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Molecular taxonomy and subgeneric classification of tapeworms of the genus *Moniezia* Blanchard, 1891 (Cestoda, Anoplocephalidae) in northern cervids (*Alces* and *Rangifer*)

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ABSTRACT

Phylogenetic relationships of tapeworms of the genus *Moniezia* Blanchard, 1891 (Cestoda, Anoplocephalidae) parasitizing the Eurasian elk *Alces alces*, the moose *A. americanus* and the reindeer/caribou *Rangifer tarandus* (Cervidae) were studied using DNA sequences of two mitochondrial genes (*cox1* and *nad1*). Several isolates from domestic ruminants, representing *Moniezia expansa* (Rudolphi, 1810) sensu lato and *M. benedeni* (Moniez, 1879) sensu lato, and one unidentified isolate from an African antelope, were also included in the analysis.

Both genes identified the same six species of *Moniezia*, but interspecific phylogenetic relationships were better resolved by the *nad1* data. The six species of *Moniezia* comprised two main clades: clade 1 that originates in bovids, with subsequent colonization of northern cervids in Eurasia, and clade 2 that originates in northern cervids, with subsequent specific divergence within these hosts. Clade 2 has a Holarctic distribution.

None of the *Moniezia* specimens in *Alces* and *Rangifer* was conspecific with the species in domestic ruminants, suggesting that the custom of identifying *Moniezia* spp. in northern cervids either as *M. expansa* or *M. benedeni* is incorrect. At least two of the species parasitizing *Alces* and *Rangifer* have not been previously recognized. These findings challenge the results of all previous studies concerning the diversity and ecology of *Moniezia* spp. in northern cervids.

The traditional classification into three subgenera (*Moniezia* Blanchard, 1891, *Blanchariezia* Skrjabin & Schultz, 1937 and *Baeriezia* Skrjabin & Schultz, 1937), based on the presence and type of interproglottidal glands, conflicts with the currently observed molecular phylogenetic relationships within the genus *Moniezia*.

1. Introduction

Tapeworms of the genus *Moniezia* Blanchard, 1891 (Cyclophyllidea, Anoplocephalidae) are predominantly parasites of ruminant artiodactyls, such as cattle, goat, sheep, antelopes and deer, although a few species are also known from suids, equids and rodents [1–5]. Presently, there are 15 valid species of *Moniezia* (see the Global Cestode Database at http://tapewormdb.uconn.edu/, accessed 2 November 2017).

The first taxonomical review of the genus [6] listed seven species as parasites of domestic ruminants, but Taylor [7] later proposed that *Moniezia expansa* (Rudolphi, 1810) and *M. benedeni* (Moniez, 1879) were the only valid species in these hosts (see also [8,9]). This view was accepted in Spasskii's [1] authoritative monograph, and adopted widely in subsequent studies.

There is now solid molecular evidence based on enzyme electrophoresis showing that cestodes morphologically identifiable as *M.*

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Table 1
Species of *Moniezia* reported in *Alces* spp. and *Rangifer tarandus* from Eurasia and North America, including the species identified in the present study (in bold). IG, interproglottidal gland.

Subgenus/species	Host genus	Eurasia (including Svalbard)	North America (including Greenland)
1) Subgenus *Moniezia* Blanchard, 1891 (IG's rosette–shaped)			
M. expansa (Rudolphi, 1810)	*Alces*	[38,39,40]	[41,42]
	Rangifer	–	[43,15]
M. rangiferina Kolmakov, 1938	*Rangifer*	[27,28]	–
M. taimyrica Semenova in Mickevich, 1967	*Rangifer*	Semenova in [26]	–
***Moniezia* sp. A**	***Alces***	**Present study**	**Present study**
***Moniezia* sp. C**	***Rangifer***	**–**	**Present study**
2) Subgenus *Blanchariezia* Skrjabin & Schultz, 1937 (IG's linear)			
M. benedeni (Moniez, 1879)	*Alces*	[38,39,44]	[45]
	Rangifer	[14]	–
M. mizkewitschi Skrjabin in Mickevich, 1967	*Rangifer*	[26]	–
***Moniezia* sp. B**	***Alces***	**Present study**	–
	Rangifer	**Present study**	–
3) Subgenus *Baeriezia* Skrjabin & Schultz, 1937 (IG's absent)			
M. baeri Skrjabin, 1931	*Rangifer*	[46]	–
M. mathevossianae (Semenova, 1972)	*Rangifer*	[47]	–

expansa or *M. benedeni* actually include multiple (apparently) cryptic species [10–12]. Diop, Yanagida, Hailemariam, Menkir, Nakao, Sako, Ba and Ito [13], who studied DNA sequence variation of *M. expansa* and *M. benedeni* in domestic ruminants in Africa, also found genetic differences indicative of interspecific variation within both species. Although the existence of (apparently) cryptic *M. expansa* and *M. benedeni*-like species in domestic ruminants is well established, there have been no attempts to assign these forms to other species, either new or existing ones.

