Taenia spp.: 18S rDNA microsatellites for molecular systematic diagnosis

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Abstract

The 18S rDNA gene of adult worms of Taenia parva found in Genetta genetta in the Iberian Peninsula and larval stages of T. pisiformis from the wild rabbit (Oryctolagus cuniculus) in Tenerife (Canary Islands) were amplified and sequenced. The sequences of the 18S rDNA gene of T. parva (1768 bp) and T. pisiformis (1760 bp) are reported for the first time (GenBank accession nos. AJ555167–AJ555168 and AJ555169–AJ555170, respectively). In 168 alignment positions microsatellites in the 18S rDNA of both taxa were detected for the first time (TGC in T. parva and TGCT in T. pisiformis) and differences in their sequences with different repetition numbers were observed. The use of nucleotide sequences of this gene in the resolution of systematic problems in cestodes is discussed with reference to the systematic status of Taenia spp. and mainly in human taeniids such as T. solium, T. saginata, and Asian human isolates of Taenia.

Introduction

Cestodes of the genus Taenia are parasites of mammals. There are more than 35 described species in the genus (Verster, 1969) but the morphological limits between the species are problematic (Hoberg et al., 2000). In spite of the considerable taxonomical work on the genus and systematic revisions (Abuladze, 1964; Verster, 1969; Rausch, 1994), few data are available on the phylogenetical relationships within Taenia. Moore & Brooks (1987) and Hoberg et al. (2000) used a variety of morphological characters to infer a comprehensive phylogenetical hypothesis of Taenia spp. Okamoto et al. (1995) estimated relationships among seven species and variants of taeniids using nucleotide sequences of the cytochrome c oxidase subunit I (COI) gene. Queiroz & Alkire (1998) inferred phylogenetical relationships within Taenia spp. using COI and 28S large subunit rDNA sequences. COI and 5’28S rDNA were used by Bowles & McManus (1994) and Fan & Chung (1998) to distinguish between T. saginata and T. asiatica. Variable regions of ribosomal DNA, such as the internal transcribed spacer (ITS) and others, have been used in phylogenetical studies and species identification of T. crassiceps and Asian taeniids (Zarlenga et al., 1991; Zarlenga & George, 1995; Eom et al., 2002). The 12S rDNA gene has been used by Nickisch-Rosewegk et al. (1999) in 11 Taenia species, together with Echinococcus granulosus and E. multilocularis to test the phylogenetical classification based on the morphology of Taenia spp. described by Verster (1969). Gonzalez et al. (2002) confirmed inter-species differences based on the sequence of the HDP2DNA fragment, specific for T. saginata and T. solium, and the sequence of the rDNA internal transcribed spacers 1 and 2.

The 18S rDNA gene has not previously been used in the genetic characterization of taeniids. The nucleotide sequence of this gene can significantly help in the resolution of systematic problems in cestodes (Mariaux, 1998) and no completed sequences of 18S rDNA of any species of Taenia is currently available. In the present work, the sequences of 18S rDNA from two species of Taenia are presented and compared and the presence of
microsatellites in these sequences is discussed from a systematic point of view.

Materials and methods

*Taenia parva* Baer, 1926 is a parasite in adult stage of the common genet, *Genetta genetta* (Carnivora, Viverridae) in Africa and the Iberian Peninsula. The larval stage of *T. pisiformis* (Bloch, 1780) is parasitic in the liver of Lagomorpha, and different species of Canidae act as definitive hosts. These two species of taeniids, *T. parva* and *T. pisiformis*, adult and larval tapeworms respectively, were collected from the small intestine of *G. genetta* and liver of *Oryctolagus cuniculus* in Tenerife (Canary Islands).

Twelve specimens of *T. parva* were collected from Spain (Montseny, Catalonia, in the north-east of the Iberian Peninsula) and 15 specimens of *T. pisiformis* from Canarian rabbits in Tenerife (Canary Islands).

Genomic DNA was extracted from ten worms of each species. Specimens were homogenized and incubated in 700 μl of extraction buffer (10 mM Tris, 100 mM Na2EDTA, 100 mM NaCl, pH 8, 0.05%, w/v SDS, 200 μg proteinase K) overnight at 55°C. Samples were then incubated with RNAse-A (100 μg ml⁻¹) for 30 min followed by a phenol/chloroform extraction. DNA was precipitated with ethanol, dried and resuspended in 50 μl TE.

The 18S rDNA gene was totally amplified from genomic DNA by Mariaux (1998) in two overlapping fragments, in 200 mM, 20 pmol of each primer, and 1 unit of Taq DNA polymerase (Applied Biosystems, New Jersey, USA). The PCR mixture and cycling conditions were: an initial denaturing phase of 5 min. Polymerase chain reactions (PCR) were performed in a Perkin Elmer GeneAmp PCR System 9600 thermal cycler, using the oligonucleotides 81 + 83 and 82 + 84 designed by Mariaux (1998). Sizes of fragments were fractionated by 1% agarose gel stained with ethidium bromide and the band was further purified using QIAEX II Gel Extraction kit (150 l TE). The ladder was 2 P. Foronda et al. for designing the flanking primer pair microsatellite, using the Gene-Runner (Hastings Software INC) computer programme. The PCR mixture and cycling conditions with microsatellite primer were identical to these used for the first PCR with an annealing temperature of 66°C.

