

Parasitology Research
vol. 87, nr. 6, p. 439 - 444
© Springer-Verlag 2001
DOI 10.1007/s004360100379

## Original Paper


# Morphological and genetic differentiation of *Rodentolepis straminea* (Goeze, 1752) and *Rodentolepis microstoma* (Dujardin, 1845) (Hymenolepididae)

J.C. Casanova<sup>1</sup>, , F. Santalla<sup>1</sup>, P. Durand<sup>2</sup>, C. Vaucher<sup>3</sup>, C. Feliu<sup>1</sup> and F. Renaud<sup>2</sup>

(1) Laboratori de Parasitologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, E-08028 Barcelona, Spain

(2) Centre d'Étude sur le Polymorphisme des Micro-Organismes CEPM/UMR CNRS-ORSTOM 9926, Montpellier, France

(3) Museum of Natural History, Route de Malagnou 1, P.O. Box 6434, 1211 Geneva 6, Switzerland

 E-mail:

[Casanova@farmacia.far.ub.es](mailto:Casanova@farmacia.far.ub.es)

Fax: +34-3-4024504

**Received:** 7 October 2000 / **Accepted:** 11 January 2001 / **Published online:** 28 March 2001

**Abstract.** The two related species, *Rodentolepis straminea* (Goeze, 1782) and *Rodentolepis microstoma* (Dujardin, 1845) (Cestoda, Hymenolepididae), both parasites of rodents, were compared morphologically and electrophoretically. Adult **worms** were isolated from three wild rodent species of the family Muridae (*Apodemus flavicollis*, *Apodemus sylvaticus*, and *Mus musculus*) from three different sites in Spain and France. Although these two species were strikingly similar in morphological appearance, some of the morphological and metrical features analysed (**scolex**, mature segments and eggs) can be used for differentiation. Fixed allelic differences were found. Of the ten enzymes detected by starch-gel electrophoresis, six (AAT, AK, **GPI**, MDH, NP, PGM) showed characteristic isoenzyme profiles in each species. Only in MPI, PEPC, PEPD, and ME enzyme loci were no differences found. The study revealed that the two taxa can be clearly differentiated.

## Introduction

There is a long history of hymenolepidid systematics. The latest important systematic arrangement of Hymenolepididae Ariola, 1899 is the classification of Czaplinski and Vaucher (1994). In the past, *Rodentolepis straminea* (Goeze, 1782) and *Rodentolepis microstoma* (Dujardin, 1845) were placed in the genera *Hymenolepis*, *Rodentolepis*, and *Vampirolepis*. Spasskii and Spasskaja (1954) placed both species in the genus *Rodentolepis* and Czaplinski and Vaucher (1994) consider *R. straminea* as a type species. *R. straminea* and *R. microstoma* have historically confused systematics and faunistics in wild rodents, partially due to the non-availability of type material, an incomplete

taxonomic description, and the multiplicity of mixed morphological and morphometric data. Tenora and Murai ( [1970](#) ) revalidated *R. straminea* and revised its morphological characters in relation to *R. microstoma*. Baer and Tenora ( [1970](#) ) suggested the synonymy of both species, and in several later works this synonymy was applied.

Faunistical reports of both species in natural rodent infections are abundant and their range of natural hosts has also been reported (genera *Mus*, *Rattus*, *Meriones*, *Apodemus*, *Mastomys*, *Promomys*, *Arctomys*, *Dendromus*, *Leggada*, *Microtus*, *Cricetus*, *Cricetulus*) (Schmidt [1986](#) ). The life-cycle of both species is identical, but in laboratory experiments the susceptibility of different definitive hosts is variable (Dvorak et al. [1961](#) ; Walkey et al. [1980](#) ). Although these studies indicated that both taxa are non-conspecific, no genetic evidence of this synonymy has been reported. In the present study, morphological and electrophoretic isoenzyme analyses were performed to provide and compare the data of *R. straminea* and *R. microstoma*. The proposed synonymy of both species is discussed.

