Molecular identification of *Taenia* spp. in the Eurasian lynx (*Lynx lynx*) from Finland

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SUMMARY

Cestodes of the genus *Taenia* are parasites of mammals, with mainly carnivores as definitive and herbivores as intermediate hosts. Various medium-sized cats, *Lynx* spp., are involved in the life cycles of several species of *Taenia*. The aim of the present study was to identify *Taenia* tapeworms in the Eurasian lynx (*Lynx lynx*) from Finland. In total, 135 tapeworms from 72 lynx were subjected to molecular identification based on sequences of 2 mtDNA regions, the cytochrome *c* oxidase subunit 1 and the NADH dehydrogenase subunit 1 genes. Available morphological characters of the rostellar hooks and strobila were compared. Two species of *Taenia* were found: *T. laticollis* (127 samples) and an unknown *Taenia* sp. (5 samples). The latter could not be identified to species based on mtDNA, and the rostellar hooks were short relative to those described among other *Taenia* spp. recorded in felids from the Holarctic region. In the phylogenetic analyses of mtDNA sequences, *T. laticollis* was placed as a sister species of *T. macrocystis*, and the unknown *Taenia* sp. was closely related to *T. hydatigena* and *T. regis*. Our analyses suggest that these distinct taeniid tapeworms represent a putative new species of *Taenia*. The only currently recognized definitive host is *L. lynx* and the intermediate host is unknown.

Key words: *Taenia laticollis*, *Taenia* sp., mtDNA, phylogeny, lynx.

INTRODUCTION

Taeniids (Cestoda: Cyclophyllidea) are characteristic parasites of terrestrial mammals. Adult taeniid tapeworms occur in the small intestine of typically carnivorous definitive hosts, and their cystic larvae (metacestodes) develop in tissues or body cavities of herbivorous or omnivorous intermediate hosts (Abuladze, 1964). A predator–prey relationship between the definitive and intermediate hosts maintains the transmission of taeniids.

The family Taeniidae Ludwig, 1886 is undergoing a taxonomic revision due to the development of molecular diagnostic methods and increasingly available DNA sequence data (Lavikainen *et al*. 2008, 2011). Currently, the largest taeniid genus, *Taenia* Linnaeus, 1758, consists of approximately 45 valid species (see Hoberg, 2006; Lavikainen *et al*. 2008; Rossin *et al*. 2010; Haukisalmi *et al*. 2011), and in addition, mitochondrial DNA (mtDNA) evidence has revealed at least 3 previously unrecognized species (Lavikainen *et al*. 2008; Galimberti *et al*. 2012). Felids serve as the definitive hosts for a minimum of 14 species of *Taenia* (Loos-Frank, 2000). In addition, several other *Taenia* spp. can occasionally parasitize felid hosts (Abuladze, 1964; Jones and Pybus, 2001).

Lynx spp. are Holarctic felids, occurring especially in boreal and temperate forests. As medium-sized cats, lynx prey on a wide range of mammals including rodents, lagomorphs and even cervids. Therefore, lynx are involved in the life cycles of several species of

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Taenia (Abuladze, 1964; Jones and Pybus, 2001). Recently, endoparasites of the Eurasian lynx (*Lynx lynx*) were examined in Finland by analysing a considerable number of fecal and intestinal samples (Deksne *et al*. 2012). Tapeworms recovered from the survey were not in adequate condition for morphological examination, and identifications were limited to the generic level. Based on rostellar hooks and the size of the strobilae, however, at least 2 species of *Taenia* were observed: *Taenia laticollis* Rudophi, 1819 and a much larger *Taenia* sp., in which specific identity has remained unclear. The focus of the present study is the molecular identification of *Taenia* tapeworms in lynx from Finland. In the current study we explore the diversity and phylogenetic placement of these *Taenia* spp., emphasizing the status of a putative unknown species.

MATERIALS AND METHODS

Intestinal samples and helminth collection

The *Taenia* tapeworms were collected from intestines of 296 lynx (*L. lynx*), which were shot during the hunting season 2010–2011 (from the beginning of December to the end of February) in Finland. This represented 80% of the legally hunter-harvested lynx, and included lynx from all of the 15 game management districts of Finland.

Skinned lynx carcasses were stored frozen until dissection and necropsy. Intestines were removed from the thawed carcasses and intestinal contents were squeezed out and washed. The helminths were preserved in 70% ethanol. The tapeworms were macroscopically identified at the generic level.

