

## What can metazoan parasites reveal about the taxonomy of *Scomber japonicus* Houttuyn in the coast of South America and Madeira Islands?

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The metazoan parasites of four populations of the chub mackerel *Scomber japonicus* were analysed from two localities in the Atlantic Ocean (Madeira Islands, Portugal, and Rio de Janeiro, Brazil) and two localities in the Pacific Ocean (Callao, Peru, and Antofagasta, Chile), collected during 2002 and 2003. A total of 373 fish specimens were studied and 34 metazoan parasite species were obtained. Parasites identified from the populations of chub mackerel studied could be separated into three categories: parasites with a wide distribution, present in the Pacific and Atlantic, parasites proper of the Pacific Ocean and parasites proper of the Atlantic Ocean. The analyses of some highly specific parasites of the genus *Scomber* (*i.e.* monogeneans of the genus *Kuhnia* and didymozoid digeneans) strongly suggest the need for a revision of the taxonomic status of chub mackerels from the Atlantic and Pacific coast of America. The results demonstrated the usefulness of parasites as adequate tools to clarify the taxonomic status of their hosts.

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Key words: Atlantic Ocean; biogeography; metazoan parasites; Pacific Ocean; *Scomber japonicus*.

### INTRODUCTION

Parasites have been used successfully not only as tools to understand the population structure of the host species but also to clarify doubtful systematic relations between closely related hosts. Szidat (1955) used the parasite fauna of hake (genus *Merluccius*) to identify their migratory routes that explained their

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current distribution. Later, Kabata & Ho (1981) proposed a new model to identify the origin and dispersal of hake but on the basis of only parasitic copepods. Similar results were obtained by Fernández (1985), who studied the parasites of the southern hake *Merluccius australis* (Hutton, 1872), and Fernández & Durán (1985) on the basis of biological information from the sanguinicolid digenean genus *Aporocotyle*. A similar biogeographic approach has been developed for *Acanthopagrus* and Sillaginidae, among other fishes (Byrnes, 1987; Byrnes & Rohde, 1992; Hayward, 1997). In a similar way Rohde & Hayward (2000) analysed the importance of oceanic barriers in the distribution of Scombridae and their ectoparasites (Copepoda and Monogenea), concluding that the East Pacific Barrier represents an efficient barrier for almost all the studied Scombridae, but a less effective barrier for species of the genus *Scomber*. All the cited examples refer to congeneric (or members of the same family) species living in discrete habitats.

Parasites have been used also as biological markers, mainly as tags for the identification of stocks (subpopulation units) and migratory movements (MacKenzie & Abaunza, 2005). Under the traditional population studies approach, parasites are used as a tool for the identification of discrete subpopulations of the same species but living within a continuous habitat. Few fish species show the alternative distributional model: the same species with an extended geographical distribution but living in discrete habitats. One of such species is chub mackerel *Scomber japonicus* Houttuyn, 1782, a widespread, circumglobal, pelagic species which is divided into geographically disjunct populations (Fig. 1) (Scoles *et al.*, 1998). This distributional pattern closely resembles the distribution of different species of the pelagic genera *Trachurus* and *Engraulis* and the demersal *Merluccius*. The main characteristic of the chub mackerel is its circumglobal distribution as a unique species, whereas the same distributional pattern is evident for other marine fishes but at the generic level.