The Eurasian elk (*Alces alces*), moose (*A. americanus*) and reindeer/caribou (*Rangifer tarandus*) are commonly parasitized by *Moniezia* spp. throughout their Holarctic range (Table 1), including the high Arctic populations of the latter host on Svalbard and Greenland [14,15]. In *Alces* spp., both *M. expansa* and *M. benedeni* (but no other species) have been reported in Eurasia and North America (Table 1). In reindeer the situation is more complicated, because, in addition to *M. expansa* (in North America) and *M. benedeni* (in Eurasia), five species of *Moniezia* have been described from reindeer from Siberia and north-western Europe (Table 1). The morphological features separating them from *M. expansa*, *M. benedeni* and from each other have been poorly defined.

It may be concluded that the taxonomical status, species diversity and host specificity of *Moniezia* spp. in northern cervids are in need of a thorough re-evaluation. In addition, the widely used subgeneric classification of Skrjabin and Schultz [16] has not been evaluated using phylogenetic methods. As a first step towards these goals, we present a phylogenetic analysis on *Moniezia* spp. in *A. alces, A. americanus* and *R. tarandus* from Finland and Alaska, based on nucleotide sequences of two mitochondrial genes (*cox1*, *nad1*). The present material also includes *M. expansa* sensu lato (hereafter "s.l.") and *M. benedeni* s.l. from sheep and cattle, respectively, and *Moniezia* sp. from an African antelope. Previous molecular systematic studies on *Moniezia* spp. in cervids do not exist, and DNA sequences (nuclear 28S rDNA) have been available only for a single isolate from reindeer [17].

The present results also allow to discuss the host-parasite cophylogeny and historical biogeography of *Moniezia* spp.

2. Materials and methods

2.1. Tapeworm material

The material of *Moniezia* spp. from cervids originates from Finland (10 specimens from five individuals of *A. alces* and four specimens from three individuals of *R. tarandus*) and Alaska (one specimen from *A. americanus* and *R. tarandus* each) (Table 2).

The bulk of the Finnish tapeworms (12 specimens) originated from two neighbouring municipalities (Kuusamo and Taivalkoski) in north-eastern Finland, collected in 2014. In addition, one specimen was obtained from *R. tarandus* from the municipality of Utsjoki in northernmost Finland (Lapland) and another one from *A. alces* from the municipality of Kuhmoinen in south-central Finland.

The Eurasian elk occurs throughout Finland, but the occurrence of the semi-domesticated reindeer is restricted to northern Finland, including the Kuusamo-Taivalkoski region and Utsjoki, but excluding Kuhmoinen. Moose and caribou have wide, overlapping distributions in Alaska.

Five specimens of *M. expansa* s.l. and one specimen of *M. benedeni* s.l., obtained from domestic ruminants in Senegal (both species; [13]) and China, (*M. expansa* s.l. only; present study), were also included in the phylogenetic analysis. See Diop, Yanagida, Hailemariam, Menkir, Nakao, Sako, Ba and Ito [13] for an extensive phylogenetic analysis of *M. expansa* s.l. and *M. benedeni* s.l. in Senegal and Ethiopia based on complete sequences of *cox1* gene (mtDNA). In addition, a single specimen (*Moniezia* sp.) from the greater kudu *Tragelaphus strepsiceros* from South Africa was available for study (Table 2).

The cervids were either legally hunted (Eurasian elk, caribou and greater kudu), culled from free-ranging herds (reindeer) or found dead (moose). In Finland and Alaska, the hunting and culling took place in autumn or early winter. All cervid hosts were calves; ten adult reindeer and four adult Eurasian elks, all without *Moniezia*, were also examined in Finland.

Tapeworms from Senegal and China were obtained from slaughterhouses. Due to slaughterhouse management procedures in Senegal, intestines from sheep and goat were not differentiated.

Mature proglottids of a few specimens of each *Moniezia* clade were stained overnight with alum carmine, destained with acid alcohol, dehydrated in ethanol, cleared in eugenol and mounted in Canada balsam. Voucher specimens (slides, ethanol specimens or both) of each species identified in the present study have been deposited in the Finnish Museum of Natural History (Table 2).

2.2. Molecular and phylogenetic methods

Total genomic DNA of specimens fixed and preserved in 70–96% ethanol was extracted using E.Z.N.A.™ Tissue Kit (OMEGA Bio-Tek) or DNA Mini Kit (Qiagen). Two mitochondrial genes (either partial or total), cytochrome *c* oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*), were analysed. See Table 3 for primers used in amplification and sequencing. The PCR procedures used are those of Haukisalmi, Wickström, Henttonen, Hantula and Gubányi [18] (COX-F + COX-R) and Diop, Yanagida, Hailemariam, Menkir, Nakao, Sako, Ba and Ito [13] (MoCox1F + MoCox1R) for *cox1*, and Littlewood, Waeschenbach and Nikolov [19] (Cyclo_Nad1F + Cyclo_trnNR) for *nad1*. Successfully amplified DNA fragments of correct size were purified using E.Z.N.A.™ Cycle Pure Kit (OMEGA Bio-Tek).

For the isolates from Finland, Alaska and South Africa, purified PCR products were directly sequenced using dye terminators and visualized with an ABI 3730XL DNA analyser at Macrogen Inc. (Seoul, Korea). For the isolates from China and Senegal, *cox1* was sequenced in full-length as reported previously [13]. In addition, the complete sequences of *nad1* were similarly determined by using new primers (Table 3). The primers used in sequencing were the same as those used in DNA amplification.