Selection of Taenia *specimens for analyses*

The majority of the *Taenia* tapeworms resembled *T. laticollis* by their general appearance and size. Length and shape of a few observed rostellar hooks supported this diagnosis. Since the worms were so numerous, it was not feasible to identify all specimens based on molecular characters. To create a general view of the species diversity among the *T. laticollis* -like specimens, we decided to identify strobilate cestodes or their fragments from one third of the infected lynx from each game management district. To cover the geographical area thoroughly, the lynx were selected in relation to maximum distances between collection sites separating host specimens, trying to avoid individuals from the same or neighbouring municipalities. Furthermore, both mildly (<10 worms per animal, $n=37$ lynx) and heavily (11–112 worms per animal, $n=30$ lynx) infected animals were selected assuming that different infection intensities might be related to different species of *Taenia*. One or 2 tapeworms per selected lynx (2 worms if more than 1 were present) were subjected to molecular identification.

Molecular identification was also performed for all specimens of *Taenia*, which differed macroscopically from typical *T. laticollis* and could be suspected to represent another species. This included 7 specimens from 5 lynx. Six of the specimens (from 4 lynx) represented the unknown large *Taenia* tapeworms, having obviously wider and longer strobilae than *T. laticollis*. Furthermore, a single tapeworm fragment having a strange dark colour was subjected to molecular identification.

Two short anterior fragments with scoleces were found in one of the lynx infected with the large unknown *Taenia* sp. The rostellar hooks indicated that these belonged to *T. laticollis*. These specimens were further identified by sequencing to demonstrate a possible mixed infection.

Comparative materials and morphological examination

Since mtDNA sequence data of *T. laticollis* have not been published, we used well-preserved morphologically verified strobilate adults as reference specimens. The samples (8 isolates) were collected in routine necropsies at the Finnish Food Safety Authority Evira from 6 Finnish lynxes found dead during the winter of 2008–2009. In addition, 2 specimens of *Taenia taeniaeformis* (Batsch, 1786) in a Finnish lynx, collected during a necropsy at Evira in 2009, were used for morphological and molecular comparison.

We also analysed a reference specimen of *Taenia omissa* Lühe, 1910, a Nearctic species of typically large dimensions which infects felids, mainly the cougar (*Puma concolor*). The sample was collected in a necropsy at the Canadian Cooperative Wildlife Health Centre from an adult male cougar, which was accidentally trapped in a coyote snare and died in the town of Pincher Creek, southern Alberta, in the spring of 2011.

Reference specimens (*T. laticollis, T. omissa* and *T. taeniaeformis*) were preserved in 70% ethanol following collection. These specimens and others representing the unknown species of *Taenia* were stained with Mayer's haemalum or Semichon's acetic carmine, cleared in eugenol and mounted in Canada balsam. The rostella were cleared in Berlese's medium. Comparative morphological studies, focusing on structural characteristics of the proglottids and rostellar hooks, were completed based on this series of specimens. The hooks were drawn with the aid of a camera lucida, and measurements were taken from these drawings using a calibrated ruler. Specimens were identified morphologically according to Verster (1969), Rausch (1981) and Loos-Frank (2000). Vouchers for entire specimens, including those that substantiate the definitive identification for

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sequences used in the study have been deposited in the Finnish Museum of Natural History, University of Helsinki, under code MZH 123001-123141.

Molecular identification and phylogenetic analysis

The molecular identification was based on 2 alternative mtDNA regions, namely partial sequences of the cytochrome *c* oxidase subunit 1 (*cox1*) and the NADH dehydrogenase subunit 1 (*nad1*) genes (396 bp and 491 bp, respectively). In addition, for the phylogenetic analysis, the complete *nad1* (894 bp) was sequenced for *T. omissa* and the unknown *Taenia* sp.

DNA extractions, enzymatic amplifications and sequencing were performed as reported previously (Lavikainen *et al*. 2003, 2011). The mtDNA regions were amplified using previously published primers (partial *cox1*: 5′-TTTTTTGGGCATCCTGAGGTTTAT-3′ and 5′-TAAAGAAAGAACATAATGAAAATG-3′ by Bowles *et al*. (1992); partial *nad1*: 5′-AGATTCGTAAGGGGCCTAATA-3′ and 5′-ACCACTAACTAATTCACTTTC-3′ by Bowles and McManus (1993); complete *nad1*: 5′-TATTAAAAATATTGAGTTTGCGTC-3′ and 5′-TCTTGAAGTTAACAGCATCACGAT-3′ by Hüttner *et al*. (2008)). For the partial *cox1* and *nad1*, the sequencing primers were the same as used for the primary PCR. For the complete *nad1*, the PCR primers and also a reverse sequencing primer (5′-CCATTAAACAAGCCTCAAACCT-3′ by Lavikainen *et al*. (2008)) were used.