## Materials and methods

### Hosts and parasites

Adult hymenolepidids were collected from natural hosts. The host-collecting sites were located in Spain and France and were visited between 1995 and 1997: Eugi (Spain) (10°30'26" W / 42°58'49" N); Granollers (Spain) (2°17'17" E / 41°36'33" N); and Moulis (France) (1°5'33" E / 42°57'35" N). A total of 34 individual murid hosts were parasitised by hymenolepidids and 69 individual [worms](#) were recovered [20 from *Mus musculus* (Granollers); 13 from *A. flavicollis* (12 from Eugi and one from Moulis); and 36 from *Apodemus sylvaticus* (30 from Eugi and six from Moulis)].

Living [cestodes](#) were rinsed several times in physiological saline to remove remains of host tissue. [Worms](#) were then separated and placed in tap-water to relax. The [scolex](#), posterior gravid segments and some mature segments were cut out and placed in tubes containing 70% ethanol for morphological determination. The remainder of the [worms](#) were frozen in liquid nitrogen and used subsequently for electrophoresis. Thus, each isoenzyme pattern was compared with the morphological characters.

### Morphological and morphometric analysis

Only fully mature [worms](#) with a [strobila](#) with gravid segment were investigated. For morphometric measurements we selected only host individuals with a parasite burden of 1-15 [worms](#) since, in the case of *R. microstoma*, a crowding effect has been reported (Smyth and McManus [1989](#) ). For morphological study, [scolex](#) and eggs were cleared with lactophenol. The scolices were studied without cover-glass pressure. Eggs were removed from the last gravid segment of the [worms](#). Mature segments were stained in iron acetocarmine, according to Georgiev et al. ( [1986](#) ). Measurements were taken of morphological characteristics of the [scolex](#), mature segments and eggs. All morphometric data are given in micrometres. For species identification, the works of Joyeux and Kobozieff ( [1928](#) ), Dvorak et al. ( [1961](#) ), Baer and Tenora ( [1970](#) ), Tenora and Murai ( [1970](#) ) and Murai ( [1989](#) ) were mainly considered. Voucher specimens are deposited in the Laboratory of Parasitology, University of Barcelona.

### Multilocus enzyme electrophoresis

All recovered [worms](#) were analysed by MEE. A total of ten enzyme systems from the parasites gave interpretable results: aspartate-amino-transferase (AAT, E.C. 2.6.11), adenylate kinase (AK, E.C. 2.7.4.3), glucose-phosphate-isomerase ([GPI](#), E.C. 5.3.1.9), malate dehydrogenase (MDH, E.C.

1.1.1.37), malic enzyme (ME, E.C. 1.1.1.40), mannose-phosphate-isomerase (MPI, E.C. 5.3.1.8), nucleoside phosphorylase (NP, E.C. 2.4.2.1), [peptidases](#) (PEPC, PEPD, E.C. 3.4.11 ó 13) and phosphoglucomutase (PGM, E.C. 2.7.5.1). Isoenzyme patterns were determined following starch-gel electrophoresis using 10% starch. The homogenate of the [worms](#) was absorbed onto chromatography paper (2×10 mm, Whatman No. 3). Electrophoresis was carried out at 37°C in a specific staining mixture for each enzyme, as described in Pasteur et al. ( [1987](#) ).

## Statistical study

Morphometric data obtained from the [scolex](#), mature segments, and eggs of the [worms](#) from *Apodemus* spp. and *M. musculus* were normally distributed and compared using Student's *t*-test. Statistical differences were significant at  $P<0.05$  and  $P>0.001$ . The genetic differences between hymenolepidids coming from *Apodemus* spp. and *M. musculus* were calculated using the Nei distance (D) (Nei [1972](#) ).

