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Mesocestoides litteratus (Batsch, 1786) (Cestoda: Cyclophyllidea: Mesocestoididae) from the red fox: morphological and 18S rDNA characterization of European isolates

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Summary

Mesocestoides litteratus (Batsch, 1786) (Cestoda: Cyclophyllidea: Mesocestoidae) is a common parasite of the red fox (*Vulpes vulpes*) and other carnivores across Europe. There has been considerable debate as to the validity of *M. litteratus* and other closely related, often sympatric species of *Mesocestoides*. We examine isolates of *M. litteratus* from red foxes in the Czech Republic, Slovakia and Spain both morphometrically and by characterization of 18S rDNA. Morphometric ranges of all isolates confirmed their identity as *M. litteratus* and were usually within the range published formerly. The sequences of 18S rDNA of one or two isolates from each country were analysed. The sequences were the same and distinct from all published *Mesocestoides* 18S sequences with the exception of tetrathyridia from a lizard in the Czech Republic, which was identical to those of *M. litteratus*.

Key words: *Mesocestoides*; red fox; *Vulpes vulpes*; Cestoda; morphological analysis; DNA

Introduction

Mesocestoides litteratus (Batsch, 1786) (Cestoda: Cyclophyllidea: Mesocestoidae), a common tapeworm of the red fox (*Vulpes vulpes* Linnaeus, 1758) and other carnivores in Europe, has had a rich history of systematic and taxonomic evaluation (e.g. Müller, 1928; Wittenberg, 1934; Voge, 1955; Tschertkowa & Kosupko, 1978; Loos-Frank, 1980; Loos-Frank & Zeyhle, 1982; Priemer, 1983; Jancev, 1986; Tenora, 2004, 2005), at least partly due to a high degree of apparently host-induced morphological variation (Rausch, 1994). In a largely nomenclatural review, Loos-Frank (1980) concluded that *M. litteratus* is a *nomen dubium* and in the same paper described a new species, *Mesocestoides leptothylacus*, with the red fox and domestic cat as hosts. In contrast, Priemer (1983) and Jancev (1986) supported Tschertkowa and Kosupko (1978) about a validity of the name *M. litteratus* for the same taxon.

Recent studies have followed Priemer (1983) in using the name *M. litteratus* (e.g. Torres *et al*., 1998; Miquel *et al.*, 1999; Andras, 2001; Miguel & Marchand, 2001; Andras & Peter, 2002) but their determinations were not supported by morphological analyses. Gubanyi and Eszterbauer (1998) and Tenora (2005) only described cestodes from red foxes in morphological detail in support of their identification as *M. litteratus*. Gubanyi and Eszterbauer (1998) declared erroneously *M. litteratus* (referred to as *M. leptothylacus*) to be a synonym of *M. lineatus* (Goeze, 1782). However, the spherical cirrus pouch in mature segments is a diagnostic feature of *M. lineatus* and based on this character, *M. lineatus* may be unambiguously distinguished from *M. litteratus*, the cirrus pouch of which is elongated and sac-shaped.

Few studies have examined variation among *Mesocestoides* spp. using molecular data. Nickisch-Rosenegk *et al.* (1999) found a small genetic difference in a partial fragment of the mitochondrial 12S rDNA (4 of 304 bases compared) of *M. lineatus* and *M. litteratus* (the latter being referred to as *M. leptothylacus*) from red foxes in Germany. In North America, Crosbie *et al.* (2000) used 18S and ITS2 rDNA to examine variation among adults and tetrathyridia of *Mesocestoides* spp. from dogs and coyotes, finding at least three distinct genotypes based on ITS2 data. Most recently, Padgett *et al.* (2005) expanded the work of Crosbie *et al.* (2000) and compared interspecific variation among *Mesocestoides* isolates with that of *Taenia* spp. using 12S

and ITS2 data together with morphology. Three strains of *Mesocestoides* were found and were concluded to represent distinct species (Padgett, 2005). Formal taxonomic assignment of the nominal species was made difficult due to morphometric ranges that were found either to overlap with multiple, or were outside the range of any, described species of *Mesocestoides* (see Padgett, 2005).
Morphological determination of *M. litteratus* in Europe is possible but difficult. In this paper we combine morphometrics with ribosomal DNA data in order to verify the conspecificity of isolates of *M. litteratus* from red foxes from Europe.

