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

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ABSTRACT

The Eurasian lynx (*Lynx*) is a medium-sized wild cat species distributed throughout Eurasia. There has been no report on *Taenia* species (Cestoda: Cyclophyllidea) infecting this felid in China. In this study, 24 tapeworms were found in two Eurasian lynxes (#1 and #2) in Xinjiang Uygur Autonomous Region (XUAR), northwestern China. Based on the number, measurements and the shape of rostellar hooks, these tapeworms belong to two *Taenia* species. According to the number (n = 32) and length (185–194 μm) of small hooks, the first *Taenia* species (n = 1, found in #2 lynx) was identified as *Taenia laticollis*. Phylogenetically, this species was clustered with *T. laticollis* genotype C (JX860623) based on its cytochrome *c* oxidase subunit 1 (*cox1*) and *16S* r*DNA* sequences. The second *Taenia* species (n = 23, provisionally named as "*Taenia* sp.") may represent a potentially novel tapeworm species, because of its obvious differences in the shape and lengths (174–182 μm, 98–113 μm) of large and small rostellar hooks in comparison with ten taxonomically related species. Molecular and phylogenetic analyses of the *cox1* gene revealed that "*Taenia* sp." has the highest rate of sequence identity (92.93%, 368/396 bp) with *Taenia hydatigena* reported from sheep (*Ovis aries*) in Slovakia. To sum up, a potentially novel tapeworm species, "*Taenia* sp.", is found in Eurasian lynx. In addition, *T. laticollis* was found for the first time in China.

1. Introduction

The Eurasian lynx (*Lynx*), is 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 taeniaeformis*, *Taenia lynciscapreoli*, *Taenia krabbei*, *Taenia rileyi*, *Taenia serialis*, *Echinococcus multilocularis*, *Diphyllobothrium 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 (Abliimiti, 2013). For instance, *Echinococcus multilocularisis* 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. *Rhombomys opimus*" were found in the great gerbil (*Rhombomys 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

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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* r*DNA* 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 (*cox*1: MW846305, MW846313 and MW843568; 16*S* rRNA: MW854635, MW854636 and MW843496). A phylogenic tree was constructed using the Neighbor-Joining method in MEGA 7.0. Amino acid sequences were compared by DNAMAN software.

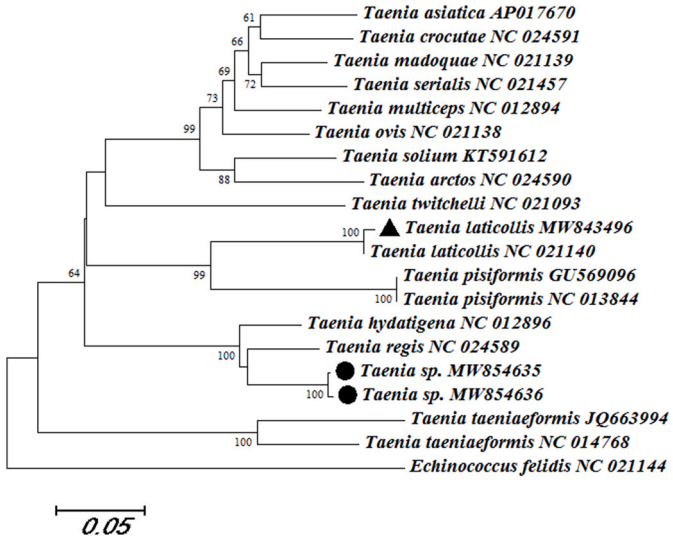

Fig. 1. Phylogenetic relationships of *Taenia* species from two Eurasian lynxes (marked with black circle and triangle) based on *16S rDNA* sequences.

