Taenia laticollis and a potentially novel Taenia species from the Eurasian lynx (Lynx) in Northwestern China

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1. Introduction

The Eurasian lynx (Lynx), a medium-sized carnivore, distributed sporadically in Europe and Asia (Castelló, 2020). To date, there are at least 13 valid tapeworm species infecting lynx, as reported from Finland, Russia, Turkey, Poland, Canada, Latvia and Estonia, including Taenia pisiformis, Taenia laticollis, Taenia hydatigena, Taenia taeniaformis, Taenia lyciscapreoli, Taenia krubbei, Taenia rileyi, Taenia serialis, Echinococcus multilocularis, Diphyllolocotrium latum, Mesocestoides lineatus, Mesocestoides spp. and Spirometra sp. (S Table 1).

Xinjiang Uygur Autonomous Region (XUAR, northwestern China), covering 1.66 million square kilometers, has numerous mammalian species that can participate in the life cycle of tapeworm species (Ablimit, 2013). For instance, Echinococcus multilocularis and Echinococcus granulosus, causing human echinococcosis, were previously found in red foxes, grey wolves, domestic dogs and wild rodents (Wu et al., 2017; Zhang et al., 2006; Wang et al., 1989; Guo et al., 2021). Recently, three genotypes of “Taenia sp. Rhombonys opimus” were found in the great gerbil (Rhombonys opimus) (Ji et al., 2021). However, data are scarce on wild felids as definitive hosts of Taenia spp. in this region. Therefore, the aim of the present study was to identify tapeworms in Eurasian lynx from XUAR.

2. Materials and methods

2.1. Sample collection

Two Eurasian lynxes were found dead during our field investigation...
on ticks and fleas in the West Junggar Mountains (north region of XUAR, S Fig. 1). One (adult female, #1) was road-killed in 2018. Another (adult male, #2) died due to natural causes in 2019. During a routine necropsy of the small intestine, 9 and 15 tapeworms were collected from lynxes #1 and #2, respectively. All tapeworms were washed in physiological saline prior to morphological identification and DNA extraction.

2.2. Morphological identification

Three representative tapeworms were selected. The scolex, neck and strobila (immature, mature and gravid proglottids) of each individual were cut and stained, respectively. The staining procedure was performed as previously reported (Li and Yang, 2009). Briefly, tapeworm specimens were sequentially fixed with 30%, 50% and 70% ethanol, and stained with acetate carmine. The decolorization was done with hydrochloric acid in alcohol (2 ml hydrochloric acid and 100 ml 70% ethanol). For the dehydration, 80%, 95% and 100% alcohol solutions were used sequentially, and then transparency was ensured with xylene. Finally, the specimens were mounted in Canada balsam. Specimens were identified morphologically according to Verster (1969), Rausch (1981) and Loos-frank (2000).

2.3. DNA extraction and molecular-phylogenetic analyses

A small part of the immature proglottids (0.2g) was ground and treated with proteinase K overnight. Individual DNA was extracted from using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). Molecular identification was performed from all tapeworm specimens based on two genetic markers of their mitochondrial genome: a 450 bp fragment of the cytochrome c oxidase subunit I (cox1) gene and a 526 bp fragment of the 16S rDNA as reported previously (Liu et al., 2011; Ali et al., 2015). Sequences from this study were compared to those in GenBank with the BLASTn program (https://blast.ncbi.nlm.nih.gov).

New sequences were deposited in GenBank (cox1: MW844630, MW8446313 and MW843568; 16S rRNA: MW854635, MW854636 and MW843496). A phylogenetic tree was constructed using the Neighbor-Joining method in MEGA 7.0. Amino acid sequences were compared by DNAMAN software.

3. Results

3.1. Morphological description

Twenty-four tapeworms were divided into two distinct Taenia species according to scolex characteristics. The first species (n = 1) from lynx #2, measuring 8 cm in length and 0.25 cm in width, was identified as Taenia laticollis according to the following morphological characteristics: the diameter of scolex (2016 μm), rostellum (847 μm) and sucker (403 μm), and the number of small rostellar hooks (n = 32). Other measurement data of small rostellar hooks included total length (TL), total width (TW), posterior length (PL), anterior length (AL) and guard number (GL), as shown in S Tables 2 and 3. The second species (n = 23) measured 60.8–67.9 cm in length and 0.51–0.64 cm in width. Based on the diameter of scolex, rostellum and sucker (738–865 μm, 321–329 μm and 192–224 μm, respectively) this species is different from taxonomically related Taenia species according to its definitive hosts, place of collection, the length and shape of the large and small hooks, suggesting that it is a potentially novel species. The above data are shown in Additional files (S Tables 2 and 3 and S PPTX).