Material and Methods

Specimens of *Mesocestoides* sp. from red foxes originating from the Czech Republic, Slovakia and Spain were collected by dissection of hosts and preserved in 70 % ethanol. Massive infections of more than 100 specimens per host were found in five red foxes in Slovakia of which five cestodes from each were selected for morphological analysis. A total of ten and two specimens were found and analyzed from red foxes in the Czech Republic and Spain, respectively (see Table 1). Specimens used for morphological analysis were stained with borax-carmine (Jírovec, 1948). After differentiation in acid alcohol and dehydration through a graded alcohol series, cestodes were cleared in xylene, or were studied in temporary mounts and mounted in Canada balsam 2:1 solution of glycerine-water (Tenora, 2005) for morphometric analysis by light microscopy. Voucher specimens of the cestodes were deposited in the collection of the Institute of Parasitology of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, under accession no. C-397.
Partial specimens were used to characterize the complete 18S rDNA gene from samples collected in Spain (n=1), Czech Republic (n=1) and Slovakia (n=2). Following replacement of EtOH in the specimens with Tris-EDTA buffer via soaking, total genomic DNA (gDNA) was extracted using a Qiagen® DNeasy™ tissue kit following manufacturer-recommended protocols, with the exceptions that the incubation period with proteinase-K was extended to overnight in a rotating incubator and the final elution volume was 200 µl. Three µl gDNA were used as a template in 25 µl PCR reactions using Ready-To-Go™ (Amersham Pharmacia Biotech) PCR beads. Due to the generally poor preservation of gDNA exhibited by these samples, the (near) complete 18S gene was amplified in two overlapping fragments using primers WormA + 1270R and 18S-8 + WormB (see Littlewood and Olson 2001 for a complete list of 18S primer definitions) and the following thermocycling conditions: 94 C/5 min denaturation hold; 40 cycles of 94 C/1 min, 52 C/1 min, 72 C/2 min; 72 C/7 min extension hold. PCR products were gel-excised and recovered using Qiagen Qiaquick™ columns and cycle-sequenced from both strands using ABI BigDye™ chemistry and a variety of internal primers (see Littlewood & Olson, 2001), alcohol precipitated and run on an ABI Prism 377™ automated sequencer. Contiguous sequences (~2,150 bps) were assembled and edited using Sequencher™ (GeneCodes Corp., ver. 4.5), BLAST-screened (Altshul *et al.*, 1997) and submitted to GenBank under accession numbers DQ642999-DQ643002. Sequences were aligned by eye with MacClade (Maddison & Maddison, 2000) together with available complete and partial 18S sequences of *Mesocestoides* from the publications of Crosbie *et al.* (2000), Olson *et al.* (2001) and Literák *et al.* (2004).

Results

Cestodes were morphologically identified as *Mesocestoides litteratus* (Batsch, 1786). Morphometric characteristics are shown in Table 1. Neck was present or absent, if present 16 in average. Testes were connected or slightly separated (in one strobila) in posterior part of segments, other testes surrounding genital organs. Cirrus pouch elongated. Ovaries consisting of two lobes, situated posteriorly. Vittelarium semiovoid. Paruterine organ more or less spherical. Eggs spherical. The determination was based mainly on these features: 1. Cirrus pouch is elongated, not ovoid. 2. Female sex organs (ovaries; vittelaria) stand apart distinctly from the posterior end of the proglottis. 3. Testes are mainly in the posterior end of the proglottis.
The 18S sequences were ~2,150 bps in length and were identical among the four isolates.

Discussion

Examined cestodes from red foxes were determined as *Mesocestoides litteratus* (synonym *M. leptothylacus*). *Mesocestoides litteratus* is distinct from the closely related species *M. lineatus* by the character of the cirrus pouch, the positions of the testes and female sex glands with regard to the posterior end of proglottis.
We compared our measurements of *M. litteratus* with data by Tschertkova and Kosupko (1978), Loos-Frank (1980) and Jancev (1986). Our measurements were usually within the range published. For example, the size of paruterine organ (length x width) was 230 – 710 × 191 – 530 in our samples and reported as 230 – 388 × 256 – 384 by Tschertkova and Kosupko (1978), 372 – 672 × 240 – 420 by Loos-Frank (1980) and 210 – 720 × 180 – 580 by Jancev (1986). Ranges for other organs also fall within those previously published and show the considerable variability of *M. litteratus* as was observed by Priemer (1983) and Jancev (1986). These two authors revised critically a work by Sadykov (1971) regarding *M. petrowi*, which they synonymized with *M. litteratus*.
We found that isolates of *M. litteratus* from Czech Republic, Slovakia and Spain were identical across the entire length of the 18S gene and differed from previously published, mostly unnamed species or strains of *Mesocestoides*. Without molecular characterization of *M. lineatus* we cannot state with certainty that the 18S gene differs between the two species occurring in red foxes in Europe. Nevertheless, variability within the gene is great enough to