Sequences were compared with previously published mtDNA data assembled for various species of *Taenia*. For the phylogenetic analyses, the partial *cox1* and complete *nad1* sequences were aligned separately using ClustalW2 (Chenna *et al*. 2003). In addition to the new sequences, 25 previously published *cox1* sequences representing 20 species of *Taenia* (Bowles and McManus, 1994; Okamoto *et al*. 1995; Le *et al*. 2000; Nakao *et al*. 2003, 2007; Jeon *et al*. 2005, 2007; Zhang *et al*. 2007; Lavikainen *et al*. 2008, 2010, 2011; Jia *et al*. 2010; Liu *et al*. 2011; Galimberti *et al*. 2012) and 13 *nad1* sequences representing 9 species of *Taenia* (Le *et al*. 2000; Nakao *et al*. 2003, 2007; Jeon *et al*. 2005, 2007; Jia *et al*. 2010, 2012; Hüttner *et al*. 2009; Liu *et al*. 2011) were included. The GenBank Accession numbers or references for the previously published sequences are presented in Fig. 2. The alignments were manually adjusted. Gaps and codons with ambiguous sites were deleted. The final alignments contained 366 and 891 nucleotides for the partial *cox1* and complete *nad1*, respectively. *Echinococcus oligarthrus* (Diesing, 1863) and *Taenia mustelae* Gmelin, 1790 were used as outgroups for *cox1*, and *E. oligarthrus* for *nad1* data sets. The phylogenetic trees were constructed by the neighbour-joining (NJ) method in PAUP* v4.0b10 (Swofford, 2002) using Kimura 2-parameter distances (Kimura, 1980), and assessed with 10 000 bootstrap replicates. Furthermore, to resolve the phylogenetic relationships of the unknown *Taenia* sp. and the closest taxa, a *nad1* sequence (894 bp) data set of 5 species (see Fig. 2C, *Taenia multiceps* Leske, 1780 as an outgroup) was addressed with the maximum likelihood (ML) method. The substitution model (HKY85 + I) and its parameters were determined for the ML analysis by Akaike Information Criterion implemented in Modeltest 3.7 (Posada and Crandall, 1998). Heuristic search was used to estimate the ML tree, which was tested by bootstrapping with 10 000 replicates.

The new DNA sequences of this study have been deposited in the DDBJ/EMBL/GenBank databases under the Accession numbers JX860621-JX860633.

RESULTS

Molecular identification

Taenia tapeworms were found in two-thirds (201/296) of the lynx, ranging from 1 to 112 worms per animal. The total number of individual worms recovered from all hosts exceeded 2700. Altogether 135 tapeworms from 72 lynx were subjected to PCR-based molecular identification. This is 5% of the *Taenia* tapeworms recovered, and 36% of the lynx infected with *Taenia*. Most of the analysed samples were from central, southern and eastern Finland (Fig. 1) coincidental with the distribution of the harvested lynx.

Two species of *Taenia* were differentiated using mtDNA sequence data. The most abundant species was *T. laticollis* represented by 127 specimens from 67 lynx. In contrast, a second putative species occurred at low prevalence and intensity, being represented by 5 specimens from 4 lynx. These latter cestodes were obviously distinguished from *T. laticollis* by the larger dimensions of the strobila, and did not correspond in mtDNA sequences with any of the reference specimens of this study nor those previously published. In a single case, a mixed infection with both species was confirmed. The lynx harbouring the unknown *Taenia* sp. were shot in western and southern Finland, whereas *T. laticollis* was found in all game management districts (Fig. 1). One specimen, classified as the unknown species macroscopically, remained unidentified due to the ambiguous sequencing result.

Among the reference specimens evaluated in the present study, *T. omissa* and *T. laticollis* showed characteristic sequences differing from previously published mtDNA data for other species of *Taenia*. The sequences of the *T. taeniaeformis* specimens were identical with those of an isolate in a domestic cat from Finland (Lavikainen *et al*. 2008).

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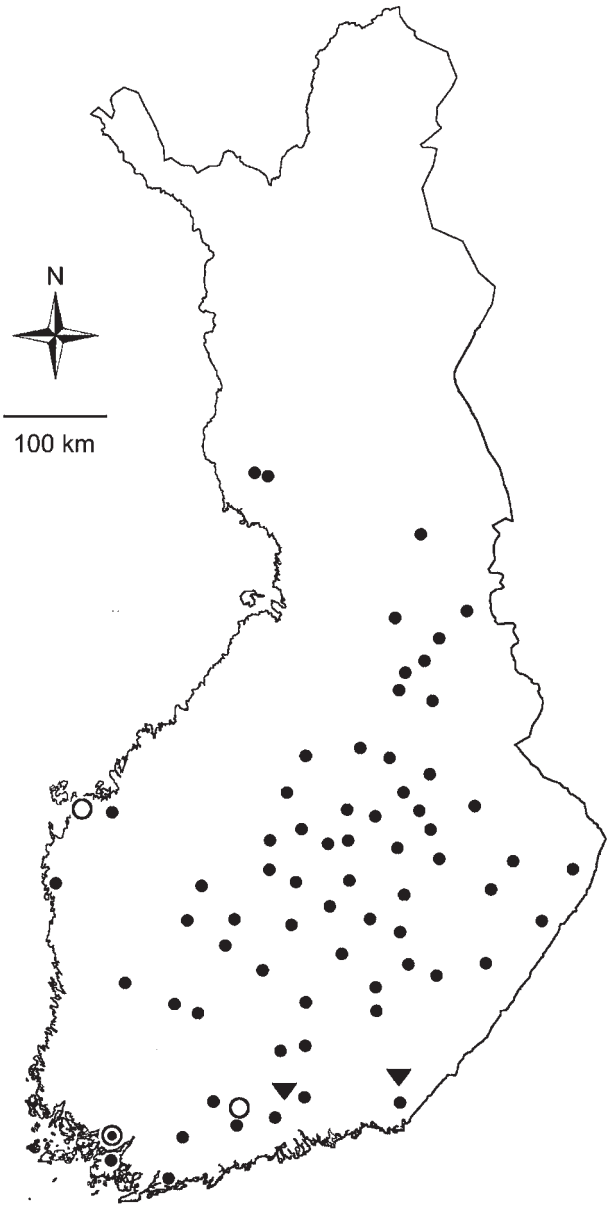

