CHARACTERIZATION BY ENZYME ELECTROPHORESIS OF SPECIMENS OF THE GENUS *HELICOMETRA* (TREMATODA, OPECOELIDAE) FROM FISH CAUGHT OFF THE COAST OF NORTHWEST SPAIN

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Summary :

Species within the genus *Helicometra* are difficult to distinguish on morphological grounds alone, and are best discriminated with the aid of biochemical techniques. In the work reported here, the electrophoretic mobility of malate dehydrogenase isoenzyme of the Mdh-1 locus was used to characterize 375 individuals of *Helicometra* obtained from various teleost species (*Anguilla anguilla*, *Conger conger*, *Gobius niger* or *Ciliata mustela*) caught off the coast of northwest Spain. The results suggest that all specimens belong to only one species, probably *H. fasciata*. Observed genotype frequencies did not differ from those expected under the assumptions of Hardy-Weinberg equilibrium, even when the genic frequencies differ considerably respecting a Mediterranean population.

KEY WORDS : *Helicometra*, malate dehydrogenase, enzyme electrophoresis, fish, trematodes.

Résumé : CARACTÉRISATION PAR ÉLECTROPHORÈSE DES ISOENZYMES DE TRÉMATODES APPARTENANT AU GENRE *HELICOMETRA* (TREMATODA, OPECOELIDAE), PARASITES DE TÉLÉOSTÉENS MARINS DU NORD-OUEST DE L'ESPAGNE

Les espèces du genre Helicometra *sont souvent indiscernables morphologiquement, mais des différences biochimiques peuvent séparer des espèces qui ne pouvaient l'être initialement sur la base de critères morphologiques. Nous avons utilisé dans ce travail la mobilité électrophorétique de la malate déshydrogénase (Mdh-1) pour la caractérisation de 375 trématodes du genre* Helicometra, *récoltés chez des téléostéens marins (*Anguilla anguilla, Conger conger, Gobius niger *et* Ciliata mustela*) capturés dans le littoral du nord-ouest de l'Espagne.*

Les résultats montrent que tous les individus analysés appartiennent à la même espèce, probablement H. fasciata. *Les fréquences génotypiques observées ne sont pas différentes de celles attendues d'après la loi de Hardy-Weinberg, même si les fréquences alléliques sont très différentes de celles d'une population méditerranéenne.*

MOTS CLÉS : Helicometra, *Malate-déshydrogénase, électrophorèse enzymatique, trématodes, poissons.*

In the genus *Helicometra*, species-level identifications are complicated by morphological polymorphism, the wide range of definitive hosts, and the wide geographical distribution. Some workers considered this genus either as a complex of species each with strict host specificity (Yamaguti, 1971), or as a single highly polymorphic species with broad host specificity (Sekerak & Arai, 1974). Life-cycle studies have produced some taxonomically valuable information (Meenakshi *et al.*, 1993; Reversat & Silan, 1991), but definitive classification has required enzyme electrophoresis. Specifically, malate dehydrogenase (locus Mdh-1) isoenzyme polymorphism has been shown to discriminate eflectively between three sympatric species from the south coast of France (Reversat *et al.*, 1989); the latter authors showed that the *Helicometra* group is neither a single very polymorphous species with broad specificity, nor a complex of species with strict specificity. These species are practically indistinguishable on morphological grounds, though their primary intermediate hosts are different (Reversat & Silan, 1991). Since these were local studies and geographically distant populations may be quite different genetically, especially because of asexual reproduction in digenids, we have used genetical analysis of the locus Mdh-1 to study specimens of genus *Helicometra* isolated from marine teleosts for identifying the species on the N.W. coast of the Iberian Peninsula, testing the discriminative capacity of this locus proposed by Reversat *et al.* (1989) in populations of our environment. The aim of this study is to assess species diversity and make a comparison between two populations from very distant geographical localities.

MATERIALS AND METHODS

Adult *Helicometra* specimens were obtained from the intestinal tracts of *Anguilla anguilla*, *Conger conger*, *Gobius niger* and *Ciliata mustela*, caught in the rías of Arosa, Noya or Ferrol (Galicia, northwest

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Site	Host	Number of host individuals	Mean intensities	Number of *Helicometra* analysed
Noya	*C. Conger*	6	39.5	66
Ferrol	*G. niger*	2	5.5	8
	A. anguilla	3	18.5	19
Arosa	*C. conger*	9	35.5	93
	A. anguilla	5	77.5	99
	C. mustela	2	33	46
	G. niger	4	12	44
Total		31		375

Table I. – Sampling data: number of host individuals for each species and site, and the mean intensities of parasites per host species and site.

Spain). The distance between Arosa and Noya is 30 km, between Noya and Ferrol is 140 km. All individuals were examined under a stereomicroscope to confirm identification to genus level, and for characterization of testes morphology. Some specimens were stained with iron acetocarmine and mounted in Canada balsam for examination of the vitelline glands and testes (Georgiev *et al.*, 1986).

A total of 375 individuals were analysed by enzyme electrophoresis. The sampling data corresponding to the number of host individuals for each species and site, and the mean intensities of parasites per host species and site are presented in Table I. These small helminths were maintained in physiological saline for one-two hours, then stored at – 80 °C until analysis. The procedure followed was: extracts obtained by crushing with a glass rod on a rectangle of Whatman paper (No. 3. 0.5 × 0.5 cm) were separated on 10 % starch gels at a constant 105 V for five hours at 4 °C. The running buffer was a 29-fold dilution of the electrode buffer (0.687 M Tris, 0.157 M citrate, pH 8) (Selander *et al.*, 1986). Specific histochemical staining of malate dehydrogenase (E.C. 1.1.1.37) activity was performed using the methods described by Pasteur *et al.* (1987).