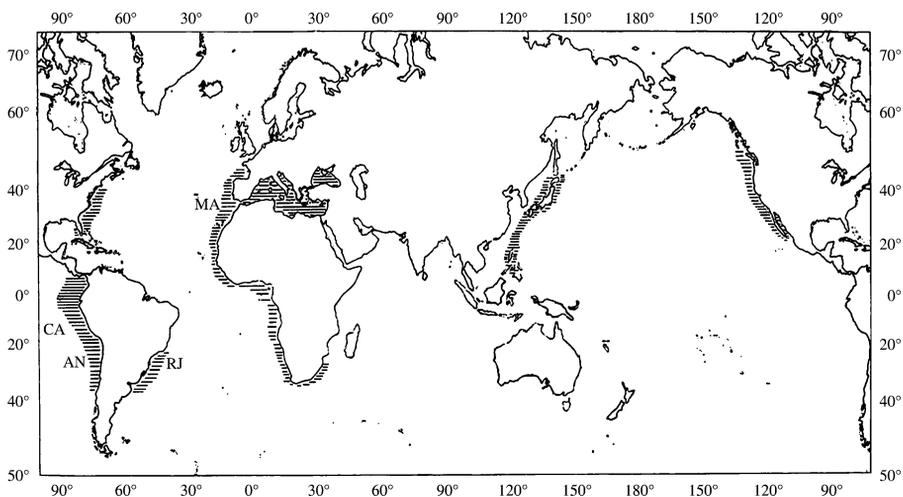


FIG. 1. Geographic distribution of *Scomber japonicus* (based on Scoles *et al.*, 1998). Approximate position of sampling localities: MA, Madeira Islands; RJ, Rio de Janeiro; CA, Callao; AN, Antofagasta.

Data on the metazoan parasite fauna of the chub mackerel, specifically the highly specific parasites of *S. japonicus* from the south-eastern Pacific (Peru and Chile), western Atlantic (Brazil) and Eastern Atlantic (Madeiran waters, Portugal) were used in order to evaluate if the four chub mackerel populations studied can be considered as members of the same species or must be considered as different taxonomic units.

## MATERIALS AND METHODS

Samples of chub mackerel were taken during, 2002 and 2003 from Callao, Peru, Antofagasta, Chile, Rio de Janeiro, Brazil, and Madeira, Portugal. Geographic co-ordinates, sample size and specimen sizes for the sampling localities are given in Table I. Fish were frozen ( $-18^{\circ}\text{C}$ ) and transported to the laboratory for parasitological analyses. After thawing, the fish total length ( $L_T$ ) was measured to the nearest 10 mm, dissected, sexed and examined for metazoan ectoparasites and endoparasites. Parasites were separated, stored in 5% formalin, and counted by species. For each parasite species the population descriptors (prevalence and mean intensity) were calculated according to Bush *et al.* (1997). Two multivariate analyses were used to detect associations between metazoan parasite fauna and locality (data reduction: correspondence analysis, CA) and a classification analysis (multivariate discriminant analyses, MDA) to determine if parasites were good discriminants of locality. Analyses were performed following the recommendations of Wilkinson (1990) using SYSTAT<sup>®</sup> as the statistical tool.

## RESULTS

A total of 373 specimens of chub mackerel were analysed and produced 13 757 parasite specimens belonging to 34 taxa (Monogenea = 5, Digenea = 10, larval Cestoda = 4, Nematoda = 6, Acanthocephala = 5, Copepoda = 2 and Isopoda = 2). *Anisakis* identified as *Anisakis* type I and type II (Berland, 1961) from Madeira, *Anisakis* sp. from Callao and *Anisakis* sp. from Rio de Janeiro and Antofagasta were pooled and considered as *Anisakis* sp. because recent studies have demonstrated that the taxonomy of members of *Anisakis* based on morphology is confused (Mattiucci & Nascetti, 2006). Sibling species in *Anisakis* from the Atlantic and Pacific waters was first demonstrated by Nascetti *et al.* (1986). The discovery of at least five species of *Anisakis* in *S. japonicus* from Madeira (Pontes *et al.*, 2005) using molecular techniques justifies this decision.

Mean  $L_T$  of host fish differed significantly between localities (ANOVA, d.f. = 3,369,  $P < 0.001$ ), with chub mackerel from the Pacific being larger than

TABLE I. Geographic co-ordinates, sample size ( $\delta$ ) and total length ( $L_T$ ) of the specimens of *Scomber japonicus* from the four localities analysed

Locality	Latitude	Longitude	$n$	Host $L_T$ (mm) mean $\pm$ s.d. (range)
Madeira, Portugal	33° N	16° W	151	287 $\pm$ 29 (230–400)
Rio de Janeiro, Brazil	23° S	43° W	100	257 $\pm$ 28 (200–320)
Callao, Peru	12° S	77° W	60	325 $\pm$ 19 (271–358)
Antofagasta, Chile	23° S	70° W	62	361 $\pm$ 31 (283–475)