## Results

### Morphology and morphometry

The [worms](#) isolated from the three host species were identified as *R. straminea* and *R. microstoma*. The comparative morphological study of *R. straminea* isolated from *A. sylvaticus* and *A. flavicollis*, and *R. microstoma* isolated from *M. musculus* had similar characteristics. Different metrical features in the [scolex](#), mature segments and eggs distinguished the two species. The most important qualitative differences between them was the presence of [polar filaments](#) in the eggs of *R. microstoma*, which were absent in the eggs of *R. straminea*. The measurements of *R. straminea* and *R. microstoma*, including the average value, range (minimal value and maximal value), and standard deviation (SD) are given in Table [1](#). The morphometric data of the two species are not generally coincident and an obvious difference was found in most morphometric features. The [strobila](#) of both species are long, craspedote, with numerous [proglottids](#) in gradual maturation. Young [proglottids](#) contain only primordia of testes. Mature [proglottids](#) contain three-lobed ovary and testes in both species. All metrical parameters studied in the [scolex](#) of both species (Table [1](#)) show statistical significant differences at  $P<0.001$ , except in [scolex](#) length and the size of the hooks. [Scolex](#) width, diameters of suckers, [rostellum](#) and rostellar sheath are higher metrical values in *R. straminea* than *R. microstoma*. Morphology (cricetoid type) and morphometry of rostellar hooks are identical in both species. The number of hooks differed significantly; in *R. microstoma* the number is always less than 30. In mature segments (Table [1](#)), the three sub-espherical testes, one poral and two aporal, are disposed aligned, or in an elongated triangle. External vesicle and seminal [reservoirs](#) show higher measures in *R. straminea*. The seminal reservoir reaches the median line of the proglottid in *R. straminea*, but not in *R. microstoma*. In contrast, the width of the [cirrus](#) sac and that of the internal vesicle are significantly greater in *R. microstoma*. Measurements of testes and ovary showed only significant differences (  $P<0.05$  ). The size of the [vitellarium](#) is significantly higher in *R. microstoma* than in *R. straminea*. The diameter of eggs did not overlap (Table [1](#)). Eggs are ovoid in both species, but larger (  $P<0.001$  ) in *R. microstoma*. Oncospheres are similar in diameter, with slight [polar plugs](#) in *R. microstoma* and 3-5 [polar filaments](#). Measurements of oncosphera hooklets show significant differences in the total length of lateral embryonic hooks in both species.

**Table 1.** Metrical data of *Rodentolepis straminea* from *Apodemus* spp and *R. microstoma* from *Mus musculus*. SD Standard deviation

	<i>Apodemus</i> spp.			<i>M. musculus</i>		
	<i>R. straminea</i> ( <i>n</i> =65)			<i>R. microstoma</i> ( <i>n</i> =21)		
	Mean	SD	Range	Mean	SD	Range