Fig. 1. Geographical origins of the genetically analysed cestode specimens from lynx collected during the hunting season of 2010–2011 in Finland. Lynx individuals infected with identified cestodes are indicated with the following symbols: dot, *Taenia laticollis*; open circle, *Taenia* sp.; open circle with a dot inside, mixed infection with *T. laticollis* and *Taenia* sp.; triangle, Diphyllobothriidae. The origin of one lynx infected with *Taenia* sp. was not documented.

Two specimens were misidentified macroscopically as *Taenia* tapeworms before the molecular identification. One of them aroused suspicion because of its dark colour while another was erroneously classified within the group of *T. laticollis*-like specimens. Both of the tapeworm specimens were short fragments of strobila. The specimens shared identical *cox1* sequences (396 bp), which were very different from those of *Taenia* spp. According to the results of a BLAST nucleotide database search (basic local alignment search tool, http://blast.ncbi.nlm.nih.gov), the *cox1* sequence showed highest similarity (89%) with those of *Sparganum proliferum* (Ijima, 1905) and *Spirometra erinaceieuropaei* (Rudolphi, 1819) (GenBank Accession numbers AB015753 and JQ267473, respectively) suggesting that these specimens may represent a taxon within the family Diphyllobothriidae Lühe, 1910.

Sequence variation and phylogenetic relationships

Taenia laticollis. The identifications of 70 *T. laticollis* specimens were based on the partial sequence of the *cox1* gene. Since several samples remained PCR negative, an alternative region (partial *nad1*) was applied for 57 specimens. Among these, 4 *cox1* haplotypes (designated as A, B, C and D; see Fig. 2) and 3 *nad1* haplotypes (NA, NB and NC) were detected. In addition, a reference specimen of *T. laticollis* had a *nad1* haplotype of its own (ND). The sequence variations were 0·3–0·5% in *cox1* (corresponding to 1–2 nucleotide differences per 396 bp) and 0·2–0·8% in *nad1* (1-4/491 bp).

In the phylogenetic analysis of the *cox1* dataset, *T. laticollis* formed a clade with *Taenia macrocystis* (Diesing, 1850) (Fig. 2A). The branching order among the haplotypes of *T. laticollis*, as well as phylogenetic relationships of *T. laticollis* with *Taenia parva* Baer, 1926 and *T. taeniaeformis*, were not well resolved. The haplotypes of *T. laticollis* differed in *cox1* sequence (366 bp) from *T. macrocystis* by 9·8–10·4%, and from the other analysed *Taenia* spp. by 10·9–15·8%.

Unknown *Taenia* sp. No intraspecific variation was detected in the partial *cox1* sequence (396 bp) of the unknown *Taenia* sp. The complete *nad1* (894 bp) was sequenced only for a single specimen, and thus it is not possible to analyse its intraspecific variation. In the phylogenetic analyses of the partial *cox1* and complete *nad1* sequence datasets, the unknown species was located as a close sister either to *Taenia hydatigena* Pallas, 1766 or to a clade formed by *T. hydatigena* and *Taenia regis* Baer, 1923 (Fig. 2). A well-supported monophyletic group including *Taenia* sp., *T. hydatigena* and *T. regis* formed a clade with *T. omissa*. This clade appears as a polytomy with the other main clades of *Taenia* when nodes with bootstrap support below 50% are ignored.

The nucleotide sequences of *Taenia* sp. differed from those of *T. hydatigena* and *T. regis* by 6·8–7·7% in *cox1* and 8·3–10·4% in *nad1* (Table 1). The level of divergence was similar between *T. hydatigena* and *T. regis*. All 3 species differed from *T. omissa* by more than 10% in the *cox1* sequence and about 20% in *nad1*.