those from the Atlantic. For each locality, parasite richness did not co-vary with host size (Madeira  $r^2 = 0.0075$ , Rio de Janeiro  $r^2 = 0.0064$ , Callao  $r^2 = 0.0118$  and Antofagasta  $r^2 = 7 \times 10^{-5}$ ). Thus,  $L_T$  of the host was not considered as a confounding factor for parasite species richness. For all localities, except Antofagasta, 100% of fish were parasitized by one or more parasite species. For Antofagasta, 92% of the fish were parasitized. An ANOVA (d.f. = 3, 369,  $P < 0.001$ ) following  $\log_{10}(n + 1)$  transformation of the data indicated that parasite richness differed significantly among the four localities. The *a posteriori* Tukey test (Zar, 1996) showed that parasite richness of samples from the Pacific ( $P > 0.05$ ) and the Atlantic do not differ significantly ( $P > 0.05$ ).

The parasitic species found from chub mackerel in each locality, and the prevalence and mean intensity of infection are given in Table II. From Table II it is evident that parasites can be included in three groups: parasites limited to the Atlantic (*i.e.* the monogeneans *Grubea cochlear* and *Kuhnia scombercolias*, non-Didymozoidae digeneans except *Nematobothrium scomбри* and the acanthocephalan *Rhadinorhynchus pristis*), parasites limited to the Pacific (the monogenean *Kuhnia sprostonae*, the didymozoids *Ovarionematobothrium saba* and *Didymocystis* sp. the isopods *Ceratothoa gaudichaudii* and *Rocinela* sp.) and parasites common to both oceans (the monogenean *Kuhnia scomбри* and the copepod *Clavellisa scomбри*).

Multivariate analyses (CA and DMA) were performed for the whole sample, for those parasites with prevalence  $>5\%$  and those with prevalence  $>10\%$  in at least one locality. For both analyses results did not differ. In CA the variance explained by the first two factors for the whole sample, parasites with prevalence  $>5\%$  and parasites with prevalence  $>10\%$  were 82.8, 83.2 and 84.6%, respectively. In a similar way the DMA also did not show dramatic differences: Wilks' lambda = 0.0091, 0.0095 and 0.0010, for the whole sample, parasites with prevalence  $>5$  and  $>10\%$ , respectively. The correct assignation was high and similar: 92, 93 and 92%, respectively. The results of both analyses corroborate that parasites correspond well with localities and can serve to discriminate the origin of the samples with high resolution. (Figs 2 and 3)

## DISCUSSION

As stated by Stepián & Rosenblatt (1996) there are many closely related marine fish species in temperate north and south Pacific, but absent from the tropical region of Central America. Representatives of *Merluccius*, *Trachurus* and *Scomber* are good examples, but other pelagic fishes like anchovies and sardines also meet this distributional pattern. Stepián & Rosenblatt (1996) concluded that the anti-tropical distribution at the species level for representatives of *Merluccius* is well supported by genetic evidence. For the genus *Trachurus* the genetic evidence is weak in support of specific differentiation but recently Poulin *et al.* (2004) and Cárdenas *et al.* (2005) demonstrated that representatives of *Trachurus* from the Pacific coast of North and South America belong to different *Trachurus* species. Finally and as stated by Stepián & Rosenblatt (1996), *Scomber* does not represent distinctly different species, but two isolated populations of *S. japonicus*.

TABLE II. Metazoan parasites of *Scomber japonicus* from four localities (see Table I). Prevalence of infection (P) and mean intensity of infection (MI) are given