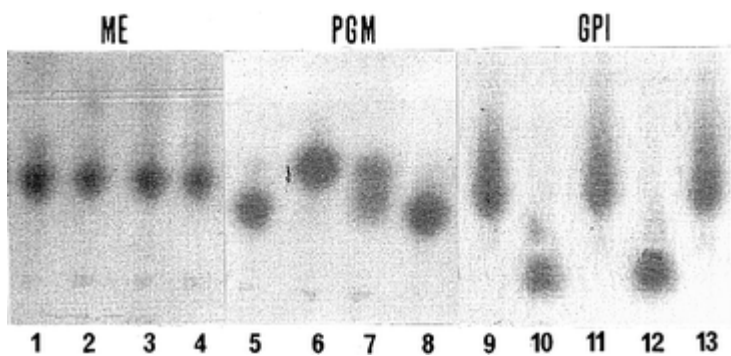
	<i>Apodemus</i> spp.			<i>M. musculus</i>		
<u>Scolex</u> width**	269.9	29.6	76.8-307.2	222.0	27.0	186.9-294
<u>Scolex</u> length	178.4	28.3	102.4-226.7	169.7	21.7	143.4-204.8
Sucker diameters**	100.2	6.9	51.2-115.2	78.0	9.6	56.3-89.6
<u>Rostellum</u> length**	82.4	12.6	38.4-107.5	54.5	17.7	38.4-58.8
<u>Rostellum</u> width**	79.4	15.4	51.2-110.1	46.8	13.0	33-66.6
Length of rostellar sac**	184.3	40.3	94.7-266.2	126.3	27.0	69.1-148.5
Width of rostellar sac **	118.7	21.0	51.2-130.6	84.1	29.5	64-125.4
Number of rostellar hooks**	30.9	3.0	22-36	25.4	1.5	23-29
Rostellar hooks length	12.4	2.0	10.1-17.9	11.0	1.3	10.2-23
Length of blade hooks	8.2	1.8	5.1-10.2	6.6	1.4	5.1-7.7
Length of testes*	87.3	9.3	70.0-110.9	79.2	14.8	50.3-117
Width of testes	62	4.1	53.8-78.5	62.6	12.1	41-85.3
Length of <u>cirrus</u> sac	117.7	15.5	89.6-153.6	124.0	24.4	97.3-181.8
Width of <u>cirrus</u> sac**	29.3	4.0	23.0-38.4	36.8	3.3	28.2-41
Length of internal seminal vesicle	91.2	13.0	64-120.3	88.7	22.0	58.9-141
Width of internal seminal vesicle**	28.8	3.4	25.6-64	35.0	3.0	28.2-41
Length of external seminal vesicle**	116.5	27.4	62.1-166.4	91.0	22.8	56.3-128
Width of external seminal vesicle**	46.3	12.0	25.6-76.8	38.3	8.1	25.6-59
Length of seminal reservoir**	222.1	42.1	128-294.4	160.8	38.4	99.8-218
Width of seminal reservoir**	83.7	12.5	56.3-102.4	60.8	19.3	28.2-90
Maximal length of ovari	230.1	47.5	163.8-307.2	201.3	76.1	102.4-358.4
Maximal width of ovari*	57.0	10.1	35.8-76.8	70.1	28.0	38.4-115.2
Vagina length	164.4	56.1	89.6-279.0	142.6	31.2	92.2-207.4
Length of vitelline gland*	57.2	15.1	35.8-89.6	67.0	17.6	38.4-110.1
Width of vitelline gland**	37.8	8.3	25.6-64	49.0	14.0	30.7-79.4
Eggs length**	51.6	3.6	44.4-56.6	72.7	6.2	62.6-98
Eggs width**	42.8	2.1	35.3-47.5	59.6	9.2	51.5-75.7
Oncosphaera length	38.2	4.4	28.3-45.4	39.4	7.6	27.3-47.5
Oncosphaera width	33.0	4.5	24.2-39.4	34.1	7.2	29.3-54.5
Length of internal lateral oncosphaera hooklets**	14.9	0.4	13.1-16.2	16.5	0.8	15.1-18.2
Length of external lateral oncosphaera hooklets**	14.8	0.5	13.0-15.6	15.9	0.9	14.1-17.6
Length of central oncosphaera hooklets	16.2	0.6	14.4-17.1	16.9	0.9	13.1-17.1

\*Significant differences at  $P < 0.05$

\*\*Significant differences at  $P < 0.001$

## Multilocus enzyme electrophoresis

No allelic variation was found between worms from *A. flavicollis*, *A. sylvaticus* and *M. musculus* for ME (Fig. 1), MPI and PEPD, all encoded by a single locus. Fixed allelic differences were observed in *R. straminea* and *R. microstoma* for AAT, AK, GPI (Fig. 1), MDH, NP1, NP2 and PGM (Table 2). The *R. straminea* zymogram showed a single band at PEPC, whereas in *R. microstoma* it was inactive. Different single loci in the zymograms of the two species were found for AAT, AK, GPI, MDH, and NP1. For NP2, two bands of activity were seen in worms from *A. flavicollis* and *A. sylvaticus*, and only a single band of different mobility was detected in specimens from *M. musculus*. NP2 isoenzyme patterns in *R. straminea* were characterised by the existence of three phenotypes. Two of these could be interpreted as the homozygotic state of a gene with two different alleles, whereas the third phenotype could correspond to a heterozygotic state of the same gene. Four phenotypes were observed in the PGM isoenzyme patterns (Fig. 1), two for *R. straminea* and two for *R. microstoma*. One phenotype for *R. straminea* and one for *R. microstoma* are encoded by a single band with different electrophoretic mobilities in the two species. The heterozygotic state of the same gene is found only in *R. microstoma*. Allele frequencies found in *R. straminea* and *R. microstoma* at the loci studied are given in Table 2. No common alleles were shared between these species at seven loci, indicating that no gene exchange occurs between them and confirming their specific rank. Nei's value of genetic distance between *R. straminea* and *R. microstoma* was found to be 1.55.