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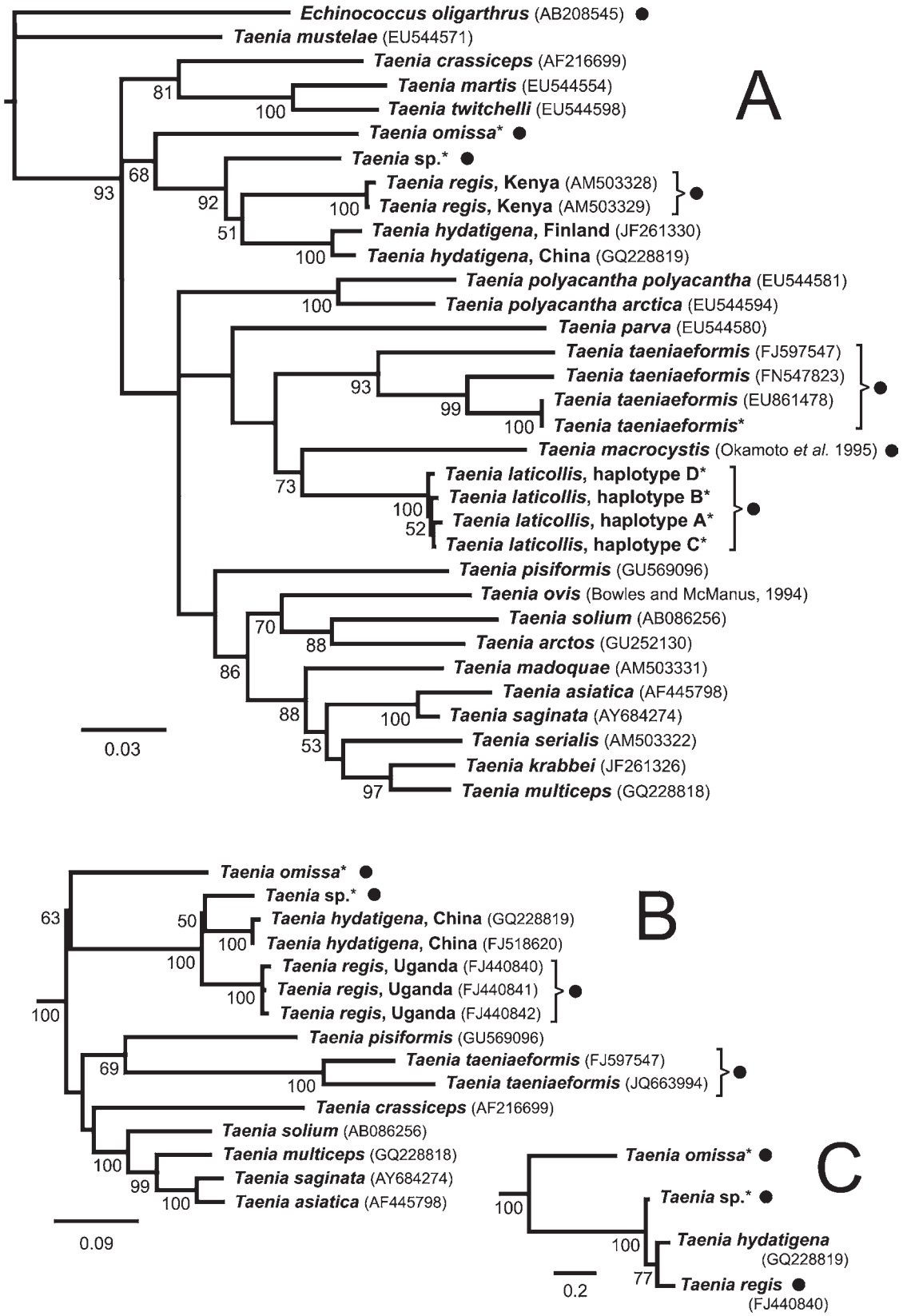

Fig. 2. (A-C) Dendrograms for *Taenia* spp. based on 2 mtDNA genes. (A) Neighbour-joining tree inferred with Kimura 2-parameter distances from the partial *cox1* gene, *Echinococcus oligarthrus* and *T. mustelae* as outgroups. (B) Neighbour-joining tree inferred with Kimura 2-parameter distances from the complete *nad1* gene, *E. oligarthrus* as an outgroup (not shown). (C) Maximum-likelihood tree inferred from the *nad1* sequences of selected taxa, *T. multiceps* as an outgroup (not shown). Accession numbers or references of the previously published sequences are shown in parentheses. The sequences obtained in the present study are marked with an asterisk. Dots indicate species using primarily felids as definitive hosts. Bootstrap values >50% are shown. The scale bars are proportional to the number of substitutions per site.