Parasite species	Madeira		Rio de Janeiro		Callao		Antofagasta	
	P	MI	P	MI	P	MI	P	MI
Monogenea								
<i>Grubea cochlear</i> *	3.3	1.0	18.0	1.6	—	—	—	—
<i>Pseudokuhnia minor</i> *	98.7	13.9	—	—	—	—	—	—
<i>Kuhnia scombercolias</i> *	39.1	1.8	46.0	8.2	—	—	—	—
<i>Kuhnia sprostonae</i> *	—	—	—	—	43.3	5.2	25.8	2.3
<i>Kuhnia scombri</i> *	43.7	2.2	11.0	2.1	91.7	5.6	45.2	2.3
Digenea								
<i>Didimocystis</i> sp.	—	—	—	—	40.0	13.4	8.1	4.2
<i>Ovarionematobothrium saba</i> *	—	—	—	—	30.0	2.7	25.8	2.8
<i>Halvorseniuss exilis</i> *	6.6	2.5	—	—	—	—	—	—
<i>Nematobothrium scombri</i>	27.8	2.1	55.0	4.6	—	—	4.8	1.3
<i>Clavogalea trachinoti</i>	6.6	1.8	—	—	—	—	—	—
<i>Prodistomum orientalis</i>	19.9	6.8	—	—	—	—	—	—
<i>Lecithocladium harpodontis</i>	—	—	80.0	12.8	—	—	—	—
<i>Opechona</i> sp.	—	—	85.0	43.7	—	—	—	—
Digenea (intestine)	31.1	5.6	—	—	—	—	—	—
Digenea (swimbladder)	—	—	—	—	—	—	4.8	1.3
Cestoda								
<i>Nybelinia</i> sp. (L)	22.5	1.6	—	—	—	—	—	—
<i>Scolex pleuronectis</i> (L)	15.2	18.1	30.0	5.1	—	—	—	—
<i>Tentacularia coryphaenae</i> (L)	22.5	1.4	—	—	8.3	1.0	1.6	1.0
Diphyllobothrid (L)	—	—	—	—	1.7	2.0	—	—
Acanthocephala								
<i>Rhadinorhynchus pristis</i>	44.4	1.4	7.0	1.9	—	—	—	—
<i>Bolbosoma vasculosum</i> (L)	2.0	1.0	—	—	—	—	—	—
<i>Bolbosoma</i> sp. (L)	—	—	—	—	—	—	6.5	6.0
<i>Corynosoma australe</i> (L)	—	—	12.0	1.4	—	—	38.7	9.3
<i>Corynosoma</i> sp. (L)	—	—	10.0	2.1	—	—	—	—
Nematoda								
<i>Hysterothylacium</i> sp.	22.5	2.6	—	—	—	—	—	—
<i>Hysterothylacium fabri</i>	7.3	1.6	—	—	—	—	—	—
<i>Oncophora</i> sp.	6.0	1.0	—	—	—	—	—	—
<i>Contraecum</i> sp. (L)	—	—	2.0	2.5	—	—	—	—
<i>Anisakis</i> sp. (L)	53.6	2.0	4.0	3.5	10.0	1.2	43.5	2.7
<i>Rhapidascaris</i> sp.	—	—	52.0	27.4	—	—	—	—
Copepoda								
<i>Caligus pelamidys</i>	—	—	2.0	1.5	1.7	1.0	17.7	1.1
<i>Clavellisa scombri</i> *	7.9	1.5	2.0	1.0	20.0	1.7	6.5	1.8
Isopoda								
<i>Rocinela</i> sp.	—	—	—	—	8.3	1.2	35.5	1.3
<i>Ceratothoa gaudichaudii</i>	—	—	—	—	1.7	1.0	17.7	1.4

L, Immature stages.

\*parasite species with records only from *Scomber* spp.

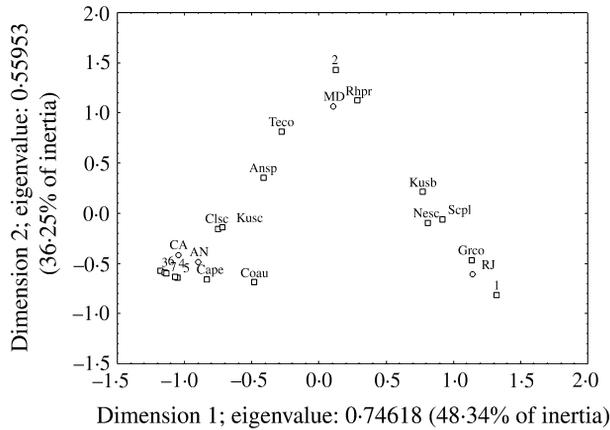


FIG. 2. Plot of the correspondence analysis. (See Fig. 1 for localities): 1, *Lecithocladium harpodontis*, *Opechona* sp., *Rhapidascaris* sp. and *Corynosoma* sp.; 2, *Pseudokuhnia minor*, *Prodistomum orientalis*, *Hysterothylacium fabri*, *Hysterothylacium* sp., *Nybelinia* sp. and a unidentified intestinal digenean from MD; 3, *Kuhnia sprostonae*; 4, *Ovarionematobothrium saba*; 5, *Ceratothoa gaudichaudii*; 6, *Didymocistis* sp.; 7, *Rocinela* sp.; Rhpr, *Rhadinorhynchus pristis*; Teco, *Tentacularia coryphaenae*; Ansp, *Anisakis* sp.; Kusc, *Kuhnia scombrisi*; Clsc, *Clavellisa scombrisi*; Cape, *Caligus pelamidis*; Coau, *Corynosoma australe*; Kusb, *Kuhnia scombercolias*; Nesc, *Nematobothrium scombrisi*; Sep1, *Scolex pleuronectis*; Grco, *Grubea cochlear*.