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 length (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). Phylogenic tree of *cox1* sequences indicated that *T. laticollis* (MW843568) collected in XUAR is most closely related to *T. laticollis* genotype C (JX860623) found in Eurasian lynx in Finland. Specimens of the second *Taenia* species (provisionally named as "*Taenia* sp.") shared 100% identities based on 16S *rDNA* sequences (MW854635 and MW854636) (Fig. 1) but 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

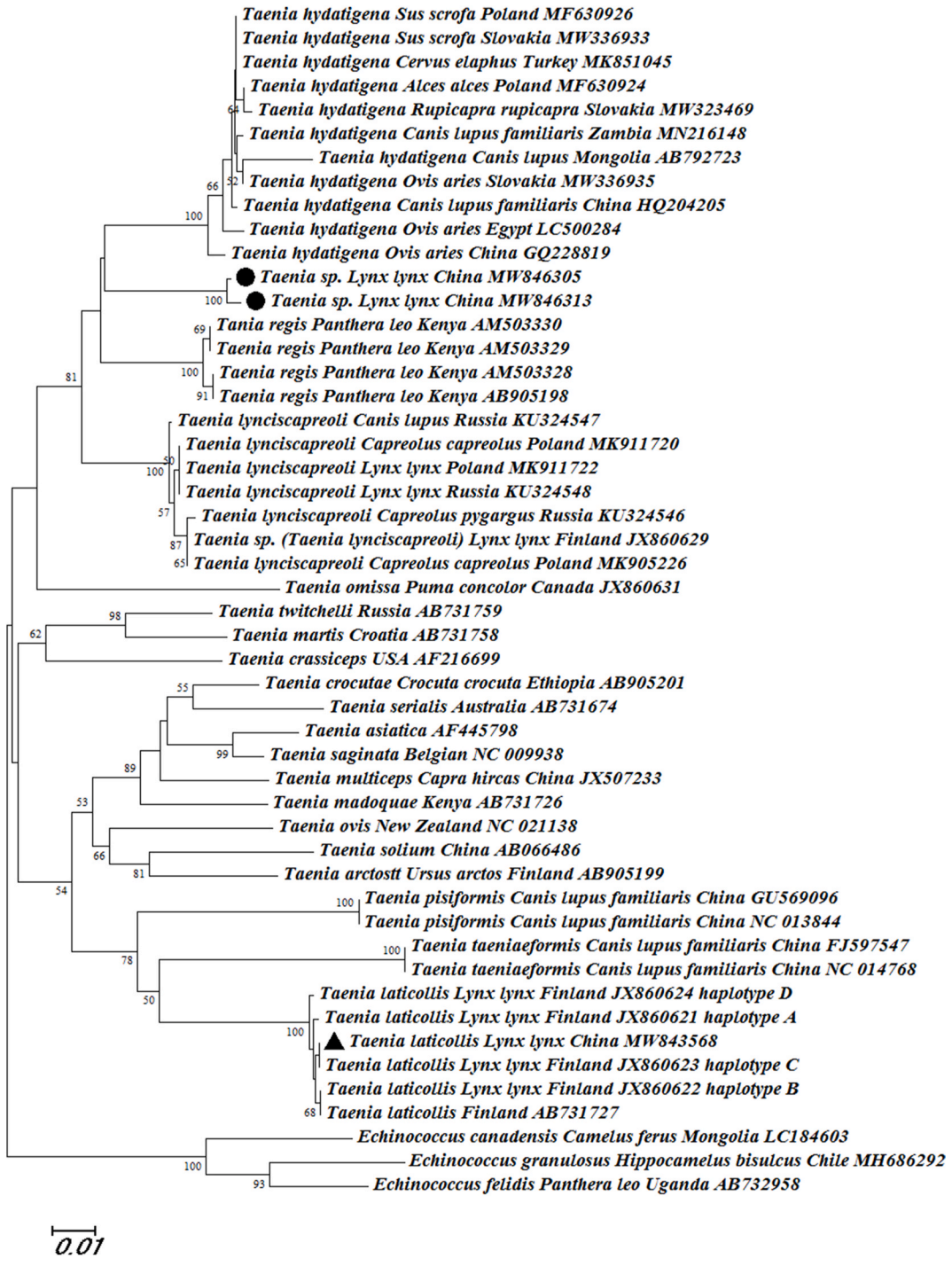

Fig. 2. Phylogenetic relationships of *Taenia* species from two Eurasian lynxes (marked with black circle and triangle) based on *cox1* sequences.

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.

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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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