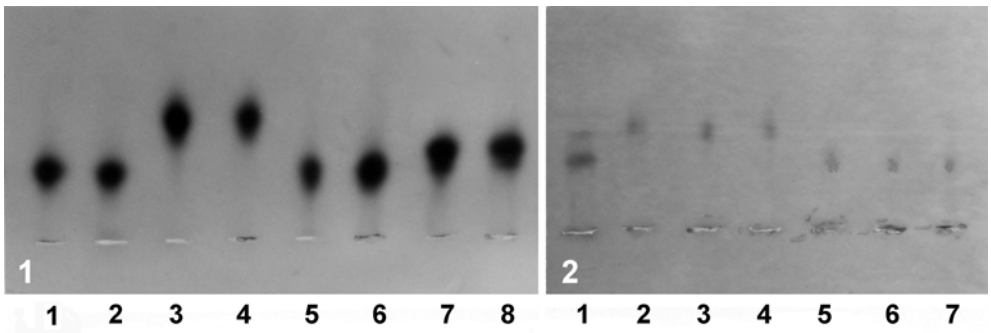

Fig. 1. Glucose phosphate dehydrogenase (GPD) pattern: *Lecithochirium fusiforme* (lanes 1, 2), *Lecithochirium musculus* (lanes 3, 4: Gpd^b/Gpd^b; lanes 5, 6: Gpd^a/Gpd^a), *Lecithochirium rufoviride* (lanes 7, 8). **Fig. 2.** Glutamate dehydrogenase (GDH) pattern: *L. rufoviride* (lane 1), *L. fusiforme* (lane 2), *L. musculus* (lanes 3, 4: Gdh^b/Gdh^b; lanes 5–7: Gdh^a/Gdh^a).

Table 2. Genotypic frequencies of different samples of *Lecithochirium musculus* from *Conger conger* and *Anguilla anguilla* within (samples A, C) and outside the coastal embayment (sample B).

Locus	Sample A (*Conger*)	Sample B (*Conger*)	Sample C (*Anguilla*)
Gdh	a/a = 8 a/b = 0 b/b = 0	a/a = 0 a/b = 0 b/b = 14	a/a = 55 a/b = 0 b/b = 6
Gpd	a/a = 0 a/b = 0 b/b = 8	a/a = 14 a/b = 0 b/b = 0	a/a = 2 a/b = 0 b/b = 51

shown in Table 1. Because the sample size was lower than 30, Mann-Whitney test was used. All morphometric measurements are given in micrometres, except for the sucker-ratios (for determination of the sucker-ratio see Mas-Coma S., Montoliu I., Valero M.A. 1984: Bull. Soc. Neuchâtel Sci. Nat. 107: 185–195). In general, the specimens taken from *C. conger* fitted the description of *L. musculus*, however, they showed smaller testes than those obtained from *A. anguilla*. Moreover, *L. musculus* from *C. conger* showed significant differences in the sucker-ratio ($U = 10.0$; $P<0.01$). In order to confirm the diagnosis at the molecular level, the samples were then analysed using starch gel electrophoresis. Two enzymes that in a preliminary analysis allowed discrimination of *L. musculus* and the other three sympatric species of the genus (*Lecithochirium fusiforme*, *Lecithochirium rufoviride* and *Lecithochirium furcolabiatum*), glucose phosphate dehydrogenase (GPD, E.C. 1.1.1.49) and glutamate dehydrogenase (GDH, E.C. 1.4.1.3), were analysed following routine procedures (Pasteur N., Pasteur G., Bonhomme F., Catalan J., Britton-Davidian J. 1987: Manuel Technique de Génétique par Électrophorèse des Protéines. Lavoisier, Paris, 211 pp.). There were no differences between the specimens isolated from *C. conger* (sample A) and those from *A. anguilla*, therefore, despite of the morphological differences detected, we can confirm the identification as *L. musculus* and verify its presence in this host in the area studied. However, *L. musculus* was not common in *C. conger* with very low values of prevalence ($P = 3.8\%$), abundance ($A = 0.10$) and mean intensity ($I = 2.5$).

