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Specific Identification of a Taeniid Cestode from Snow Leopard, *Uncia uncia* Schreber, 1776 (Felidae) in Mongolia

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Abstract

An unknown taeniid cestode, resembling *Taenia hydatigena*, was recovered from a snow leopard, *Uncia uncia* in Mongolia. Morphology and nucleotide sequence of the mitochondrial cytochrome *c* oxidase subunit I gene (mt DNA COI) of the cestode found was examined. The cestode is differed from *T. hydatigena* both morphologically and genetically. The differences between two species were in the gross length, different number of testes, presence of vaginal sphincter and in egg size. The nucleotide sequence of this cestode differed from that of *T. hydatigena* at 34 of the 384 (8.6%) nucleotide positions examined. The present cestode is very close to *T. kotlani* in morphology and size of rostellar hooks. However, the adult stages of the latter species are unknown, and further comparison was unfeasible.

Key words: Mongolia, snow leopard, *Taenia*, taxonomy, mt DNA, cestode, Taeniidae

Introduction

The snow leopard, *Uncia uncia* Schreber, 1776 (Felidae) is an endangered species within Mongolia and throughout its range. It is listed in the IUCN Red Data Book, Mongolian Red Book and included in CITES (Convention on International Trade in Endangered Species of Fauna and Flora) on Appendix I. The helminth fauna of this carnivore is almost unknown. Only two species of nematode, namely *Toxascaris leonina* and *Toxocara cati* were reported from this host in Russia (Mozgovoi, 1953) and India (Maity *et al.*, 1994), respectively.

In 1986, we were able to obtain a digestive tract from one snow leopard that was shot under special permission to the Academy of Sciences of Mongolia. During dissection, the cestodes resembling *Taenia hydatigena* Pallas, 1766 in terms of rostellar hook lengths, hook shape and number were recovered from the small intestine of a snow leopard (Ganzorig & Amarsanaa, unpublished). However, a more recent examination has revealed that the specimens are differing from *T. hydatigena* in the number of morphological traits.

The specific identification of taeniid cestodes based on morphological characters only is often inadequate. Because of the characters of *Taenia* species are subject to variations which necessitate the use of more than one character for specific identification (Edwards & Herbert, 1981). Identification based on hook morphology and measurements are difficult because of overlap in the hook lengths between different species. That based on the gross morphology of the strobila and segments are invalid because of distortion due to poor fixation (Verster, 1969; Beveridge & Gregory, 1976; Edwards & Herbert, 1981). Beveridge & Gregory (1976) found that gross strobilar morphology and anatomy of the mature proglottid were reliable methods of differentiating 4 species of *Taenia* in suitably relaxed, fixed and stained specimens. Identification of taeniid cestodes requires well-relaxed and subsequently fixed complete cestodes with mature segments and scoleces.

To date, the nucleotide sequences of the mitochondrial DNA cytochrome *c* oxidase subunit I (mt DNA COI) gene were used to distinguish and resolve phylogenetic relationships in the strain variation of *Echinococcus granulosus* and *E. multilocularis* (Bowles *et al.*, 1995); between species of Taeniidae (Okamoto *et al.*, 1995). Using this approach, it has been indicated that the Asian *Taenia* recently described as *Taenia asiatica*, is closely related to *T. saginata* and taxonomic classification as a subspecies or strain of *T. saginata*

is more appropriate than formal designation as a new species (Bowles & McManus, 1994).

In this study we used both morphological and DNA approach for exact determination of the snow leopard's cestode.

Materials and Methods

Parasite specimens. Helminths were collected from one snow leopard that was shot on 30th December 1986, by special permission to the Academy of Sciences of Mongolia, in Mt. Burhanbuudai uul of Govi Altai Province, Mongolia (intestines and other internal organs were provided to us by Mr. G. Amarsanaa, Institute of Biology, Academy of Sciences, Mongolia). The esophagus, stomach, small and large intestines were dissected. In total, 81 specimens of nematode and 62 specimens of cestodes were collected from small intestine. Nematodes were identified as *Toxascaris leonina*. The cestodes are consisted of 5 matured and 57 young unmatured specimens. Voucher specimens deposited in Helminthological Collection of the Department of Zoology of the National University of Mongolia and at the Laboratory of Parasitology of the Graduate School of Veterinary Medicine, Hokkaido University, Japan.