For restriction fragment analysis, 5 μl of PCR products with microsatellite primers were digested in a 10 μl reaction mixture containing 2 U of *SphI* restriction endonuclease and 5 μl of the appropriate restriction buffer at 37°C for 4 h. The digested products were fractionated on a 2% agarose gel, stained with a solution of ethidium bromide and visualized and photographed under an UV transilluminator.

**Results and discussion**

Sequences from the 18S rDNA of *T. parva* (1768 bp) and *T. pisiformis* larvae (1760 bp) were identified and submitted to GenBank under the accession nos. AJ555167–AJ555168 and AJ555169–AJ555170 respectively. These sequences have not been previously reported. We detected microsatellites in the 18S rDNA gene sequence, in 168 alignment positions in both taxa (fig. 1). Differences in the repetition number and in the sequences of microsatellites between the two species were observed. *Taenia parva* presents eight repetitions of three base pairs (TGC), while the microsatellite in *T. pisiformis* is constituted by seven repetitions of four base pairs (TGCT).

The primer pair flanking microsatellite sequences for PCR reactions were 5’AACGCTATCATGTCCTCAGTCAG and 5’GCCCTCAATTGATCTCGTG, and were designed from regions of 18S rDNA that are highly conserved in a wide range of cestodes between the cyclophyllids deposited in the GenBank database, i.e. *Andria cuniculi* AJ555162, *Anoplocephaloides dentata* Z98356–Z98358, *Mosgovoyia ctenoides* AJ555164 (Anoplocephaloidae), *Choanotaenia infundibulum* AJ555171 (Dilepididae), *Lyretina nigropunctata* AJ555173 (Paruterinidae), *Lyruterina nigropunctata* AJ555173 (Paruterinidae), *Kailietina australis* AF286980, *Kailietina microcantha* AJ555178, *Pseudihogenes nana* AJ555175 (Davaineidae) [Cyclophyllidea]; and between the tetrabothrids, i.e. *Tetrabothrius* sp. AJ555181, *Tetrabothrius* sp. AJ287582, *Tetrabothrius erosi* AJ287581, *Tetrabothrius forsteri* AF124473 (Tetrabothriidae) [Tetrabothriidea].

The PCR products that were amplified with this primer pair contain a highly conserved region and another hypervariable region, both in sequence and length. The microsatellites are located inside this second variable region in the two *Taenia* species and are not present in cestode sequences deposited in the Gen Bank database.

![Fig. 1. Fragment of the alignment of 18S rDNA sequences of *Taenia parva* and *T. pisiformis*. The number (230) refers to positions obtained in the alignment made with CLUSTAL X. Shaded regions correspond to the microsatellites.](image-url)
Microsatellites in \textit{Taenia} spp.

For these primers, the PCR products were 640 and 636 bp long for \textit{T. parva} and \textit{T. pisiformis}, respectively. The PCR products were digested with \textit{Sphl}. \textit{Taenia parva} generated two visible bands of 170 and 470 bp, and \textit{T. pisiformis} generated one visible band (fig. 2). Differences in the restriction site in this region permit a distinction between the two species with a \textit{Sphl} restriction enzyme.

Species of the genus \textit{Taenia} are cestodes with much medical and veterinary significance. In the present study, adult worms in \textit{G. genetta} were compared with larval stages of \textit{T. pisiformis} from the wild rabbit. \textit{Taenia parva} is a typical parasite of \textit{Genetta} spp. in Africa and \textit{G. genetta} in the Iberian Peninsula. \textit{Taenia parva} was introduced with the common genet from Africa to Iberia (Casanova et al., 2000). Species of Canidae harbour adult stages of \textit{T. pisiformis} (Verster, 1969; Opuni, 1970; Casanova et al.), and dogs are definitive hosts of this species (Valladares et al., 1996). Differences in the number and sequences could be used as specific markers in \textit{Taenia} systematics as they are found in the same position in the two phylogenetically diverse species.

Microsatellites, tested by PCR, are currently used for analyses in human genetics (Weissenbach et al., 1992). In vertebrates and invertebrates, microsatellites in the 18S rDNA are distributed in constant regions of this gene (Foronda et al., 2002). Microsatellites found in both \textit{T. parva} and \textit{T. pisiformis} sequences could be used as specific markers in \textit{Taenia} systematics as they are found in the same position in the two phylogenetically diverse species.

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Acknowledgements

We wish to thank Dr Mariano Hernandez Ferrer for help with this work and the ‘Excelentísimo Cabildo Insular de Tenerife’ and 2001SGRR00088 project by the ‘Comisionat por la Recerca y Universitats de la Generalitat de Catalunya’ (Catalonia Government) for supporting this study.

References

Abuladze, K.I. (1964) 


(Accepted 7 January 2005) © CAB International, 2005
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JOB NUMBER: 280
JOURNAL: JOH

Q1 In reference Raush, 1994 has been changed to Rausch, 1994 kindly check.
Q2 Please note that et al. has been added to Nickisch-Rosenegk, 1999.
Q3 Bloch, 1780 has not cited in the reference list.