Surprisingly, a single fish caught outside the ría was a host of 14 specimens that were identified as *L. musculus* by their size, sucker-ratio, well-developed ecsoma and vitellarium composed of two slightly lobed masses (sample B). All of the helminths isolated from this individual fish revealed the same monomorphic pattern for GPD and GDH, which unexpectedly differed from that corresponding to *L. musculus* obtained from fishes from the ría (Fig. 1: lanes 5, 6, and Fig. 2: lanes 3, 4). In the light of these results, we decided to examine the population of *L. musculus* collected from the main definitive host *A. anguilla* in greater detail (sample C). Therefore, the sample size of the population was increased and significance of deviations of observed genotype frequencies from those expected under Hardy-Weinberg equilibrium was estimated using Chi-square test. Both enzymatic systems were coded by a single locus. Each showed two alleles in the population but the expected heterozygotes were not detected (Table 2). Thus, the population did not conform to Hardy-Weinberg predictions (GDH: $\chi^2 = 59.06$, $P<0.001$; GPD: $\chi^2 = 75.47$, $P<0.001$). For both loci, the least frequent allozyme in the population that parasitized *A. anguilla* was the same as that shown by all individuals that parasitized the conger eel caught outside the ría. Although this electrophoretic pattern was the same as for *L. fusiforme* (see Figs. 1 and 2) it is unlikely that the helminths that presented allozymes *a* and *b* for GPD and GDH respectively, actually corresponded to *L. fusiforme* because they differed in terms of morphological characteristics. In particular, the shape of the vitellarium differed and in all specimens analysed it consisted of two lateral lobed masses instead of being digitiform. Furthermore, to our knowledge, *L. fusiforme* has not been reported as occurring in *A. anguilla*.

The results of a previous study of the same population showed that there was a strong genetic subdivision for the *Pgm-1* locus (Vilas R., Paniagua E., Sanlés D.G., Sanmartín M.L. 2000: Parasitol. Res. 86: 419–421). The findings of the present study confirm the existence of this subdivision for another two loci. This is consistent with the existence of at least two cryptic species that may show a certain degree of differential predilection for the definitive host. However, these results should be interpreted cautiously because there may be other explanations for the genetic subdivision detected. Although a geographically small sampling area reduces the

probability of a mixture of genetically different populations, other factors should be taken into account. Asexual amplification of the parasite, habitat patchiness, host mobility and the existence of a complex life-cycle make it very difficult to determine what comprises a local population. In our case, it is possible that the conger eel caught outside the ría was host to a sample of a different geographical population of *L. musculus* that differed significantly in its allele frequencies for the *Gpd* and *Gdh* loci, probably due to strong genetic drift. The putative effect of interpopulational genetic differentiation due to genetic drift is intensified by the fact that the infra-populations of *L. musculus* in *C. conger* are very small. It is also possible that disruptive selection takes place, due to the different selective pressures imposed by different hosts or attributable to different environmental conditions that prevail inside and outside of the ría. Another possibility is that the absence of heterozygotes reflects a certain clonal structure and that the agreement with the assumptions of the Hardy-Weinberg equilibrium for the *Gpi* locus (see Vilas et al. 2000, op. cit.) are the result of antagonist evolutionary forces or natural selection. Although these results do not preclude the possibility that there exist two different sibling species identified as *L. musculus*, which are potential parasites of both *C. conger* and *A. anguilla*, the presence of *L. musculus* is confirmed in *C. conger*. In addition, the results show that there is a strong genetic subdivision within the population studied. However, further studies are required to clarify whether this subdivision is the result of the presence of cryptic species, or whether it is due to other factors associated with the mode of distribution of the genetic variability over time and space in populations of marine digenetic trematodes. In the latter case the implications for systematics are clear and indicate the suitability of a multidisciplinary approach in identifying these groups.

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