Cárdenas *et al.* (2005) suggested that *Trachurus* species entered the Pacific across the submerged Isthmus of Panama. It is not surprising that the hypothesis that explains the presence of *Merluccius* in the Pacific coast of America refers to the crossing of the submerged Isthmus of Panama, as suggested by Quinteiros *et al.* (2000), Kabata & Ho (1981) and Ho (1989) using molecular analyses, parasitological data and a cladistic analysis, respectively. Unfortunately,

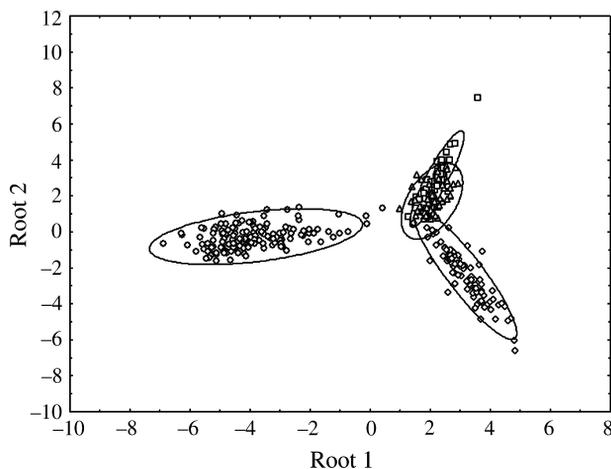


FIG. 3. Plot of a multivariate discriminant analysis. [Madeira Islands (○), Rio de Janeiro (◇), Callao (□) and Antofagasta (Δ).]

this approach has not been applied to other marine fishes like anchovies. The submerged Isthmus of Panama can probably explain the distribution pattern for other fish species (of the same genus) that are found in the Pacific and Atlantic coast of America, such as *Trachurus* and *Engraulis*. Members of those genera have a similar global distribution, with species in the Pacific and Atlantic coast of America, Atlantic coast of Africa, the Mediterranean Sea, North Atlantic (Europe) and the Indo-Pacific. The genus *Scomber* includes four species: *S. japonicus*, the Atlantic mackerel *Scomber scombrus* L., 1758, the blue mackerel *Scomber australasicus* Cuvier, 1832, and the Atlantic chub mackerel *Scomber colias* Gmelin, 1789 (Scoles *et al.*, 1998; Infante *et al.*, 2006). An important biogeographic characteristic of this genus is the global distribution of *S. japonicus* (Fig. 1) with a distribution that closely resembles those of species of the genus *Trachurus*, *Engraulis* and *Merluccius* among others.

The metazoan parasite fauna of chub mackerel from the Atlantic (Madeira and Brazil) and the Pacific (Peru and Chile) coast of America includes a total of 34 species (Table II), of which eight species (six ectoparasites and two endoparasites (Table II) are specific to *Scomber* spp. and at least one (*Ovarionematobothrium saba*) is specific to *S. japonicus* in Japan and the Pacific coast of South America.

Parasites of *S. japonicus* from the studied localities can be pooled in three categories: parasites found only in the Atlantic, parasites found only in the Pacific and parasites common to all localities (Table II). Only two parasites specific to *Scomber* (the monogenean *K. scombri* and the copepod *C. scombri*) are present in all the localities. According to Kabata (1979) the copepod *C. scombri* 'offers one of the rare instances among parasitic copepods of discontinuous distribution'. This comment also applies to *K. scombri*. The presence of both ectoparasites demonstrates a common origin for the four studied populations of *S. japonicus*, but the presence of *K. scombercolias* in the Atlantic population and *K. sprostonae* in the Pacific one suggests an early separation of both host populations. Recently, Costa *et al.* (2007) recorded just one specimen of *K. sprostonae* in one of 151 specimens of *S. japonicus* from Madeiran waters. Mazocraeids have been defined as primitive parasites specific to Clupeiformes and the presence of mazocraeids (*Grubea* spp. and *Kuhnia* spp.) are considered to be the consequence of a host shift (Mollaret *et al.*, 2000; Jovelín & Justine, 2001), but specificity for the new host apparently remains as a characteristic of this family of monogenean worms.