Fisher's exact test was used to detect significant departures in genotypic frequencies from the predictions of the Hardy-Weinberg model, using the GenePop version 3.0 program (Raymond & Rousset, 1995).

RESULTS AND DISCUSSION

Morphological characteristics suggested that all individuals were of a single type, with lobed testes (although to differing extent) and the specimens examined after iron acetocarmine staining, showed a lateral vitelline field not confluent in the hindbody. This type has been found in *H. fasciata* and *H. gobii* (Reversat *et al.*,1991), which makes both species morphologically indistinguishable, but different from *H. pulchella.*

In enzyme electrophoresis, all individuals showed a band pattern consistent with two loci (Mdh-1 and Mdh-2). Mdh-2 was monomorphic in all cases, represented by a single band (the fastest-migrating band in the gels shown in Figure 1).

As regards Mdh-1, all individuals showed one of two banding patterns. In the first pattern (observed in 359 (96 %) of the 375 individuals), a single band with malate dehydrogenase activity was observed (band 1). In the second pattern, observed in the remaining 16 individuals (eight *Helicometra* from two *C. conger* caught in the ría of Noya, one *Helicometra* from one *C. conger* caught in the ría of Arosa and seven *Helicometra* from three *A. anguilla* caught in the ría of Arosa), three bands were observed: one band migrating to the band-1 position, and two faster-migrating bands (bands 2 and 3, band 2 being the most intensely stained of the three) (Fig. 1).

The observed genotypic frequencies were consistent with Hardy-Weinberg expectation (Table II).

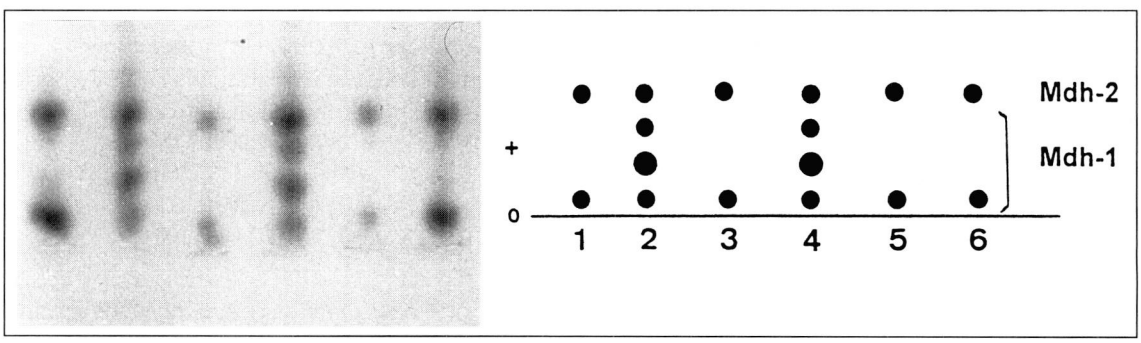

Fig. 1. – Mdh (malate dehydrogenase) photograph and diagram of *Helicometra* isoenzyme patterns 1, 3, 5 and 6: genotype Mdh-1^a/Mdh-1^a. Patterns 2 and 4: genotype Mdh-1^a/Mdh-1^b.

Allele Frequencies		Genotype		
f(a) = 0.979 f(b) = 0.021		a/a	a/b	b/b
	Observed	359	16	0
	Expected	359.42	15.42	0.17
N = 375		p = 1.00		

Table II. – Fisher's exact test of correspondence between observed genotype frequencies and frequencies expected under the assumptions of Hardy-Weinberg equilibrium.

Assuming that the malate dehydrogenases of *Helicometra* are dimeric (Reversat *et al.*, 1989), these electrophoretic results are consistent with the existence in the studied population of the two alleles for the locus Mdh-1: one of them only present in heterozygosity, indicating that this allele is rare.

In their study of *Helicometra* species from the Etang de Thau (Hérault, France), Reversat *et al.* (1991) found that only one species, *H. fasciata*, showed heterozygosity at the Mdh-1 locus. The presence of heterozygosity in our population doesn't mean necessarily that our specimens belong to *H. fasciata*, but we can conclude that all of them belong to only one species, polymorphic for Mdh-1, like those of *H. fasciata* from the Etang de Thau population. Note that we have sampled *C. conger* and *C. mustela* and that, as far as we know, *Helicometra* spp. haven't been isolated in the latter host, in spite of their being present in gadids (Bray & Cribb, 1989).

lt should be stressed that the allele frequencies detected in the present study (0.98 and 0.02) differ dramatically from those detected by Reversat *et al.* (1989) (0.52 and 0.58), which might be due to the need of differential adaptation to the environmental conditions in the other host species and geographic localities, or to chance.

Reversat *et al.* (1989) suggested that the Etang de Thau populations of *H. fasciata* must reproduce by random mating since the observed genotype frequencies did not differ significantly from those predicted under the assumptions of Hardy-Weinberg equilibrium. In the present study, Mdh-1 genotype frequencies likewise did not differ significantly from equilibrium (Table II). However, this result does not necessarily indicate that the study population is a single panmictic unit. Further studies are necessary to clarify this point.

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