Table 1. Morphometric comparison of *Mesocestoides litteratus* isolates from red foxes. All measurements in μm

Strain designation Collection locality	ML1 Bacov, Czech Republic	ML2 Rozhanovce, Slovakia	ML3 Rozhanovce, Slovakia	ML4 Rozhanovce, Slovakia	ML5 Rozhanovce, Slovakia	ML6 Rozhanovce, Slovakia	ML7 Solsona, Spain
Date of collection	1985	26 Nov 2003	10 Dec 2003	29 Jan 2004	4 June 2004	28 Jan 2005	Oct 2004
No. worms collected	10	>100	>100	>100	>100	>100	2
No. worms examined	10	5	5	5	5	5	2
Strobila, length	62 – 194	68 – 106	72 – 130	61 – 152	78 – 98	64 – 76	55 – 79
Mature segments,	800 – 850	444 – 720	380 – 420	400 – 520	710 – 760	620 – 710	721 – 732
width × length	× 1,350 – 1,550	× 600 – 640	× 520 – 980	× 810 – 1,002	× 1,110 – 1,210	× 1,153 – 1,160	× 1,120 – 1,130
Gravid segments,	920 – 1,235	823 – 1,310	903 – 940	890 – 1,100	720 – 910	740 – 921	699 – 905
width × length	× 1,543 – 1,890	× 1,500 – 1,870	× 2,010 – 2,100	× 1,340 – 1,530	× 1,311 – 1,428	× 1,112 – 1,431	× 1,320 – 1,340
Scolex	380 – 620	584 – 622	376 – 400	693 – 700	430 – 720	405 – 604	550 – 570
	× 475 – 680	× 320 – 480	× 650 – 680	× 510 – 620	× 390 – 500	× 312 – 440	× 500 – 530
Suckers	171 – 220	314 – 316	210 – 240	216 – 256	212 – 220	203 – 252	350 – 352
	× 285 – 320	× 314 – 320	× 210 – 240	× 216 – 236	× 150 – 175	× 211 – 290	× 115 – 119
No. of testes	72 – 94	52 – 84	56 – 96	76 – 86	64 – 90	68 – 102	78 – 88
Size of testes	33 – 42	43 – 45	52 – 59	34 – 40	38 – 42	42 – 46	30 – 41
Size of cirrus pouch	270 – 320	166 – 274	281 – 286	210 – 290	160 – 205	240 – 310	260 – 290
	× 80 – 120	× 80 – 86	× 61 – 81	× 90 – 96	× 51 – 80	× 70 – 190	× 90 – 110
Size of ovaries	150 – 210	123 – 212	165 – 200	110 – 250	103 – 230	180 – 210	140 – 210
	× 80 – 220	× 56 – 89	× 53 – 81	× 60 – 72	× 52 – 83	× 41 – 61	× 48 – 81
Size of vitellaria	80 – 130	123 – 215	81 – 110	91 – 191	69 – 200	73 – 140	93 – 109
	× 60 – 112	× 53 – 90	× 61 – 79	× 53 – 98	× 72 – 81	× 56 – 89	× 68 – 93
Paruterine organ	380 – 420	230 – 340	510 – 601	231 – 380	369 – 596	280 – 710	351 – 610
	× 220 – 470	× 241 – 346	× 480 – 530	× 256 – 379	× 239 – 410	× 191 – 460	× 280 – 475
Size of eggs,	28 – 35	28 – 30	26 – 34	24 – 26	27 – 30	28 – 35	26 – 30
size of oncosphere	16 – 18	16 – 18	16 – 18	14 – 19	17 – 20	18 – 20	17 – 19

distinguish among other strains (e.g. Crosbie *et al.*, 2000) and species (e.g. Literák *et al.*, 2004) and we anticipate that it will readily differentiate *M. lineatus* from *M. litteratus*.

The only previously published complete 18S sequence of *Mesocestoides* is that of a laboratory isolate of *M. corti* (AF286984) which showed 97.3 % similarity to that of *M. litteratus*, excluding gapped positions in the V4 and V7 variable regions of the gene where length differences precluded meaningful comparison: an additional 79 out of 2,171 total positions were represented by gaps in one or the other sequence, significantly increasing the difference between the primary sequence of these taxa. Other publicshed sequences characterize approximately the first half of the gene encompassing the V2 and V4 regions. Among these, only a tetrathyridial isolate (AY426257) from the sand lizard (*Lacerta agilis* Linnaeus, 1758) collected in the Czech Republic (see Literák *et al.*, 2004) showed an exact match to this region of the 18S, confirming the identity of this larval stage as *M. litteratus*.

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