Didymozoids are common parasites of pelagic and oceanic fishes and four species were found in the samples. *Ovarionematobothrium saba* and *Didymocystis* sp. are present only in the Pacific coast of South America, *Halvorsenius exilis* is restricted to the Atlantic (but not Brazil) and *N. scombri* occurs in both Atlantic and Pacific host populations (but was not found in Peru). Didymozoid digeneans also can show high host specificity. The presence of specific (or specialist) parasites limited to the Atlantic and Pacific in the same host species raises some questions: either some parasites speciate at a higher rate than their host, or the *S. japonicus* populations from the Pacific coast of South America and Atlantic coast of South America and Madeira really represent cryptic species, that in turn can explain the presence of different parasite species. *Merluccius* spp. and *Engraulis* spp. harbour the monogenean *Anthocotyle merlucci* and

*Pseudanthocotyloides heterocotyle*, respectively, along their geographical distribution as a genus, suggesting that the hosts speciate at a rate higher than their monogenean parasites.

The current distribution of *S. japonicus* in the Atlantic and Pacific Ocean can be explained by a migratory route that closely resembles those of the genera *Merluccius* and *Trachurus*, specifically using the passage over the submerged Isthmus of Panama. But, why did *Merluccius* and *Trachurus* (and other fish species) produce different species in an anti-tropical distributional pattern along the coast of South America whereas *Scomber* did not? A speculative point of view suggests a very low speciation rate for *Scomber*. Both, the genera *Trachurus* and *Merluccius* have speciated since the Isthmus of Panama closed as a passage between the Atlantic and Pacific Ocean. Any other possible explanation must consider a migratory route throughout the Pacific Ocean. Rohde & Hayward (2000) suggested that the eastern Pacific was a good barrier for most scombrids but not for the genus *Scomber*. According to Zardoya *et al.* (2004) marine migratory fishes with high dispersal capabilities and large effective population size have high levels of gene flow and consequently a low degree of differentiation. From this perspective, Mediterranean populations of mackerel (*S. scombrus* and *S. japonicus*) are genetically well differentiated. Specifically, the population of *S. japonicus* is considered as panmictic in the Mediterranean Sea and adjacent Atlantic waters. In addition, Nesbo *et al.* (2000) pointed out that the distribution and level of divergence of different genetic lineages in highly mobile species can reflect both present and historical events of gene flow. The presumed high mobility of *S. japonicus* has not been proved (at least for the Pacific coast of America). Moreover, it is well known that populations from the South Pacific and North Pacific coast of America do not overlap (Stepian & Rosenblatt, 1996; Scoles *et al.*, 1998) and gene flow between both populations remains undefined.

Unfortunately, the most comprehensive study of the phylogeography of the genus *Scomber* does not include samples from the Pacific coast of South America, Brazil and Madeira (Scoles *et al.*, 1998). The particular distributional pattern of *S. japonicus* in addition to the parasitological evidence, based on the presence of highly specific parasites for Atlantic and Pacific populations of *S. japonicus*, strongly supports the recommendation of a revision of the taxonomy of *S. japonicus* that may actually be a complex of species along its extended and discontinuous distribution. Recently Infante *et al.* (2006) have suggested, on the basis of nuclear DNA sequences, the phylogenetic differentiation between *S. colias*, a species from the Atlantic (Gulf of Cádiz, Spain) and *S. japonicus* from the Pacific (Kochi Prefecture, Japan). In addition, they strongly suggest the need for a revision of the classical taxonomic status of mackerels of the genus *Scomber*, a suggestion also proposed by Collete *et al.* (2001) who give evidence that supports the division of *Scomber* into two genera. The suggested revision is now supported not only by molecular evidence, but also by parasitological evidence. The agreement between molecular and parasitological evidence reinforces the use of parasites as adequate tools to clarify taxonomic problems of their hosts.

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