**Fig. 1.** Electrophoretic patterns of ME, PGM and GPI in *Rodentolepis straminea* and *R. microstoma* isolates generated by starch gel electrophoresis. Lanes 1-6, 10, 12: *R. straminea*; lanes 7-9, 11, 13: *R. microstoma*

**Table 2.** Allele frequencies at 12 loci tested in *R. straminea* and *R. microstoma*

		<i>R. straminea</i>	<i>R. microstoma</i>
		<i>Apodemus</i> spp	<i>M. musculus</i>
AAT	A	1	
	B		1
AK1	A	1	
	B		1
AK2	A	1	
GPI	A	1	
	B		1

		<i>R. straminea</i>	<i>R. microstoma</i>
MDH	A	1	
	B		1
ME	A	1	
MPI	A	1	
NP1	A	1	
	B		1
NP2	A	0.47	
	B	0.53	
	C		1
PEPC	A	1	
PEPD	A	1	1
PGM	A	0.99	0.05
	B	0.01	
	C		0.95

## Discussion

*R. straminea* and *R. microstoma* are commonly reported in wild rodents in Europe, the USSR, America and Africa (Dvorak et al. [1961](#) ; Baer and Tenora [1970](#) ; Prokopic and Genov [1974](#) ; Schmidt [1986](#) ). In these hosts, partial and mixed descriptions of both species are reported. It could be deduced, in view of our results, that their faunistical data are not in accordance with their real distribution and true definitive hosts. This is surprising, especially in the case of *R. microstoma*, because this [worm](#) is widely used as a laboratory model in studies of experimental hymenolepidiasis, and several aspects of its morphoanatomy, growth, biology, physiology, immunology and pathology are well known (Smyth and McManus [1989](#) ).

*R. straminea* has been studied less , but several aspects of their morphology, biology and immunology have also been investigated (Walkey et al. [1980](#) ). The life-cycles of *R. straminea* and *R. microstoma* are known in both natural and experimental conditions. Both species infest beetles (mainly *Tribolium* spp.) as [intermediate hosts](#) and several differences have been found in their infection susceptibility (Dvorak et al. [1961](#) ; Vaucher and Hunkeler [1967](#) ; Walh [1967](#) ; Walkey et al. [1980](#) ). In view of these studies, the conspecificity of the two species is doubtful; however, elsewhere, their synonymy has been proposed (Baer and Tenora [1970](#) ).

Dvorak et al. ( [1961](#) ) studied the biology of *R. microstoma* in *Mus musculus*, *Rattus norvegicus* and *Mesocricetus auratus*, and concluded that *M. musculus* is the most suitable species of definitive host besides the golden hamster. These authors failed to infect *R. norvegicus* with *R. microstoma* , which were, however, reported as compatible by Joyeux and KovoziEFF ( [1928](#) ), and attributed this refractory character to biological strain differences of the parasite. Observations of Walkey et al. ( [1980](#) ) on the specificity of *R. straminea*, showed that the preferred hosts are *A. flavicollis*, *A. sylvaticus* and *Mesocricetus auratus* , and that *Mus musculus* could be infective only in individuals having potentially reduced immunological competence. These authors failed to infect *R. norvegicus* and other rodents ( *Meriones unguiculatus*, *Clethrionomys glareolus* and *Microtus agrestis*) with [cysticercoids](#) from experimental infections in *Tribolium confusum*, from eggs to natural infections

of *R. straminea* in *A. sylvaticus*. In our study, *A. sylvaticus* and *M. musculus* were captured several times at the same sites. No cross-infections of *R. straminea* in *M. musculus* and *R. microstoma* in *A. sylvaticus* were found. This seems to indicate that each parasite species exhibited a stronger preference in natural infections than that suggested by faunistical data.

Murai ( [1989](#) ) reported cricetids as a common definitive host for *R. straminea* and also *Apodemus* spp. in eastern Europe (Prokopic and Genov [1974](#) ; Merkusheva and Bobkova [1981](#) ). In Spain and the South of France, the preferred definitive host for this species is *Apodemus* spp.