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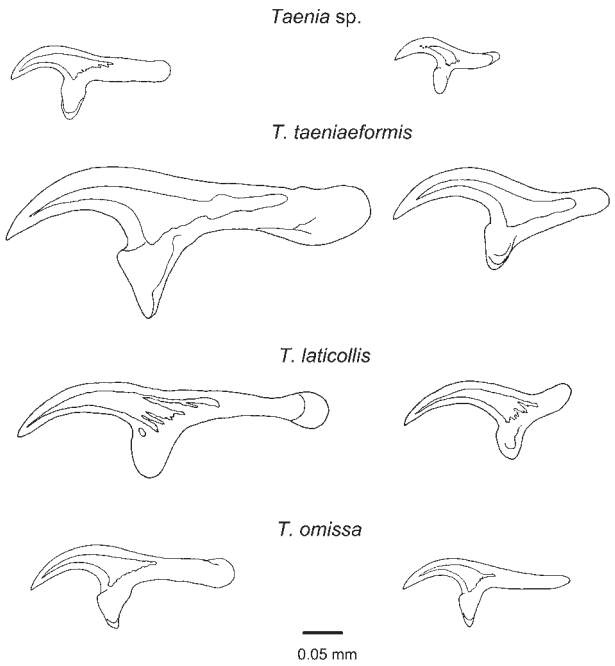

Fig. 3. Large and small hooks of *Taenia* sp., *T. taeniaeformis* and *T. laticollis* from lynx, and *T. omissa* from the cougar.

Morphological characters of Taenia *sp. and differential diagnosis*

Specimens of the unknown *Taenia* sp. were not in adequate condition for morphological examination. Strobilae could not be measured exactly due to the fragmentation of the specimens. The estimated length of the strobila in individual cestodes was at least 100 cm and the maximum width 7–8 mm. The samples were decomposed, and the only internal structures detected were the cirrus sac, terminal vagina and a vaginal sphincter. The cirrus sac is short, not overlapping the ventral longitudinal osmoregulatory canal. The vagina is dilated before the sphincter. Only 3 scoleces with a few rostellar hooks were found. Altogether 5 large and 6 small well-aligned hooks were measured. The large hooks are 202–217 μm and the small 124–132 μm in length. The large hooks are characteristic in shape having a short strongly curved blade and a long guard (Fig. 3). The hooks can easily be distinguished from those of the reference samples (*T. laticollis*, *T. omissa* and *T. taeniaeformis*) by shape and size (Fig. 3).

Taenia sp. can be distinguished from those species that occur in felids across the Holarctic region (Table 2). This includes 9 valid species (Verster, 1969; Loos-Frank, 2000) and 1 species of undetermined status, *Taenia wyolagus* Shults, 1982 (published as a master's thesis), which had passed into oblivion and was omitted from the latest revision of the genus (Loos-Frank, 2000). *Taenia* sp. is distinct from those species by its relatively small rostellar hooks. Dimensions of the small hooks overlap with those of *Taenia pisiformis* (Bloch, 1780) and *Taenia bubesei* Ortlepp, 1938 (considered as a synonym of *T. regis* by Verster, 1969), the latter of which has been recorded once in a Caspian tiger, *Panthera tigris virgata* (Petrov and Potekhina, 1953); the large hooks are clearly different in structure relative to these species. Additionally, 4 species, *T. bubesei*, *T. omissa*, *T. rileyi* Loewen, 1929 and *T. taeniaeformis* have a vaginal sphincter.

Taenia kotlani Murai, Gubányi & Sugár, 1993, which has been described based only on the metacestode, differs from *Taenia* sp. by the hook shape, although the hooks are similar in length; the blade of the large hooks is clearly shorter in *Taenia* sp. Although *T. hydatigena* is genetically closely related and has similar-sized hooks, it differs from *Taenia* sp. by having longer and less curved blades in the large hooks. In addition, *T. hydatigena* lacks a vaginal sphincter. This structure is present in another genetically closely related species, *T. regis*. The measurements of the small hooks of *Taenia* sp. overlap those of *T. regis* if *T. bubesei* is considered conspecific. The large hooks of *T. regis* sensu lato, however, are highly variable in shape (Verster, 1969) and are longer than those of *Taenia* sp.

DISCUSSION

In the current survey, the presence of 2 species of *Taenia* in lynx collected during the hunting season 2010–2011 in Finland was demonstrated by mtDNA sequencing and comparative morphology. *Taenia laticollis*, a lynx tapeworm with a Holarctic distribution (Loos-Frank, 2000), was the numerically dominant species in this material. A second, and apparently undescribed species, was rare, and its unique mtDNA sequences clearly differed from all available sequences of *Taenia* (22 species; including *T. laticollis* and *T. omissa*, sequences of which were obtained in the present study). In addition, this unknown *Taenia* sp. is clearly distinct from the other species of *Taenia* recorded in felids (including *Lynx*) from the northern hemisphere, based on the morphology of the rostellar hooks. In a recent study, this putative new species was not found in wolves (*Canis lupus*) from Finland or Sweden, nor brown bears (*Ursus arctos*) in Finland (Lavikainen *et al*. 2011). In the 4 infected lynx of the present study, the strobilae appeared to be fully developed. Evidently, lynx serve as a primary definitive host for this species. According to the present results, *Taenia* sp. demonstrated here has not been reported previously or described. Well-preserved adult specimens and metacestodes, however, would be needed to further compare this species with the previously described members of the genus.