Microscopical study. All the cestodes were fixed and preserved in 70% alcohol. Four matured cestodes were stained with aceto-carmine, dehydrated in alcohol, cleared in xylene, and mounted in Canada balsam. Rostellum of 15 cestodes was mounted in Hoyer's medium. All measurements were made with aid of an Olympus video micrometer (Model VM-30). At the time of dissection, helminths recovered were largely contracted; the cestodes are mostly poorly stained. Moreover, numerous calcareous bodies have been intensively stained with aceto-carmine making observation of internal organs difficult. Measurements and examination of internal organs were done using phase-contrast microscopy.

DNA study. Nucleotide sequence of the mitochondrial COI gene of the specimens was examined. Total DNA was extracted from alcohol fixed specimens using the Easy-DNA isolation kit (Invitrogen). PCR amplifications were performed according to manufacturer's instructions. The oligonucleotide primers used (pr-A 5′ TGGTTTTTTGTGCATCCTGAGGTTTA 3′ and pr-B 5′ AGAAAGAACGTAATGAAAATGAGC AAC 3′) were that of Okamoto *et al.* (1995). PCR products were purified with QIAquick-spin PCR purification Kit (Amicon, USA) and CENTRI-SEP Columns (Princeton Separations, Inc.). PCR products were sequenced using a Dye terminator cycle sequencing kit and a model 373A DNA sequencer (Applied Biosystems). DNA-sequence data were aligned using the CLUSTAL V and the phylogenetic tree was constructed with the neighbor-joining and maximum-likelihood methods (Saitou & Ney, 1987) using sequence data on other taeniid cestodes (Okamoto *et al.*, 1995).

Results

Taenia sp. (Taeniidae: Cestoda)

Morphological description. Mature cestodes with gravid segments 32.6 to 39 cm long, consisted of 250-285 segments. Scolex 0.723-0.884 mm in diameter, that of rostellum 0.401-0.408 mm. Suckers 0.253x0.209 mm in diameter. Rostellum armed with 30-35 hooks arranged in two rows (Fig. 1). Large hooks 0.190-0.209 mm (0.200±0.001) in length with it blade and handle 0.096-0.102; and 0.112-0.123 mm long, respectively. Small hooks with bifid guard, measured 0.127-0.144 mm (0.133±0.001) in length. Blade and handle were 0.076-0.080 and 0.070-0.83 mm long, respectively. Segments are wider than long. Mature segments 2.0-2.2 mm long by 5.9-7.1 mm wide. That of gravid segments 2.8-3.9x5.8-6.2 mm. There are 400-480 testes, 0.052-0.068 mm in size, its number larger in aporal part, 220-280 vs 179-200 in poral part. Testes are does not connect posteriorly and distributed between ventral excretory canals only. Cirrus sac 0.287-0.359 mm long by 0.085-0.090 mm wide. Vagina posterior to bursa sac, with a copulative part 0.281 mm long. Vagina has a well-developed sphincter, 34 to 58 μm in diameter. Mature uterus possessed 8-10 primary lateral branches. Eggs 0.027-0.032 mm in diameter. Embryonal hooks 0.006-0.007 mm in length.

DNA sequence. The nucleotide sequence of the mitochondrial COI gene (Fig. 2) is differed from that of the *T. hydatigena* (in Okamoto *et al.*, 1995) at 34 of the 384 nucleotide positions examined. None of that nucleotide changes causes a coding change i.e. changes that do not affect the amino acid sequences. The maximum-likelihood tree inferred for taeniid cestodes showed that genetically, the present species is close to *T.*

hydatigena (Fig. 3).