Morphological features, such as the size and shape of rostellar hooks, that have been used as the important systematic characteristics in armed hymenolepidids (see Mas-Coma and Galán-Puchades [1991](#) ) have little value in discriminating between *R. straminea* and *R. microstoma*. The only morphological characters differentiating both species are the constancy in the dimensions of the eggs and oncosphera hooklets, and the presence of [polar filaments](#). The rostellar armature is important for distinguishing *R. straminea* and *R. microstoma* from other related species, e.g. *R. nana*, which has hooks of the fraternoid type (Baer and Tenora [1970](#) ) and uses Muridae as preferential hosts. This differentiation is particularly interesting in the case of *R. microstoma* , because the eggs of *R. nana* also have [polar filaments](#) (3-5 [polar filaments](#) in *R. microstoma* compared with 5-6 in *R. nana* ) and measurements have lead to successful differentiation (Dvorak et al. [1961](#) ).

Isoenzymatic studies have been performed in several species of hymenolepidids from laboratory rodents. The most frequently studied species is *H. diminuta*. Different isoenzyme techniques (isoelectrofocusing, cellulose acetate-gel, starch-gel) were applied to characterise different species or strains (Walkey and Fairbairn [1973](#) ; Logan et al. [1979](#) ; Kholhagen et al. [1985](#) ; Pappas et al. [1986](#) ; Dixon and Arai [1987](#) , [1989](#) ; Montgomery et al. [1987](#) ; Andrews et al. [1989](#) ; Novak et al. [1989](#) ). The results obtained in these works show that protein electrophoretic methods give reliable results for differentiation of species and species strains in hymenolepidids. Dixon and Arai ( [1987](#) , [1989](#) ) found characteristic patterns in *Hymenolepis diminuta*, *H. citelli* and *R. microstoma*. Noticeable differences were found in enzymatic profiles of the two morphologically similar species, *H. diminuta* and *H. citelli*, both while maintained in their preferred and alternative definitive hosts. Novak et al. ( [1989](#) ) differentiated four species of hymenolepidids by starch-gel electrophoresis: *R. microstoma*, *R. nana*, *H. citelli* and *H. diminuta* , finding a large number of banding patterns for *R. microstoma*. They clearly distinguished this species from the unarmed species *Hymenolepis* spp. Biochemical studies of *R. straminea* are unknown and few morphological and biological data are available for this species. In our study, the comparison of the enzyme pattern of *R. straminea* and *R. microstoma* revealed a remarkable degree of genetic difference. The dissimilarity in the zymograms indicates that the taxa studied belong to different species.

Alternative methods to morpho-anatomy are essential for testing the validity of closely related or sibling species. Isoenzymes proved to be a powerful tool for clarifying the taxonomic status of closely related species in various groups of [cestodes](#), particularly: Renaud et al. ( [1983](#) ) (bothriocephalids); Renaud and Gabrion ( [1988](#) ) (cyathocephalids); de Chambier et al. ( [1992](#) ); de Chambier and Vaucher ( [1994](#) ); Hanzelová et al. ( [1995](#) ); Snabel et al. ( [1994](#) ); Zehnder et al. ( [2000](#) ) (proteocephalids); Ba et al. ( [1993](#) ); Baverstock et al. ( [1985](#) ) (anoplocephalids); and Montgomery et al. ( [1987](#) ) (hymenolepidids) (see also Beveridge [1998](#) and Andrews and Chilton [1999](#) , for reviews). In our case, the taxa considered are sympatric. Consequently, as emphasised by Beveridge ( [1998](#) ), the results of isoenzyme electrophoresis are particularly useful, because the fixed diagnostic differences strongly support the absence of gene-flow between the two taxa *R. straminea* and *R. microstoma*. The synonymy between *R. straminea* and *R. microstoma* has already been strongly questioned following the results of the karyological study of Spakulová and Casanova ( [1998](#) ). The present study confirms the conclusions of these authors.

Finally, although statistically significant metric differences have been demonstrated in this present paper, they are not totally reliable in identification because of their high degree of overlap. The

actual morphological diagnostic character remains the presence or absence of egg filaments in *R. microstoma*.