Consistent with a morphological phylogeny by Hoberg *et al*. (2001), *T. macrocystis* is the closest relative of *T. laticollis* in the present phylogenetic analysis. A close relationship of the new *Taenia* sp.

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Table 1. Pairwise comparison of percentage nucleotide sequence differences in *nad1* (891 bp, above the diagonal) and cox1 (366 bp, below the diagonal) among the unknown *Taenia sp.* and closely related species

Species	*Taenia* sp.	*Taenia hydatigena*[1]	*Taenia regis*[1]	*Taenia omissa*
Taenia sp.		8·3	10·1–10·4	19·4
Taenia hydatigena[1]	6·8–7·1		8·5–9·3	20·1–20·2
Taenia regis[1]	7·4–7·7	6·8–7·4		20·8–20·9
Taenia omissa	10·1	11·7–12·6	10·4–10·7	

[1] Selected previously published sequences, for Accession numbers, see Fig. 2A and B.

with a tapeworm of canids, *T. hydatigena*, and a tapeworm of *Panthera* spp., *T. regis*, is shown in the present analysis. Although closely related, the level of sequence difference, as well as the morphological characters, supports their recognition as distinct species. A monophyletic group containing these 3 species is distantly related to a tapeworm of the cougar, *T. omissa*. The phylogenetic position of *T. hydatigena* among those species of *Taenia* characteristic of felids suggests that host-switching to canids has occurred during the evolutionary history of *T. hydatigena*. Such is consistent with the developing view of the importance of guild structure and foraging habits and host colonization in the context of regional faunas as determinants of diversification among taeniid tapeworms (e.g. Hoberg, 2006).

Both *T. laticollis* and *T. macrocystis* are adapted to a predator–prey relationship between felids and lagomorphs (Rausch, 1981; Zyll de Jong, 1966). The intermediate host of the new *Taenia* sp. remains unknown. The lynx harbouring *Taenia* sp. were from southern and western Finland, where the preferred prey include hares (*Lepus* spp.), European roe deer (*Capreolus capreolus*) and an introduced cervid species, the white-tailed deer (*Odocoileus virginianus*) (Pulliainen, 1981; Pulliainen *et al*. 1995). In contrast, in eastern Finland the mountain hare (*Lepus timidus*) forms the most important part of the diet of the lynx. Judging by the phylogenetic position of *Taenia* sp., the intermediate host might be a ruminant. Close phylogenetic relationships with species parasitizing ruminants, however, do not automatically indicate that similar intermediate hosts should be involved in transmission. For example, *Taenia serialis* (Gervais, 1847), which occurs in lagomorphs as a metacestode, is closely related to species parasitizing ungulates based on phylogenetic trees inferred from both mt and nuclear DNA data (e.g. Zhang *et al*. 2007; Lavikainen *et al*. 2008; Knapp *et al*. 2011).

The prevalence of the putative new species is 1·4% in the present material. The exact prevalence of *T. laticollis* cannot be calculated since only a subsample of the specimens was subjected to molecular identification. The prevalence would be about 66%, if the remaining and morphologically similar specimens all represented *T. laticollis*. In previous studies, *T. laticollis* has been frequently found in *Lynx* spp. from Europe and North America (e.g. Smith *et al*. 1986; Valdmann *et al*. 2004), even occurring as the most common species (Zyll de Jong, 1966). In addition to *T. laticollis* and the new species, *T. taeniaeformis* evidently occurs sporadically in lynx from Finland, as 2 of our reference samples represented this species. Specimens of *T. taeniaeformis*, however, were not found in the material collected in 2010–2011.