Discussion

Morphologically, in terms of hook size and shape, the present cestode species is close to *T. kotlani* Murai, Gubanyi & Sugar, 1993 and *T. hydatigena*. The former species was first described based on metacestodes recovered from lungs and abdominal cavity of ibex, *Capra sibirica* Pallas, 1776 in Mongolia (Murai *et al.*, 1993). The descriptions of the latter species largely complicated, considering large ranges of main characters (see Verster, 1969; Loos-Frank, 2000) one may suppose that more than one species is involved. Of the description available on this species, the present cestode has close similarities with the cestode described as *T. hydatigena* reported by Sawada & Shogaki (1975) from wild dog in Nepal. However, the present cestode can be distinguished from the *T. hydatigena* by the possessing vaginal sphincter, less number of testes (400-480 vs 600-700), and in smaller eggs (0.027-0.032 mm versus 0.038-0.039 mm). Also, differences are in gross length of the cestodes, which is about 40 cm in present cestode and 50-250 cm in *T. hydatigena* (see Edwards & Herbert, 1981). Other *Taenia* species with the same hook dimensions or internal characters included only *T. parenchymatosa*. But, *T. parenchymatosa* has very distinct type of the large hook that is with very prominent handle (Murai *et al.*, 1993). Thus, the present cestode is close to *T. kotlani* and *T. hydatigena* in means of the hook dimensions and shape, and to *T. parenchymatosa* in the anatomy of the mature and gravid segments. The results of the present study showed that the snow leopard's cestode is distinguishable based on morphological characters, from both *T. hydatigena* and *T. parenchymatosa*, at adult stage. However, we have not full confidence in that our specimens are belong to *T. kotlani*, because, the adult stages of *T. kotlani* are unknown. Only, direct comparison of the DNA sequences from the *T. kotlani* and the present material could solve this question.

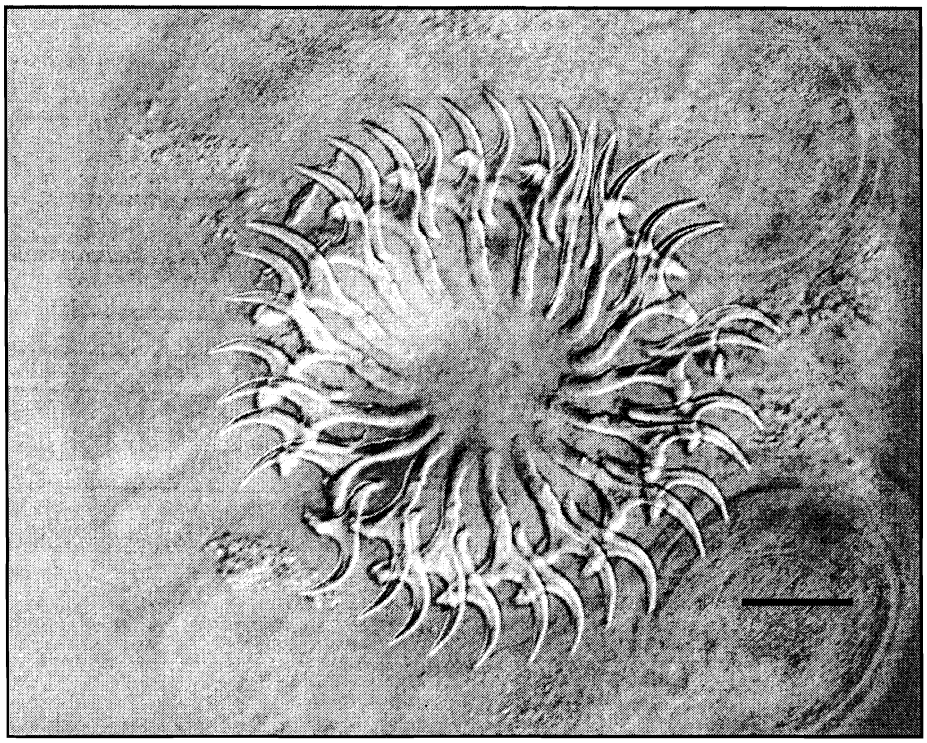

Fig. 1. Scolex and rostellar hooks of the snow leopard's cestode (scale bar = 0.10 mm)

The level of nucleotide variation in the COI gene within the genus *Taenia* is about 6.3-15.6% (McManus & Bowles, 1994). Although, these are 4.6-9.3% between several strains of *Echinococcus granulosus* (Bowles *et al.*, 1992). In the present study, a value of 8.6% sequence variation was observed between the snow leopard's cestode and *T. hydatigena*. The mitochondrial COI gene sequence data supported the morphological differences between two species.

In this study, we provisionally identified the snow leopard's cestode as *T. kotlani* ? Murai, Gubanyi & Sugar, 1993.