**Acknowledgements.** This study was supported by the PICASSO initiative (HF 96-186, HF 97-114), DGYCIT project PB 96-0401-C02 (Ministerio de Educación y Cultura) and the Comissionat per Universitat i Recerca (1998 SGR 0003).

## References

- Andrews RH, Chilton NB (1999) Multilocus enzyme electrophoresis: a valuable technique for providing answers to problems in parasite systematics. *Int J Parasitol* 29:213-225
- Andrews RH, Adams M, Baverstock PR, Behm CA, Bryant C (1989) Genetic characterisation of three strains of *Hymenolepis diminuta* at 39 enzyme loci. *Int J Parasitol* 19:515-518
- Ba CT, Wang XQ, Renaud F, Euzet L, Marchand B, De Meeüs T (1993) Diversity and specificity in *cestodes* of the genus *Moniezia*: genetic evidence. *Int J Parasitol* 23:853-857
- Baer JG, Tenora F (1970) Some species of *Hymenolepis* (Cestoidea) from rodents and from primates. *Acta Sci Nat Brno* 4:1-32
- Baverstock PR, Adams M, Beveridge I (1985) Biochemical differentiation in bile duct *cestodes* and their marsupial hosts. *Mol Biol Evol* 2:321-337
- Beveridge I (1998) Allozyme electrophoresis-difficulties encountered in studies on *helminths*. *Int J Parasitol* 28:973-979
- Czaplinski B, Vaucher C (1994) Family Hymenolepididae Ariola, 1899. In: Khalil LF, Jones A, Bray RA (eds) *Keys to the cestode parasites of vertebrates*. CAB International, Wallington, UK, pp 595-663
- Chambier A de, Vaucher C, Renaud F (1992) Étude des caractères morpho-anatomiques et des flux géniques chez quatre *Proteocephalus* (Cestoda: Proteocephalidae) parasites de *Bothrops jararaca* au Brésil et description de trois espèces nouvelles. *Syst Parasitol* 23:141-156
- Chambier A de, Vaucher C (1994) Étude morpho-anatomique et génétique de deux nouveaux *Proteocephalus* Weinland, 1858 (Cestoda: Proteocephalidae) parasites de *Platydoras costatus* (L.), poisson siluriforme de Paraguay. *Syst Parasitol* 27:173-185
- Dixon BR, Arai HP (1987) An investigation of host influence on soluble protein banding profiles of *Hymenolepis* spp. (Cestoidea), using isoelectric focusing. *Can J Zool* 65:2471-2474
- Dixon BR, Arai HP (1989) Differentiation of three species of *Hymenolepis* (Cestoidea) using enzyme isoelectric focusing on thin-layer agarose gels. *Can J Zool* 67:51-54
- Dvorak JA, Jones AW, Kuhlman HH (1961) Studies on the biology of *Hymenolepis microstoma* (Dujardin, 1845). *J Parasitol* 39:128-132
- Georgiev BB, Biserkov V, Genof T (1986) In toto staining method for *cestodes* with iron acetocarmine. *Helminthologia* 62:235-240
- Hanzelová V, Snabel V, Spakulová M, Kralova I, Fagerholm HP (1995) A comparative study of the fish parasites *Proteocephalus exiguus* and *P. percae* (Cestoda: Proteocephalidae): morphology, isoenzymes, and karyotype. *Can J Zool* 73:1191-1198
- Joyeux CH, Kobozieff NI (1928) Recherches sur l' *Hymenolepis microstoma*. *C R Soc Biol* 97:12-13
- Kholhagen S, Behn CA, Bryant C (1985) Strain variation in *Hymenolepis diminuta*: enzyme profiles. *Int J Parasitol* 15:479-483
- Logan J, Ubelaker JE, Vrijenhoek RC (1979) Isozymes of L(+)-LDH in *Hymenolepis diminuta*.