The present results contrast with those in the Baltic countries, where *T. pisiformis*, primarily a parasite of canids (Loos-Frank, 2000; Jones and Pybus, 2001), is common in lynx (Kazlauskas and Matuzevicius, 1981; Bagrade *et al*. 2003; Valdmann *et al*. 2004). In Latvia, *T. pisiformis* was detected in all examined lynx (42 individuals), and no other species of *Taenia* were found (Bagrade *et al*. 2003). In Estonia, *T. pisiformis* was also found in all examined lynx (37), but *T. laticollis* was common as well with a prevalence of 41%, and single cases of *T. taeniaeformis* and *T. hydatigena* were detected (Valdmann *et al*. 2004). In 39 lynx from Lithuania, *T. taeniaformis* and *T. pisiformis* were the most common species, and *Taenia crassiceps* (Zeder, 1800), *T. krabbei* and *T. laticollis* were rare (Kazlauskas and Matuzevicius, 1981). In all these studies, the specimens were identified based on morphology. Different diets cannot explain the difference between the lynx tapeworms in Finland and the Baltic countries nearby, since *T. pisiformis* uses the same intermediate hosts as *T. laticollis*, i.e. lagomorphs. Instead, these observations suggest that *T. pisiformis* is absent from Finland and may demonstrate the distinct nature of the helminth fauna in this region of northern Europe. Similar patterns of species diversity for taeniids in wolves have also been observed (Lavikainen *et al*. 2011), perhaps reflecting some general historical and ecological mechanisms that have influenced diversity.

Tapeworms examined during the present study were damaged due to freezing, thawing and processing, and morphological characters were mostly unidentifiable. Such challenges are not uncommon when conducting surveys of helminths among wildlife species. Consequently, we applied a molecular-based method, mtDNA sequencing, for accurate identification of *Taenia* spp. Although mtDNA sequencing has been successfully applied to identification of taeniids (e.g. Zhang *et al*. 2007; Lavikainen *et al*. 2011), it is relatively costly and time consuming,

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Table 2. *Taenia spp*. of felids recorded in the Holarctic region

(Hook lengths, presence of the vaginal sphincter, geographical distribution and hosts (Abuladze, 1964; Verster, 1969; Rausch, 1981; Shults, 1982; Loos-Frank, 2000), and published mtDNA data.)

Species	Large hooks μm	Small hooks μm	Vaginal sphincter	Geographical distribution	Felid definitive hosts	Intermediate hosts	mtDNA data published by
Taenia sp.	202–217	124–132	+	Finland	*Lynx lynx*	Unknown	This study
Taenia bubesei Ortlepp, 1938[1]	223–273	128–180	+	Tadzhikistan (mainly Africa)	*Panthera*	Ruminants	
Taenia krepkogorski (Schultz & Landa, 1934)	265–354	182–222	–	Southwest central Asia	*Felis*	Rodents	
Taenia laticollis Rudophi, 1819	370–420	150–247	–	Holarctic	*Lynx*, *Leopardus*	Lagomorphs	This study
Taenia macrocystis (Diesing, 1850)	297–430	180–247	–	America, Asia	*Lynx*, *Leopardus, Puma*	Lagomorphs, rodents	Okamoto *et al*. (1995)
Taenia omissa Lühe, 1910	223–297	165–223	+	America	*Puma*, *Leopardus*	*Odocoileus*	This study
Taenia pisiformis (Bloch, 1780)	220–300	114–177	–	Cosmopolitan	Felids, including *Lynx* (primarily canids)	Lagomorphs, rodents	e.g. Jia *et al*. (2010)
Taenia pseudolaticollis Verster, 1969	352–415	214–240	–	America	*Leopardus*, *Lynx*	Unknown	
Taenia rileyi Loewen, 1929	238–258	145–198	+	North America	*Lynx*, *Puma*	Rodents	
Taenia taeniaeformis (Batsch, 1786)	300–530	187–360	+	Cosmopolitan	Felids, including *Lynx*	Rodents, lagomorphs	e.g. Okamoto *et al*. (1995) Lavikainen *et al*. (2008)
Taenia wyolagus Shults, 1982	325–362	181–209	?	North America	*Lynx*, *Felis*	Lagomorphs	

[1] Considered as a synonym of *Taenia regis* Baer, 1923, by Verster (1969).

which limits its use when a large number of specimens are under evaluation. In large-scale epidemiological studies, another method, for example multiplex PCR (e.g. Al-Sabi and Kapel, 2011) or restriction fragment length polymorphism PCR (e.g. Hüttner *et al*. 2009) would be more practical, if the sole need is to provide an accurate diagnosis. Sequencing is essential, however, for characterization of an unknown species, phylogenetic analyses, or validation of other molecular methods. For example, recently, an unknown species of *Taenia* was discovered by mtDNA sequencing in brown bears and elk (*Alces alces*) from Finland (Lavikainen *et al*. 2010, 2011). Subsequently it was described as a new species, *Taenia arctos* Haukisalmi, Lavikainen, Laaksonen & Meri, 2011, and was shown to have a considerably broad distribution across the Holarctic. The present discovery in lynx suggests that the true diversity of the genus *Taenia*, although being the focus of extensive studies since the 1700s, is still insufficiently known. Further, our study clearly highlights the need for integrated analyses using molecular and morphological characters, and the assembly of archival collections that form the foundations for assessment of biotic structure and faunal history (e.g. Hoberg *et al*. 2012).

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