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TspUncia          ——ATTCTTCCTGGGTXTGGAATTATTAGTCATATATGTTTAAGAA
Thydatigena       TGTTTTAATTCTTCCTGGATTTGGAATTATTAGTCATATATGTTTGAGAA
                         *********** * ********************** ****
TspUncia          TAAGTATGAGTCCTGATGCTTTTGGGTTTTATGGATTGTTATTTGCTATG
Thydatigena       TAAGCATGAGTCCTGATGCTTTTGGATTCTATGGATTATTATTTGCTATG
                  **** ******************** ** ******** ************
TspUncia          TTTTCGATAGTTTGTTTAGGTAGAAGTGTGTGAGGACATCATATGTXTAC
Thydatigena       TTTTCAATAGTCTGTTTGGGTAGAAGTGTGTGGGGTCATCATATGTTTAC
                  ***** ***** ***** ************** ** ********** ***
TspUncia          TGTTGGGTTGGATGTTAAGACTGCTGTTTTTTTTAGTTCCGTTACTATGA
Thydatigena       TGTTGGATTAGATGTTAAGACTGCTGTTTTTTTTAGTTCTGTCACTATGA
                  ****** ** ***************************** ** *******
TspUncia          TTATAGGTGTGCCTACTGGTATAAAGGTATTTACTTGACTATACATGCTT
Thydatigena       TTATAGGTGTGCCTACTGGTATAAAGGTGTTTACTTGGTTATATATGCTT
                  *************************** ********  **** ******
TspUncia          TTAAATTCTCATGTGAATAAGAGTGATCCTGTTGTTTGGTGAATTGTCTC
Thydatigena       TTAAACTCTCATGTGAATAAGAGTGATCCTGTTGTTTGATGAATTGTTTC
                  ***** ********************************* ******** **
TspUncia          TTTTATAGTTTTATTTACTTTXGGGGGGGTCACTGGCATTGTGTTATCAG
Thydatigena       TTTTATAGTTTTGTTTACTTTTGGTGGGGTTACTGGTATTGTGTTGTCAG
                  ************ ******** ** ***** ***** ******** ****
TspUncia          CATGTGTGTTAGATAAAGTTCTGCATGATACTTGGTTTGTG
Thydatigena       CATGTGTATTAGATAAAGTTCTTCATGATACCTGGTTTGTA
                  ******* ************** ******** ********
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Fig. 2. CLUSTAL V multiple sequence alignment of the mitochondrial COI gene from *Taenia* sp. and *T. hydatigena*. TspUncia - *Taenia* sp. from snow leopard

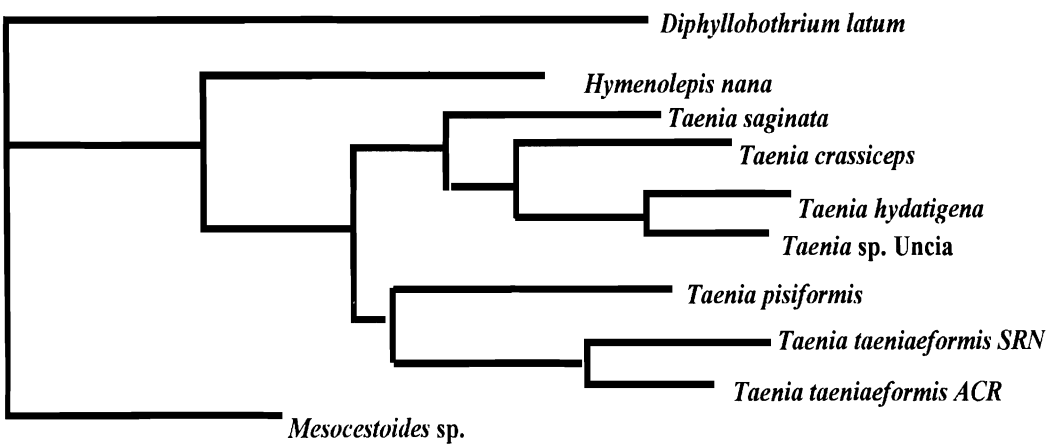

Fig. 3. Phylogenetic tree of cestodes constructed from maximum-likelihood analysis of the mt DNA COI gene nucleotide sequences

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