Comp Biochem Physiol 57B:51-53

Mas-Coma S, Galán-Puchades MT (1991) A methodology for the morphoanatomic and systematic study of the species of the family Hymenolepididae Railliet et Henry, 1909 (Cestoda: Cyclophyllidea). Res Rev Parasitol 51:139-173

Merkusheva YV, Bobkova AF (1981) [Helminths](#) of domestic and wild animals of Bielorrussia. Catalogue. Nauk TechMinsk: 118

Montgomery SSJ, Montgomery WI, Dunn TS (1987) Biochemical, physiological and morphological variation in unarmed hymenolepids ([Eucestoda](#): Cyclophyllidea). Zool J Linn Soc 91:293-324

Murai E (1989) *Ceratozetes gracilis* (Michael, 1884) (Acari: Oribatida), an [intermediate host](#) of *Vampirolepis asymmetrica* (Janicki, 1904) (Cestoda: Hymenolepididae). Misc Zool Hung 5:13-19

Nei M (1972) Genetic distance between populations. Am Nat 106: 283-291

Novak M, Taylor WR, Pip E (1989) Interspecific variation of isoenzyme patterns in four *Hymenolepis* species (Cestoda). Can J Zool 67:2052-2055

Pappas PW, D. Leiby A (1986) Alkaline phosphatase and phosphodiesterase activities of the brush border membrane of four strains of the tapeworm *Hymenolepis diminuta*. J Parasitol 72:809-811

Pasteur N, Pasteur G, Bonhomme F, Catalan J, Britton-Davidian J (1987) Manuel technique de génétique par électrophorèse des protéines. Technique et documentation (Lavoisier), Paris Cedex p. 217

Prokopic J, Genov T (1974) Distribution of [helminths](#) in micromammals (Insectivora and Rodentia) under different ecological and geographical conditions. Csek Ak, Praha: 159

Renaud F, Gabrion C, Pasteur N (1983) Le complexe *Bothriocephalus scorpii* (Mueller, 1776): différenciation par électrophorèse enzymatique des espèces parasites du turbot (*Psetta maxima*) et de la barbu (*Scophthalmus rhombus*). C R Acad Sci Ser III 296:127-129

Renaud F, Gabrion C (1988) Evidence for two sibling species in the complex *Bothriomonus nylandicus* (Schneider, 1902) (Cestoda: Cyathocephalidae). Parasitology 30:139-147

Schmidt GD (1986) Handbook of tapeworm identification. CRC Press, Boca Raton, Fla, p. 675

Smyth JD, McManus DP (1989) The physiology and biochemistry of [cestodes](#). Cambridge University Press, Cambridge, p 398

Snabel V, Hanzelova V, Fagerholm HP (1994) Morphological and genetic comparison of two *Proteocephalus* species (Cestoda: Proteocephalidae). Parasitol Res 80:141-146

Spakulová M, Casanova JC (1998) Chromosome analysis of *Rodentolepis straminea* (Cestoda: Hymenolepididae) parasiting wood mice (*Apodemus* spp.) in Spain. Helminthologica 35:185-188

Spasskii AA, Spasskaja LP (1954) Systematic structure of the Hymenolepidids parasitic in birds: Tr Gelmintol Lab. Akad Nauk SSSR 7:55-119

Tenora F, Murai E (1970) *Hymenolepis straminea* (Goeze, 1752) (Cestoda, Hymenolepididae), Parasite of *Cricetus cricetus* L. in Hungary. Parasitol Hung 3:33-42

Vaucher C, Hunkeler P (1967) Contribution a l'étude des [cestodes](#) et des trématodes parasites des micromammifères de Suisse. I. Bull Soc Neuchâtel Sci Nat 90:161-184

Wahl E (1967) Étude parasito-écologique des petits Mammifères (Insectivores et Rongeurs) du Val de l'Allodon (Genève). Rev Suisse Zool 74:129-188

Walkey M, FairBairn D (1973) L (+) lactate dehydrogenases from *Hymenolepis diminuta* (Cestode). J Exp Zool 183:365-373

Walkey M, Simmons DJC, Nasher AK (1980) Observations on the host specificity of *Hymenolepis*

*straminea* (Goeze). J Parasitol 66:420-423

Zehnder MP, de Chambier A, Vaucher C, Mariaux J (2000) *Nomimoscolex suspectus* n. sp. ([Eucestoda](#): Proteocephalidea, Zygobothriinae) with morphological and molecular phylogenetic analyses of the genus. Syst Parasitol (in press)