3.2. Molecular identification

Analysis of 16S rDNA sequences showed that T. laticollis from this study (GenBank accession no. MW843496) clustered with T. laticollis from Finland (NC_021140) (Fig. 1). Phylogenetic tree of cox1 sequences indicated that T. laticollis (MW843568) collected in XUAR is most closely related to T. laticollis genotype C (JX806023) found in Eurasian lynx in Finland. Sequences of the second Taenia species ( provisionally named as “Taenia sp.”) shared 100% identities between 16S rDNA sequences (MW854635 and MW854636) (Fig. 1) and had two nucleotide substitutions in cox1 sequences (MW846305, MW846313). Due to lack of sufficient number of 16S rDNA reference sequences in GenBank, here their cox1 sequences were used to analyze their genetic diversity and taxonomy. The results showed that i) the cox1 sequences of this Taenia species had 92.93% (368/396 bp) and 92.42% (366/396 bp) sequence identities to T. hydatigena (MW336935) from sheep (Ovis aries) reported in Slovakia, respectively (S Fig. 2); ii) the phylogenetic analysis suggested that this Taenia species is divided into two haplotypes (haplotype-1, n = 15; haplotype-2, n = 8), and forms a sister group to Taenia hydatigena (Fig. 2). Analysis of the COX1 protein amino acid sequences showed that i) these are identical between the two haplotypes of “Taenia sp.”, and ii) “Taenia sp.” shared 98.49% (131/133), 96.99% (129/133), and 97.74% (130/133) identities compared with T. hydatigena (GQ228819), Taenia regis (AB905198) and T. lynciscapreoli (MK905226), respectively (S Fig. 3).

4. Discussion

Here we report a potentially novel Taenia species, provisionally named as “Taenia sp.”, from the Eurasian lynx. This species is phylogenetically closely related to T. hydatigena, and together these form a sister clade to T. regis reported from lion (Panthera leo) in Kenya and T. lynciscapreoli from the grey wolf, Eurasian lynx in Russia, Finland and Poland (Lavikainen et al., 2013a,b; Loos-frank, 2000; Myczka et al., 2020; Haukisalmi et al., 2016; Verster, 1969). Analysis of the COX1 protein amino acid sequences showed that in comparison with T. hydatigena, T. regis and T. lynciscapreoli, “Taenia sp.” has 2–4 amino acids substitutions (S Fig. 3). These findings confirm “Taenia sp.” as a potentially novel tapeworm species, the taxonomic status of which needs to be further clarified by data on morphological characteristics of larvae, the range of definitive/intermediate hosts and geographic distribution.

As previously reported, the definitive hosts of T. laticollis include the Eurasian lynx in Finland and Estonia, the Canada lynx (Lynx canadensis) in Canada, the timber wolf (Canis lupus) and the coyote (Canis latrans) in Canada (Skinker, 1935; Grundmann, 1958; Freeman et al., 1961; Smith
et al., 1986; Lavikainen et al., 2013a,b). Here T. laticollis was found for the first time in Eurasian lynx in China. It’s worth noting that the shape of small rostellar hooks showed slight difference from those of T. laticollis in Finland (S PPTX), although T. laticollis from XUAR shared 100% identity to T. laticollis haplotype C based on cox1 sequences. While four haplotypes (A, B, C and D) of T. laticollis were identified from Eurasian lynx in Finland, it is unclear which of them had the published shape of small rostellar hooks as reported by Lavikainen et al. (2013a,b). In the future, the relationships between the shape of small/large rostellar hooks and haplotypes should be further investigated.

The West Junggar Mountains, between the Tianshan and Altai mountain belts, are located on the western rim of the Gurbantunggut Desert in northwestern China (S Fig. 1), and its altitudes range from 2000 to 3000 m above sea level (Ablimiti, 2013). In this region, Pallas’s cat (Felis manul pallas), Eurasian lynx (Lynx), snow leopard (Uncia uncia), grey wolf (Canis lupus), red fox (Vulpes vulpes), corsac fox (Vulpes corsac), wild rabbit (Lepus capensis), wild boar (Sus scrofa) and several wild ruminant species are indigenous (Ablimiti, 2013), which, as definitive/intermediate hosts, probably play an important role in life cycles of various Taenia species. Here only two tapeworm species, T. laticollis and “Taenia sp.”, were found in two Eurasian lynxes. Therefore, in the future, tapeworms should be investigated systematically from more wildlife species in XUAR.
5. Conclusion

“Taenia sp.” is a potentially novel tapeworm species found in Eurasian lynx. In addition, *T. laticollis* was found in this wild felid for the first time in China.

Declaration of competing interest

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81960379 and 82060297), High-Level Talent Initiative Foundation of Shihezi University (RCZK202033) and Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2020-PT330-003).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.10.001.

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